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2024 REGIONAL SYMPOSIA

September 19–20, 2024

FINAL PROGRAM

- **Northeast: Northeastern University**
- **Midwest: Case Western Reserve University**
- **Southeast: Georgia Institute of Technology**
- **Southwest: University of Texas at Austin**
- **Western: University of Colorado, Denver | Anschutz Medical Campus**
- **Northwest: University of Washington**

Thank You to Our Speakers

PLENARY SESSION I
Thursday, September 19

Dr. Shana Kelley
Northwestern University

PLENARY SESSION I
Thursday, September 19

Dr. Joel Collier
Duke University
(2024 Clemson Award for Basic Research)

PLENARY SESSION II
Thursday, September 19

Dr. Sarah Stabenfeldt
Arizona State University

PLENARY SESSION II
Thursday, September 19

Dr. Danielle Benoit
University of Oregon

PLENARY SESSION III
Friday, September 20

Dr. Elazer Edelman
Massachusetts Institute of Technology
(2024 Founders Award)

PLENARY SESSION III
Friday, September 20

Dr. Cynthia Reinhart-King
Rice University

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2024 REGIONAL SYMPOSIA

September 19–20, 2024

FINAL PROGRAM

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2024 Regional Symposia

Northeast Symposium: Northeastern University

September 19 - 20, 2024

Program Agenda

Thursday, September 19, 2024

8:00 AM - 8:30 AM	Outside Indoor Pit	Registration / Check-in
8:30 AM - 8:45 AM	Indoor Pit	Welcome by Site Chairs
8:45 AM - 10:00 AM	McLeod Suites Room 318, Senate Chambers Room 333, & Meeting Room 340	Concurrent Session I-A: Drug Delivery 1 ***Invited Speaker:*** ***Tim O-'Shea, Boston University***
8:45 AM - 10:00 AM	McLeod 318, Senate Chambers 333 & Meeting Room 340	Concurrent Session I-B: Biomaterials-Interface Interaction 1 ***Invited Speaker:*** ***Jeff Ruberti, Northeastern University***
8:45 AM - 10:00 AM	McLeod 318, Senate Chambers 333 & Meeting Room 340	Concurrent Session I-C: Immune Engineering 1 ***Invited Speaker:*** ***Kara Spiller, Drexel University***
10:00 AM - 11:15 AM	McLeod Suites Room 318, Senate Chambers Room 333, & Meeting Room 340	Concurrent Session II-A: Drug Delivery 2
10:00 AM - 11:15 AM	McLeod 318, Senate Chambers 333 & Meeting Room 340	Concurrent Session II-B: Biomaterials-Interface Interaction 2

Program Agenda

Thursday, September 19, 2024

10:00 AM - 11:15 AM	McLeod 318, Senate Chambers 333 & Meeting Room 340	Concurrent Session II-C: Immune Engineering 2 ***Invited Speaker:*** ***David Mooney, Harvard University***
11:15 AM - 11:30 AM	Indoor Pit	Coffee Break
11:30 AM - 12:45 PM	McLeod Suites Room 322	Plenary Session I: ***Shana Kelley, Northwestern University*** ***Joel Collier, Duke University***
12:45 PM - 1:45 PM	Indoor Pit	Lunch
1:45 PM - 3:00 PM	McLeod Suites Room 318, Senate Chambers Room 333, & Meeting Room 340	Concurrent Session III-A: BioInterfaces ***Invited Speaker:*** ***Shelly Peyton, Tufts University***
1:45 PM - 3:00 PM	McLeod 318, Senate Chambers 333 & Meeting Room 340	Concurrent Session III-B: Regenerative Medicine ***Invited Speaker:*** ***Anita Shukla, Brown University***
1:45 PM - 3:00 PM	McLeod 318, Senate Chambers 333 & Meeting Room 340	Concurrent Session III-C: Biomaterials and Medical Product Commercialization ***Invited Speaker:*** ***Helen Za, Rensselaer Polytechnic Institute***

Program Agenda

Thursday, September 19, 2024

3:00 PM - 4:00 PM	McLeod Suites Room 318	Rapid Fire Presentations
3:00 PM - 4:00 PM	1-Minute Poster Talks in Senate Chambers	1-Minute Poster Talks
4:00 PM - 4:15 PM	Indoor Pit	Coffee Break
4:15 PM - 5:30 PM	McLeod Suite Room 322	Plenary Session II: ***Sarah Stabenfeldt, Arizona State University*** ***Danielle Benoit, University of Oregon***
5:30 PM - 6:30 PM	Robinson Quad Tents	Poster Session
6:30 PM - 7:30 PM	Robinson Quad Tents	Mixer

Program Agenda

Friday, September 20, 2024

8:15 AM - 8:45 AM	Outside Indoor Pit	Registration / Check-in
8:45 AM - 10:00 AM	McLeod Suites Room 318 & Senate Chambers	Concurrent Session IV-A: Engineering Cells and Their Microenvironments 2 ***Invited Speaker:*** ***Chris Chen, Boston University***
8:45 AM - 10:00 AM	McLeod Suites Room 318 & Senate Chambers	Concurrent Session IV-B: Orthopedic Biomaterials 1 ***Invited Speaker:*** ***Treena Arinzeh, Columbia University***
10:00 AM - 10:15 AM	McLeod Suites Room 318 & Senate Chambers	Coffee Break
10:15 AM - 11:30 AM	McLeod Suites Room 318 & Senate Chambers	Concurrent Session V-A: Engineering Cells and Their Microenvironments 2
10:15 AM - 11:30 AM	McLeod Suites Room 318 & Senate Chambers	Concurrent Session V-B: Orthopedic Biomaterials 2 ***Invited Speaker:*** ***Lin Han, Drexel University***

Program Agenda

Friday, September 20, 2024

11:30 AM - 12:45 PM	McLeod Suites Room 322	Plenary Session III: ***Elazer Edelman, Massachusetts Institute of Technology*** ***Cynthia Reinhart-King, Rice University***
12:45 PM - 1:45 PM	Indoor Pit	Lunch
1:45 PM - 3:00 PM	McLeod Suites Room 318 & Senate Chambers	Concurrent Session VI-A: Bioelectric Materials ***Invited Speaker:*** ***Rebecca Carrier, Northeastern University***
1:45 PM - 3:00 PM	McLeod Suites Room 318 & Senate Chambers	Concurrent Session VI-B: Tissue Engineering 1 ***Invited Speaker:*** ***Mehmet Toner, Harvard University***
3:00 PM - 4:15 PM	McLeod Suites Room 318 & Senate Chambers	Concurrent Session VII-A: Nanomaterials ***Invited Speaker:*** ***Samir Mitragotri, Harvard University***
3:00 PM - 4:15 PM	McLeod Suites Room 318 & Senate Chambers	Consurrent Session VII-B: Tissue Engineering 2
4:15 PM - 5:30 PM	Robinson Quad Tents	Student Award Ceremony
5:30 PM - 7:30 PM	Robinson Quad Tents	Mixer

2024 Regional Symposia Chair:

Anita Shukla, PhD. Brown University

Northeast Regional Symposium Co-Chairs:

Ambika Bajpayee, PhD, Northeastern University
Abigail Koppes, PhD, Northeastern University

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Tannin Schmidt, PhD, University of Connecticut

CONCURRENT SESSION I-A: DRUG DELIVERY 1

9:15 AM - 9:30 AM

Intra-joint sustained release of Neosaxitoxin for Long-Term Osteoarthritis Pain Relief

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INTRODUCTION: Osteoarthritis (OA) induces chronic pain with an upregulation of inflammatory mediators by activating synovial joint sensory nerves and nociceptors1. Specific voltage-gated sodium channels on nociceptors are potential therapeutic targets for chronic OA pain relief. Neosaxitoxin (NSTX), a small hydrophilic paralytic shellfish toxin can suppress pain signaling by blocking Nav1.7 channels on nociceptors2. Lower dosage of NSTX has similar analgesic effect and potentially fewer side effects than anesthetics like bupivacaine and lidocaine3. However, its efficacy in OA pain management has never been clinically evaluated, and its rapid clearance from joints post-intra-articular (IA) administration limits its therapeutic duration4,5. Here we examine NSTX's pain-suppressive capabilities in post-traumatic OA (PTOA) animal models, employing Poly (lactic-co-glycolic acid) (PLGA) microparticles for sustained release, with an innovative addition of alginate for improved encapsulation of NSTX and surface modification with Avidin for increased in vivo joint retention.

RESULTS: Our in vivo studies exhibited a short pain relief effect lasting 1–2-hour post-IA administration of free NSTX in DMM mice and ACLT rats, highlighting the necessity for prolonged drug delivery strategies. Alginate-PLGA microparticles with an average diameter of 9.9 ± 5.8 µm, demonstrated a remarkable 92.6% encapsulation efficiency for NSTX when incorporating 2% alginate. This drug-delivery-system achieved a sustained NSTX in vitro release over a month, with 4.1% burst release, maintaining their bioactivity comparable to its unencapsulated form. Surface modification with Avidin reversed the microparticles' charge from -24.2 ± 2.9 mV to +8.4 ± 0.3

mV, enhancing retention in rat joints and indicating a successful strategy for extending NSTX's therapeutic in OA pain management.

SIGNIFICANCE: NSTX can effectively suppress pain in PTOA rodent models, however, suffers rapid clearance from the joint. Alginate-PLGA microparticles can enhance its intra-joint retention time and enable controlled release for long-term pain relief with a single dose. The ongoing experiment focuses on evaluating the pain relief performance of Avidin-Alginate-PLGA-NSTX in in vivo experiments with dose-dependent studies.

REFERENCES: [1] Miller+, Cytokine 2014 [2] Stevens+, Frontiers in pharmacology 2011 [3] Neal+, Reg Anesth Pain Med 2012 [4] Bajpayee+, Nature Rheum 2017 [5] Evans+, Nature Rheum 2013

ACKNOWLEDGEMENTS: NIH Tufts CTSI Award #5UL1TR002544, NIH R01AR077019, P30AR079206

Concurrent Session I-A: Drug Delivery 1
9:30 AM - 9:45 AM

Engineering potent mRNA vaccines through innovation in delivery material and antigen design

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Infectious diseases remain the biggest threat to public health claiming thousands of lives annually, hence development of protective vaccines is a global priority. Strategies that can address challenges such as viral resistance, waning immunity, pan-strain efficacy and reactogenicity (adverse reactions), can offer lasting and sustainable resolution to this issue. Notably, success of COVID-19 vaccines highlights the potential of LNP-mRNA technology as a versatile platform for rapid and scalable vaccine development. These emerging vaccine platforms typically comprise of two major components - (i) mRNA sequence for antigen production, and (ii) lipid nanoparticles for RNA delivery. While mRNA transcripts can be engineered to generate novel antigen constructs, LNPs can be engineered to overcome delivery challenges and generate desired adaptive immune response. Here, we have taken a multi-pronged approach to develop next-generation vaccines through innovation in both delivery vehicle and mRNA antigen design. To develop potent lipid nanoparticles, we have utilized combinatorial chemistry to generate a vast array of novel ionizable lipids which exhibit high mRNA transfection in vivo upon intramuscular administration. Subsequently, we optimized the molecular structure of top ionizable lipid using medicinal chemistry approach and perform structure-immunogenicity studies to generate potent vaccine LNP formulations. These LNPs generate 10-100-fold higher humoral and cellular response as compared to FDA approved LNP formulations. This superior immune response was attributed to enhanced antigen production combined with the immunostimulatory nature of the ionizable lipids that generated strong and persistent germinal centers, necessary for a stronger immune response. Subsequently, we vaccinated humanized mice using a germline targeting immunogen which generated 5-10-fold higher bnAb (broadly neutralizing antibody) precursor B-cells as compared to clinically used immunogens. Finally, we designed novel mRNA sequences encoding networked peptide epitopes to generate T-cell based vaccines. By varying the copy number for each epitope in these cassettes, we were able to generate potent cellular response against sub-dominant viral epitopes. Overall, our study offers novel insights into engineering of delivery vehicles and antigen design to develop mRNA-vaccines that generate potent and durable immunity against multiple infectious diseases.

Concurrent Session I-A: Drug Delivery 1
9:45 AM - 10:00 AM

Charge-Reversed Exosome mediated mRNA delivery for Cartilage Targeting and Repair

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INTRODUCTION: Osteoarthritis (OA) is a chronic degenerative joint condition characterized by pain, inflammation, and cartilage degradation1. Previous studies have leveraged the negative fixed charge density of cartilage to develop a specialized arginine-rich short-length cationic peptide carrier with a net charge of +14 with superior transport and retention in cartilage2. Exosomes (Exos), being native lipid nanoparticles (LNPs), possess inherent therapeutic potential and exhibit lower immunogenicity3. However, their relatively large size (40-200 nm) and negatively charged lipid bilayer impede effective penetration into the deep layers of negatively charged cartilage. We anchored cartilage-targeting cationic peptide on the surface of Exos to modify their surface charge using buffer pH as a charge-reversal switch. In this study, we employed eGFP mRNA as a proof-of-concept to assess the effectiveness of cationic Exos as gene delivery vehicles. Additionally, we utilized IL-1Ra mRNA as a disease-modifying gene, delivered via cationic Exos, and demonstrated their efficacy in mitigating cartilage degradation.

RESULTS: We successfully reversed the surface charge of Exos from -25.4 ± 1.3 to -2.5 ± 1.5 mV, with no observable change in their size. Cationic Exos exhibited full-thickness cartilage penetration in an OA mouse model. Importantly, our research demonstrated the promise of utilizing cationic Exo for efficient mRNA delivery, as evidenced by the delivery of eGFP/IL-1Ra mRNA in mouse joints and OA-conditioned human cartilage explants. Additionally, levels of prostaglandin E2 confirmed potential disease-modifying effects of IL-1Ra mRNA in OA-conditioned human cartilage.

SIGNIFICANCE AND FUTURE DIRECTIONS: Cationic Exos have the capacity to accommodate sizable nucleic acids and facilitate the precise transport of eGFP/IL-1RA mRNA to chondrocytes in the deep layers of cartilage tissue. Consequently, cationic Exos exhibit promise as an optimal non-viral vehicle for delivering genes aimed at modifying diseases. We plan to evaluate the

efficacy of IL-1RA mRNA-loaded cationic Exos in a medial meniscus transection (MMT) rat model of post-traumatic OA.

REFERENCES: [1] Bajpayee+, Nature Rheum 2017; [2] Vedadghavami+, Acta Biomater 2019; [3] Tian+, Nature BioMed Eng 2021

ACKNOWLEDGMENTS: NIH NIBIB Trailblazer R21 (EB028385-01) and NIAMS (P30AR079206)

CONCURRENT SESSION II-A: DRUG DELIVERY 2

10:00 AM - 10:15 AM

WITHDRAWN

10:15 AM - 10:30 AM

Dual coil-coiled protein domain mimic and drug delivery vehicle for SARS-CoV-2

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Since severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) depends on the helical protein-protein interaction (PPI) between the N- terminal α-helix of angiotensin converting enzyme 2 (ACE2) and the SARS-CoV-2 receptor binding domain (RBD), it has become a prime target for the creation of protein domain mimics (PDMs). Our latest creation, ACE-MAP, is a multivalent assembled protein based on ACEBINDER (Britton et al., 2022) that is dependent on the fusion of a binding domain with the coiled-coil domain of cartilage oligomeric matrix protein (COMPcc) via a kinked rigid linker (Britton et al., 2024). We develop ACE-MAP-2 for resilient binding across SARS-CoV-2 variants, such as D614G, B.1.617.2, BA.2, and XBB1.5, using an optimized binding sequence, ACEBINDER2, and an optimized kinked linker for greater solvent exposure. Furthermore, we show that ACE-MAP-2 can be employed in concert with other strategies to neutralize SARS-CoV-2 by utilizing its coiled-coil pore for small molecule encapsulation of ritonavir. This gives ACE-MAP-2 the dual function of acting as a PDM antagonist and a drug delivery system. These qualities of ACEMAP-2 allow it to have distinct but

similar characteristics to ADCs in situations when there are no antibody-drug conjugates for SARS-CoV-2. These properties allow ACE-MAP-2 to encapsulate and deliver a targeted therapeutic payload without the need for covalent linkage.

Concurrent Session II-A: Drug Delivery 2
10:30 AM - 10:45 AM

Dual stimuli-responsive silver loaded nanoparticles eliminate Staphylococcus biofilms

Zhaowei Jiang, Brown University, Zhaowei Jiang 1, Yingying Wang 2 and Anita Shukla 1,*

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Staphylococcus biofilm infections pose a significant challenge due to their prevalence and resilience. Nanoparticles (NPs) are promising drug-delivery vehicles for the targeted delivery of antimicrobials into biofilms. In this work, we engineered gelatin nanoparticles (GNPs) that exhibit pH- and bacterial enzyme-triggered drug release, resulting in excellent antibiofilm efficacy. The GNPs are hypothesized to degrade by bacterial gelatinases at the site of infection, releasing the therapeutic payload. Silver nanoparticles (AgNPs) were synthesized within the gelatinase-responsive core, serving as a broad-spectrum antibiotic alternative. To enhance antibiofilm properties, we conjugated deoxyribonuclease I (DNase) to the GNPs to form the complete responsive NP formulation (R-GNP). DNase degrades extracellular DNA, a major component of the biofilm matrix. To ensure stability, we conjugated a pH-degradable poly(ethylene glycol) derivative to the DNase-coated GNPs. We hypothesized that the low pH at the biofilm site would cause the coating to be shed, exposing the DNase. Dynamic light scattering measurements showed an increase in NP hydrodynamic diameter with the addition of AgNP in the GNPs and a further increase with surface functionalization. Transmission electron microscopy confirmed the successful formation of AgNPs within the GNPs, while inductively coupled plasma-optical emission spectrometry suggested 17.7 ± 0.1 % (w/w) Ag ions within the R-GNPs. The conjugation of DNase was confirmed by monitoring plasmid degradation upon incubation with the R-GNPs via gel electrophoresis, while the conjugation and pH-responsiveness of the co-polymer were verified via Fourier-transform infrared spectroscopy, following overnight incubation of NPs in an acidic pH. Antibiofilm efficacy of the NP formulations was evaluated using Staphylococcus aureus 25923 (SA), Staphylococcus epidermidis 12228 (SE), and Pseudomonas aeruginosa PA01 (PA). SA and SE form acidic biofilms, while PA forms basic biofilms, and only SA and PA produce gelatinases. The R-GNPs led to significant eradication of SA and SE biofilms, with an 80% reduction in biofilm biomass at 32 μg/mL for SA and SE, and 128 μg/mL for PA. In a murine superficial S. aureus biofilm infection model, treatment with R-GNPs led to a significant reduction

in bacterial burden compared to non-treated controls. This study introduces a stimuli-responsive nanoparticle treatment as a novel strategy to combat bacterial biofilms.

Concurrent Session II-A: Drug Delivery 2
10:45 AM - 11:00 AM

Milk Exosomes Anchored with Hydrophilic and Zwitterionic Motifs Enhance Mucus Permeability for Applications in Oral Gene Delivery

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INTRODUCTION: Exosomes have emerged as a promising tool for drug and gene materials delivery, owing to their biocompatibility and non-immunogenic nature1,2. However, challenges persist in achieving successful oral delivery due to their susceptibility to degradation in the harsh gastrointestinal (GI) environment and impeded transport across the mucus-epithelium barrier1,3. To overcome these challenges, we have developed high-purity bovine milk exosomes (mExo) as a scalable and efficient oral drug delivery system, which can be customized by incorporating hydrophilic and zwitterionic motifs on their surface with click-chemistry-based post-insertion technique4. PEGylation and mucin-mimicking peptide (MP) was introduced to increase the hydrophilicity of mExo and prevent hydrophobic interactions. Alternating peptide (AP), block peptide (BP), and DLPC zwitterionic motifs were anchored on the mExo surface to imitate mucus-penetrating viruses.

RESULTS: Surface-modified mExo significantly improved transport rates by 2.5–4.5-fold in native porcine intestinal mucus after the introduction of hydrophilic and zwitterionic surface modifications, as demonstrated by transwell setup and fluorescence recovery after photobleaching (FRAP) analysis. Remarkably, mExo functionalized by BP, consisting of cationic and anionic amino acids arranged in blocks at the two ends, demonstrated superior tolerability in the acidic gastric environment (with a protein recovery rate of 84.8 ± 7.7%) and exhibited a 2.5-fold increase in uptake by intestinal epithelial cells. Both mExo and mExo-BP demonstrated successful intracellular delivery of functional siRNA, resulting in up to 65% suppression of the target green fluorescence protein (GFP) gene expression at a low dose of siRNA (5 pmol) without causing significant toxicity.

SIGNIFICANCE: Here we demonstrated that surface modification of mExos with hydrophilic and zwitterionic motifs confers particle stability, enhances permeability through intestinal mucus, and improves uptake by intestinal epithelial cells. mExo-BP has significant potential to serve as a stable oral drug delivery system with high bioactivity. In future work we will introduce enteric coatings on mExo surfaces.

REFERENCE: [1] Warren+, Biomater. Sci. 2020 [2] Luan+, Acta Pharmacol. Sin. 2017 [3] Lock+ Adv. Drug Delivery Rev. 2018 [4] Zhang and Zhang+, Biomater. Sci. 2023

ACKNOWLEDGEMENTS: NSF Career Award 2141841 and NIH Trailblazer R21 grant EB028385

CONCURRENT SESSION II-A: DRUG DELIVERY 3

11:00 AM - 11:15 AM

Mucin mimics and impacts the function of polymeric inhibitors in stabilizing drug supersaturation.

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Many drugs entering clinical trials today rely on supersaturating formulations such as amorphous solid dispersions (ASD) to enhance the bioavailability of poorly water-soluble active pharmaceutical ingredients (API). The in vivo performance of these formulations is often investigated through biorelevant dissolution testing using simulated intestinal fluid. Often overlooked in biorelevant dissolution is the presence of mucus within the intestinal environment and its possible role in affecting formulation performance. In this study, the impact of mucus on the precipitation of weakly basic and acidic model compounds from their supersaturated solutions was investigated using commercially available porcine mucin type (III). A combination of analytical approaches including high performance liquid chromatography and polarized light microscopy were used to evaluate the kinetics of crystallization from supersaturated solutions generated by the solvent shift approach. Crystal growth rate was quantified by measuring the rate of de-supersaturation in the absence and presence of mucin and polymeric additives.

The presence of mucin within the supersaturated environment was demonstrated to significantly alter the rate of drug precipitation in vitro. The impact of mucin on precipitation was then compared to commercially used polymer precipitation inhibitors hydroxypropyl methyl cellulose (HPMC) and Kollidon® VA 64 which are commonly used in ASD formulations. Surprisingly, mucin at concentrations as low as 0.2% (w/v) was able to significantly reduce drug precipitation similar to polymer precipitation inhibitors. Additionally, we observed that the presence of mucin in the supersaturated environment altered the precipitation inhibitory effects of HPMC and Kollidon® VA64, suggesting that mucin could play an important and complicated role in formulation performance in the intestine.

This work suggests an important role of mucus in formulation performance which is largely overlooked in biorelevant dissolution systems used to evaluate today's formulations. A rational

modification to common biorelevant dissolution testing to include appropriate mucus activity is critical for developing supersaturated drug delivery formulations and improving the prediction of drug precipitation risk.

CONCURRENT SESSION III-A: BIOINTERFACES

2:15 PM - 2:30 PM

Development of a Biohybrid Tendon Interface for Engineered Muscle

Nicolas Castro, MIT, Nicolas Castro, MIT, castrnx@mit.edu

Unlike metal and plastic, biological materials can communicate with their surroundings, adapt to stimuli, and self-repair damage. Incorporating these materials into engineered systems could foster smarter, more adaptable machines. We have shown that engineered skeletal muscle stretched around an elastomer 'skeleton' can generate force and drive locomotion. However, the interface between the biotic and abiotic components of this robotic system are friction-based, leading to inefficient force transmission. In the body, muscle is covalently tethered to bone via tendons, which efficiently transmit force. Thus, we have developed a bioinspired synthetic tendon to act as a biohybrid interface, enabling the design of more modular and adaptive biohybrid machines.

We developed a tough adhesive hydrogel tendon, composed of a poly(acrylic acid) hydrogel functionalized for tissue adhesion with N-Hydroxysuccinimide ester groups, in collaboration with the Zhao Lab at MIT. Muscle tissues were manufactured from C2C12 mouse myoblasts seeded into a fibrin and Matrigel matrix. Peel tests of these differentiated and undifferentiated muscle tissues bound to the synthetic tendon revealed that the biotic-abiotic interface could withstand forces >500mN before breaking. This is significantly greater than those generated from the contraction of engineered muscle (~300uN), demonstrating a robust binding. A cell viability assay and pH exposure test confirmed that the synthetic tendon had no significant impact on muscle health, indicating general biocompatibility.

Furthermore, we have been able to bind strips of mature engineered skeletal muscle tissue between two strips of synthetic tendon, akin to myotendinous junctions in vivo. We have leveraged this tendon-muscle-tendon (TMT) construct as a modular actuator that can be mechanically coupled to robotic skeletons to generate force and produce motion. We have evaluated the effects of varying synthetic tendon stiffness and preload tension on the force production capability of these units, enabling the optimized design and deployment of TMT actuators in untethered machines. In short, we have developed a hydrogel tendon system that serves as a robust biocompatible musculoskeletal interface. In addition to robotics, we anticipate the future application of our system as implantable muscle grafts with suturable tendons to repair injured and diseased muscle in vivo.

Concurrent Session III-A: BioInterfaces
2:30 PM - 2:45 PM

Piezo1 Facilitates Shear-Stress-Priming of the NLRP3 Inflammasome

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The effect of shear stress on cells is widely studied in literature where it alters cell morphology, cytokine secretion, and gene expression, however, little is known about how shear stress plays a role in inflammatory responses of innate immune cells. No studies have mechanistically investigated how physiologically relevant shear stress magnitudes in a microfluidic device system can prime NLRP3 inflammasome activation. We utilized qPCR for observing changes in the gene expression of NLRP3-associated proteins and a small-molecule inhibitor to determine the mechanism through which shear stress induces priming for NLRP3 inflammasome activation. In this study, we aim to gain a deeper understanding of how shear stress regulates the inflammatory response in innate immune cells.

To determine the effect of shear-stress exposure on NLRP3 inflammasome activation, iBMDMs were primed with physiologically relevant magnitudes of shear stress followed by nigericin treatment. We discovered that ASC-CFP speck formation was positively correlated to shear stress magnitude in wild-type cells, with no significant changes in knock-out cells. This observation indicated that NLRP3 and caspase-1 are both necessary in the mechanism of shear-stress-primed NLRP3 inflammasome activation in macrophages. The mechanism of shear-stress-primed NLRP3 inflammasome activation was investigated through performing qPCR which determined an increase in the relative expression of known players for NLRP3 inflammasome activation. Additionally, we performed an inhibitor study using Dooku1, a well-known inhibitor of mechanosensitive ion channel Piezo1, where 50 μM treatments showed significant reductions in the formation of NLRP3 and Piezo1 gene expression.

In conclusion, we determined the mechanism through which shear stress can selectively prime for NLRP3 inflammasome activation. We supported these observations with imaging, knock-out cell studies, IL-1β ELISA, qPCR analysis, and a study with a Piezo1 inhibitor indicating the involvement of both NLRP3 and Piezo1 in shear stress priming of the NLRP3 inflammasome. This study determined that physiologically relevant shear stress exhibits a positive correlation on intracellular signaling mechanisms and the activation of the inflammatory response in innate immune cells.

Concurrent Session III-A: BioInterfaces
2:45 PM - 3:00 PM

Biodegradable Polyester Coacervates and Electrospun Polyester Mats for Topical Hemorrhage Control

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Non-compressible hemorrhage accounts for 90% of deaths following traumatic injury in military settings. Hemorrhage control products need to stop the bleeding in <3 minutes in a military setting and less than 40 minutes in civilian settings and is easy to use by first responders. Ideal hemostatic biomaterials mimic, amplify, and leverage natural hemostatic mechanisms. They should ideally be biocompatible, and biodegradable to avoid cumbersome operative removal of these materials. Ease of use, low cost, and storage stability are other considerations for translational purposes. Existing hemostatic materials do not meet all the above requirements.

In this study, we have fabricated biodegradable polyester electrospun mats and coacervating polyester tissue adhesives. We are investigating the effect of these materials with diverse chemistries in accelerating the clotting cascade and crosslinking components of blood and tissue to form an adhesive hemostatic plug. We hypothesize that selected functional groups combined with specific geometrical features of the fibers will address hemorrhage. Electrospun mats are chosen due to their high surface area, porosity, possibility to control fiber diameters, and their material resemblance to widely used gauze products. The coacervates are chosen as tissue adhesives due to their low interfacial tension which will help them flow into wound crevices. Our current in vitro results show that the mats accelerate the clotting cascade while our in vivo experiments show demonstrate that both material types reduce bleeding time individually and in combination.

CONCURRENT SESSION IV-A: ENGINEERING CELLS AND THEIR MICROENVIRONMENTS 1

9:15 AM - 9:30 AM

Matrix Stiffness and Viscoelasticity Influence Human Mesenchymal Stem Cell Immunomodulation

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Chronic, non-healing wounds are a growing financial and medical burden, with the global wound care market valued at approximately $20B in 2022. Human mesenchymal stem cells (hMSCs) implanted at the wound site are an attractive therapy for chronic, non-healing wounds due to their immunomodulatory behavior. However, hMSCs have high heterogeneity, low viability, poor engraftment, and a risk of eliciting an immune response, thereby reducing therapeutic potential. Prior studies have demonstrated that substrate stiffness and viscoelasticity have been shown to modulate hMSC differentiation. However, the effects of stiffness and viscoelasticity on hMSC immunomodulation remain largely unknown. In addition, the mechanisms by which these biophysical cues affect immunomodulation have yet to be elucidated. Thus, we seek to elucidate the synergistic effects of substrate stiffness and viscoelasticity on hMSC immunomodulatory behavior using highly tunable polyacrylamide (PAAm) gels. Three gels were fabricated, a low-stiffness gel, a medium-stiffness gel, and a high-stiffness gel, with expected Young's Moduli (E) of 1.16 ± 0.54, 4.47 ± 1.19, and 8.73 ± 0.79 kPa, respectively. To impart viscoelastic properties to the gels, linear acrylamide was fabricated and mixed with the acrylamide and bis-acrylamide monomer mixture prior to gelation. Mechanical characterization confirmed that observed Young's moduli match expected values for both non-viscoelastic and viscoelastic gels. The storage modulus, which relates to the elastic behavior of materials, increases proportionally with Young's modulus, and the loss modulus, related to the viscous behavior of materials, increases, as expected, from the non-viscoelastic to viscoelastic low- and medium-stiffness PAAm gels (40.1 to 110 and 109 to 190 Pa, respectively; n=1). The PAAm gels were found to be cytocompatible, and F-actin and nuclei staining demonstrated noticeable differences in cell morphology. As differences in hMSC morphology are known to affect hMSC behavior, this demonstrates the potential for variations in immunomodulatory potential due to differences in hMSC interactions with the different PAAm gels. Current work is focused on quantifying viscoelastic properties, viability, focal adhesion formation, morphology changes over time, and immunomodulatory potential changes over time by investigating the production of immunomodulatory cytokines by hMSCs cultured on these gels.

Concurrent Session IV-A: Engineering Cells and Their Microenvironments 1
9:30 AM - 9:45 AM

Soft Poly(ethylene) Glycol Hydrogels Support Morphogenesis of 3D Cultured Human Pluripotent Stem Cells

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Models of the peri-implantation embryo assembled from human pluripotent stem cells (hPSCs) have enjoyed a recent explosion in popularity, as these cells can capture developmental events that are impossible to study in vivo. However, most models rely on matrigel to provide a 3D microenvironment, which is poorly defined, varies between batches, and does not permit tuning of biochemical or physical cues, such as cell-matrix adhesion or matrix stiffness. While models assembled in chemically defined matrices have been established, all current methods rely on matrix adhesion to drive hPSC morphogenesis. To create a more modular platform for investigating how matrix cues influence early development, we first designed a “blank-slate”, non-adhesive hydrogel matrix through the crosslinking of PEG thiol and acrylate macromers, which could support primed hPSC morphogenesis into lumenal, epiblast-like structures without additional biochemical cues. To better understand the role of matrix stiffness in primed hPSC morphogenesis, we next encapsulated cells into soft (~250 Pa), intermediate, (~1000 Pa), and stiff (~3000 Pa) hydrogels. Aggregates formed under these different matrix stiffnesses were then characterized for their viability, prevalence of epiblast morphological and molecular characteristics, and germ-layer differentiation capacity. Matrix stiffness was found to influence aggregate growth and morphogenesis, with soft and intermediate stiffness hydrogels yielding the highest aggregate viability. Intermediate stiffness hydrogels were found to be the most conducive to lumenogenesis, as aggregates cultured in these matrices had the highest rate of lumen formation and expansion. While aggregates in soft and intermediate hydrogels underwent apico-basal polarization, aggregates in stiff hydrogels did not. Immunofluorescence revealed that aggregates from intermediate hydrogels expressed stemness markers after six days of culture. Like the epiblast, these aggregates could further differentiate into ectoderm, mesoderm, and endoderm progenitors. As our matrices were able to support hPSC viability, lumenogenesis, pluripotency, and differentiation, they hold potential as a platform for modeling the peri-implantation epiblast. Observed differences in lumen morphology suggest that matrix biophysical cues affect lumenogenesis, the mechanisms of which warrant further investigation. As Michael-addition chemistry allows the facile incorporation of adhesion ligands, this platform can additionally be used to investigate the role of biochemical cues in hPSC morphogenesis.

Concurrent Session IV-A: Engineering Cells and Their Microenvironments 1
9:45 AM - 10:00 AM

Differential Gene Expression of Patient Circulating Tumor Cells on Engineered Biomaterials

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Introduction: Circulating tumor cells (CTCs) isolated from the bloodstream represent a "missing link" between primary tumors and metastatic colonies, and may yield new insights into "homing" and "dormancy" at preferred dissemination sites. Estrogen receptor positive breast cancers preferentially metastasize to bone, with some metastases to liver and lung, which represent profoundly different metastatic niches with varied matrix, stromal, and immune components. In order to understand how CTC gene expression and mechanophenotype are regulated by matrix or stromal interactions, we seek to reverse engineer the metastatic niche using a tissue-mimetic microenvironment.

Methods: Patient-derived breast cancer CTC lines were kindly provided by S. Maheswaran, and isolated from metastatic breast cancer patients with informed consent and IRB approval. CTC lines were cultured in suspension on Ultra Low Adhesion (ULA) plates or on lung, bone, or liver decellularized extracellular matrix (ECM) in a 96-well plate for four days, with regular imaging using confocal microscopy. After four days, CTCs were isolated from the media and ECM, using collagenase, then sent for RNA-seq at Genewiz / Azenta. Gene expression profiling was performed using over representation analysis (ORA) and gene set enrichment analysis (GSEA). The ORA provided stringent and relaxed gene lists based on a significance threshold of FDR < 0.1 and $p < 0.05$, respectively.

Results and Discussion: CTCs exhibited profound differences in clustering behavior when cultured on different ECM, with dispersed few-cell clusters, relative to low adhesion conditions, where they formed larger aggregated clusters. Further, CTCs exhibit differences in cellular morphology when seeded on different ECM, including protrusions and multinucleation. Previous RNA sequencing results from CTCs dispersed on Matrigel vs ULA revealed differences in gene expression, including gene sets associated with surface receptors, ECM proteins, cytokines, leukocytes, and proteins involved in extracellular signal-related kinases signaling.

Conclusion: We show that patient CTCs respond to different culture conditions by altering their mechanophenotype based on cell-cell and cell-matrix adhesions. Ongoing work will profile CTCs on different engineered matrices at varied stiffnesses, and the effect of chemotherapeutic drugs and targeted inhibitors.

Acknowledgements: S. Maheswaran (Massachusetts General Hospital) for the gift of CTC lines (BRx-68), funding from NIGMS under P20GM109035.

CONCURRENT SESSION V-A: ENGINEERING CELLS AND THEIR MICROENVIRONMETS 2

10:30 AM - 10:45 AM

A simple and versatile method for integrating microscale topographical cues in engineered muscle from a variety of cell sources

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Skeletal muscle is a key player of the locomotor system. Reduced muscle contractility is associated with diseases like DMD and ALS. In vitro muscle models producing physiologically-relevant forces allow to explore new therapeutic strategies. Skeletal muscle is also an excellent candidate actuator for soft robotics. However, these applications are limited by the difficulty of controlling muscle fiber alignment in vitro. For example, random alignment reduces comparability between biological replicates in therapeutic screens. Controlling muscle orientation would also permit complex motions for robotics, like actuating circular valves. Existing strategies to align muscle tissue often rely on microfabricated scaffolds that impair microscopy, in addition to requiring complex fabrication protocols. Alternative methods in which proteins are patterned on stiff abiotic substrates lack important cues from the extracellular matrix, and ultimately result in muscle delamination. Directly patterning grooves in hydrogels overcomes these limitations. However, current protocols are tedious and comprise manual handling steps, which limits their accessibility and yield. Here, we developed a fast and simple approach to generate 2.5D sheets of aligned muscle. We used 3D printed stamps to pattern microscopic ridges onto a fibrin gel before seeding human or murine muscle cells. We explored the relation between cell size and groove size by varying the width and depth of the pattern. Dimensions comparable to the size of single cells yielded the most aligned muscle fibers, likely by constraining the direction of growth and fusion of myoblasts. Upon electrical stimulation, muscle contracted along the direction of fiber alignment, as quantified with our custom computational framework. Next, we applied our method to different culture formats and complex geometries, like a microfluidic channel and a multilayered ring configuration mimicking ocular muscle. We seeded the latter with murine muscle cells which had been engineered to express light-sensitive channelrhodopsin. We controlled the contraction of the concentric and radial layers separately by selectively shining blue light on either region, thereby reproducing how the iris opens and closes the pupil. Overall, our method provides the community with a versatile way of introducing microscale topographical cues in extracellular matrix hydrogels, which could eventually be extended to patterning tissues beyond muscle.

Concurrent Session V-A: Engineering Cells and Their Microenvironmets 2
10:45 AM - 11:00 AM

Investigating region specific mechanical properties of the spinal cord on cellular diversity and function

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Tissue mechanics in the central nervous system (CNS) play a crucial role in guiding differentiation and fostering cellular diversity, essential for its complex structure and function. Our lab has recently developed methods to produce region-specific neurons from hindbrain to lumbar spinal cord in adherent 2D cultures. Here, we are interested in understanding how varying the mechanical properties of hyaluronic acid-based (HA) hydrogels affect region-specific neuronal differentiation in 3D. HA is a major component of native spinal cord ECM and is widely studied as a biomaterial in neural systems. Different brain regions demonstrate significantly lower stiffness (0.1-1kPA) compared to the spinal cord, which also has varying stiffness between cervical (4 to 6 kPa), thoracic (10 to 12 kPa), and lumbar (6 to 8 kPa) regions. We hypothesize that culturing region-specific spinal progenitors on mimetic substrates will improve neuronal differentiation efficiency, which has significant implications for design of advanced in vitro models and compatible transplant materials. H9 human embryonic stem cells were differentiated to cervical (HOXC6+), thoracic (HOXC9+), or lumbar (HOXD10+) progenitor motor neurons with tri-lineage (astrocyte, oligodendrocyte, motor neurons (MNs)) potential, and cryopreserved for experimental consistency. We developed HA hydrogels supplemented with collagen and laminin to match the mechanical properties of the brain and these three spinal regions. Stiffness of the prepared HA hydrogels is validated by rheology and microindentation. Ongoing work will assess cell viability and differentiation to MNs (TUJ1, Isl1), astrocytes (SOX9, GFAP), and oligodendrocytes (Olig2, O4) over 1 to 3 weeks on these different substrates. Outcomes from this work will inform biomaterial design strategies for targeted therapies that cater to the unique cellular environments of different spinal regions, including for therapeutic use after spinal cord injury.

Concurrent Session V-A: Engineering Cells and Their Microenvironmets 2
11:00 AM - 11:15 AM

Investigating the role of collagen stiffness in modulating immune and cancer trafficking markers on lymphatic endothelial cells

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Lymphatic vasculature actively facilitates cell transport and regulates physiological and pathophysiological conditions, including cancer (e.g. breast, pancreatic), where fibrosis alters biophysical properties (e.g., increased stiffness) of the extracellular matrix (ECM) surrounding peritumoral lymphatic capillaries. ECM stiffening to pathological levels can impact lymphatic vessels trafficking of cells from the immune system to tumor sites and aids in cancer metastasis away from the primary tumor, through trafficking markers and soluble factors. Our study aims to tune the stiffness of the ECM environment around lymphatic capillaries by photo-crosslinking methacrylated collagen (ColMA) with ruthenium/sodium persulfate at 405 nm. This approach allows us to investigate stiffness-mediated changes in the expression of immune cell trafficking markers (ICAM-1, VCAM-1, E-selectin) on lymphatic endothelial cells (LEC). Normal and pathological ECM stiffnesses were obtained by exposing ColMA gels to 0 seconds (uncrosslinked, ~1.4 kPa), 30 seconds (~5.7 kPa), and 90 seconds (~8.6 kPa) of 405nm light. Qualitative assessment of immunostained LECs cultured on fibronectin-coated gels showed ICAM expression was highest on 30 second photo-crosslinked ColMA, and consistent VCAM expression across 30 and 90 second crosslinked ColMA. E-selectin was expressed the most on 90 second photo-crosslinked ColMA. There was also noticeable upregulation of VCAM, ICAM, and E-selectin in LECs cultured with TNF-α (10 ng/mL) on 2D, which could relate to immune and cancer cell activity and trafficking since macrophages and pancreatic cancer cells are known to secrete TNF-α and other cytokines. Next steps are to quantify differences in trafficking molecule expression. We have also differentiated THP-1 monocytes into mature dendritic cells and M1 macrophages in anticipation of future studies that are designed to evaluate functional trafficking of dendritic cells and macrophages across LEC monolayers on ColMA of varied stiffness. In addition, we will investigate stiffness related expression of migration markers CXCR4 and Mac-1 on dendritic and pancreatic cancer cells to apply our model to tumor cell trafficking with and without immune cells. By leveraging photo-crosslinked ColMA, the trafficking capabilities of lymphatic vessels for immune and cancer cells in a fibrotic environment can be explored and better understood.

Concurrent Session V-A: Engineering Cells and Their Microenvironmets 2
11:15 AM - 11:30 AM

Protein corona formation on lipid nanoparticles alters the NLRP3 inflammasome activation

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Nanoparticles when in the blood stream absorb proteins in the surroundings to form a complex termed as protein corona. While it has been elucidated that the new identity in the form of protein corona can affect the nanoparticle-cell interaction in several ways (Oh, J. Y. Nat. Commun. 2018), but the effect of protein corona on innate immune system is yet to be explored. In this study, we described the potential impacts of the corona formation on surface interactions as well as intracellular signaling pathways by explaining the shift in immunogenicity involved in lipid nanoparticles (LNPs).

Two systems of LNPs were synthesized via ethanol dilution method, by varying the molar ratios of each constituent lipids. The protein corona was formed by incubating the LNPs with normal rat serum and the composition was quantified by LC-MS, where the top 20 proteins formed around 70 percent of the entire protein corona for both the LNPs. LNP2, having 50 molar percent ionizable lipid was found to significantly activate higher NLRP3 inflammasome on LPS primed iBMDMs as compared to LNP1, which has no ionizable lipid. Interestingly, the corona formation caused a notable decrease in the

inflammasome activation. This was verified by IL-1β release from ELISA and ASC specks

formation from confocal microscopy. The reduced lysosomal rupture was described as the major

mechanism behind the suppressed NLRP3 activation. As a next step, the cellular uptake studies helped us to realize the involvement of multiple endocytic pathways of LNPs in macrophages. Also, the lipid constituents and the corona formation were found to be major factors in deciding the potential internalization pathways.

In conclusion, we were able to demonstrate the inverse correlation of protein corona with the

NLRP3 inflammasome complex. The reduced lysosomal disruption by the corona as compared to pristine LNPs was reported as the primary cause of the corona based NLRP3 inflammasome suppression. The findings described the mechanism of internalization followed by the nanoparticles in the absence and presence of corona. Overall, the results suggest the lipid formulation and corona composition to play a vital

role in regulating internalization mechanisms as well as intracellular signaling pathways.

CONCURRENT SESSION VI-A: BIOELECTRIC MATERIALS

2:15 PM - 2:30 PM

Surface modified exosomes for targeted delivery of mRNA to the retina via topical application

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INTRODUCTION: Vitreoretinal diseases remain a challenge due to a lack of reliable retinal-targeting treatment options1. Topical treatment could avoid the risks from invasive methods but is currently unfeasible due to slow corneal uptake resulting in ~90% drug loss1-3. Exosomes are cell-derived extracellular vesicles that facilitate intercellular communication via genetic material4. Their intrinsic physiochemical properties make them a promising retinal-targeting carrier but are insufficient to permit topical delivery5. Their anionic lipid bilayer eases intracellular penetration into the anionic cornea but cannot cling to its surface to prevent flushing. Their size might allow them to circumnavigate the crystalline lens to reach the vitreous humor (VH), but the VH's high water content hinders hydrophobic particle-diffusion. Therefore, we anchored cationic motifs on milk-derived exosomes to increase their surface charge and hydrophilicity allowing them to cling onto the corneal surface and effectively diffuse through the VH thus enabling topical-route retinal targeting.

RESULTS: Avidin or cationic peptide carrier (RRRR(NNRRR)3R) motifs conjugated to DSPE-PEG lipid were anchored into the exosome's bilayer. Cationic-motif-modification increased exosome surface charge from -24 to -2 mV and improved corneal transport by 2-fold. Exosomes did not impact the electro-mechano-chemical properties of corneal explants or crystalline lens. In

healthy VH, cationic-motif-modified exosomes were retained ~3x longer than native which would suit sustained delivery of small anti-inflammatory drugs in younger patients. In glycosaminoglycan-depleted VH, they diffused ~2x faster which would favor delivery of genomic material to photoreceptors in older patients. Cationic-motif-modified exosomes had a 20-fold stronger presence at the superficial retina compared to native and, unlike native, penetrated to the photoreceptors. Cationic-motif-modified exosome-delivered eGFP mRNA had 3x stronger signal compared to native and did not impact tissue viability.

SIGNIFICANCE AND FUTURE DIRECTIONS: These findings, for the first time, demonstrate the potential of cationic-motif-modified exosomes for mRNA delivery to photoreceptors in the retina via topical delivery. Future studies will explore their use in a mouse animal model.

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ACKNOWLEDGEMENTS: NIH NIBIB Trailblazer R21 EB028385 and NSF CAREER Award 2141841

Concurrent Session VI-A: Bioelectric Materials
2:30 PM - 2:45 PM

Formulation and delivery of tissue-engineered bioelectric threads with hiPSC-derived cardiomyocytes for regenerating electrical conduction in the heart

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Deficits in cardiac conduction underlie different types of arrhythmias, such as atrioventricular block and reentrant circuits due to fibrosis in the setting of atrial fibrillation or ventricular tachycardia after heart attack. Current treatments include pacemaker implantation to replace electrical activation of the ventricles or ablation procedures to burn conduction circuits, but they are fraught with complications and do not restore conduction in the diseased heart. Thus, implanting an engineered cardiac tissue (ECT) that acts as a wire or “bioelectric thread” to provide a direct conduction path to restore electrical coordination around/across the affected myocardium is a promising novel therapeutic approach. To that end, we developed a bioelectric thread and a custom delivery device leveraging biomaterials to improve coupling between two regions of electrically active cardiomyocytes. We differentiated ventricular cardiomyocytes (CMs) from human induced pluripotent stem cells (hiPSCs) by small molecule modulation of Wnt and metabolic-based lactate purification (CM purity >80%). Coextrusion of fibrinogen and thrombin into a HEPES bath enabled polymerization of fibrin threads which were then stretched and dried, coated in Matrigel, and seeded with hiPSC-CMs mixed with 5% human ventricular cardiac fibroblasts at 5x105 cells/cm. Conductive and contractile bioelectric threads were cultured for 2 weeks under 1 Hz electrical stimulation prior to analysis of action potential duration (APD) and conduction velocity (CV) by optical mapping. Bioelectric threads had maximal APD = 547 ± 29 ms and CV = 2.51 ± 0.59 cm/s. Immunofluorescence for "α" -actinin and connexin-43 showed hiPSC-CM alignment with the fibrin thread core with disperse gap junctions, suggesting an electrical syncytium. Bioelectric threads are loaded into tubing and a needle-based delivery system was used ex vivo to demonstrate insertion and placement into muscle. When positioned in vitro on top of two 3D ECTs separated by 1 cm, bioelectric threads electrically coupled within 3 days, enabling direct electrical propagation from one ECT to the other. Collectively, these results demonstrate that bioelectric threads can establish a CM bridge for electrical propagation, be delivered into muscle, and have the potential to improve conduction to enable the development of therapies to treat diseases of cardiac electrical conduction.

Concurrent Session VI-A: Bioelectric Materials
2:45 PM - 3:00 PM

Silicon Nanowired Motor Neurospheres for Improved Electrophysiological Maturation

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Spinal motor circuits are constructed through complex networks of chemical synapses, primarily between spinal motor neurons and interneurons. However, electrical synapses, mediated through gap junctions, are also hypothesized to play an important role in the generation of locomotive patterns and are implicated in the pathophysiology of diseases like amyotrophic lateral sclerosis and incomplete spinal cord injury. To date, few studies have examined the relevance of gap junctions to human motor neuron networks in vitro. Moreover, current methods of neuronal differentiation are hindered by poor electrophysiological maturity, which may affect the potential for functional recovery and circuit reformation in neuron transplantation therapies.

To address this gap, we introduced quasi-1-dimensional silicon nanowires into motor neurospheres. Silicon nanowires are high-aspect-ratio crystalline nanostructures that are biodegradable, possess tunable electrical conductivity, and can infiltrate intracellular environments without compromising cell viability. These unique properties allow silicon nanowires to act as conductive bridges between the intracellular spaces of neurons, forming synthetic gap junctions that eventually degrade over time.

We thus hypothesized that the integration of silicon nanowires into neuronal cultures could mimic synaptic pruning that occurs in normal embryonic and postnatal development. These synthetic gap junctions could thus accelerate electrophysiological maturation, increase synaptic connectivity, and encourage the development of physiologically relevant oscillatory circuits. Heavily doped silicon nanowires were introduced into cervico-thoracic motor neurosphere cultures derived from human embryonic stem cells. The use of 3D culture systems facilitated the self-organization of these nanowired neurospheres, while also maximizing the physiological relevance of our culture system for future expansion into in vivo applications.

As an initial exploratory study into the effects of nanowire integration, we characterized the time course of nanowire degradation, assessed the cytoskeletal responses of neurons to silicon nanowires, identified effects of chemical and electrical synaptic remodeling, and quantified functional electrophysiological maturity and circuit behavior.

This work thus lays the foundation for further advancements in the biological interfaces between high-aspect-ratio nanostructures and neurons. Our studies also suggest possible future applications for silicon nanowires as a therapeutic nanomaterial, by enabling the generation of 3D neuronal cultures that can better integrate with host neuronal circuits.

CONCURRENT SESSION VII-A: NANOMATERIALS

3:30 PM - 3:45 PM

A universal polymer-based non-viral gene delivery system with enhanced stability

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mRNA-based non-viral gene therapy is gaining enormous attention due its potential to either treat incurable diseases arising from genetic diseases or work as a powerful vaccine as demonstrated by the Moderna and Pfizer COVID19 vaccines. Both vaccines utilize lipid nanoparticles (LNPs) as their delivery system, but this system is currently a liquid suspension that requires stringent storage conditions and has a relatively short shelf life (6 months). Furthermore, such technology requires encapsulating the genetic material inside the LNPs which often affects the stability of the loaded genetic material. These limitations are associated with higher production, storage, and distribution costs. We propose the use of polymer-lipid hybrid nanoparticles that can be stored in a powdered state alongside powdered genetic material in the same vial, and reconstituted just before administration. Our delivery system will be available in a powdered (lyophilized) format and will be conveniently mixed with powdered genetic material in the same vial. This approach eliminates any need to preload the mRNA in the delivery system, thus there is no possibility to compromise its stability. Therefore, the genetic material is expected to retain at least the 2 years of shelf life if stored at -20°C. Our delivery system is a powdered polymer-lipid hybrid nanoparticles that is made of three biocompatible, safe and translatable components: poly(lactic-co-glycolic acid); cationic polymer and ionizable lipid. Through the precise control of the manufacturing technique and the ratio of each component we managed to prepare more than 80 different formulations with different sizes and surface properties. The size of our lead formulation is <100 nm with net positive charge. We were then successfully able to lyophilize the NPs in the presence of sucrose without affecting the NP properties. Furthermore, our lyophilized lead formulation was capable of complexing and retaining mRNA following reconstitution via simple hand shaking. Importantly, our lead formulation once complexed with EGFP encoded mRNA was capable of achieving up to 65% transfection efficiency in HEK293 without affecting cell viability. Our proposed technology is expected to act as a universal non-viral gene delivery system that allows for broad global distribution and accessibility.

Concurrent Session VII-A: Nanomaterials
3:45 PM - 4:00 PM

NanoBiomaterial Inspired Technologies for Healthcare Automation

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Microneedle (MN) assays have been extensively reported and applied toward a variety of wearable biosensing for continuous health tracking. These devices can penetrate through the skin, allowing access to the interstitial fluid (ISF) which contains biomolecules of great clinical significance. Traditional MN biosensors utilize solid and rigid MNs as the device electrodes which are not compatible with mechanically soft and curved skin. Rectifying this significant mismatch in physical properties using functional polymeric and flexible biomaterials holds the potential to open extensive opportunities in the development of biocompatible systems that seamlessly integrate with human skin with unique capabilities in clinical healthcare.

To address this unmet need, my group has developed novel wearable platforms that unlike previous rigid MN biosensors use, for the first time, flexible, hydrogel microneedle (HMN) electrodes for continuous, real-time measurement of patient health status. While HMNs have been used in drug delivery and cosmetic applications, their potential for in-situ sensing has remained unexploited until now. Our electrodes utilize state-of-the-art conductive and flexible yet mechanically strong polymers to tackle the problems associated with rigid MN biosensors. The backbone of our HMN electrodes is hyaluronic acid (HA), a biocompatibility polymer. To enhance the electrical conductivity of the electrode patches, we introduced doping into the polymer by incorporating PEDOT:PSS, a conductive and biocompatible polymer. For the first time, we leverage the functional groups found in HA and bind them with various molecules for a variety of sensing applications. For example, we employed methacrylate functionalization to create a crosslinked hydrogel, as well as to covalently attach aptamer probes for rapid and reagentless biosensing. This covalent bonding effectively addresses the long-lasting issue of biorecognition detachment and enhances the sensor's longevity. We linked dopamine (DA) with HA to create a HMN-based pH meter, capitalizing on DA's pH-responsive behavior. Additionally, by exploiting DA's redox properties, we generated platinum nanoparticles (Pt NP) in situ for non-enzymatic glucose detection and reported the first HMN-continuous glucose monitoring (CGM) device. We also recently employed the DA of DA-HA as a redox mediator to measure ketone bodies, developing a first-of-its-kind HMN-continuous ketone monitoring (CKM) device.

Concurrent Session VII-A: Nanomaterials
4:00 PM - 4:15 PM

Hybrid Exosomes for Non-Viral Gene Therapy in Osteoarthritis

Andrew Selvadoss

CONCURRENT SESSION I-B: BIOMATERIALS-INTERFACE INTERACTION-1

9:15 AM - 9:30 AM

In situ force probe characteristics influence measurements of cellular mechanical microenvironments

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The mechanical microenvironment of cells is a critical determinant in how they behave. This is especially true for cells in musculoskeletal tissues, which experience a variety of static and dynamic forces during normal activity. Despite the importance of cellular-level forces, their direct measurement has been challenging. To address this gap in knowledge, our group developed hydrogel-based force probes mimicking the size and elasticity of living cells. By embedding the probes within a self-assembling population of stem cells, we could monitor the microenvironmental forces present during processes such as mesenchymal condensation. The current study implemented this test platform across >600 self-assembled spheroids to investigate the impact of probe size (small/large), elasticity (soft/stiff), and surface coating (uncoated/collagen-I/N-cadherin) on force measurements. Results for average pressure, elastic energy, and elastic energy density showed that soft probes measured similar forces regardless of their size (15-35 μm), whereas stiff probes exhibited large variability and overall inaccuracy. Uncoated probes experienced primarily compressive forces within spheroids (-170 ± 170 Pa), whereas collagen-I- and N-cadherin-coated probes exhibited both compressive and tensile forces (-120 ± 170 Pa and 40 ± 350 Pa, respectively) facilitated by cell adhesions. When spheroids were treated with cytochalasin D, which depolymerizes actin, average pressures reverted closer to zero. Treatment with nocodazole, which interferes with microtubule polymerization, elicited no significant change. In addition to establishing the feasibility of this platform for a highly arrayed format, we also found that using “virtual Time 0 probes” introduced minimal error into the measurements, provided the probes used were monodisperse. This approach greatly increased the number of probes that could be used for measurements and eliminated the need to track their movement across time points. While microenvironmental force measurements at present are primarily of value for basic science investigations, they can potentially be used as indicators of tissue assembly since cellular-level forces can change as matrix is deposited and remodeled. The findings of the current study can further these investigations by recommending soft, large probes as a starting point for acquiring accurate microenvironmental force measurements, with custom ligand coatings to interrogate specific cell-cell/matrix interactions within developing neotissues.

Concurrent Session I-B: Biomaterials-Interface Interaction - 1
9:30 AM - 9:45 AM

One-step in situ printing of macroporous scaffolds for treatment of full-thickness wounds

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Introduction: Skin wounds exert significant burdens on military personnel, civilians, and the healthcare system. Current treatments rely on dressings that seal the wound with poor regeneration capacity. Treatments that provide supporting scaffolds that enable cellular infiltration, vascularization, and remodeling of the wound bed without upregulating the inflammation can improve the rate and quality of healing. Applying temporary scaffolds like hydrogels that support tissue regeneration is a promising strategy for wound treatment. However, conventional hydrogels' small pores limit cell ingrowth and vascularization, while inducing inflammation. Although macroporous hydrogels can solve these challenges, their fabrication, and implantation into irregularly shaped wounds is challenging.

Materials and Methods: We developed a handheld apparatus for reliable, facile, and continuous formation, deposition, and in situ crosslinking of hydrogel foams to form macroporous scaffolds directly within the wound. Gelatin methacryloyl (GelMA) was mixed with air at a controlled ratio to form GelMA foams. Different process parameters were optimized for the production of a homogeneous macroporous structure. Mechanical properties, biodegradation, printability, and cellular permissibility of the scaffolds were analyzed in vitro. The functionality of the strategy was then tested in a full-thickness murine wound model and wound healing was evaluated for up to 12 days through gross image and histological analyses.

Results: The device was able to reliably form and deposit foams using the optimized conditions, which resulted in the formation of a macroporous scaffold with ~5 kPa compression modulus and a significantly reduced biodegradation time compared to normal GelMA. Furthermore, the scaffolds enabled increased cell infiltration and spreading when cells were cultured on and within the macroporous scaffolds. Interestingly, while bulk GelMA scaffolds inhibited wound closure due to the minimal cell infiltration capacity, foams applied to the wounds significantly increased the healing rate. A thicker granulation tissue with a matured skin structure was observed in vivo for the foam group compared to no treatment and normal GelMA scaffold administration.

Conclusions: One-step in situ printing of macroporous scaffold developed in this work presents a highly translational, simple, and robust strategy for the treatment of large deep wounds when compared to current clinical practices.

CONCURRENT SESSION I-B: BIOMATERIALS-INTERFACE INTERACTION - 2

9:45 AM - 10:00 AM

Microneedle puncture criteria for ex vivo bacteria infected porcine skin tissue

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Skin is the largest organ of the body and serves as the main protective barrier against harmful chemicals and pathogens encountered daily. Skin has been widely characterized in its healthy state; however, the properties of skin once infected by pathogens, like bacteria, have not been thoroughly investigated. Current treatments for bacterial skin infections including debridement, topical ointments, and systemic antibiotics have various limitations that can lower their efficacy. There is growing interest in the use of microneedles (MNs) to enhance the treatment of these infections. For example, MNs can be used as localized antibiotic delivery systems, taking advantage of their ability to physically penetrate skin. The forces required to puncture healthy skin with MNs have been characterized for different parts of the body and for diverse demographics. To the best of our knowledge, there have been no studies focused on exploring how bacterial skin infections affect MN puncture forces. Therefore, this study aimed to investigate mechanical force criteria, such as compressive and puncture forces, required for a solid MN to puncture infected versus non-infected skin. Porcine dorsal skin was infected with Pseudomonas aeruginosa, a Gram-negative pathogen that readily infects skin. Bacteria density was determined at 24-, 48-, and 72-hrs post-skin inoculation via homogenization of the tissue and colony enumeration. We determined that there was a consistent bacteria burden at each time point for infected skin (3.63×107, 9.40×106, 8.90×108 CFU/mL for 24, 48, and 72 hr, respectively), while non-infected skin showed no bacteria growth. Next, the elastic modulus of bacteria infected and non-infected skin was determined via indentation. We found that uninfected skin exhibited a significantly greater elastic modulus than non-infected skin. A wide distribution of point puncture forces of the MN through 48-hr infected skin was observed, while MN puncture in uninfected skin was 409.9 ± 96.2 mN at a depth of 1.3 ± 0.07 mm. These findings indicate that MNs do not exhibit high puncture forces on infected skin and that MNs that exhibit low fracture forces during fabrication and optimization may be used for drug delivery to infected skin.

Concurrent Session II-B: Biomaterials-Interface Interaction - 2
10:00 AM - 10:15 AM

Evaluating the Effects of Adipocytes on Pancreatic Cells in Cancer Development

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Obesity is a known risk factor for developing pancreatic cancer, yet biological associations between obesity and pancreatic cancer initiation are not fully understood. In patients with obesity, adipocytes are present in higher quantities, and the surrounding extracellular matrix (ECM) experiences increased stiffness. ECM stiffening is involved in pancreatic cancer progression, but its role in cancer initiation alongside obesity is understudied. The current study examines individual and combined effects of adipocyte signaling and ECM stiffness on normal human pancreatic cell phenotype to monitor any characteristics related to malignant transformation. Adipocyte signaling is provided using adipocyte conditioned media (ACM), and ECM stiffening was produced by photo-crosslinking methacrylated type I collagen with lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) at 450 nm. First, pancreatic cell viability in adipocyte culture media was evaluated by culturing human pancreatic duct epithelial cells (H6c7) in 2D with varying volumetric ratios (100%, 75%, 50%) of regular adipocyte media or ACM to pancreatic cell media. An AlamarBlue metabolic assay was then used to determine the highest adipocyte media concentration that maintained high cell viability for use in subsequent 3D culture experiments. Results showed no significant change in viability with 50% ACM:pancreatic media compared to control pancreatic media. H6c7 cells were then suspended within low-stiffness (~0.5 kPa) and high-stiffness (~4 kPa) methacrylated type I collagen matrices to simulate normal and diseased ECM stiffness, respectively, and cultured for three days in 50% ACM. Cell staining was performed to assess changes in proliferation (Ki67) and actin morphology (Phalloidin) that could indicate phenotypic changes similar to what is observed in early malignancy. CellProfiler image analysis was used to count Ki67-positive cells and evaluate cells on elongation shape parameters. H6c7 cells exposed to ACM exhibited reduced proliferation and slightly more elongated morphology, suggesting a tendency toward a migratory phenotype. Proliferation further decreased at higher collagen stiffness. Study results suggest that ACM influences normal pancreatic cell behavior, and next steps are to compare results to ongoing parallel studies with pancreatic cancer cell lines and modified H6c7 cells. Overall, this work offers an important initial understanding of the effects of obesity on pancreatic tissue to provide insights into pancreatic cancer.

Concurrent Session II-B: Biomaterials-Interface Interaction - 2
10:15 AM - 10:30 AM

Tunable equine osteoarthritis in vitro model improved by cartilage electromechanical properties

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Introduction: Cartilage lesions can occur after traumatic over-loading of joints and progress into chronic disorders like osteoarthritis (OA). This study aims to develop a tunable ex vivo OA model using equine osteochondral plugs to effectively screen therapeutics and lubricants, and to use cartilage electromechanical properties to guide plug extraction and alleviate sample-to-sample variability.

Methods: Equine osteochondral blocks (N=4) harvested postmortem were subjected to electromechanical quantitative parameter (QP) assessment with a Benchtop Arthro-BST probe. Plugs (D=4.8mm) were then extracted with a drill press and assigned to Control (n=5), Injured (n=6), or Injured+TNFα (n=6) groups. Plugs were fitted in custom 2-chamber systems and incubated for 6-days at 37°C/5%CO2 in DMEM/F12/0.1% BSA/13nM Dexamethasone media. Mechanical injury (50% strain at 100%/s rate) was applied to Injured samples (n=12) at day-0, with Tumor Necrosis Factor Alpha (TNFα, 100ng/mL) added to the cartilage chamber of the Injured+TNFα group. Cartilage chamber media was sampled on day-3 and day-6. Cartilage thickness and surface alterations were assessed with stereomicroscope images. Unconfined compression and rotational friction-on-glass was performed aseptically with a mechanical tester prior to injury on day-0 and again 6-days later. Cell viability, glycosaminoglycans (GAG) tissue content and histology was analyzed post-mechanical tests. Statistical significance was determined with ANOVA post-hoc or Kruskal-Wallis tests.

Results: Initial QP values confirmed high variability in cartilage properties among osteochondral samples, correlating with both the acute severity of macroscopic cracks at the cartilage surface ($p=0.00094$), and with changes in mechanical and tribological properties of plugs 6-days post-injury. Low QP (QP≤12) plugs exhibited greater fibril modulus decline, while High QP plugs (QP>12) plugs showed more drastic changes in permeability and relaxation constant. High-QP plugs showed higher responsiveness to TNFα, doubling GAG release compared to Low-QP plugs ($p=0.0029$), leading to visible gradient of GAG depletion in cartilage histology.

Conclusion This study demonstrated electromechanical QP as a promising tool in refining in vitro OA models using equine osteochondral plugs and proved to be instrumental in understanding sample-to-sample variability. A tunable OA model holds promise in supporting the development of OA therapeutics tailored to the diverse stages and severity of this disease.

Concurrent Session II-B: Biomaterials-Interface Interaction - 2
10:30 AM - 10:45 AM

Antibacterial and immunomodulatory colloidal scaffolds for the treatment of burns

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Introduction: The management of high-energy blast injuries and burns is complicated by high rates of soft tissue contamination and prolonged delays to bedside care. Such burns often result in infections, as such the timely delivery of antimicrobials, along with debridement and wound covering with healing agents is crucial. Yet, this is not feasible in an austere battlefield environment and poorly healed burns can significantly reduce quality of life. Therefore, immediate treatment of burns using antimicrobial and immunomodulatory scaffolds is a significant but unmet need.

Materials and Methods: We developed a simple and robust strategy for in situ fabrication and delivery of macroporous gelatin methacryloyl (GelMA) scaffolds releasing minocycline, a broad-spectrum antibiotic, and proteoglycan 4 (PRG4), an immunomodulatory protein. The scaffold was fabricated by supplementation of LAP photoinitiator, followed by foaming using a micromesh-equipped double-syringe approach. The foam was then deposited in situ and crosslinked with blue light. The scaffolds were characterized in vitro using scanning electron microscopy, mechanical testing, stability evaluation, degradation assessment, release kinetics, biocompatibility testing, antimicrobial activity against methicillin-resistant Staphylococcus aureus (MRSA), and anti-inflammatory effects on RAW macrophages. The optimized concentration was used for the treatment of porcine burn wounds and the quality and rate of the healing were assessed up to 21 days using gross images and histology.

Results: An optimized precursor containing 15% GelMA, 0.4 mg/ml minocycline, 1mg/ml PRG4, and 0.67% LAP was identified with ~100 µm pore size, ~67% porosity, ~5 kPa compression modulus, biodegradability, long term stability (>14 days), a sustained release of PRG4 (>7 days), and a rapid release of minocycline (~24 hr). The optimized composite was biocompatible when exposed to human dermal fibroblasts and murine myoblast, and significantly reduced inflammatory markers (TNF-α) secretion from activated macrophages. The scaffolds were able to reduce MRSA bacterial load by >5X. Porcine studies confirmed scaffolds prevented infection and improved the quality of healing.

Conclusions: We developed a highly translational robust strategy for the immediate treatment of burn wounds via in situ fabrication of adherent macroporous scaffolds enabling cell migration and regeneration, while releasing compounds to prevent infection and reduce inflammation-associated scarring.

Concurrent Session II-B: Biomaterials-Interface Interaction - 2
10:45 AM - 11:00 AM

Enteric neurons modulate epithelial barrier properties in a microphysiological system

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Microphysiological systems (MPS), or organ-chip devices, provide a physiologically relevant in vitro model that are able to better recapitulate the microenvironment of the body. Gut-chip devices have become a promising method for researching drug delivery, disease mechanisms, and developmental biology. However, current MPSs of the gut mainly feature the gut lining, or epithelium, and lack the enteric nervous system (ENS) component that is responsible for critical digestion and sensation functions. In addition, the relationship between the ENS and epithelium is an understudied area with several unknown mechanisms regarding epithelium barrier dysfunction, a symptom of inflammatory bowel disease and irritable bowel syndrome. Together, these disorders affect over 15% of the global population. The ENS is also increasingly becoming implicated in neurological disorders like anxiety, depression, chronic pain, and even Parkinson's disease, theorized to have a gut-first origin. To allow for well-controlled probing of the mechanisms behind ENS dysfunction, we developed an MPS that features interfacing epithelial cell and enteric neuron populations. The device is fabricated using laser cut thermoplastics assembled by layer. The neuro-epithelial interface features a semi-permeable membrane that can support the passage of media components, metabolites, and neurite extension. The MPS design also supports a high throughput, pumpless design that is still capable of producing physiologically relevant shear. This work highlights some microenvironmental differences in epithelial cell properties that occur in the presence or absence of enteric neurons. We showed that enteric neurons contribute to an increased epithelial barrier strength through fluorescent molecule diffusion and transepithelial resistance measurements. We also saw changes in growth factor consumption and gene expression related to innate immunity with enteric neuron's present. These findings open new inquiries into the mechanisms behind neuro-epithelial interactions, how exogenous factors like the microbiome influence them, and how these relationships relate to disorders of both the gastrointestinal tract and nervous systems. The design used here has the ability to incorporate additional cell types of interest including immune or smooth muscle cells and could extend to other organ systems that feature innervated epithelial or endothelial layers.

CONCURRENT SESSION II-B: BIOMATERIALS-INTERFACE INTERACTION - 3

11:00 AM - 11:15 AM

Cell behavior enhancement by collagen/heparin layered coatings

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In this talk, collagen (COL) and heparin (HEP) layered coatings prepared via the layer-by-layer assembly will be presented as a robust coating to enhance cell behavior. We performed an exhaustive characterization of the COL/HEP coatings confirming their construction, chemistry, and their stability at body temperature. We evaluated the response of both human mesenchymal stromal cells (hMSCs) and human Schwann cells (hSCs) cultured on these coatings as well as in the presence of modulatory cytokines. Our results show that hMSCs cultured on COL/HEP coatings have a better response to soluble interferon-gamma regarding proliferation, protein expression, immunosuppressive properties and cell morphology as compared to the uncoated culture plates. hSCs cultured on COL/HEP coatings also demonstrated an enhancement in growth, protein expression, cell migration, and response to the nerve growth factor as compared to the uncoated culture plates. These COL/HEP coatings have strong potential to enhance the manufacturing of hMSCs or to serve as coatings for nerve implants.

CONCURRENT SESSION III-B: REGENERATIVE MEDICINE

2:15 PM - 2:30 PM

Tissue Geometry Impacts the Arrhythmogenic State of Engineered Heart Tissues Derived from Human Induced Pluripotent Stem Cells

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Background: Cardiac arrhythmias often manifest from and are exacerbated by pathological geometrical remodeling of the heart. However, their mechanism remains elusive. Therefore, our objective is to investigate the role that geometry and tissue tension play in arrhythmogenesis of engineered heart tissues (EHTs) in vitro. Methods: We differentiated high purity cardiomyocytes (CMs, >80% cTnT+) from human-induced pluripotent stem cells (hiPSCs) via Wnt-modulation and metabolic-based lactate purification. hiPSC-CMs were mixed with 5% human cardiac fibroblasts in collagen-1 and casted onto custom-designed PDMS molds to generate EHTs with unique geometries. Square, rectangular, and teardrop-shaped molds with isotropic or anisotropic peripheral post-spacing for tension distribution were evaluated. We cultured EHTs for 1 week under 1Hz electrical field stimulation to promote tissue compaction and electromechanical maturation before optical mapping of action potentials (APs) from a point electrode stimulation. Results: EHTs constructed in square molds with isotropic post-spacing were non-arrhythmic while those created in molds with alternative geometries were highly arrhythmic with spiral wave reentrant arrhythmias (0% vs. 86% arrhythmia incidence). This increase in arrhythmogenicity was associated with a 1.4-fold increase in AP duration (APD) dispersion throughout the EHTs, with activation maps showing conduction blocks across regions with large APD gradients, establishing reentrant pathways. We found that posts positioned on opposing sides of tissues to induce anisotropy increased conduction velocity along the perpendicular axis of the post by 2-fold, compared to the parallel axis. Interestingly, culturing EHTs in maturation media that supports CM bioenergetics reduced arrhythmia incidence and increased the pacing rate threshold for arrhythmogenesis. Conclusion and Future Works: Ongoing work investigates how mold geometries alter CM architecture in EHTs via immunohistochemical staining. Collectively, tissue geometries and tension distribution influence the arrhythmogenic state of EHTs by affecting APD heterogeneity and conduction, which can be leveraged to investigate substrate-level arrhythmia mechanisms. Funding: This work is funded by an AHA postdoctoral fellowship awarded to AHS.

Concurrent Session III-B: Regenerative Medicine
2:30 PM - 2:45 PM

Development and characterization of a geometrically-tunable blood shunt for pediatric heart reconstruction

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Congenital heart defects affect more than 40,000 babies born in the US each year; one in four will require surgery in the first year of life. In the most severe form of these heart defects, children are born with only half of their heart properly developed and undergo life-saving heart reconstruction surgery. The procedure includes the placement of a blood shunt – a fixed-diameter tube that diverts blood to the lungs to be oxygenated. Mortality rates following shunt implantation remain high despite decades of treatment optimization. This is because current shunts are unable to grow with the child in the coming months, resulting in hypoxia and a need for repeated invasive thoracotomy to place a larger shunt. Here, we develop a geometrically tunable blood shunt that can change in diameter on demand, allowing blood flow through the shunt to be tuned to meet the child's needs without surgical intervention.

Dextran was methacrylated by glycidyl methacrylate (50% substitution) and crosslinked into hydrogels (10%w/v) by Michael-addition with dithiothreitol (DTT; DTT/methacrylate ratios of 20-50%). Hydrogel volumes and moduli were assessed at baseline and after secondary photopolymerization that was performed to induce de-swelling. 40% DTT/methacrylate afforded excellent volume reduction (40%) with moduli (8.46±0.67kPa) comparable to soft connective tissues and was selected for prototype development. Biocompatibility with a blood-contacting environment was assessed in vitro. Hydrogels did not hinder cell proliferation (PrestoBlue), induce inflammatory response (RAW-Blue reporter assay) or hemolysis (ASTM 756-13, relative to non-hemolytic controls [LDPE] and clinically-used shunt tubing [polytetrafluoroethylene, PTFE]). PTFE tubing was surface modified by polydopamine to enable hydrogel adhesion. When irradiated via a fiberoptic catheter, hydrogels contracted towards the outer tubing. Clinically-indicated increases in lumen diameter (15-18%) necessary to support infant growth were obtained in <1min; changes far-exceeding clinical demands (40%) were possible. The dual-stage crosslinking approach is a promising strategy to create responsive biomedical implants that change in size with childhood growth, and the geometrically tunable shunt design may prevent repeated open thoracotomy in this fragile patient population.

Concurrent Session III-B: Regenerative Medicine
2:45 PM - 3:00 PM

Cardiac extracellular matrix-derived matrikine to modulate healing response post-myocardial infarction

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Introduction: Cardiovascular disease (CVD) remains the leading cause of death worldwide and the primary cause of CVD-related death is myocardial infarction (MI). Patients who experience a moderate-to-severe MI will eventually progress to heart failure where only transplantation is an effective treatment. Therefore, the development of regenerative means to repair the heart is vital. A major challenge in repairing the heart is the limited proliferative capacity of postnatal cardiomyocytes (CMs), where they shift from hyperplastic to hypertrophic growth following birth. Studies have demonstrated that the cardiac extracellular matrix (cECM) and matricellular proteins play an important role in promoting cardiac differentiation and CM proliferation. Our lab has previously identified a peptide (F1R1) derived from proteolytically digested fibrillin-1 that leads to improved proliferation in cardiomyocytes. Here, we investigate the potential of F1R1 peptide, a fragment of fibrillin-1 previously identified in our lab, as a therapeutic method to improve cardiac regeneration post-MI.

Methods: MI was induced in rats via left anterior descending artery (LAD) ligation and animals were randomly assigned to one of 5 different groups: F1R1 peptide, scrambled F1R1 peptide, fetal pig cECM, adult pig cECM and no treatment control. The fetal and adult pig cECM were as previously described by our lab and others. All treatments were solubilized in phosphate-buffered saline and injected post-MI. Echocardiography was employed to monitor cardiac function for 2 months post-treatment. Following isolation of the hearts, tissue was analyzed via histology and our novel ECM structure-function analysis method.

Results and Discussion: In vitro studies indicated that not only does F1R1 promote cardiomyocyte proliferation but it also reduces cardiac fibroblast activation in response to TGF-beta. Our first cohort of animals for the in vivo were recently finished and we are currently analyzing the functional data (echocardiography and pressure-volume loops) and processing the tissue for histology and our ECM structure-function analysis. Early results support the potential of cECM-derived matrikines as a promising strategy for cardiac regeneration.

CONCURRENT SESSION IV-B: ORTHOPEDIC BIOMATERIALS - 1

9:15 AM - 9:30 AM

Hybrid Double Network with Cryogels Tunable Degradation Rates for Cartilage Tissue Engineering

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Trauma or repeated injuries to joints can lead to localized cartilage damage, significantly elevating the risk of osteoarthritis—a debilitating joint condition. Unfortunately, the inherent capacity for self-repair of damaged articular cartilage is limited, presenting a critical clinical challenge yet to be addressed. Current clinical strategies for treating localized cartilage damage primarily involve surgical interventions, which have been met with limited success. As a result, there's an increasing exploration into biodegradable hydrogels as viable scaffolds for the repair or reconstruction of cartilage defects. Of particular interest are hybrid double network (DN) hydrogels, distinguished by their superior toughness compared to traditional hydrogels. However, the applicability of current DN hydrogels in cartilage tissue engineering is constrained by their nano-porous, non-degradable, and the absence of an interconnected macro-porous architecture, which hinders cell and tissue integration. Cryogels are a type of hydrogels, and they are gel matrices with interconnected macroporous structures synthesized by “cryogelation”, which is the crosslinking of gel precursors at subzero temperatures.

In this study, we have engineered a biodegradable, macroporous hybrid DN cryogel by synergizing two distinct crosslinked networks: multi-arm polyethylene glycol (PEG) acrylate and alginate. This hybrid DN cryogel is synthesized through a combination of a highly biocompatible click reaction for the PEG network and ionic bonding for the alginate network. By selecting structurally similar crosslinkers to establish the PEG network, we can tailor hybrid DN cryogels with customizable degradation kinetics. The resulting PEG-alginate hybrid DN cryogels exhibit an interconnected macroporous structure, enhanced mechanical strength, and rapid swelling behavior. These interconnected macropores facilitate the deep penetration of mesenchymal stem cells (MSCs) at high densities. Furthermore, we have achieved sustained release of growth factors IGF-1 and TGF-β1 from the cryogels. We also demonstrated the potential of these cryogel scaffolds to induce differentiation of MSCs into chondrocytes by leveraging growth factor stimulation. This investigation introduces an innovative approach to create macroporous hybrid DN cryogels with adjustable degradation rates, offering a promising scaffold solution for cartilage tissue engineering.

Concurrent Session IV-B: Orthopedic Biomaterials - 1
9:30 AM - 9:45 AM

Cartilage-Targeting Cationic Exosomes for the Delivery of Receptor Antagonist of Interleukin-1

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INTRODUCTION: Osteoarthritis is a degenerative joint disease that affects tissues like cartilage. MSC-derived exosomes (Exos) have recently been shown to facilitate cartilage repair in injury-stimulated OA animal models1. However, the negatively charged lipid bilayer of Exos is repelled by the anionic cartilage matrix which hinders their transport in the tissue. The high negative charge along with rapid clearance from the intra-articular joint space makes drug delivery to cartilage extremely challenging2. Our lab has previously designed a cartilage-targeting arginine-rich cationic peptide carrier with a net charge of +14 (CPC+14) that showed full-depth penetration in cartilage and long retention time3. We hypothesize that by modifying the surface of Exos to make them positively charged for delivering IL-1RA into the cartilage, sustained administration will be possible with a single dose via depot delivery. We have synthesized cationic Exos by conjugating CPC+14 and a cationic glycoprotein Avidin to their lipid bilayer for effective targeting in the cartilage along with IL-1RA anchored for therapeutic effects.

RESULTS: For the first time, we engineered charge-reversed cationic Exos by anchoring cartilage-targeting cationic motifs on their surface via click chemistry. We successfully synthesized cationic Exos by anchoring cationic motifs like CPC+14 and Avidin on the surface of Exos along with IL-1RA and reversed the zeta potential from -27.96 ± 3.0 to -1.6 ± 1.1 and -2.3 ± 0.85 respectively. Exo-CPC+14 exhibited superior transport, retention, and uptake in early-stage arthritic cartilage explants compared to native Exos. Exo-CPC+14 effectively targeted early-stage arthritic cartilage and successfully delivered the loaded IL-1RA to chondrocytes, thus suppressing IL-1-induced catabolism over 8 days with a single dose.

SIGNIFICANCE AND FUTURE DIRECTIONS: These Exo-based delivery systems have the potential to create intra-cartilage drug depots following their administration and facilitate delivery of any loaded drug or gene to its targets. Ongoing work focuses on evaluating the effectiveness of IL-1RA delivered using cationic Exos in injury-induced small animal arthritic models.

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ACKNOWLEDGEMENTS: NIH NIBIB Trailblazer R21 EB028385 and NSF Career Award 2141841

Concurrent Session IV-B: Orthopedic Biomaterials - 1
9:45 AM - 10:00 AM

Designing Cartilage-Targeting and Drug Depot-Forming Cationic Fusion Protein of Insulin-Like Growth Factor 1

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Introduction: Drug delivery to cartilage for osteoarthritis (OA) treatment suffers from rapid clearance from synovial fluid (SF) and hindrance by the dense cartilage matrix. Cationic nanocarriers can overcome these challenges by elevating drug uptake via electrostatic interactions with the high negative fixed charge density (FCD) of aggrecan-rich cartilage[1]. Previously, we designed a cartilage-targeting arginine-rich cationic peptide carrier (CPC) with an optimal +14 net charge that enables 400x higher intra-cartilage uptake than its neutral counterparts. Here we (i) investigate the effect of spatial arrangement of cationic and hydrophobic residues of CPC on their transport and binding properties in healthy and OA cartilage, and (ii) use this knowledge to optimize CPC design for sustained insulin-like growth factor 1 (IGF-1) delivery to cartilage for OA treatment.

Results: Spatially concentrated cationic and hydrophobic residues exhibited stronger intra-cartilage binding of CPCs resulting in reduced transport rates compared to peptides of the same net charge but with spatially distributed cationic or hydrophobic residues. Their uptake in arthritic cartilage of diminished FCD, however, was enhanced owing to short-range effects of H-bonds, charge-dipole, and hydrophobic interactions that synergistically stabilize intra-cartilage charge-based binding[2]. In the presence of SF, CPCs with higher hydrophobicity and clustered hydrophobic residues exhibited lower intra-cartilage uptake owing to competitive binding interactions with SF constituents like globulin and albumin. The work demonstrates that it is necessary to spatially distribute charges along the peptide length while minimizing its hydrophobicity index for effective targeting of arthritic cartilage. CPC+14 with alternating arginine and asparagine exhibited the fastest intra-cartilage transport, >40x intra-cartilage uptake in the presence of SF, and long-term retention. CPC was conjugated to an OA anabolic drug, IGF-1, to synthesize a cationic fusion protein, CPC-IGF-1. Molecular modeling confirmed that CPC-IGF-1 retained its bioactivity as indicated by proper IGF-1 domain folding and minimum steric clashes with IGF-1 receptor.

Significance and future works: CPCs can electrically charge OA therapeutics enabling effective cartilage-targeting and drug depot-forming properties. CPC-IGF-1 will be tested in vivo to evaluate its cartilage repair ability with only a one-time injection.

Acknowledgment: NSF CAREER AWARD 2141841

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CONCURRENT SESSION V-B: ORTHOPEDIC BIOMATERIALS - 2

10:45 AM - 11:00 AM

A Biocompatible Injectable Cytokine Trap For Local Immunomodulation Of Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a chronic autoimmune disorder that represents a public health concern. It is the major cause of mobility-related functionality loss among adults in the US. Although antibody-based products for neutralizing inflammatory cytokines have made remarkable improvement in RA treatment, a substantial portion of RA patients do not respond to those therapies. There is a clinical demand for new treatment modalities which can locally restore the cytokine balance.

In this project, we designed an injectable biomaterial scaffold as a cytokine delivery system with a prolonged residence time at the disease site for sustained delivery of therapeutically relevant cytokine levels to locally program the immune response to protect against cartilage and bone destruction while avoiding the off-target effects. We fabricated & optimized polymeric polycaprolactone nanowires using a nano-templating technique utilizing an anodized aluminum oxide (AAO) membrane with a specific pore size as the template for the formation of with nanowires with lengths ranging from 2–25 μm while keeping the diameter of 200 nm. The nanowires showed size dependent interactions with macrophages. Nanowires were further loaded with IL-4 using alternating layers of oppositely charged bioactive polymers. The levels of anti-inflammatory and proinflammatory cytokine in the cell culture media were assessed by ELISA. The polarization behavior of macrophages were evaluated by staining for cell surface and intracellular M1 & M2 markers and flow cytometry analysis on the consecutive days to evaluate the sustained polarization capability of IL-4 loaded NWs The IL-4 loaded nanowires showed sustained release of IL-4 for a period of one week. Treatment of bone marrow derived macrophages prestimulated with lipopolysaccharide with IL-4 nanowires shifted them to an anti-inflammatory phenotype as demonstrated by increase levels of CD206 and Arginase-1. The therapeutic efficacy of the cytokine loaded nanowires for their ability to potentiate cytokine balance and mitigate the disease symptoms are under investigation in-vivo in a collagen induced arthritis mouse model.

Concurrent Session V-B: Orthopedic Biomaterials - 2
11:00 AM - 11:15 AM

Nucleus Pulposus-Targeted Drug Delivery using Avidin Functionalized Polymeric Microcarriers for Intervertebral Disc Degeneration

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INTRODUCTION: Intervertebral disc (IVD) degeneration is a major cause of low back pain, characterized by alterations in biochemical and biomechanical functions of disc [1]. Cytoskeletal F-actin fibers regulate cell mechanobiology in degenerated discs through the RhoA pathway [2]. Activation of RhoA using a recombinant protein CN03 confers protection against degradative effects in nucleus pulposus (NP) [3]. Systemic delivery of CN03 is challenging due to avascular nature of NP. Although intradiscal injection enhances CN03 bioavailability, its rapid diffusion results in short-lived benefits and requires repeated dosing [4]. Our previous research has leveraged the negative fixed charge density of NP to develop a cationic avidin-dextran conjugate for month-long retention in NP [5]. In this study, we surface-engineered PLGA MPs by conjugating Avidin to make them cationic to mitigate the rapid clearance from NP and as a carrier for CN03. Our goal is to enable a month-long NP retention of cationic PLGA MPs and achieve a sustained CN03 release with a single dose.

RESULTS: Encapsulation efficiency of CN03 in our optimized PLGA MPs was 80.7 ± 1.5% with a reduced burst of 13% and a sustained release of about 77.2 ± 10.6 ng/day for a month. The bioactivity of CN03 at different time points of release kinetics was confirmed in terms of its potency to activate Rho pathway in NP. The net negative charge of PLGA MPs was reversed to 0.6 ± 0.4 mV after avidin conjugation. Cationic PLGA MPs demonstrated a localized drug distribution in the NP tissue for a month without diffusing to the surrounding tissue.

SIGNIFICANCE AND FUTURE DIRECTIONS: Our engineered cationic PLGA MPs enhance intra-NP retention and enable controlled CN03 release for long-term therapeutic benefits with a single dose. Hence, our system obviates the frequency of intra-discal injections. We plan to evaluate the efficacy of CN03-loaded cationic PLGA MPs in an injury-induced IVD degeneration rat model.

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ACKNOWLEDGMENTS: Supported in part by NIH NIAMS R21 AR080516, R01 AR077760, T32 AR080744.

Concurrent Session V-B: Orthopedic Biomaterials - 2
11:15 AM - 11:30 AM

Fabrication of Nanoparticles Loaded Microparticles for Sustained and Targeted Drug Delivery of Zoledronate to Activated Macrophages for Osteoarthritis Treatment.

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Osteoarthritis (OA) is a debilitating joint disease marked by cartilage degradation. Despite being a major contributor to functional disability and projected to impact ~1 billion people globally by 2050, there is no cure for OA. Mounting evidence indicates that cartilage debris resulting from joint overuse, injury, or aging often triggers an immune response, leading to macrophage recruitment, activation, and subsequent secretion of proinflammatory and catabolic mediators in the joint. This local inflammation further drives the degradation of remaining cartilage, consequently creating a deteriorating cascade leading to disease progression. Thus, specifically targeting macrophages holds promise for modulating inflammation, and in effect, halting OA progression. Zoledronate (Zol), a drug with high affinity and toxicity to macrophages, shows promise in abating macrophage activity. However, its early synovial clearance limits its intraarticular targeting efficacy. To address this limitation, we have developed a novel drug delivery system: nanoparticle in microparticle (NiM) to sustainably deliver Zol to target activated macrophages for OA therapy.

Zol was complexed with calcium to form nanoparticles (CaZol-NPs,~45nm) via reverse microemulsion technique and loaded into polymeric microparticles (PEG-PLGA-MPs) to obtain our NiM formulation (CaZol-NiM,~7µm). Zol-loaded particles were confirmed and characterized with analytical and microscopy techniques. The toxicity of Zol-loaded particles to activated macrophages was studied with MTT while their release was determined spectrophotometrically. To investigate targeted cell uptake, a folic acid(FA) conjugated formulation(CaZol-NiM-FA) was synthesized. Additionally, a mouse model of OA was established by ACL rupture via cyclic loading of the knee joint, and the macrophage population was characterized.

Compared to CaZol-NP, we observed that CaZol-NiM exhibited lower cytotoxicity and showed sustained release over 10 days. Confocal imaging and flow cytometry validated cellular uptake of all particle formulations, with enhanced uptake demonstrated by CaZol-NiM-FA. Furthermore, we identified folate-receptor2 (FR-2)-positive populations of macrophages in the OA mouse model.

Overall, CaZol-NiM-FA shows promise for sustained and targeted delivery of Zol to activated macrophages, emphasizing our system's capacity for longer joint residence time. Notably, the overexpression of FR-2-positive macrophages in the OA mice model, coupled with the strong affinity between FR-2 and folate derivatives underscores the potential of CaZol-NiM-FA as an immunomodulatory therapy for OA.

CONCURRENT SESSION VI-B: TISSUE ENGINEERING - 1

2:15 PM - 2:30 PM

WITHDRAWN

2:30 PM - 2:45 PM

Developing Multistage Fluidic Chips for Muscle and Neuron Co-Culture

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In vivo studies have shown that muscle exercise is correlated with improved motor function and control in both physiological and pathological states, indicating a potential role for muscle fiber contraction in modulating motor neuron growth [1]. However, as cell-specific signaling can be hard to isolate and control in vivo, the relationship between motor neuron recruitment and muscle activation remains understudied due to lack of relevant mesoscale in vitro model systems. We have designed and fabricated a multistage modular neuromuscular fluidic chip design consisting of a central “spinal cord”, containing independently controllable motor neuron spheroids, and distal “limbs” containing independent mature muscle tissue. State-of-the-art neuromuscular co-culture microfluidic systems only enable monitoring single motor units in isolation and physically separate motor neurons and skeletal muscle into separate compartments, thereby minimizing biochemical and mechanical communication. By contrast, our mesoscale chip enables monitoring multiple independent motor units in parallel and has an open-well format that encourages biochemical and mechanical crosstalk between skeletal muscle and motor neurons, as both modes of exercise signaling have been shown to modulate innervation [2]. Moreover, our device is designed to facilitate media replacement for highly metabolically active neuromuscular cultures and to precisely observe neurite extension towards muscle and synaptic pruning during development and in response to injury. Our chips consist of 3D-printed stages that leverage kinematic couplings to align stages with high precision and shows promise in supporting live muscle and neuron cultures to further understand neuromuscular interactions for applications in tissue engineering and regenerative medicine.

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[2] Bu, A., Afghah, F., Castro, N., Bawa, M., Kohli, S., Shah, K., Rios, B., Butty, V., and Raman, R., 2024, “2.5D Actuating Substrates Enable Decoupling the Mechanical and Biochemical Effects of Muscle Exercise on Motor Neurons,” p. 2024.03.02.583091.

Concurrent Session VI-B:Tissue Engineering - 1
2:45 PM - 3:00 PM

An organoid-derived model to explore the spatiotemporal expression of leukemia inhibitory factor in patients with and without endometriosis

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Endometriosis is a medical condition characterized by the ectopic growth of endometrial-like tissue outside the uterine cavity for which limited treatment options exist. Globally, it is estimated that up to 190 million women and people with a uterus of reproductive age are impacted by endometriosis. The symptoms of endometriosis include debilitating pelvic pain, dyspareunia, and it is the leading cause of infertility. Of patients with endometriosis, up to of 50% of patients have endometriosis-associated infertility. Leukemia inhibitory factor (LIF) is a pleiotropic cytokine and fertility marker associated with endometrial receptivity. Research suggests that LIF expression is downregulated in the eutopic endometrium of patients with endometriosis, however the role of LIF in cellular mechanism by which this occurs is not understood. The use of our recently developed poly-ethylene glycol (PEG)-based hydrogel provides a unique platform to perform long-term analysis of complex hormonal stimulation without interference from exogenous factors present in naturally derived hydrogels. I build on this platform by modeling both lumenal and glandular epithelial structures within this hydrogel and dissecting the spatial and temporal expression of LIF via high resolution imaging and morphometric analysis.

In this study, we utilized our synthetic 8-arm PEG-vinyl sulfone hydrogel functionalized with integrin binding peptides and cell matrix binders to generate lumenal (LE) and glandular (GE) structures of the endometrial epithelium from patient-derived endometrial organoids (EEOs). Utilizing this model, we assessed spatiotemporal LIF and LIF receptor expression in organoids derived from the eutopic endometrium of patients with endometriosis and control patients. Alongside LIF, other markers of receptivity such as pinopode formation, ciliation, and MUC1 staining was assessed via immunostaining. To validate the formation of lumenal and glandular structures, cultures were be fixed and immunostained with an antibody panel to confirm the presence of endometrial lumenal (WNT7A, MSLN, VTCN1) and glandular epithelial markers (FOXA2, SCGB2A2).

Given that LIF is a marker associated with endometrial receptivity, strategies to rescue dysregulated LIF in endometriotic tissue might offer therapies to enhance endometrial receptivity in the patient populations that are impacted by infertility. Future directions aim to explore how LIF signaling is regulated by epithelial-stromal cell crosstalk.

CONCURRENT SESSION VII-B: TISSUE ENGINEERING - 2

3:00 PM - 3:15 PM

Enhancement of Cartilage Regeneration in an Injured Murine Growth Plate Using a Cytokine-Loaded Nano-Matrix

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Background: The growth plate cartilage responsible for long bone elongation among children and teenagers represents the most vulnerable site of long bones, injuries of which can induce mesenchymal stem cell (MSC) infiltration, causing bony bridge formation and growth arrest. Surgical intervention has a failure rate of up to 38%. Thus, our focus is a localized cytokine delivery approach to guide recruited MSCs to reparative chondrogenesis. Stromal cell-derived factor 1 (SDF1) is a potent chemoattractant driving MSC migration, while the transforming growth factor-beta2 (TGFβ2) promotes chondrogenic differentiation of MSCs and inhibits chondrocyte hypertrophy. The Janus base Nano-Matrix (NM), a biomimetic scaffold comprising DNA-inspired Janus base nanotubes (JBNts) and matrilin-1 (Matn1), facilitates an optimal microenvironment conducive to cartilage regeneration.

Objectives: We aimed to inhibit bony bridge formation and enhance cartilage regeneration in a murine growth plate injury model through local delivery of a Layer-by-Layer (LbL) Janus Base Nano-Matrix (NM) loaded with SDF1 and/or TGFβ2 all carried within fibrin gel.

Methods: A lateral 0.5 mm diameter defect was created under fluorescence stereo microscopy in the right proximal tibia growth plate of two-week-old transgenic mice expressing tri-lineage fluorescent reporters for collagen types I, II, and X, using a dental bur. The groups were: untreated control, uninjured contralateral control, NM/fibrin vehicle, or NM/fibrin with SDF1 and/or TGFβ2. X-ray imaging one day before and 21 days post-surgery at the study endpoint was used to quantify the tibia growth. Micro-computed tomography (μCT) and histology were applied to evaluate the volume and spatial distribution of the bony bridge within the injured growth plate and chondrogenesis.

Results: Three weeks post-surgery, NM/TGFβ2 significantly reduced bony bridge volume compared to the untreated injury control. NM/TGFβ2/SDF1 did not reduce bony bridge volume but shifted the formation to the growth plate periphery relative to untreated and NM/TGFβ2 groups. This would be easier to remove surgically, so it is perceived as a clinically beneficial outcome.

Conclusions: NM/TGFβ2 treatment enhanced chondrogenesis and inhibited bony bridge development. The addition of SDF1 further facilitated this process by relocating the bony bridge from the interior of the growth plate to its periphery, thereby simplifying its surgical extraction.

Concurrent Session VII-B: Tissue Engineering - 2
3:15 PM - 3:30 PM

Humanized Organ-on-Chip for the Investigation of Multi-Tissue Systems

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Humanized microphysiological organ-on-chip systems have the potential to serve as a patient-relevant and cost-effective alternative to traditional 2D-cell culture or animal models. However, current organ-on-chip designs often lack capabilities to specifically access cell types for endpoint analyses, which limits their use with biological analysis techniques. Here we present a multi-layer organ chip for modeling complex multi-tissue systems, which features a 2D-cell culture and 3D-cell culture layer which are removable from each other. To our knowledge, our organ-on-chip design is the first to include multiple removable cell culture layers.

This organ-on-chip design includes three removable layers: a glass-bottom 3D-cell culture layer, a 2D-cell culture layer, and a media reservoir. Each cell culture layer can contain up to three chambers. The 2D and 3D layers contact each other through a semi-permeable membrane, and the 3D chambers contact each other through GelPins [1-3], which allow the contact of adjacent cell types. Additionally, the media reservoir can be replaced with a flow component to allow fluid flow over the 2D-cell culture layer. This organ chip is fabricated using a cut-and-assemble method [1-3], which avoids the tedious workflow of traditional soft lithography and allows rapid design modifications. Briefly, polyethylene terephthalate, polymethyl methacrylate, Viton, and clear resin pieces are attached together with 3M 966 tape to form the three layers, which are then

assembled using stainless-steel screws and nuts [4]. As each layer of this organ-on-chip is removable, each cell type can be conveniently accessed for biological assays.

To verify feasibility, a variety of human cell types have been cultured on this organ chip, including aortic smooth muscle cells, endothelial cells, intestinal smooth muscle cells, and differentiating neural progenitor cells. These preliminary studies have demonstrated the aptitude of the organ-on-chip for modeling multi-tissue systems, potentially with innervation or vascularization. With further investigation, this versatile organ-on-chip has immense potential for research in drug screening, biomaterials development, and/or mechanistic studies.

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Concurrent Session VII-B: Tissue Engineering - 2
3:30 PM - 3:45 PM

Stimulating In Vivo Angiogenesis with Sustained Local Release of an Optimized Proangiogenic Protein Cocktail

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Vascular perfusion, largely controlled by the high surface area and altered resistance provided by the microcirculation, is vital to maintain tissue integrity and function. An impaired microcirculatory network due to injury, ischemia, or disease stimulates maladaptive remodeling and disrupts broader tissue function. Microvascular regeneration remains an elusive therapy in the clinic, defining a critical need to regenerate the local microvasculature of target tissues. We hypothesize that sustained, local delivery of an optimized combination of growth factors will stimulate angiogenesis and facilitate vascular remodeling to increase perfused microvascular density. We developed and characterized a fully defined, injectable collagen I and alginate hydrogel that sustains developmentally relevant, local protein release for >14 days in vitro. Leveraging biomimicry of the native ECM with covalent heparin-modification of the alginate and shear-thinning behavior, the defined biomaterial alone enhanced average vessel size 15-fold and endothelial cell recruitment 12-fold when compared to Matrigel. Second harmonic generation using multiphoton microscopy revealed isotropic early collagen fibril organization and in vitro, endothelial cell-based 3D network formation assays confirmed proangiogenic function of released proteins. We used an iterative fractional factorial design of experiments approach and an in vivo subcutaneous gel plug assay to identify VEGF, Shh, IGF-1, and PDGF as the most potent proangiogenic factors from 10 initial candidates. Empirical evaluation of 1-, 2-, and 3-protein combinations of these factors identified VEGF, IGF-1, and PDGF (VIP) as an optimal protein cocktail for local, in vivo vascularization, enhancing perfused vascular density by 6-fold vs. the biomaterial alone. Thus, we optimized a highly angiogenic protein cocktail and developed an injectable, defined biomaterial for its local, sustained delivery, providing a promising revascularization therapy for local microvascular regeneration.

Concurrent Session VII-B: Tissue Engineering - 2
3:45 PM - 4:00 PM

Comparison of Traditional and Supercritical CO2 Sterilization Methods on Grafts: A Pig Full Thickness Wound Model Study

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Introduction: Graft sterilization is a critical step in biomaterials processing, ensuring safety and efficacy in clinical applications. Traditional methods, such as e-beam, gamma irradiation and ethylene oxide (ETO) gas sterilization, may impact graft properties. Supercritical carbon dioxide (CO2) sterilization presents a novel approach with potential benefits for preserving graft integrity. This study investigates the effects of traditional versus supercritical CO2 sterilization methods on graft performance using a pig full thickness wound model.

Methods: Full thickness wounds were created in a pig model, and grafts were sterilized using either a traditional method (e-beam) or supercritical CO2. Wound healing progress and graft integration were assessed through histological analysis, including evaluation of epithelialization, granulation tissue formation, and collagen deposition. Tensile strength and biomechanical properties of the integrated grafts were also evaluated.

Results: Preliminary results indicate comparable wound healing outcomes between traditional and supercritical CO2 sterilized grafts, with similar levels of epithelialization, granulation tissue formation, and collagen deposition observed in both groups. However, grafts sterilized using supercritical CO2 exhibited enhanced biomechanical properties, including increased tensile strength and elasticity, compared to traditionally sterilized grafts.

Conclusion: Our findings suggest that supercritical CO2 sterilization may offer advantages over traditional methods in preserving graft biomechanical properties while maintaining comparable wound healing outcomes. Further investigation is warranted to elucidate the underlying mechanisms and optimize the use of supercritical CO2 sterilization for biomaterial grafts in clinical applications.

Concurrent Session VII-B: Tissue Engineering - 2
4:00 PM - 4:15 PM

Decoupling the effects of mechanical and biochemical exercise stimuli on neurite outgrowth on an actuating hydrogel

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New in vivo findings indicate that muscle exercise affects peripheral nerves, yet the challenges of separating cell-specific biochemical and mechanical influences in the in vivo environment encourage further investigation of this phenomenon in vitro. In this study, we optimized a tunable hydrogel that mimicked the stiffness of native muscle to sustain long-term 2.5D muscle contraction in vitro, without delamination. The ability to culture contractile muscle for longer periods of time allows us to leverage muscle's function as an endocrine organ and harvest its exercise-secreted cytokines, termed "myokines". Motor neurons stimulated with myokines from "low intensity" and "high intensity" exercise groups demonstrated a significant and dose-dependent increase in morphological metrics such as neurite outgrowth and migration area. Bulk RNA sequencing of our motor neurons revealed an upregulation in signaling pathways and gene expression associated with axonal growth cones and neuron-neuron synaptic transmission. Principal component analysis was able to distinctly separate the transcriptomic data into the control, low-intensity, and high-intensity groups. To understand the mechanical effects of exercise on motor neurons, we utilized our established methodology for Magnetic Matrix Actuation (MagMA) to embed actuatable magnetic microparticles in fibrin substrates. This enabled us to dynamically stretch our motor neurons to mimic forces generated during muscle exercise. Similar to biochemical stimulation, we observed that mechanical stimulation equivalently increased neurite outgrowth and migration area as compared to controls, though the transcriptomic impact of mechanical stimulation was minimal. We believe this discrepancy to be caused by the large amount of RNA released from the spheroids which only the surface is dynamically stretched during magnetic actuation. Our actuating hydrogel platform thus enabled us to investigate the impact of muscle exercise on motor neuron growth and maturation through both biochemical and mechanical signaling pathways, robustly validating a previously only hypothesized role for exercise in mediating nerve growth.

CONCURRENT SESSION I-C: IMMMUNE ENGINEERING - 1

9:15 AM - 9:30 AM

Itaconate-based nanoparticles alter macrophage polarization to improve the treatment of inflammatory diseases

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Inflammation plays a key role in protecting the body against foreign invaders. When the triggering event is poorly controlled, severe inflammation and global dysregulation of immune responses can occur. These overreactive responses are often tied to infection, autoimmune disorders, cancer, and other chronic conditions. Macrophages play a pivotal role in sepsis progression, functioning as APCs that identify pathogens, initiate the immune response, engage in phagocytosis to eliminate foreign material, and spear inflammation resolution. A dysregulated balance between proinflammatory (M1-like) and anti-inflammatory (M2-like) macrophages is present in many inflammatory diseases and identifying strategies to skew their phenotype towards and anti-inflammatory state, holds significant therapeutic potential. Itaconate (ITA) is a key metabolite of activated macrophages and is well known to possess immunomodulatory and cytoprotective properties in inflammatory and infectious diseases and have antimicrobial properties. Here, we developed itaconate-based polymers and formulated them into nanoparticles (NPs) to allow for precise intracellular delivery to innate immune cells to mitigate inflammation, alter macrophage polarization, and improve survival in a lethal mouse model of LPS-induced endotoxemia.

ITA polymers were synthesized via polycondensation by reacting ITA with 1,10- decanediol and formulated into NPs using the single emulsion method. Polymers of different molecular weights were characterized using 1H-NMR and GPC. NPs displayed sizes between 300-500 nm and negative zeta potentials as determined by dynamic light scattering and nanoparticle tracking analysis. Bone marrow-derived macrophages were treated with NPs at various concentrations in the presence or absence of LPS stimulation. Luminex assays showed decreases in inflammatory cytokines and flow cytometry demonstrated NP treatment induced significant changes in macrophage phenotype using CD206 as a marker for M2-like and MCHII was decreased. In addition, treatment with NPs indicated little toxicity. Prophylactic treatment (i.p. administration; 2 mg) of mice with NPs followed by 20 mg/kg LPS challenge, led to a significant survival enhancement.

The goal of this research was to create a novel treatment strategy for anti-inflammatory immunomodulation through alteration of macrophage polarization. Dysfunctional macrophage polarization is at the crux of many diseases, thus this treatment has the potential to be employed for the treatment of multiple inflammation-associated diseases and conditions.

Concurrent Session I-C: Immmune Engineering - 1
9:30 AM - 9:45 AM

Alginate hydrogels for recruiting or activating muscle-specific regulatory T-cells

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Regulatory T-cells (Tregs) are potent anti-inflammatory immune cells that have been shown to broadly suppress pro-inflammatory immune cells during tissue injury and regeneration. Muscle-specific Tregs, a subset of Tregs that are recruited to skeletal muscle injuries, have also been shown to secrete growth factors that can drive satellite cell differentiation, innervation, and vascularization. However, Tregs are recruited to severely injured muscles at low frequencies, resulting in degenerating pro-inflammatory microenvironments and fibrosis. We engineered injectable, oxidized (degradable by hydrolysis), calcium crosslinked alginate hydrogels to provide sustained release of interleukin-33 (IL-33), to recruit ST2+ muscle-specific Tregs, or amphiregulin (AREG), to activate EGFR+ Tregs, for application in treating severe, ischemic muscle injuries. IL-33 demonstrated strong electrostatic affinity for the alginate hydrogel, as demonstrated by minimal burst release of the protein and absence of prolonged release of IL-33 by the hydrogel. Interestingly, incorporation of charged laponite nanodiscs into the hydrogel with IL-33 dramatically increased the burst release of IL-33 and resulted in moderate prolonged release of IL-33 for over 10 days in vitro. Incorporation of AREG in the alginate hydrogel resulted in moderate burst release, followed by more sustained release of AREG over 10 days in vitro. Incorporation of laponite reduced the burst release and rate of release of AREG from the hydrogel over the course of 10 days. Reducing the calcium crosslinking concentration of the hydrogel enhanced the rate of release of AREG. On-going and future work will evaluate how IL-33 or AREG releasing alginate hydrogels can be injected into the ischemic muscles of mice with severe hindlimb ischemia to 1) locally recruit or activate muscle-specific Tregs and 2) enhance the innervation, vascularization, and regeneration of the ischemic muscle.

Concurrent Session I-C: Immmune Engineering - 1
9:45 AM - 10:00 AM

HDM-Encapsulated PLGA Nanoparticle Mitigates Th2-Mediated Immune Responses in a Model of House Dust Mite Allergy

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House dust mite (HDM) is one of the prominent causes of indoor aeroallergens affecting approximately 100 million people worldwide. Untreated allergy to HDM leads to disease presentations such as atopic dermatitis and asthma. Symptom management of HDM allergies combine the use of antihistamines, corticosteroids and leukotriene inhibitors, all non-specific immunomodulators. Allergen specific immunotherapies (AIT) represents an antigen-specific treatment option to modulate the dysregulated Th2- mediated immune response that contributes to HDM allergy. However, AITs require repeated and long-term administration of HDM extracts, which pose serious side effects to patients such as anaphylactic shock. Polymeric nanoparticles (NPs) can be used to encapsulate allergenic proteins to provide a safer alternative to delivery of HDM extracts and provides a therapeutic platform for antigen specific tolerance. Here, we encapsulated HDM allergens into poly(lactic-co-glycolic acid)-based NPs (PLGA(HDM)) and assessed its efficacy using a clinically relevant mouse model of HDM-induced allergic airway disease induced by intranasal immunization. We found that intravenous administration of PLGA(HDM) efficiently suppressed Th2-mediated cytokines such as IL-4, IL-5, and IL-13. PLGA(HDM) also caused an increase in IFNy as a function of HDM loading, and decreased IL-17A. Histopathological examination revealed a considerable reduction in inflammation in PLGA(HDM) treated mice, with significant reduction in eosinophils and macrophages in the lung. Together these results indicate that PLGA(HDM), without the need for adjuvants, is a potential therapeutic approach to modulate Th2-mediated immune responses, reduce immune cell infiltration, and ameliorate airway inflammation in an HDM model of allergic airway disease.

CONCURRENT SESSION II-C: IMMMUNE ENGINEERING - 2

10:30 AM - 10:45 AM

Lymph node biophysics change during and after inflammation

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The lymph node (LN) is a critical immune tissue whose main function is to coordinate our adaptive immune response. During infections or other inflammatory challenges, LNs rapidly expand to withstand the influx of B and T cells through migration and proliferation. During resolution of the inflammation, the LNs then slowly contract again. We have previously shown that nanoparticles can be used to assess the mechanical properties of LN extracellular spaces, including elastic and viscous moduli, and here we used this technology to assess how the extracellular spaces of LNs change over the course of an inflammatory response. We found that by day 3 (peak inflammation) nodes have approximately doubled in size and gradually go back to their original size by day 14 (full resolution). We correlated day 1 to be initial inflammation and saw average pore sizes to be largest on this day 1030 ± 10 (SD) nm, which also exhibited the largest range of pore sizes 500-8900nm. At peak inflammation (day 3) the LNs also exhibit the highest elastic (G') and viscous (G") moduli (1.33 and 0.94 Pa, respectively) with G'/G" >> 1 indicating that the tissue has a more elastic (solid-like) response. As the inflammatory response continued to peak inflammation (day 3), pore sizes began to decrease to an average of 880 ± 10 (SD) nm, with a range of 500-8700nm. At full resolution (day 14), the node has returned to its uninflamed size and we found an average pore size of 690 ± 2 (SD) nm, with a range of 500-4150nm. Interestingly, when we compare viscoelasticity and pore size changes in B and T cell zones, they have similar trends but pore size starts much higher in the B cell zone and does not return to its original size. Similarly, elastic and loss moduli are both slightly increased at recovery. Overall our work is the first to assess biophysical changes in the LN during inflammation and suggests that a single inflammatory course may permanently alter the LN microenvironment. Future studies include investigating how these changes in LN extracellular mechanical properties affect cell behavior within the tissue.

Concurrent Session II-C: Immmune Engineering - 2
10:45 AM - 11:00 AM

Injectable granular hydrogels enable avidity-controlled biotherapeutic delivery

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Biotherapeutics exhibit excellent target specificity and are used to treat a range of diseases from cancer to autoimmune dysfunction. Their use benefits from sustained local delivery to concentrate drugs at the site of action and prolong therapeutic effects. The delivery of multiple biotherapeutic signals at different rates, such as to recapitulate natural developmental or healing processes, remains challenging. Here, we develop locally-injectable hydrogels composed of a supramolecular host, β-cyclodextrin, that undergoes guest-host complexation with guest-modified proteins to enable their tunable release.

Dextran and β-cyclodextrin were methacrylated by glycidyl methacrylate and co-polymerized (5mM LAP, 10mW/cm2) to yield hydrogels (G'~15kPa) with a high host concentration. Hydrogels were processed into microgels (34.016.7μm) by extrusion fragmentation and centrifuged to yield an injectable granular hydrogel. Bovine serum albumin (BSA, a model biomolecule) and cytokines were conjugated to guests (adamantane, Ad) via EDC in near-quantitative yield (1-10 Ad:BSA), with maintenance of guest–host affinity (12.01.81μM, isothermal calorimetry); avidity was assessed by surface plasmon resonance and release monitored in vitro. The avidity of Ad-BSA conjugates increased with the extent of guest-modification (kd=1.58x10-4 to 3.95x10-5 1/s). Guest-modification attenuated burst release (>5-fold) and sustained BSA release for over one month with release rates inversely related to avidity. Guest-modification of cytokines (IL-10, IL-4, IFN) did not alter their bioactivity (qPCR, bone marrow-derived macrophages) and enabled their controlled release (>2-fold less cumulative release, relative to unmodified controls). Cytokine-loaded hydrogels remained bioactive at the endpoint. Biocompatibility was assessed in healthy BalbC mice (15μL kidney subcapsular injection) relative to saline injection controls; kidney function was assessed (transdermal glomerular filtration rate, blood urea nitrogen, and serum creatinine) over 28 days. Hydrogel injection did not negatively impact kidney function. In sum, the bioconjugation proceeds under mild conditions, maintains therapeutic bioactivity, and is therefore applicable to a range of biotherapeutics (e.g., chemokines, cytokines, and antibodies) that may benefit from sustained local delivery, including for tissue repair following acute kidney injury.

Concurrent Session II-C: Immmune Engineering - 2
11:00 AM - 11:15 AM

Therapeutic macrophage activation using RNA-lipid nanoparticles in metastatic ovarian cancer

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Inhibiting checkpoints of T cell activation has proven therapeutic efficacy, however only a minority of patients respond. Macrophages, the predominant immune cell in many solid tumors, are potent immunosuppressors. Here, we hypothesized that siRNA-driven checkpoint silencing in macrophages would (i) promote their activation, (ii) enhance cancer phagocytosis and (iii) antigen presentation, to ultimately promote a therapeutic antitumor T cell response.

siRNA was designed in silico to silence a macrophage phagocytosis-checkpoint, and siRNAs with various sequences and methylation patterns were screened in vitro to assess silencing efficiency (qPCR). Lipid nanoparticles (LNPs) were formulated with a novel, biodegradable, ionizable lipid, and LNPs screened to optimize macrophage transfection. Protein-level silencing of the phagocytosis-checkpoint, and macrophage phenotype, were quantified using flow cytometry.

Results: Optimized siRNA-LNPs allowed high levels of silencing (~90%) in peritoneal macrophages following low-dose (0.01 mg/kg), intraperitoneal, administration. The novel siRNA-LNPs demonstrated macrophage-tropism in vivo, with uptake mediated by clathrin-mediated endocytosis and macropinocytosis, independent of ApoE and LDLR. siRNA-LNP driven silencing shifted murine and primary human macrophages towards an immunostimulatory phenotype, in vivo and ex vivo, respectively; and, compared to antibody-based checkpoint-blocking, minimized counterproductive mutual phagocytosis (macrophage-macrophage attack triggered by antibody-opsonization). siRNA-driven macrophage-checkpoint silencing was synergistic with cancer-opsonizing antibody therapy, the combination yielding ~70% cancer phagocytosis vs ≤10% phagocytosis by cells lacking either opsonizing-treatment or siRNA-silencing, ex vivo. Following macrophage coculture with ovalbumin-expressing melanoma cells, siRNA-LNP lead to a 2-fold increase in MHCI cross-presentation of the ovalbumin antigen. Ultimately, in a murine model of peritoneal metastasized ovarian cancer, intraperitoneal administration of siRNA-LNP + a cancer opsonizing-antibody significantly prolonged survival. Administration of siRNA-LNP with standard chemotherapy, significantly slowed tumor growth even following treatment cessation. The slowed growth was concomitant with increases in effector memory CD4 and CD8 T cells. These data demonstrate that siRNA-LNP elicits potent checkpoint silencing in macrophages, and that such macrophage activation ultimately drives the development of antitumor adaptive immunity. Together this data indicate that RNA-LNPs, and innate immune checkpoint inhibition, are promising platforms for cancer immunotherapy.

CONCURRENT SESSION III-C: BIOMATERIALS AND MEDICAL PRODUCT COMMERCIALIZATION

2:15 PM - 2:30 PM

MECHANOCHROMIC POLYURETHANE SHAPE MEMORY POLYMERS FOR INFECTION SURVELLANCE IN CHRONIC WOUNDS

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Background: Chronic wounds are difficult to resolve due to a combination of impaired wound physiology and bacterial infections. To that end, our lab developed a polyurethane (PUR) shape memory polymer that changes shape in the presence of bacteria to aid in infection surveillance in chronic wounds. To improve this capability, this study explores the incorporation of spiropyran (SP) mechanophores into PURs. These chromogenic compounds reversibly generate optical variations in fluorescence and color in response to external stimuli (such as force or light), due to the conversion of the closed SP structure to the corresponding open merocyanine (MC) structure, thereby acting as molecular force sensors. We hypothesize that SP-containing PUR (PUR/SP) wound dressings could be designed to undergo simultaneous shape and color changes in the presence of bacteria to enhance visible detection of infected wounds.

Methods: To incorporate SP, 0.31, 0.63, 1.25 and 2.5 mg of SP were dissolved in a solution of our control PUR in chloroform. The solutions were poured into Teflon dishes, and the solvent was evaporated, leaving PUR/SP films. Films were exposed to visible light for 48 hours. Rectangular samples were cut from the films and heated in an isothermal oven, strained, and cooled. A second set of samples was irradiated with UV light for 24 hours. After imaging on the bench, under fluorescence, and under UV, samples were heated to recover their original shapes and imaged again. Dog bones were cut from films and exposed to tensile force (2 mm/min strain rate) with a 24 N load cell with imaging before/after.

Results: Strained samples and samples irradiated by UV light showed a visible color change from red to yellow-brown. In addition, these samples showed increased fluorescence. Thus, application of UV light or force to PUR/SP films resulted in conversion between the red SP and yellow-brown MC forms of the mechanophore. Upon exposure to visible light, there was a conversion back to the original SP form.

Conclusions: Current work is focused on incorporating SP into our bacteria-responsive PUR to act as a molecular force probe, providing color-based surveillance of infection for future use in chronic wound dressings.

Concurrent Session III-C: Biomaterials and Medical Product Commercialization
2:30 PM - 2:45 PM

Flexible Electronics as a Companion Digital Diagnostic for Dysphagia: Needs Assessment Drives Novel Polymeric Nanocomposites for Surface Stimulation Applications

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Dysphagia, or difficulty in swallowing, can result from different etiologies such as stroke, neuromuscular disorders, including myasthenia gravis and amyotrophic lateral sclerosis, blunt force trauma, or extrinsic compression of the esophagus. Diagnosed patients deal with financial strain, with individuals having to spend more than $6,000 on average compared to the general patient population, due to factors such as longer length of stay, and additional diagnostic testing and treatment. Patients with dysphagia are at an extreme health risk as dysphagia can result in other conditions such as aspiration and malnutrition. Current clinical therapies consist of dietary modifications, postural adjustments, and resistance-based exercises. Neuromuscular electrostimulation (NMES) provides rapid treatment that promotes neuroplasticity, angiogenesis, and skeletal muscle hypertrophy and repair through tetanic muscle contraction. However, gaps in NMES treatment, including homogenization of procedures, such as determining optimal electrode placement, electrical frequency, and intensity, present clinical challenges, consisting of skin irritation, risk of erythema, and non-specific cathodal vasodilation. Despite standard-of-care commercial devices utilizing NMES, there remains an unmet engineering need for the development of soft flexible wearable electroceutical devices that facilitate delivery of electroceutical therapy for long-term applications. The development of flexible electrodes with low through thickness impedance, necessary biocompatibility, and long-term adhesion are required to address current short comings. This study focuses on the development of a platform electrode for applications warranting electroceutical therapy. Herein this poster evaluates the structure property relationship of a polymeric nanocomposite for surface stimulation applications towards treating dysphagia. This work will present preliminary results from this development with a key focus on correlating clinical needs to benchtop experimental design.

Concurrent Session III-C: Biomaterials and Medical Product Commercialization
2:45 PM - 3:00 PM

Hands-on Engineering Exercises Positively Impact Student Learning in Nanotechnology

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The 'Nanoengineering and Nanomedicine course at Brown University focuses on teaching new developments in the field of nanotechnology for biomedical applications, ranging from drug delivery to diagnostics. The goal is for students to understand and critically analyze state-of-the-art research in this field, and gain exposure to tools and methods used in nanomaterials fabrication and characterization. This study aimed to determine whether coupling of hands-on laboratory experiments in nanofabrication and characterization with traditional classroom-based learning approaches (e.g., lectures, homework, exams, discussion) in this course, would support student understanding of lipid-based nanomaterials. The Fall 2023 cohort consisted of 26 biomedical engineering students (8 engineering undergraduates, 14 Master of Science graduate students, and 4 Doctor of Philosophy graduate students). Undergraduate and graduate students were divided into four groups (6-7 students per group). Two of these groups fabricated and characterized liposomes using the thin film hydration approach and extrusion, followed by the use of dynamic light scattering to examine hydrodynamic size. The remaining two groups monitored and characterized lipid bilayer formation using quartz crystal microbalance with dissipation monitoring (QCM-D). Students were assigned three surveys (pre-laboratory, post-laboratory, and post-laboratory report) to assess the effectiveness of combining hands-on experiences with lecture-based learning modalities. A comparison of the pre-and post-laboratory surveys revealed an overall positive increase in understanding of liposome fabrication and QCM-D as a characterization tool for in situ bilayer formation across all educational levels, indicating that the laboratory exercises enhanced student understanding of these nanoengineering concepts. Furthermore, preparation of the laboratory report following the hands-on experiments aided student learning of key nanotechnology concepts introduced in the experiments and deepened their understanding beyond what was achieved through participation in the lab exercises alone.

CONCURRENT SESSION VII-A: NANOMATERIALS

4:00 PM - 4:15 PM

Towards developing optimal hyper-elastic liposomes for drug delivery: Combined molecular simulation and experimental studies

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Liposomes have gained prominence in drug delivery due to their enhanced therapeutic outcomes, biocompatibility, and ability to bolster drug stability, amplify solubility, and facilitate controlled release. In cancer research, however, the challenge of enhancing drug delivery efficacy to tumor sites remains a central topic. While the effects of properties such as liposome size, shape and surface charge on drug delivery applications have been widely studied, mechanical properties such as liposome elasticity and rigidity remain significantly underexplored. Results from a recent experimental study (1) indicate that elastic liposomes made of DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), where their stiffness was modulated by fillers inside the liposomes, had increased uptake by human breast cancer cells in vitro, and had significantly larger tumor accumulation in vivo compared to more rigid liposomes. It was proposed that soft liposomes can squeeze through pores and penetrate fibrous tissue, bind surface receptors, and enter cells primarily by fusion with their membranes, whereas more rigid liposomes would be taken up primarily by cell endocytosis. These results suggest that liposome elasticity can be optimally tuned to enhance drug delivery in cancer treatment. In this talk we will provide an overview of our recent (2,3) and ongoing combined simulation-experimental studies, which aimed at fundamentally understanding how the mechanical properties of the liposomes are affected by variables such as the molecular structure of the lipids (tails, headgroups), the composition of the liposome, and the presence of embedded hydrophobic drugs.

(1) Guo, P.; Liu, D.; Subramanyam, K.; Wang, B.; Yang, J.; Huang, J.; Auguste, D. T.; Moses, M. A. Nanoparticle Elasticity Directs Tumor Uptake. Nat. Commun. 2018, 9 (1), 130. https://doi.org/10.1038/s41467-017-02588-9.

(2) Xu, J.; Karra, V.; Large, D. E.; Auguste, D. T.; Hung, F. R. Understanding the Mechanical Properties of Ultradeformable Liposomes Using Molecular Dynamics Simulations. J. Phys. Chem. B 2023, 127 (44), 9496–9512. https://doi.org/10.1021/acs.jpcb.3c04386.

(3) Abdelmessih, R. G.; Xu, J.; Hung, F. R.; Auguste, D. T. Integration of an LPAR1 Antagonist into Liposomes Enhances Their Internalization and Tumor Accumulation in an Animal Model of Human Metastatic Breast Cancer. Mol. Pharm. 2023, 20 (11), 5500–5514. https://doi.org/10.1021/acs.molpharmaceut.3c00348.

RAPID FIRES

Rapid Fire #: 1

Comparison of Sterilization Methods on Biomarker Libraries of Dehydrated Placenta Membrane Products: E-beam vs Supercritical CO2

Mora Melican, Tides Medical

Olivia Logan BS, Babak Safavieh PhD, Isabella Sledge MD

Introduction: Human Amniotic Membrane (hAM) is a valuable biomaterial utilized for wound coverage in various clinical settings. Dehydration is a common processing step to ensure product stability and shelf life. Two widely used dehydrated membrane products, Artacent Wound® and Artacent Wound Pro®, are terminally sterilized using different methods, namely E-beam and Supercritical CO2, respectively. This study aims to characterize and compare the protein content and biomarker libraries of these products.

Methods: Total protein content, SDS-Page Gel analysis, and Microarray techniques were employed to assess protein composition in Artacent Wound® and Artacent Wound Pro®. Additionally, biomarkers and cytokines were categorized into angiogenic, immune-modulating, and regenerative aspects for comprehensive analysis.

Results: Analysis of protein content and biomarker libraries revealed differences between Artacent Wound® and Artacent Wound Pro®. While both products exhibited distinct protein profiles, variations in biomarker composition were observed, particularly in angiogenic, immune-modulating, and regenerative biomolecules. The sterilization method appeared to influence the retention of specific biomarkers and cytokines within the hAM products.

Conclusion: This study provides valuable insights into the impact of sterilization techniques on the protein content and biomarker libraries of dehydrated placenta membrane products used in wound coverings. Understanding the effect of sterilization methods on biomarker retention is essential for optimizing the clinical efficacy of hAM products and advancing wound care strategies. Further investigation is warranted to elucidate the underlying mechanisms and refine sterilization protocols for enhanced biomarker preservation in hAM-based therapies.

Rapid Fire #: 2

Collective Transitions from Orbiting to Matrix Invasion in 3D Multicellular Spheroids

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Epithelial morphogenesis is governed by the dynamic reciprocity between groups of cells and the extracellular matrix, but this emergent phenomenon remains poorly understood. Here, we utilize live cell confocal microscopy to simultaneously interrogate collective cell migration and matrix deformation in 3D biomaterials. Remarkably, we show that multicellular spheroids of mammary epithelial cells exhibit highly coordinated circumferential orbiting, which transitions towards invasive strands oriented radially as the biomaterial is progressively remodeled. Intriguingly, the initial morphology of the multicellular spheroid also exhibits geometric alterations that are predictive of subsequent invasion. We further show these morphological dynamics can be linked to spatially non-uniform tractions within the biomaterial. Finally, we physically and biochemically perturb these collective dynamics to gain mechanistic insight into the underlying processes. Overall, this work establishes design principles for how epithelial tissues can be shaped by biomaterials to model development and disease.

Rapid Fire #: 3

Development of Multi-Drug Resistant Tumor Model Using 3D-Hydrogel Matrices

Ira Hysi, Northeastern University

Aditya Banga, Deeksha Diwan, Venus Sahu, Mansoor Amiji, Lara Milane

A major contributor of poor patient prognosis in cancer, including ovarian cancer, is the development of disease relapse and resistance to chemotherapy. The clinical manifestation of therapeutic resistance occurs through multiple pathways and is termed multi-drug resistance or MDR To develop a model of MDR tumors in vitro, we have used human ovarian cancer cell line (SKOV-3) and evaluated their response to stress introduced by overlaying hydrogels with different thickness. The hydrogel layer was manually added above adherent SKOV-3 cells using a syringe to make our model biomimetic to the tumor microenvironment, while maintaining cell viability. The hydrogel was composed of 10% gelatin methacrylate (GelMA) and 1% gellan gum for enhanced viscosity and was ionically crosslinked by submersion in a calcium chloride solution for improved structural integrity. The hydrogel underwent resistance tests to examine its shear stress limits and swelling tests to calculate the crosslinking density for a range of crosslinking solution concentrations and duration. To investigate the mechanical effects of hydrogel on the cell layer, the cells were stained with f-actin and imaged to detect cell stress. Diffusion tests were also conducted to evaluate the diffusion of oxygen through the hydrogel layer to the cells as validation of the hydrogel-induced hypoxia. Furthermore, the hydrogel-based model aims to draw parallels with other hypoxic related work, a condition of low oxygen concentration facilitating reactions within mitochondrial networks that promote tumor survival and progression. The results of the Mitochondrial Network Analysis (MiNA) showed an increase in mitochondrial expression as the cells' response to the introduction of a hypoxic microenvironment, mimicking the response demonstrated by in vivo models and reflecting the effectiveness of the in vitro model. Thus, this affordable and accessible model by application of hydrogels can help scientists better understand the development of MDR and response to therapy in different tumor models

Rapid Fire #: 4

Leveraging synthetic polyurethanes for the modulation of bacterial surface motility and physiological behaviors

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Modulating bacteria surface behavior can increase the efficiency of industrial biomanufacturing and clinical therapies. While genetic engineering methods have been extensively applied to manipulate microorganisms for supporting engineering living materials, there is only nascent research on the use of chemical approaches to modulate bacterial behavior. Here, we report a straightforward and simple approach to modulate bacteria's surface motility and physiological behaviors via a platform of biosurfactant mimetic and water-soluble polyurethanes. Our results showed that -COOH-containing polyurethanes significantly promoted the swarming and twitching area of P. aeruginosa PAO1 by 17-fold and 80-fold, respectively. Conversely, the polymer with -NH2 functional groups restricted the swarming area of P. aeruginosa by 58%. This modulation of bacterial motility results from the synergistic regulatory effects of the polyurethane platform on surface wetting, bacteria proliferation, and the production of the second messenger cyclic di-GMP. Moreover, we found a 4-fold increase in the production of the extracellular polymeric substance (EPS) yield in the presence of -COOH-containing polyurethane. Taken together, combining the inhibitory effect of P4-NH2 and the pro-motility effect of P2-COOH, we successfully achieved selective bacterial migration on agar to generate the desired bacterial migration pattern over time. Therefore, this novel biosurfactant-mimetic polyurethane platform emerges as a simple means of controlling microbial systems as well as enhancing the downstream effects of EPS production.

Rapid Fire #: 5

Quantifying Donor-Dependent Morphological and Functional Differences in Primary Human Muscle Cultures

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Engineering in vitro platforms enable high-throughput modeling of living systems and are prevalent in efforts to discover and test therapies for diseases. Many in vitro models of skeletal muscle rely on immortalized murine cell lines that do not always generate accurate models of native tissue. Using primary muscle cells from human donors could thus provide a more accurate method of engineering tissues that model native architecture and function. However, the long-standing issue of inter-patient variability in modeling and treatment of diseases remains an issue for in vitro systems derived from primary muscle cells. There is thus a strong need to first characterize inter-patient differences in the morphology and function of engineered muscle derived from primary cells before attempting to model and/or treat any system or disease. To our knowledge, a detailed description of donor variability contributions to the morphology and function of human skeletal muscle tissue has not been investigated. We have conducted a thorough analysis of diverse donors that span the age and health spectrum to establish how much functional variability can be attributed to normal inter-donor variability versus specific contributions due to pathology. Our database enables us to make quantitative comparisons of muscle fiber type, length, width, fusion index, and frequency-dependent force generation capacity across many individuals. Unlike iPS-derived models, which often lack the maturity of native tissue and are largely suited to modeling diseases of known genetic origin, our database enables quantifying, for the first time, the functional impact of disease with unknown or multivariate causes that affect aging adults. We show, for example, that our database enables benchmarking muscle health and strength for patients with amyotrophic lateral sclerosis (ALS) as compared to an array of healthy age-matched controls. Our research will provide novel methods and open-source databases that enable benchmarking human muscle cultures for mechanistic studies of human disease and high-throughput drug testing.

Rapid Fire #: 6

Comparison between rodent and human heart chips' response to dosed pharmaceuticals

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Cardiovascular disease is the leading cause of death in the Western world, with 931 578 deaths in the US during 2021. Furthermore, pharmaceuticals designed to manage cardiovascular disease have the lowest likelihood of FDA approval based on leading indications during Phase 1 testing. There is a need for cardiovascular pharmaceuticals; however, based on the Phase 1 data, there are issues with the animal models used to develop these pharmaceuticals. An alternative model is a microfluidic platform known as Organs-on-a-chip (OOC). This in vitro platform leverages a three-dimensional culture space, heterogeneous cell population, and biophysical stimuli to mimic in vivo conditions and features. We have previously developed and validated a heart chip of isolated rat cardiomyocytes and sympathetic neurons. Recent work has led to creating a humanized model consisting of human stem cell-derived cardiomyocytes. The rodent and human heart chip was used to screen the effects of epinephrine, milirone, and isoproterenol and determine the adverse effects of the listed compounds. We used video edge capture to determine the cardiomyocytes' beat rate, contractility force, and synchronicity before and after dosing with the pharmaceuticals. The results show that each system behaves similarly to the reported effects of each compound. Further work towards an innervated human heart chip and more complex pharmaceuticals can highlight the species differences in the two systems. Success in a human in vitro heart model can aid in the development of novel cardiovascular drugs, leading to a reduction in death and an increase in approved medications.

Rapid Fire #: 7

Injectable Synthetic Platelet-Based Therapy Enhances Healing Potential in Stem Cells Delivered for Joint Injuries

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Anterior cruciate ligament (ACL) injuries can occur due to sports or other activities and constitute a major clinical problem worldwide. While extra-articular injuries of the joint are able to heal, intra-articular injuries, such as ACL injuries, have a limited healing capacity. This is believed to be due, in part, to the proteolytic environment of the synovial fluid (SF) with a high clearance rate surrounding the ligament and the low presence of necessary clotting factors such as fibrinogen and thrombin, which hinders the healing process by not allowing fibrin scaffolds to form. For the same reason, therapeutics aiming to use mesenchymal stem cells (MSCs) for promoting intraarticular healing and joint cartilage regeneration, for example in the case of early term osteoarthritis, suffer from lack of cell persistence in the area. In previous studies, platelet-like-particles (PLPs), designed to mimic activated platelets, have successfully established stable formation of a fibrin matrix in synovial fluid from injured joints. A PLP-enhanced fibrin clot could be an effective delivery system for MSCs to the joint area. This study tests MSC activity within PLP-enhanced fibrin clots. Activity was determined by incorporating bovine fetal MSCs within fibrin clots +/- PLPs and assessing cell viability, cell migration, and exosome release and uptake. The presence of exosomes is

believed to enhance healing through delivery of healing cytokines, proteins, and by promoting signals for recruitment of further healing factors. PLP-enhanced clots induced higher cell viability and retention, and significantly higher concentrations of released exosomes relative to non-PLP-driven exosomes. These findings underscore the potential of PLPs in promoting further stages of healing beyond clot stability, to allow enhanced MSC activity for remodeling of tissue. The study provides valuable insights into the application of PLP-enhanced fibrin-based injectable therapeutics for successful delivery of MSCs to intra-articular injuries and early term osteoarthritis.

Rapid Fire #: 8

Hybrid LNP prime dendritic cells for nucleotide delivery

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Efficient activation of professional antigen-presenting cells—such as dendritic cells (DC)—in tumors and lymph nodes is critical for the design of next-generation cancer vaccines and may be able to provide anti-tumor effects by itself through immune stimulation. The challenge is to stimulate these cells without causing excessive toxicity. We hypothesized that a multi-pronged combinatorial approach to DC stimulation would allow dose reductions of innate immune receptor-stimulating agonists while enhancing drug efficacy. To achieve this goal, we developed a hybrid lipid nanoparticle (LNP) platform featuring "nanoparticle-in-a-nanoparticle" design that contains i) a toll like receptor (TLR) agonist poly I:C and ii) a second nanoparticle based on cyclodextrin as a "sponge" for encapsulating small molecule therapeutics to enhance DC stimulation. We show that a single dose of hybrid LNPs alone, effectively eradicate tumors, and generated long-lasting, durable anti-tumor immunity in mouse models. This approach lead to vastly enhanced efficacy over LNP/TLR3 alone while showing negligible toxicity at pharmacological concentration.

Rapid Fire #: 9

Macrophage-targeted drug delivery for systemic and local immune modulation

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Macrophages (MF) are mediators of the immune microenvironment that span diverse behaviors, ranging from prototypical inflammatory (M1-like) to pro-healing (M2-like) phenotypes. While small-molecule drugs are available to treat many diseases by modulating MF behavior, their efficacy remains limited by a poor understanding of which drugs can promote desirable MF cell-state transitions and inadequate drug pharmacokinetics (poor solubility, non-specific cell uptake, rapid blood clearance, and off-target effects). Biomaterial-based drug delivery systems enable cell- and tissue-targeting strategies to overcome these challenges. Furthermore, the recognition of drugs by macrocyclic hosts provides a readily adaptable method to encapsulate a variety of therapeutics for delivery, including within soluble nanoparticles (for systemic cell-targeted therapies) or injectable hydrogels (for local applications, such as focal tissue injury). This talk will discuss two applications of these systems, which include in the context of cancer immunotherapy and the prevention of post-ischemic heart failure.

Tumor-associated macrophages (TAMs) are abundant in solid cancers, assuming a pro-angiogenic and immunosuppressive M2-like phenotype that supports tumor growth and immune escape. Recent methods have focused on identifying therapeutic drugs that re-polarize TAMs to a tumor-destructive M1-like phenotype. Through high-content screening and qPCR analysis in primary murine and human cells, we identified a drug (R848) that potently induced a tumor-destructive state (IC50=7.2nM). Cyclodextrin nanoparticles (CDNPs) were prepared by crosslinking cyclodextrin with lysine. Drug-loaded CDNPs (CDNP-R848, >10%w/w drug loading) accumulated in tumors by MF-specific update and eradicated tumors in multiple models (MC38, Gl261). Treatment synergized with frontline anti-PD1 checkpoint therapy.

In contrast, tissue injuries such as myocardial infarction are characterized by exuberant local inflammation. We therefore developed hydrogels composed of CDNPs crosslinked by polymer-nanoparticle interactions. The hydrogels are shear-thinning for ease of injection and self-healing for local retention. Through cell-reporter assays, we identified celastrol's ability to induce a pro-healing MF state (IC50<100nM). Hydrogels released CDNP-celastrol for >14 days with >80% inhibition of inflammatory response. In a murine model of ischemia-reperfusion injury, targeted delivery of celastrol prevented left-ventricular remodeling and preserved heart function. In sum, small-molecules can potently modulate MF phenotype and their therapeutic efficacy is improved through cell- and tissue-targeted delivery to aid clinical translation.

Rapid Fire #: 10

Targeted delivery of nanoparticles to hematopoietic stem cells

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Drug delivery strategies based on nanoparticles (NPs) have significantly advanced the therapeutic landscape in the recent years. Layer-by-layer (LbL) NPs can deliver a great variety of drug cargos allowing tunable biodistributions. Genetic blood disorders have posed an important therapeutic challenge, where so far only ex vivo cell therapies have resulted in effective cures. Although promising, these strategies carry high production and implementation costs, limiting the accessibility in areas and populations of higher incidence. The ability to reach hematopoietic stem and progenitor cells (HSPCs) in vivo would contribute to advancing the field toward more translatable and accessible treatments. This work aimed at evaluating the potential of the LbL platform to develop a targeted NP system that can direct therapies to HSPCs in vivo.

Given the low numbers of HSPCs in vivo, a highly specific targeting system will increase its efficacy. We first hypothesized that we could leverage the modularity of LbL NPs to decrease unwanted interactions with circulating cells by rationally selecting the outer layer. We compared three different outer layers and observed that some of them associated more with myeloid cells, and even T cells, while Layer1 stood out as the most inert surface. We then used this polymer as a scaffold to conjugate different HSPC-targeting antibodies (Abs) to. In vitro studies in mouse progenitor cells demonstrated different NP uptake and trafficking patterns depending on the Ab, with two outstanding leading candidates. Interestingly, in vivo studies of these Ab-LbL NPs pointed to an alternative and understudied candidate (Ab5) as the one with the highest HSC targeting in vivo, suggesting the role of other physiological parameters not recapitulated in vitro. We further confirmed the translatability of targeting this receptor to human HSPCs (CD34+ cells) in vitro, showing an enhanced cell uptake over the non-targeted formulations.

In conclusion, we have designed a drug delivery platform based on the LbL assembly and engineered it to decrease unspecific interactions with circulating blood cells, while targeting HSPCs in vivo.

Rapid Fire #: 11

Enzyme-responsive hydrogel tissue expanders for guiding facial growth in microphthalmia patients

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Microphthalmia and anophthalmia, conditions where a child is born with at least one abnormally small or missing eyeball, results in abnormal facial development. A current standard of care is a hydrogel-based osmotic expander that mimics the missing or underdeveloped eyeball to guide bone and soft tissue expansion in the orbital socket. One challenge with these devices is a rapid initial rate of expansion, which can lead to inflammation of the surrounding tissue. To overcome this limitation, we created a library of hydrogel compositions in combination with an enzyme-responsive interpenetrating network (IPN) of chitosan to control the rate and extent of swelling. Chitosan is degraded by lysozyme, an enzyme that occurs naturally in tears. The IPN was designed to control the initial rate of expansion, and enzymatic degradation of the network would result in a linear rate of swelling. Hydrogels with varying ratios of methyl methacrylate, n-vinyl pyrrolidone, and crosslinker were synthesized via bulk free radical polymerization. Saponification was used to generate an ionic hydrogel. Dried hydrogels were swollen in an acidic solution of chitosan, then incubated in an aqueous solution of genipin to induce crosslinking. To quantify composition-dependent swelling, gels were incubated in saline at 37C under constant shaking. For swelling of gels with IPNs, gels were incubated in PBS or PBS with lysozyme at physiological concentrations. Swelling potential of the base hydrogel was controlled by monomer and initiator percentage and degree of ionization. The chitosan IPN degraded over time in the presence of lysozyme, leading to a linear, enzyme- and crosslinker concentration-dependent increase in hydrogel swelling over time. Preliminary data from subcutaneous implantation of the hydrogels in rats demonstrate translation of composition-driven swelling behavior from in vitro to in vivo, thus validating the utility of these hydrogels as tissue expanders for a range of applications.

Rapid Fire #: 12

A Sprayable Hydrogel-Based Wound Dressing for Burn Treatment

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Second- and third-degree burns cause severe skin damage which cannot be regenerated without medical attention. The treatment of these wounds is challenging since the irregular size and depth, location, inflammation, reactive oxygen species production, microbial invasion, and ischemic condition can hinder the healing. The limited oxygen supply and infection in the wound area are critical for the wound-healing process. Hydrogel-based dressings are promising for these types of wounds by presenting multiple functions for the treatment.

A hydrogel-based wound dressing containing hyaluronic acid and gelatin was developed to maintain homeostasis in the wound microenvironment and promote regeneration by enhancing cell migration and proliferation. HAMA and GelMA enable controllable photocrosslinking and form a durable hydrogel on the wound surface. A sprayable formulation was assessed for their easy administration in situ. GelMA precursor solution within a range of 5-15 % (w/v) was evaluated to have a sprayable formulation using different types of spray bottles. An ex vivo pig skin model was used to demonstrate sprayability of precursor solution and formation of stable hydrogel after administration. HAMA (1% w/v) was added into the precursor solution to provide elasticity and structural strength to the dressing. Calcium peroxide was also added to provide self-oxygenation and antibacterial properties. Various concentrations of CaO2 were added to the determined precursor solution. The composite hydrogels were characterized based on their mechanical, swelling, oxygen-releasing, and antibacterial properties.

A composite hydrogel with 10 % GelMA and 1 % HAMA was found as a sprayable formulation. Mechanical and swelling behaviors exhibited typical characteristics of hydrogel-based wound dressings, aiding in maintaining wound area homeostasis and absorbing exudates. CaO2 decomposition provided oxygen, particularly at concentrations of at least 12 mg/mL for up to two weeks in vitro. The released oxygen relieved metabolic stress in fibroblasts and reduced cell death under hypoxia. Furthermore, the zone of inhibition test showed that the composite hydrogel-based dressing exhibited antibacterial properties against common wound infectious bacteria including P.aeruginosa and S.aureus. The effective activity changed depending on the concentration of CaO2.

Overall, developed multifunctional sprayable hydrogel is self-oxygenating and antibacterial, enhancing wound healing and skin regeneration for second- and third-degree burns.

Rapid Fire #: 13

Photocurable citrate-based elastomers for tissue engineering

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Biodegradable citrate-based polymers (CBPs) have emerged as a distinctive biomaterial platform with significant potential for various biomedical applications, including regenerative engineering. The highly versatile mechanical properties, which can be tailored to match surrounding tissues, are crucial for their use in implantable biomedical devices, ensuring mechanical stability, structural integrity, and tissue integration. A photocurable methacrylate CBP (mCBP) has been developed to enable the high-resolution and high-speed fabrication of biodegradable scaffolds via a light-projection-based 3D printing technique. However, current mCBP inks often produce rigid and brittle polymers due to a high degree of crosslinking. In this study, we aim to modify the crosslinking network of mCBPs through the introduction of reactive diluents and/or chain transfer agents into the mCBP ink and thereby modulate the mechanical properties of photocured polymers. It was found that the addition of isobornyl acrylate (IBOA) resulted in remarkably tougher polymers. Specifically, it increased the Young's modulus by 2 folds (56 MPa vs. 29 MPa) and enhanced the ductility, measured by maximum elongation at fracture, by 2 folds (34% vs. 16%). Further addition of 3,6-dioxa-1,8-octanedithiol (DOD) produced a more ductile polymer, with ductility increasing to 56% compared to 16% for the pristine polymer, while the Young's modulus decreased to 9.2 MPa. Moreover, the modified inks could be successfully 3D printed into scaffolds with complex structures. In summary, this work demonstrates that by introducing reactive diluents and/or chain transfer agents into the mCBP inks, a wide range of mechanical behaviors of degradable polymers are readily achieved while retaining the same 3D printability to produce scaffolds with anatomically accurate structures.

POSTERS

Poster #: 1

Antimicrobial Hemostatic Shape Memory Polymer Foams for Infection Prevention in Traumatic Wounds

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The primary cause of trauma-related deaths prior to arrival at medical centers remains uncontrolled bleeding. Within a week following their injury, 39% of patients with traumatic wounds develop a polymicrobial infection. Hemostatic materials with antibacterial and antibiofilm properties that reduce infection rates could therefore enhance the healing of traumatic wounds. Previous studies have shown the successful incorporation of phenolic acids (PAs) into hemostatic shape memory polymer (SMP) foams by two mechanisms (chemical and physical) to produce dual PCA (DPCA) foams, which demonstrate excellent antimicrobial and antibiofilm properties against native E. coli, S. aureus, and S. epidermidis; co-cultures of E. coli and S. aureus; and drug-resistant S. aureus and S. epidermidis, compared with clinical controls. To build on this work in efforts to improve coagulation processes in antimicrobial foams, dual phenolic acid (DPA) foams containing vanillic acid (DVA), ferulic acid (DFA), and p-coumaric acid (DPCA) were synthesized. Biofilm formation on foam surfaces will be quantified using confocal laser scanning microscopy and metabolic activity analysis through intracellular adenosine triphosphate levels. These analyses will provide quantitative insights into the antimicrobial and antibiofilm performance of the foams. Subsequently, biofilm formation, metabolic activity, and colony forming units (CFUs) will be monitored over a 7-day period in an ex vivo model using porcine skin wounds that have been inoculated with bacteria. In parallel, blood/material interactions will be characterized using whole porcine blood in static and dynamic models of traumatic wounds in terms of platelet interactions, coagulation times, and hemorrhage control.

Poster #: 2

Polyurethane shape memory polymer foam hemostatic dressings with enhanced cell attachment to improve wound healing

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Motivation: Polyurethane (PUr) shape memory polymer (SMP) foam dressings have increased clotting efficacy compared to the clinically available treatment options for traumatic wounds. They can be tailored to degrade if left in a wound after stopping bleeding to serve as a scaffold for tissue regeneration. The incorporation of a bioactive component can further promote healing by improving cell attachment and proliferation on the porous foam scaffolds. Here, we physically adsorbed gelatin and collagen to PUr SMP foams and studied the effect it had on cell-material interactions.

Methods: PUr SMP foams were synthesized using a gas-blowing process. Then, foam samples were incubated in a 200 ug/mL solution of Type A Gelatin or Type I Collagen for 1 hour. Control foams (no bioactive coating) and foams with the physically adsorbed gelatin and collagen were sterilized and seeded with NIH/3T3 GFP cells. The samples were imaged with fluorescent microscopy at 24, 48, and 72 hours. Cell attachment was quantified for each time point using ImageJ. The effect of the bioactive coatings on shape memory properties (shape fixity and shape recovery) was also tested.

Results: PUr SMP foams coated in collagen and gelatin had increased cell attachment compared to the control foam after 24 h. Moreover, the collagen and gelatin-coated samples demonstrated cell proliferation, with increasing cell attachment at 48 and 72 h, compared to the control samples which had a decrease in cell attachment after 24 h. The physical adsorption of both collagen and gelatin had no effect on shape recovery, yet improved shape fixity compared to the control foam due to increased hydrogen bonding interactions.

Conclusions: The physical adsorption of gelatin and collagen to the PUr SMP foams improved cell attachment and proliferation and provides a method for tailoring our system without changing chemistry during foam synthesis. Future work will compare physical incorporation to the chemical incorporation of methacrylated gelatin into the PUr SMP foam network. Since gelatin and collagen are commonly used in the design of hemostatic dressings for their procoagulant properties, we will also explore blood-material interactions, expecting improved clotting capabilities which can further improve overall wound healing outcomes.

Poster #: 3

POLYVINYL ALCOHOL/GELATIN HYDROGELS WITH PHYSICALLY INCORPORATED CURCUMIN FOR CROHN'S FISTULA TREATMENT

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Background: Crohn's disease (CD) is an inflammatory bowel disease that affects ~1M people in the US. A major complication in CD is fistulas, wound tracts that cause pain and infection. Surgery and plugs can be used to treat fistulas, but they have limited efficacy. To address this need, we developed a highly tunable polyvinyl alcohol/gelatin (PVAGel) hydrogel system with potential for use in healing applications. Curcumin is a natural compound with unique features, including anti-inflammatory and antibacterial properties, that contribute towards wound healing. Here, we physically incorporate curcumin into our PVAGel hydrogel system and characterize its potential for Crohn's fistula healing in vitro and ex vivo.

Methods: We synthesized hydrogels using the thiol-ene reaction between methacrylated PVA (PVAMA), thiolated PVA (TPVA), and methacrylated gelatin (GelMA). Hydrogels were prepared by mixing TPVA/PVAMA/GelMA (ratio 1:4:2) and TPVA/PVAMA (ratio 1:4) in cell media and then adding lithium phenyl-2,4,6-trimethylbenzoylphosphinate. Curcumin was dissolved in dimethyl sulfoxide (75 mg/ml) and blended into the two hydrogel solutions at varying concentrations (0.34, 0.68, and 1.02 µM). The solutions were crosslinked under UV light for 9 minutes and then characterized in terms of surface chemistry, mechanical properties, curcumin release profiles, cytocompatibility, in vitro scratch closure, ex vivo wound healing, and antimicrobial properties.

Results: Infrared spectroscopy demonstrated successful incorporation of curcumin into PVAGel hydrogels. Initial tensile testing demonstrates that the system has high toughness and flexibility, indicating suitability for fabricating fistula plugs. A live/dead assay with NIH/3T3 cells showed that all samples have high cell viability (≥96%). Curcumin release rates were higher in GelMA-containing gels, and they depended on curcumin incorporation amounts. Current work is focused on characterizing effects of curcumin on the epithelial-to-mesenchymal transition, in vitro scratch closure, ex vivo pig skin wound closure, and bacterial growth inhibition.

Conclusions and Future Work: Our work presents a scalable method to incorporate curcumin into PVAGel hydrogels for potential use in Crohn's fistula treatment and wound healing applications. Curcumin-containing gels show adequate mechanical properties for fistula plugs, high cytocompatibility, and effective curcumin release. Future work includes incorporation of mesenchymal stem cells into these hydrogels to provide a cellular-based cue for healing.

Poster #: 4

Challenges in MUC2 Hydrogel Simulation Modeling

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Intestinal mucus, a natural hydrogel, modulates interactions between our underlying tissues and the contents of our gastrointestinal track. Understanding the molecular interactions of mucin glycoprotein, a critical building block of mucus, with signaling molecules such as bile salts, phospholipids, and fatty acids, is important to comprehend their significance in intestinal inflammation and associated diseases and facilitate the development of related therapeutics. In this study, we performed all-atom(AA)and coarse-grained (CG)classical molecular dynamics simulations of simplistic molecular models of the intestinal mucin MUC2 and its interaction with bile salts (sodium taurocholate and sodium taurodeoxycholate) and phospholipids (DOPC- 1,2-dioleoyl-sn-glycero-3-phosphocholine). The complete atomistic structure of MUC2 was assembled using AlphaFold2, homology modeling, and cryoEM maps, and used the model to study interaction patterns of the signaling molecules throughout the length of MUC2. In order to understand the aggregation properties of these glycoproteins which lead to their hydrogel behavior, we have constructed a 3D prototype peptide-glycan network, based on consensus MUC2 Variable Number of Tandem Repeats (VNTR)sequence, and native glycoform distribution. Intestinal signaling molecules have been introduced at physiological concentration to the mesh to elucidate the structural determinants that mediate binding and signaling. Key drivers of intermolecular associations such as hydrogen bonds, salt bridges, hydrophobic packing, as well as effects on rheological properties of mucin with the signaling molecules have been determined. CG simulations are also reported to study bile micelles (formed by bile salts, phospholipids, and fatty acids) with the mucin. The outcomes of this double-pronged approach of combined AA and CG simulations will be presented and discussed.

Poster #: 5

Investigating the effects of AXL as a driving force of dormant behavior

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Metastatic breast cancer remains one of the leading causes of cancer-related deaths. Despite treatment or removal of the primary tumor, many patients experience a relapse event after a latency period known as dormancy. In many cases, by the time a primary tumor is detected a select number of cancer cells will have detached and either be circulating through the body or inhabiting pre-metastatic niches at distant tissue sites. This poses a challenging issue as current therapeutics are unable to target and eliminate these single dormant cells, more pressingly, there are few physiologically relevant in vitro models to study this crucial part. Current models contain several limitations including high proliferation rates, lack of standardized dormancy markers, and utilizing cellular dormancy over existing tumor dormancy models. To combat this problem, we use live cell lineage approach to distinguish and track potential dormant cells across a variety of microenvironments and conditions. From previous Peyton lab members, we modified an existing induced-dormancy protocol by subjecting MDA-MB-231, MCF7, and HCC1954 cells to serum deprivation and imaging for 48 hours before mimicking their 'reawakening' with serum additions. By noting which cells started dividing after the 48-hour mark, we were able to postulate that these cells contained a dormant phenotype where proliferation began again once conditions were ideal. Past studies have confirmed that the distribution of dormant cells is heavily reliant on intrinsic and extrinsic factors, such as extracellular matrix composition and cell-to-cell interactions, where greater amounts of these dormant cells were found in 3D collagen gels over other environments. Currently, we're studying the effects of knocking out and inhibiting AXL, a receptor that aids in immune evasion, in triple negative MDA-MB-231 breast cancer cells. Due to its importance in a variety of signaling pathways, AXL has become a more popular clinical target. Preliminary data suggests that AXL knockout cells lead to greater quiescent cell distribution and in this way, we propose that AXL is a driving force for senescent and dormant behavior and provides a novel clinical target with greater specificity for dormant cells.

Poster #: 6

Investigating Tumor Microenvironment Drivers of Drug-Resistant Non-Small Cell Lung Cancer

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Non-small cell lung cancer (NSCLC) is a global health challenge. Despite significant advances, patients succumb to NSCLC most often because the disease becomes multi-drug resistant (MDR). One of the mechanisms by which this disease attains MDR is due to factors from the tumor microenvironment (TME) that promote cell survival even under pressure from otherwise powerful chemotherapy and targeted drug therapies. Conventional 2D tissue culture methods inadequately mimic this TME, which limits our ability to understand how the TME promotes MDR in preclinical models and in patients. I am developing 3D biomaterials and 3D spheroids to model cell-matrix and cell-cell interactions important to NSCLC in the TME. In collaboration with the Pritchard lab, I am culturing drug-sensitive, drug-resistant, and gene drive-modified NSCLC cells in 3D environments to understand how the TME regulates their relative growth and drug response rates. My preliminary results have revealed a striking phenomenon: drug-resistant cells proliferate faster in 3D clusters compared to 2D single cell cultures, even without drug pressure. This finding suggests that a therapy or a dosage that may seem effective in a 2D environment may give different results in a 3D setting. My ongoing work is optimizing the ratio of the three PC9 cell populations within spheroids to effectively eradicate the PC9 drug-resistant within a 3D environment using the gene drive cells, pairing 3D environments and a mouse model. This research has the potential to help develop more effective therapies for NSCLC patients and ultimately bridge the gap between preclinical and clinical results.

Poster #: 7

Polycaprolactone can be acutely toxic to developing zebrafish and activate nuclear receptors in mammalian cells

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Moldable plastics are sold, in the form of pellets as consumer products and over-the-counter dental materials with seemingly little regulatory oversight. Despite the anticipated dermal and oral contact, manufacturers share little information with consumers about these materials. Inherent to their function, moldable plastics pose a risk of dermal and oral exposure to unknown leachable substances. Moldable plastics advertised for modeling and dental applications were determined to be polycaprolactone (PCL) or thermoplastic polyurethane. The bioactivities of the most popular brands advertised for modeling applications of each type of polymer were evaluated using a zebrafish embryo bioassay. Water-borne exposure to the PCL pellets proved acutely toxic above 1 pellet/mL while the TPU pellets did not affect the targeted developmental endpoints at any concentration tested. Aqueous leachates of the PCL pellets were similarly toxic. Extracts from the PCL pellets were assayed for their bioactivity using the Attagene FACTORIAL platform. The extracts activated nuclear receptors and transcription factors for xenobiotic metabolism (pregnane X receptor, PXR), lipid metabolism (peroxisome proliferator-activated receptor gamma, PPARg), and oxidative stress (nuclear factor erythroid 2-related factor 2, NRF2). Chemical analyses by non-targeted high-resolution comprehensive two-dimensional gas chromatography (GC×GC-HRT), tentatively identified PCL oligomers, a phenolic antioxidant, and residues of suspected anti-hydrolysis and crosslinking additives in the extracts. In a follow-up zebrafish embryo bioassay, biomedical grade PCL was tested and elicited comparable acute toxicity to the PCL pellets. It is suggested that the toxicity was due to oligomers and nanoplastics released from the PCL rather than chemical additives. These results challenge the perceived and assumed inertness of plastics and highlight their multiple sources of toxicity.

Poster #: 8

Adhesive Implant-Tissue Interface without Fibrous Capsule Formation on Diverse Organs

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Implanted biomaterials and devices face compromised functionality and efficacy in the long term owing to foreign body reactions and subsequent formation of fibrous capsules at the implant–tissue interfaces. To alleviate the formation of the fibrous capsule at the implant–tissue interface, various approaches have been developed, including drug-eluting coatings, hydrophilic or zwitterionic polymer coatings, active surfaces and controlling the stiffness and/or size of the implants. However, despite recent advances, the mitigation of fibrous capsule formation for implanted biomaterials and devices remains an ongoing challenge in the field, highlighting the importance of developing new solutions and strategies. Here we demonstrate that an adhesive implant–tissue interface can mitigate fibrous capsule formation in diverse animal models, including rats, mice, humanized mice and pigs, by reducing the level of infiltration of inflammatory cells into the adhesive implant–tissue interface compared to the non-adhesive implant–tissue interface. Histological analysis shows that the adhesive implant–tissue interface does not form observable fibrous capsules on diverse organs, including the abdominal wall, colon, stomach, lung and heart, over 12 weeks in vivo. In vitro protein adsorption, multiplex Luminex assays, quantitative PCR, immunofluorescence analysis and RNA sequencing are additionally carried out to validate the hypothesis. We further demonstrate long-term bidirectional electrical communication enabled by implantable electrodes with an adhesive interface over 12 weeks in a rat model in vivo. These findings may offer a promising strategy for long-term anti-fibrotic implant–tissue interfaces.

Poster #: 9

Comparison of Sterilization Methods on Biomarker Libraries of Dehydrated Placenta Membrane Products: E-beam vs Supercritical CO2

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Introduction: Human Amniotic Membrane (hAM) is a valuable biomaterial utilized for wound coverage in various clinical settings. Dehydration is a common processing step to ensure product stability and shelf life. Two widely used dehydrated membrane products, Artacent Wound® and Artacent Wound Pro®, are terminally sterilized using different methods, namely E-beam and Supercritical CO2, respectively. This study aims to characterize and compare the protein content and biomarker libraries of these products.

Methods: Total protein content, SDS-Page Gel analysis, and Microarray techniques were employed to assess protein composition in Artacent Wound® and Artacent Wound Pro®. Additionally, biomarkers and cytokines were categorized into angiogenic, immune-modulating, and regenerative aspects for comprehensive analysis.

Results: Analysis of protein content and biomarker libraries revealed differences between Artacent Wound® and Artacent Wound Pro®. While both products exhibited distinct protein profiles, variations in biomarker composition were observed, particularly in angiogenic, immune-modulating, and regenerative biomolecules. The sterilization method appeared to influence the retention of specific biomarkers and cytokines within the hAM products.

Conclusion: This study provides valuable insights into the impact of sterilization techniques on the protein content and biomarker libraries of dehydrated placenta membrane products used in wound coverings. Understanding the effect of sterilization methods on biomarker retention is essential for optimizing the clinical efficacy of hAM products and advancing wound care strategies. Further investigation is warranted to elucidate the underlying mechanisms and refine sterilization protocols for enhanced biomarker preservation in hAM-based therapies.

Poster #: 10

Degradation of polyurethane foams for traumatic wound healing

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Motivation: To combat the large number of deaths from uncontrolled hemorrhage, we developed a polyurethane (PUr) foam dressing that can be inserted into a non-compressible wound to stabilize the bleed and remain there for the duration of the healing process. To ensure that the tissue regrows properly, the foam must degrade in ~8 weeks to match the expected traumatic wound closure rate. Dressing degradation also eliminates the need for additional medical attention related to dressing removal, which can cause re-bleeds and tissue damage. To that end, we incorporated degradable monomers containing sulfides and esters to increase the rate of PUr foam degradation.

Methods: Degradable monomers were synthesized using click chemistry. Briefly, 1 mole of trimethylolpropane tris(3-mercaptopropionate) or dipentaerythritol hexakis was reacted with 3 or 6 moles of 3-buten-1-ol, respectively, using a photoinitiator and UV light. The resulting trithiol and hexathiol monomers were added during PUr foam synthesis in 15% or 30% of the total hydroxyl components. Foam degradation was studied in 3% hydrogen peroxide (H2O2) to characterize oxidative degradation and phosphate buffered saline (PBS) for hydrolytic degradation analysis over 12 weeks at 37°C. Samples were analyzed at weeks 2, 4, and 5-12 in terms of mass loss, erosion, thermal, and mechanical properties.

Results: After 8 weeks, the PUr foams underwent negligible degradation in PBS. In 3% H2O2, the 30% trithiol foam experienced the most degradation, with approximately 53% mass remaining, and the 30% hexathiol foam degraded slightly slower, with 62% mass remaining at 8 weeks. The 15% trithiol and hexathiol foams had 73% and 68% mass remaining at 8 weeks. Corollary decreases in size (erosion), mechanical, and thermal properties were observed. The control foam had a remaining mass of 92%, indicating that the new degradable monomers effectively increased degradation rates.

Conclusions: Although, full degradation was not observed at 8 weeks, the addition of monomers with sulfide and ester groups did increase degradation rate. Current work is focused on synthesizing additional sulfide/ester-containing monomers to further increase degradation rates and enable PUr placement during hemostasis and healing.

Poster #: 11

Chemical Characterization of Extractables in a Radiation Stressed Yttrium-90 (Y-90) Radioembolic Microsphere Container-Closure

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Chemical characterization of extractable residues and degradation products for medical devices are an important aspect of biological evaluation. To address challenges with characterizing radioactive materials, novel container-closures (ABK Biomedical), without β-emitting Yttrium-90 (Y-90) microspheres, were radiation stressed by E-beam at a dose rate of ~60kGy/h up to >450kGy (total exposure); to simulate exposure to the inner-surfaces in direct contact with 600mg Y-90 microspheres during normal production/shelf-life.

Container-closure degradation was assessed at variable dose rates (~60kGy/h to ~285kGy/h) by Oxidation Induction Time testing according to ISO 11357-6:2018 using Differential Scanning Calorimetry (DSC) and Thermal Gravimetric Analysis (TGA). Fill-volume extractions were performed at surface area to volume ratios of ~4cm2/mL in purified water at 50°C for 24h iterative extractions under mild agitation (80 rpm). Exhaustive endpoint was determined by gravimetric non-volatile residue (NVR) for two pooled device-extracts (n=3 replicates per extraction), with exhaustive endpoint defined as NVR below the LOD (<0.03mg) or <10% the first extraction according to ISO 10993-18:2020 (requiring at minimum three 24h iterative extractions). Pooled device-extracts (24h, 48h, and 72h) and contained saline were analyzed by gas chromatography/liquid chromatography-mass spectrometry (GC/LC-MS), headspace GC-MS, inductively coupled plasma-mass spectrometry (ICP-MS) and ion chromatography (IC). Toxicological Risk (TR) was assessed according to ISO10993-17:2023 for long-term effects (based on contact with long-term implantable microspheres) including acute/subacute/subchronic/chronic toxicity, genotoxicity, carcinogenicity, developmental and reproductive toxicity.

DSC and TGA demonstrated no evidence of container-closure degradation post exposure to E-beam. Zero extractable mass by gravimetric NVR was detected after 24h, 48h, and 72h extractions (below LOD (0.03mg)), thus meeting the exhaustive endpoint criterion. TR for the biological endpoints assessed based on worst-case clinical use were deemed tolerable based on Margin of Safety (MOS) values > 1 (5 to 60,000,000) for elemental extractables (19) identified above the LOQ, with estimated exposure for the organic extractable (Trifluoroacetic Acid) identified and semi-quantitated above the analytical evaluation threshold, determined to be below the 120 µg/day Toxicological Screening Limit.

Achievement of these biological endpoints support the safety of this novel container-closure to house Y-90 microspheres intended for use as internal radiation brachytherapy for local tumor control in patients with unresectable Hepatocellular Carcinoma.

Poster #: 12

In Vitro Derived Cardiac Extracellular matrix

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Introduction: Heart disease including myocardial infarction represents the largest cause of mortality globally. This is partially due to the poor regenerative capacity of the heart, mainly owing to the post-mitotic nature of cardiomyocytes (CMs) after birth. While fetal cardiac extracellular matrix (ECM) has been shown to induce cell-cycle reentry by adult CMs, the identification of specific peptides required for this effect and how to cultivate them in a scalable way has not been fully developed. This research seeks to investigate the potential of in vitro derived cardiac ECM (ivECM) as a therapeutic biomaterial.

Methods: First, the culture primary cardiac fibroblasts from fetal and neonatal rat were optimized for maximum matrix deposition. Cultures were then decellularized and the resulting ivECM was characterized via LC-MS/MS and compared to native whole ECM. Finally, the fetal and neonatal ivECM and native ECM were assessed in terms of their influence on CM proliferation and maturation via immunohistochemistry.

Results and Discussion: Experiments and analysis are still ongoing, but early results indicate that we can optimize ECM production by cardiac fibroblasts by altering serum concentration and the addition of macromolecular crowding. Current studies are focused on analyzing the LC-MS/MS data of in vitro culture derived ECM and assessing the impacts of this ECM on cell function in the context of the heart.

Poster #: 13

Macrophage-targeted drug delivery for systemic and local immune modulation

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Macrophages (MF) are mediators of the immune microenvironment that span diverse behaviors, ranging from prototypical inflammatory (M1-like) to pro-healing (M2-like) phenotypes. While small-molecule drugs are available to treat many diseases by modulating MF behavior, their efficacy remains limited by a poor understanding of which drugs can promote desirable MF cell-state transitions and inadequate drug pharmacokinetics (poor solubility, non-specific cell uptake, rapid blood clearance, and off-target effects). Biomaterial-based drug delivery systems enable cell- and tissue-targeting strategies to overcome these challenges. Furthermore, the recognition of drugs by macrocyclic hosts provides a readily adaptable method to encapsulate a variety of therapeutics for delivery, including within soluble nanoparticles (for systemic cell-targeted therapies) or injectable hydrogels (for local applications, such as focal tissue injury). This talk will discuss two applications of these systems, which include in the context of cancer immunotherapy and the prevention of post-ischemic heart failure.

Tumor-associated macrophages (TAMs) are abundant in solid cancers, assuming a pro-angiogenic and immunosuppressive M2-like phenotype that supports tumor growth and immune escape. Recent methods have focused on identifying therapeutic drugs that re-polarize TAMs to a tumor-destructive M1-like phenotype. Through high-content screening and qPCR analysis in primary murine and human cells, we identified a drug (R848) that potently induced a tumor-destructive state (IC50=7.2nM). Cyclodextrin nanoparticles (CDNPs) were prepared by crosslinking cyclodextrin with lysine. Drug-loaded CDNPs (CDNP-R848, >10%w/w drug loading) accumulated in tumors by MF-specific update and eradicated tumors in multiple models (MC38, Gl261). Treatment synergized with frontline anti-PD1 checkpoint therapy.

In contrast, tissue injuries such as myocardial infarction are characterized by exuberant local inflammation. We therefore developed hydrogels composed of CDNPs crosslinked by polymer-nanoparticle interactions. The hydrogels are shear-thinning for ease of injection and self-healing for local retention. Through cell-reporter assays, we identified celastrol's ability to induce a pro-healing MF state (IC50<100nM). Hydrogels released CDNP-celastrol for >14 days with >80% inhibition of inflammatory response. In a murine model of ischemia-reperfusion injury, targeted delivery of celastrol prevented left-ventricular remodeling and preserved heart function. In sum, small-molecules can potently modulate MF phenotype and their therapeutic efficacy is improved through cell- and tissue-targeted delivery to aid clinical translation.

Poster #: 14

Hybrid LNP prime dendritic cells for nucleotide delivery

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Efficient activation of professional antigen-presenting cells—such as dendritic cells (DC)—in tumors and lymph nodes is critical for the design of next-generation cancer vaccines and may be able to provide anti-tumor effects by itself through immune stimulation. The challenge is to stimulate these cells without causing excessive toxicity. We hypothesized that a multi-pronged combinatorial approach to DC stimulation would allow dose reductions of innate immune receptor-stimulating agonists while enhancing drug efficacy. To achieve this goal, we developed a hybrid lipid nanoparticle (LNP) platform featuring "nanoparticle-in-a-nanoparticle" design that contains i) a toll like receptor (TLR) agonist poly I:C and ii) a second nanoparticle based on cyclodextrin as a "sponge" for encapsulating small molecule therapeutics to enhance DC stimulation. We show that a single dose of hybrid LNPs alone, effectively eradicate tumors, and generated long-lasting, durable anti-tumor immunity in mouse models. This approach lead to vastly enhanced efficacy over LNP/TLR3 alone while showing negligible toxicity at pharmacological concentration.

Poster #: 15

Computational Characterization of Glycosylated Peptides Towards the Development of Glycan Based Drug Delivery Systems

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With the increasing necessity of tissue-specific and often patient-specific drug therapies for the treatment of complex diseases, there has been a commensurate increase in the need for fine-tuned and reliable molecular packaging materials to help ensure the efficacy of these drugs. Many well researched materials such as lipid nanoparticles and viral vectors have proven particularly effective in the delivery of nucleic acid gene therapies; however, some somatic tissues exhibit molecular environments which pose great challenges to gene therapy even with current delivery methods. Somatic environments like the intestinal lining and lung epithelium can develop a thick layer of mucus in a patient suffering from diseases like Crohn's or cystic fibrosis respectively. Cystic fibrosis specifically could theoretically be cured by supplying a functional copy of the CFTR gene to a patient, but all attempts to deliver such a gene using currently favored methods have failed due to the delivery vehicles becoming lodged in the thick pulmonary mucus instead of reaching their intended cellular targets. Here, we propose utilizing a novel composite drug carrier consisting of glycosylated structural cationic polymers and cell penetrating peptides (CPPs) in a polyplex formulation with an mRNA payload to effectively deliver a gene to a fibrotic lung. These components are suggested to solve three specific problems: cationic polymers to ionically associate with mRNA strands, CPPs to initiate endocytosis, and glycans to penetrate the mucus layer. Due to the highly modular and combinatoric nature of polymers, peptides, and glycans, high-throughput molecular dynamics simulations and machine learning driven algorithms are proposed to quickly and efficiently identify viable candidate combinations before experimental validation. This work will ideally contribute to a computational, data driven platform capable of predicting an optimal combination of monomers in a polymer-based drug delivery system for delivery of a certain drug to a specified somatic environment.

Poster #: 16

Ionizable lipid content of messenger RNA loaded lipid nanoparticles impacts delivery and gene expression in trophoblast cells

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Maternal mortality rates in the United States have risen over the last five years, nearly doubling from 17.4 to 32.9 deaths per 100,000 live births [1]. Defined as "the death of a woman while pregnant or within 42 days of termination of pregnancy" [2], maternal deaths are disproportionately higher in black women and women 40 or older [1]. This aligns with an increase in chronic health conditions in women when they become pregnant, which exacerbate pre-existing conditions and increases risk for development of pregnancy-related complications. The limited information on pharmaceutical interactions during pregnancy drives the need for research on the interactions of drug and gene delivery vehicles with the placental barrier for the development of therapies for pregnancy-related complications, such as preeclampsia, and pre-existing conditions, such as bipolar disorder, during pregnancy. The potential of lipid vesicles as a delivery mechanism has risen in recent years, notably with the use of lipid nanoparticles (LNPs) for the Moderna and Pfizer-BioNTech COVID-19 vaccines [3]. Our work aims to determine the optimal ionizable lipid that has been used in an FDA-approved LNP formulations to now be used for nucleic acid delivery to placental trophoblast cells. LNPs were formulated through microfluidic mixing to contain ionizable lipids, helper lipids, cholesterol, and polyethylene glycol(PEG)-conjugated lipids. These LNP formulations contained either SM-102, ALC-0315, or Dlin-MC3-DMA (MC3) ionizable lipids, which are used in Moderna's Spikevax™ COVID-19 vaccine, Pfizer-BioNTech's Comirnaty® COVID-19 vaccine, and Alnylam's Onpattro® polyneuropathy medication, respectively. All LNPs were loaded with luciferase-producing messenger RNA (mRNA). HTR-8/SVneo trophoblast cells were dosed with mRNA loaded LNPs, and gene expression was assessed at various timepoints. The luminescence results demonstrated all LNPs had successful uptake by trophoblast cells and subsequent gene expression. The SM-102 formulation resulted in a ~10-fold increased gene expression compared to the formulations containing ALC-0315 and MC3. GFP-tagged mRNA loaded LNPs were formulated and characterized to confirm luciferase results. Based on this data, SM-102 will be further investigated for mRNA delivery in 3D trophoblast cultures.

[1] Hoyert et. al., NCHS Health E-Stats, 2023. [2] World Health Organization, International stats., 2009. [3] Tenchov et. al., ACS Nano, 2021.

Poster #: 17

Exogeneous Mucin as a Muco-Adhesive or Diffusive Biomaterial Impacting Absorption of Poorly Water-Soluble Drugs

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Mucus, a highly complex biological material composed of glycosylated proteins, serves as a vital protective barrier on mucosal surfaces throughout the body. Understanding mucus barrier properties is fundamental for the development of effective drug delivery systems. The observation that native milk emulsion droplets are coated in mucins and are highly effective in delivery of a variety of types of molecular cargo motivates exploration of the potential role of the mucin coating. This work describes the exploration of the impact of incorporation of mucin on transport of colloidal particles through intestinal mucus. Diffusion coefficients of nanoparticles with incorporated mucin were measured to understand the effect of mucin as a biomaterial for oral delivery formulations. Multiple particle tracking experiments were conducted to characterize muco-adhesive or muco-diffusive characteristics of particles with and without incorporated mucins to gain a deeper understanding of how mucins may impact efficiency of nanoparticle movement through the mucus layer. In addition, the absorption of a series of poorly water-soluble drugs (PWSD) with differing physicochemical properties was assessed from media containing bile micelles with or without mucin and fatty acid incorporation. Exploring the individual effects of exogenous mucin and fatty acids can help delineate their roles in modulating the behavior of nanoparticles within the mucus layer, offering potential insight into effective development of muco-adhesive or muco-diffusive drug delivery systems.

Poster #: 18

Entrapment of pyruvate dehydrogenase in alginate hydrogel sheets towards a biofilm-eliminating wound dressing

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Introduction: Biofilm infections pose a significant challenge in wound management due to biofilms' tolerance to antimicrobial agents. Present in 90% of chronic wounds, biofilms affect 2% of the US population within their lifetime. Our previous work demonstrated that the metabolite pyruvate's presence is crucial for both biofilm formation and maintenance, and its enzymatic depletion leads to dispersion of both gram-positive and negative biofilms.

Methods: We have synthesized a novel hydrogel-based wound dressing that varies in sodium alginate concentrations, using calcium chloride as the crosslinker, to entrap pyruvate dehydrogenase (PDH) for depleting pyruvate. Hydrogels mechanical/structural properties were investigated via compression testing, swell testing, and scanning electron microscopy (SEM) for morphology and porosity. PDH (0, 2084, 2778, or 3473mU) was encapsulated into hydrogels of 2%(w/v) sodium-alginate and 0.3M calcium chloride (Alg2-Ca3). The presence of PDH in the hydrogel and its enzymatic activity were quantified by bicinchoninic acid (BCA) protein assay and ß-NADH absorption, respectively.

Results and discussion: The hydrogel's elastic modulus was 50KPa, which is slightly higher than that of human skin, important for durability. SEM quantified Alg2-Ca3 pores at 88.37±39.3nm, large enough to hold ~45nm PDH, but their tortuosity blocked PDH escape. Hydrophilicity and swelling are essential for the hydrogel to adsorb exudate and pyruvate to interact with the encapsulated PDH. Alg2-Ca3 demonstrated significant swelling (1579.8%) in phosphate buffered saline (PBS) over 24 hours. The average activity of the PDH before encapsulation was 9.07±3.2 mU/ml, which was within the range (5-10 mU/ml) to induce biofilm dispersion. By increasing PDH encapsulated from 2084 to 3473mU, hydrogels activity increased from 0.7 to 2.02mU/ml. The BCA assay quantified enzyme encapsulated in the synthesized beads, showing a concentration-dependent trend, and the average entrapment efficiency was 8.88%, which is similar to the efficiency seen with PLGA nanoparticles (9%).

Conclusion: These findings suggest that the encapsulation of PDH in sodium alginate can potentially serve as a novel wound dressing that prevents and eliminates biofilm infections by pyruvate depletion. Future studies will focus on increasing the concentration of encapsulated PDH, optimizing PDH loading and efficiency, conducting biofilm studies in-vitro and in-vivo, and evaluating cytotoxicity in human cells studies.

Poster #: 19

Targeted delivery of nanoparticles to hematopoietic stem cells

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Drug delivery strategies based on nanoparticles (NPs) have significantly advanced the therapeutic landscape in the recent years. Layer-by-layer (LbL) NPs can deliver a great variety of drug cargos allowing tunable biodistributions. Genetic blood disorders have posed an important therapeutic challenge, where so far only ex vivo cell therapies have resulted in effective cures. Although promising, these strategies carry high production and implementation costs, limiting the accessibility in areas and populations of higher incidence. The ability to reach hematopoietic stem and progenitor cells (HSPCs) in vivo would contribute to advancing the field toward more translatable and accessible treatments. This work aimed at evaluating the potential of the LbL platform to develop a targeted NP system that can direct therapies to HSPCs in vivo.

Given the low numbers of HSPCs in vivo, a highly specific targeting system will increase its efficacy. We first hypothesized that we could leverage the modularity of LbL NPs to decrease unwanted interactions with circulating cells by rationally selecting the outer layer. We compared three different outer layers and observed that some of them associated more with myeloid cells, and even T cells, while Layer1 stood out as the most inert surface. We then used this polymer as a scaffold to conjugate different HSPC-targeting antibodies (Abs) to. In vitro studies in mouse progenitor cells demonstrated different NP uptake and trafficking patterns depending on the Ab, with two outstanding leading candidates. Interestingly, in vivo studies of these Ab-LbL NPs pointed to an alternative and understudied candidate (Ab5) as the one with the highest HSC targeting in vivo, suggesting the role of other physiological parameters not recapitulated in vitro. We further confirmed the translatability of targeting this receptor to human HSPCs (CD34+ cells) in vitro, showing an enhanced cell uptake over the non-targeted formulations.

In conclusion, we have designed a drug delivery platform based on the LbL assembly and engineered it to decrease unspecific interactions with circulating blood cells, while targeting HSPCs in vivo.

Poster #: 20

Collective Transitions from Orbiting to Matrix Invasion in 3D Multicellular Spheroids

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Epithelial morphogenesis is governed by the dynamic reciprocity between groups of cells and the extracellular matrix, but this emergent phenomenon remains poorly understood. Here, we utilize live cell confocal microscopy to simultaneously interrogate collective cell migration and matrix deformation in 3D biomaterials. Remarkably, we show that multicellular spheroids of mammary epithelial cells exhibit highly coordinated circumferential orbiting, which transitions towards invasive strands oriented radially as the biomaterial is progressively remodeled. Intriguingly, the initial morphology of the multicellular spheroid also exhibits geometric alterations that are predictive of subsequent invasion. We further show these morphological dynamics can be linked to spatially non-uniform tractions within the biomaterial. Finally, we physically and biochemically perturb these collective dynamics to gain mechanistic insight into the underlying processes. Overall, this work establishes design principles for how epithelial tissues can be shaped by biomaterials to model development and disease.

Poster #: 21

Development of Multi-Drug Resistant Tumor Model Using 3D-Hydrogel Matrices

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A major contributor of poor patient prognosis in cancer, including ovarian cancer, is the development of disease relapse and resistance to chemotherapy. The clinical manifestation of therapeutic resistance occurs through multiple pathways and is termed multi-drug resistance or MDR To develop a model of MDR tumors in vitro, we have used human ovarian cancer cell line (SKOV-3) and evaluated their response to stress introduced by overlaying hydrogels with different thickness. The hydrogel layer was manually added above adherent SKOV-3 cells using a syringe to make our model biomimetic to the tumor microenvironment, while maintaining cell viability. The hydrogel was composed of 10% gelatin methacrylate (GelMA) and 1% gellan gum for enhanced viscosity and was ionically crosslinked by submersion in a calcium chloride solution for improved structural integrity. The hydrogel underwent resistance tests to examine its shear stress limits and swelling tests to calculate the crosslinking density for a range of crosslinking solution concentrations and duration. To investigate the mechanical effects of hydrogel on the cell layer, the cells were stained with f-actin and imaged to detect cell stress. Diffusion tests were also conducted to evaluate the diffusion of oxygen through the hydrogel layer to the cells as validation of the hydrogel-induced hypoxia. Furthermore, the hydrogel-based model aims to draw parallels with other hypoxic related work, a condition of low oxygen concentration facilitating reactions within mitochondrial networks that promote tumor survival and progression. The results of the Mitochondrial Network Analysis (MiNA) showed an increase in mitochondrial expression as the cells' response to the introduction of a hypoxic microenvironment, mimicking the response demonstrated by in vivo models and reflecting the effectiveness of the in vitro model. Thus, this affordable and accessible model by application of hydrogels can help scientists better understand the development of MDR and response to therapy in different tumor models

Poster #: 22

Investigating the effect of integrin-mediated mechanotransduction on neural stem cell morphology and differentiation

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In the treatment of neurodegenerative diseases, neural stem cells play a pivotal role, yet there remains much to unravel about their mechanisms of neurogenesis and astrogenesis, particularly in adulthood. Given the presence of integrin α6β1 within the subventricular (SVZ) niche, we selected differently-sized laminin 511 fragments, a primary extracellular matrix (ECM) component within this niche, to directly engage with integrin α6β1 to better understand their role. Our objective was to investigate the impact of mechanotransduction mediated by binding of laminin 511 subunits with integrin α6β1 and its possible link to the differentiation status of neural stem cells. The substrates were prepared by conjugating laminin 511 fragments onto the glass coverslips, and neural stem cells were subsequently seeded onto these treated substrates. Our findings revealed that neural stem cells exhibited robust adhesion and proliferation across all substrate types. Intriguingly, their morphology exhibited significant variations on different substrates, a phenomenon further influenced by integrin β1 blockade. Moreover, the study identified significant differences in YAP translocation to the nucleus upon integrin β1 blockage, resulting in a significant increase in astrocytic differentiation. Overall, this study's outcomes provide a promising avenue for developing novel strategies aimed at mitigating astrocytic differentiation during aging and injury while promoting neurogenesis.

Poster #: 23

Modular, biomimetic poly(ethylene glycol)-vinylsulfone hydrogels regulate encapsulated cell growth by restricting protein transport

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Hydrogels are water-swollen polymer networks that are increasingly used as tissue-mimicking biomaterials for 3D cell culture. Hydrogels in this context can serve as in vitro models of disease and/or as drug testing platforms that can capture the influence of 3D cell-microenvironment interactions. However, the 3D microenvironments introduce design challenges, including matching the stiffness, transport properties, and functional biochemical features of native tissue extracellular matrix. Prior work in our lab has established hydrogel formulations that match the stiffness and biochemical features of human tissues including brain, lung, and bone marrow, but solute transport effects in these hydrogels have not been investigated. Since synthetic polymer hydrogels typically have relatively homogeneous mesh sizes that are orders of magnitude smaller than those of gelatin, collagen, and Matrigel, they are likely much more restrictive to solute transport than those protein-based hydrogels, even when matching stiffness. Here, we investigated the role of microenvironment-restricted solute transport on hydrogel-encapsulated cell behaviors. We encapsulated breast cancer cells in homogeneous, thiol-vinylsulfone-crosslinked poly(ethylene glycol) (PEG) hydrogels with matrix metalloproteinase-degradable crosslinks and integrin-binding pendant peptides. These hydrogels spontaneously crosslink in 5-20 minutes under pH-neutral, physiological conditions without cytotoxic catalysts, making them ideal for encapsulating stress-sensitive cell types. Our formulation matches the stiffness of Matrigel, and encapsulated cells are well-distributed throughout the 10-uL gels. However, we found that solute transport is greatly restricted in PEG hydrogels compared to Matrigel, especially for large solutes such as cytokines, serum proteins, and antibodies. Because our lab has previously established that serum deprivation can induce a dormancy-like state in breast cancer cells, we are currently investigating whether hydrogel-restricted serum protein transport during cell encapsulation is sufficient to stimulate dormancy. In this way, we propose solute transport restriction as a novel physical microenvironmental contribution to breast cancer dormancy, which can be controlled, tailored, and optimized in hydrogels design alongside stiffness, confinement, and bioactivity.

Poster #: 24

Influence of Polyester Matrices on Macrophage Polarization: Unraveling Immune Responses to M1 and M2 Phenotypes

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The immune response to biomaterials is a critical area of contemporary research, particularly in light of numerous implant failures reported in medical applications. Macrophages are primary responders to foreign materials and are known to polarize into M1 or M2 phenotypes, assuming pro-inflammatory or anti-inflammatory functions, respectively. When unable to phagocytose or endocytose materials due to factors such as size, macrophages may fuse into multinucleated giant cells (MGCs) for degradation purposes. Our study involved conjugating various small molecules, including 3-phenyl propanoic acid (A), thianaphthene-2-carboxylic acid (S), 2-(3-chloro-2-methylanilino) benzoic acid (W), 2-[4-(4-Chlorobenzoyl)phenoxy]-2-methyl propanoic acid (U) and 2-(p-Chlorophenoxy)-2-methyl propanoic acid (V). These molecules were incorporated into a polyester matrix, applied onto coverslips using spin-coating techniques, and formulated into solutions with dimethyl sulfoxide (DMSO) to examine the influence of different material forms on macrophage behavior. In this study, we utilized RAW 264.7 cells, a monocyte/macrophage cell line derived from an Abelson murine leukemia virus-transformed lineage of BALB/c mice origin. The macrophage responses were assessed via RT-qPCR for genes including Il-1b, TNF-α as M1 markers, and Arg1 as M2 marker. Findings indicated that polyester conjugates with A, S, and W directed RAW cell polarization towards M1 phenotypes, whereas U and V influenced M2 polarization. Additional immunocytochemistry analysis was conducted for CD68, CD86, CD206, and Arginase-1. Soluble polymer forms elicited comparable responses in the RAW cells, with certain cases showing significantly higher gene expression levels than their filmed counterparts. These observed effects on macrophages may arise from a variety of mechanisms, such as immune checkpoint receptor or ligand binding to the functional group of polymers, downstream signaling pathway activation, cytokine production modulation, cellular metabolism alteration, etc. Future research will focus on elucidating the specific mechanisms responsible for macrophage polarization induced by these substances.

Poster #: 25

Remodeling the tumor immune microenvironment with tunable supramolecular hydrogels

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Immunotherapy has had a tremendous impact on cancer burden, however as many as 75-80% of cancer patients do not respond to current treatments. In these cases, single-agent immunotherapy is unable to activate a robust immune response due to immunosuppressive microenvironments featuring multiple, non-redundant immunosuppressive adaptations. While combination immunotherapy may enhance outcomes in these so-called 'cold' tumors, traditional delivery methods (e.g., systemic infusion) typically result in severe autoimmune side effects, thereby limiting the maximum tolerated dosage and practicality of such approaches. To address this, we are developing an injectable hydrogel engineered to reprogram the immune microenvironment within the tumor. This will enhance the efficacy of immunotherapy while limiting its systemic toxicity. The hydrogel system is modular, and we have shown that it is able to both tether immunostimulatory cytokines and encapsulate multiple immunostimulatory molecules. Our approach combines cytokines and antibody therapeutics to engage both the myeloid and lymphoid compartments of the tumor microenvironment (TME). Our results thus far indicate this biomaterial enhances the potency of immunostimulatory IL-2 family cytokines and also enhances retention of biologic drugs. We anticipate that this approach will transform the local immune microenvironment to sensitize tumors to immune checkpoint blockade.

We will present results of this strategy in the context of the B16F10 mouse model of melanoma, a well-characterized immunosuppressive 'cold' TME. TME changes will be assessed with flow cytometry, multiplexed immunofluorescence, and cytokine assays. We will assess the safety profile of our system through bodyweight monitoring, blood tests, and histology of major organs. Further, we will investigate synergistic outcomes upon combination with PD1/PDL1 checkpoint blockade. We anticipate our approach will provide a translationally relevant route towards enhancing immunotherapy in 'cold' tumors, potentially broadening the clinical impact of cancer immunotherapy.

Poster #: 26

Enhanced Cellular Targeting by Precision Control of DNA Scaffolded Nanoparticle Ligand Presentation

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Targeted nanoparticles have been extensively explored for their unique ability to deliver their payload to a selective cell population while reducing off-target side effects. The design of actively targeted nanoparticles requires the grafting of a ligand that specifically binds to a highly expressed receptor on the surface of the targeted cell population. Optimizing the interactions between the targeting ligand and the receptor can maximize cellular uptake of the nanoparticles and subsequently improve their activity. Here, we evaluated how the density and presentation of targeting ligands dictate the cellular uptake of nanoparticles. To do so, we used a DNA scaffolded PLGA nanoparticle system to achieve efficient and tunable ligand conjugation.

A prostate-specific membrane antigen (PSMA) expressing prostate cancer cell line was used as the first model. The density and presentation of PSMA targeting ligand ACUPA was precisely tuned on the DNA scaffolded nanoparticle surface and their impact on cellular uptake was evaluated. It was found that matching the ligand density with the cell receptor density achieved the maximum cellular uptake and specificity. Furthermore, DNA hybridization mediated targeting chain rigidity of the DNA scaffolded nanoparticle offered ~3 times higher cellular uptake compared to ACUPA-terminated PLGA nanoparticle. Our findings also indicated a ~3.7-fold reduction in the cellular uptake for the DNA hybridization of the non-targeting chain.

In another study, a-Clec9A antibody was grafted on the nanoparticle surface for conventional dendritic cell 1 (cDC1) targeting. The nanoparticles deliver STING agonist MSA-2 efficiently to this dendritic cell subset for enhanced antitumor immune response; derived by in vitro and in vivo studies.

Overall, our results provide a rational guideline for designing actively targeted nanoparticles and highlight the application of DNA scaffolded nanoparticles as an efficient active targeting platform.

Poster #: 27

Enzyme-responsive hydrogel tissue expanders for guiding facial growth in microphthalmia patients

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Microphthalmia and anophthalmia, conditions where a child is born with at least one abnormally small or missing eyeball, results in abnormal facial development. A current standard of care is a hydrogel-based osmotic expander that mimics the missing or underdeveloped eyeball to guide bone and soft tissue expansion in the orbital socket. One challenge with these devices is a rapid initial rate of expansion, which can lead to inflammation of the surrounding tissue. To overcome this limitation, we created a library of hydrogel compositions in combination with an enzyme-responsive interpenetrating network (IPN) of chitosan to control the rate and extent of swelling. Chitosan is degraded by lysozyme, an enzyme that occurs naturally in tears. The IPN was designed to control the initial rate of expansion, and enzymatic degradation of the network would result in a linear rate of swelling. Hydrogels with varying ratios of methyl methacrylate, n-vinyl pyrrolidone, and crosslinker were synthesized via bulk free radical polymerization. Saponification was used to generate an ionic hydrogel. Dried hydrogels were swollen in an acidic solution of chitosan, then incubated in an aqueous solution of genipin to induce crosslinking. To quantify composition-dependent swelling, gels were incubated in saline at 37C under constant shaking. For swelling of gels with IPNs, gels were incubated in PBS or PBS with lysozyme at physiological concentrations. Swelling potential of the base hydrogel was controlled by monomer and initiator percentage and degree of ionization. The chitosan IPN degraded over time in the presence of lysozyme, leading to a linear, enzyme- and crosslinker concentration-dependent increase in hydrogel swelling over time. Preliminary data from subcutaneous implantation of the hydrogels in rats demonstrate translation of composition-driven swelling behavior from in vitro to in vivo, thus validating the utility of these hydrogels as tissue expanders for a range of applications.

Poster #: 28

Hydrolytically Degradable and Biocompataible Highly Entangled Hydrogels

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Highly entangled (HE) hydrogels, utilizing long acrylamide chains with minimal crosslinking, hold potential as orthopedic adhesives and drug-delivery devices due to heightened stiffness compared to traditional hydrogels, often embrittled by higher crosslinking ratios. However, two critical factors for in-vivo applications—biocompatibility and degradation in biological liquids—are not understood for HE hydrogels. Therefore, this study aims to assess cell viability and develop a hydrolytically-degradable variant compatible with high-water-content biofluids. We hypothesize that acrylamide polymerization during hydrogel formulation promotes cell viability. Additionally, we speculate that low ratios of hydrolytically-degradable crosslinks would result in slow degradation while maintaining similar mechanical properties due to higher degrees of entanglements. 3T3 fibroblasts were exposed to conditioned media from HE hydrogels with nondegradable N, N'-Methylenebisacrylamide crosslinks (MBAA-HE) using a WST-1 assay to evaluate cell viability. The degradation of HE hydrogels with polyethylene glycol diacrylate crosslinks (PEGDA-HE) was tracked through dry weight measurements following lyophilization for a 6-week period. Mechanical properties were assessed with tensile testing at 1, 2, and 7 days. WST-1 analysis at 480 and 485 nm wavelengths revealed no cytotoxicity for MBAA-HE hydrogels. Initial findings indicated that PEGDA-HE hydrogels were slowly degrading. However, over 7 days, PEGDA-HE hydrogels exhibited elevated toughness and elasticity. These results suggested that higher degrees of polymer entanglements may impede degradation, improving toughness for longer deformation periods without fracture but decrease stiffness. Continued investigation into a PEGDA-HE hydrogel formulation balancing toughness and stiffness is needed, as toughness is relevant in mitigating continual mechanical loading and stress for orthopedic adhesives.

Poster #: 29

Three-dimensional magnetic scaffolds: Fe3O4 particles, β-tricalcium phosphate (β-TCP), and polycaprolactone (PCL) a 3D-printing approach

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Three-dimensional magnetic scaffolds comprised of Fe3O4 nanoparticles, β-tricalcium phosphate (β-TCP), and polycaprolactone (PCL) were fabricated using a 3D-printing technique. A systematic investigation was conducted to assess the rheological, physical, and chemical properties of the Fe3O4/β-TCP/PCL composite scaffolds. The influence of varying PCL (10-50 wt%) and β-TCP (85-45 wt%) concentrations, with a constant Fe3O4 content (5 wt%), was evaluated. All scaffold compositions exhibited shear thinning behavior, with viscosity increasing proportionally to the polymer (PCL) content. X-ray diffraction (XRD) analysis confirmed the presence of both Fe3O4 and PCL within the scaffolds. Additionally, the magnetic properties of the scaffolds suggest potential applications in magnetic hyperthermia for anti-cancer therapy.

Poster #: 30

Polymer-driven neutralization of candidalysin cytotoxicity

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Fungal infections are a significant public health threat. Candida albicans is one of the most common pathogens causing these infections, with over 46,000 annual cases of invasive candidiasis in the United States and mortality rates as high as 40%. The pathogenicity of this fungi is dependent on a shift from yeast to hyphal fungal cell morphology and subsequent release of virulence factors into the invasion pocket created during filamentation. Candidalysin is one of the key virulence factors released by C. albicans hyphae; it permeabilizes host cell membranes, leading to cytotoxicity and subsequent release of pro-inflammatory cytokines, recruiting immune cells to the infection site. Despite the increasing evidence that candidalysin directly contributes to the pathogenicity of C. albicans, there has been little effort to mitigate its damaging effects. Here, we explored the binding of various natural and synthetic polymers with candidalysin, and the impact of this binding on the damaging effects of candidalysin on host cells. These polymers included hyaluronic acid, chondroitin sulfate, poly(acrylic) acid, heparin, and albumin. To quantitively monitor potential interactions, we utilized quartz crystal microbalance with dissipation (QCM-D) monitoring. Here, a decrease in frequency corresponds to mass adsorption onto a piezoelectric gold-coated sensor. Hyaluronic acid, albumin, and chondroitin sulfate exhibited similar adsorption behavior of candidalysin following polymer interaction with a poly(L-lysine) priming layer on the QCM-D sensors, indicated by comparable frequency changes following the introduction of candidalysin (−20.6 ± −2.1 Hz, −14.1 ± −1.4 Hz, and −18.1 ± −1.7 Hz, respectively). Poly(acrylic) acid exhibited the most significant interaction with candidalysin, with frequency changes approximately four times that of what was observed with other polymers. In contrast, heparin demonstrated minimal changes in frequency between the polymer and candidalysin layers, likely due to strong electrostatic interactions between heparin and the poly-L-lysine priming layer. We observed that binding with heparin, poly(acrylic) acid and chondroitin sulfate was able to reduce candidalysin cytotoxicity against fibroblasts, human umbilical vein endothelial cells, and vaginal epithelial cells in a concentration-dependent manner. These studies suggest that both natural and synthetic polymers can inhibit candidalysin activity, driven by electrostatic interaction with polyanions.

Poster #: 31

Leveraging synthetic polyurethanes for the modulation of bacterial surface motility and physiological behaviors

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Modulating bacteria surface behavior can increase the efficiency of industrial biomanufacturing and clinical therapies. While genetic engineering methods have been extensively applied to manipulate microorganisms for supporting engineering living materials, there is only nascent research on the use of chemical approaches to modulate bacterial behavior. Here, we report a straightforward and simple approach to modulate bacteria's surface motility and physiological behaviors via a platform of biosurfactant mimetic and water-soluble polyurethanes. Our results showed that -COOH-containing polyurethanes significantly promoted the swarming and twitching area of P. aeruginosa PAO1 by 17-fold and 80-fold, respectively. Conversely, the polymer with -NH2 functional groups restricted the swarming area of P. aeruginosa by 58%. This modulation of bacterial motility results from the synergistic regulatory effects of the polyurethane platform on surface wetting, bacteria proliferation, and the production of the second messenger cyclic di-GMP. Moreover, we found a 4-fold increase in the production of the extracellular polymeric substance (EPS) yield in the presence of -COOH-containing polyurethane. Taken together, combining the inhibitory effect of P4-NH2 and the pro-motility effect of P2-COOH, we successfully achieved selective bacterial migration on agar to generate the desired bacterial migration pattern over time. Therefore, this novel biosurfactant-mimetic polyurethane platform emerges as a simple means of controlling microbial systems as well as enhancing the downstream effects of EPS production.

Poster #: 32

Functionalization of PEDOT:PSS for Controlled Electrical Stimulation of Skeletal Muscle

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Exercise helps promote and accelerate the differentiation of tissue engineered skeletal muscle, and we have shown that targeted light stimulation of engineered optogenetic muscle in vitro and in vivo enhances force production. While light stimulation enables non-invasively accelerating muscle maturation, this method of stimulation is not readily translatable to in vivo contexts, such as cm-scale muscle grafts implanted at traumatic injury sites, due to the limited penetration depth of light into dense 3D tissues and the requirement for genetic modification of transplanted cells. While electrical stimulation could offer a more translatable approach to stimulating muscle without requiring genetic modification, current methods to electrically stimulate engineered muscle via electrodes placed in culture media are designed to enable whole muscle twitch, rather than enabling spatial control of contraction in different regions of tissue. Moreover, these methods generate significant electrolysis of the media, and trigger delamination of muscle fibers from the substrate, thus significantly reducing viability. We have developed a method for culturing C2C12 murine myoblast-derived muscle on a bi-continuous conducting polymer hydrogel, namely, poly(3,4-ethylenedioxythiophene): polystyrene sulfonate (PEDOT: PSS) by coating these polymers with extracellular matrix hydrogels known to promote muscle differentiation, such as Matrigel and fibrin. Our preliminary results show that myoblasts can attach to functionalized PEDOT: PSS and differentiate into contractile multinucleated muscle fibers that are stable over weeks in culture. Leveraging PEDOT: PSS conductive hydrogels to activate contraction of engineered muscle tissues prolongs cell viability and enables precise spatial control of muscle fiber activation, mimicking in vivo stimulation via upstream motor neurons. In contrast to traditional metal electrode-based methods of exercising muscle, targeted stimulation with PEDOT: PSS substrates enable long-term exercise stimulation of engineered muscle and improved precision in data acquisition. We aim to leverage our new methodology to understand how a variety of exercise training regimens impact muscle health, strength, and fatigue characteristics.

Poster #: 33

Morphological and Mechanical Characterization of 3D Printed Adipose Grafts for Skin Tissue Regeneration

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Introduction: Personalized grafts, derived from the patient's own adipose tissue using 3D printing technology, hold promise for enhancing wound healing and skin tissue repair. This innovative approach utilizes homogenized and micronized liposuction-derived aspirate as bioink, enriched with autologous growth factors and cells, to fabricate scaffolds tailored to individual patients. The Aplicor 3D bioprinter, specifically designed for biofabricating wound tissue grafts, facilitates the creation of these customized grafts, promoting granulation and re-epithelialization in wound beds.

Methods: Control of scaffold characteristics such as mechanical properties, morphology, and nanoscale porosity is essential for optimizing tissue regeneration and remodeling. This study focuses on characterizing the morphology and porosity of 3D printed adipose scaffolds using scanning electron microscopy (SEM). Additionally, mechanobiological properties, including elasticity, surface roughness, and adipocyte interaction with the scaffold, are assessed through nanoindentation. Establishing target ranges for these critical material properties is crucial for standardizing skin tissue regeneration.

Results: Scanning electron microscopy reveals the intricate morphology and nanoscale porosity of 3D printed adipose scaffolds, providing insights into their structural integrity and potential for cell infiltration. Nanoindentation analysis elucidates the mechanobiological properties of the scaffolds, including their elasticity, surface roughness, and compatibility with adipocytes. These findings contribute to establishing standardized ranges for scaffold characteristics essential for effective skin tissue regeneration.

Conclusion: Characterization of 3D printed adipose grafts highlights their morphological intricacies and mechanobiological properties crucial for skin tissue regeneration. Standardizing scaffold characteristics aids in optimizing wound healing outcomes and advancing personalized approaches in biomaterials-based therapy. Further research is warranted to refine and enhance the performance of these innovative grafts for clinical applications in skin tissue repair.

Poster #: 34

PET-RAFT Synthesis of Hydrogels

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Hydrogels are 3D polymer networks that can better replicate the natural environment of cells compared to more traditional cell culture methods such as flat, 2D plastic flasks. Current methods for hydrogel synthesis in cell culture often utilize exogenous radical initiators or high energy light, harming cells in the process. Here, we demonstrate a new strategy utilizing photoinduced electron transfer – reversible addition-fragmentation chain transfer (PET-RAFT) to crosslink several PEGs bearing norbornene pendant groups in response to blue light and at physiological conditions. We synthesized bifunctional PEG 1,2-(butylthiocarbonothioylthio) propanoic acid, bearing end groups that are able to be reduced by excited Eosin Y in the presence of triethanolamine under 465 nm light to form RAFT adduct radicals. These radicals are able to form permanent bonds with a variety of norbornene-functionalized PEGs, yielding a solid gel in ten minutes. Without the presence of any light, PEG-norbornene solutions remain liquid for at least 48 hours. Once these stored solutions were placed under 465 nm light, a sol-gel transition was observed in twenty minutes. Crosslinking occurs rapidly even in the presence of oxygen and is compatible with a wide variety of olefinic macromonomers, including (meth)acrylates and vinyl sulfones. As a result, this visible light crosslinking technique is well suited to cell culture applications, which have strict conditions needed to preserve cell viability, as well as facilitating high-throughput experiments. Additionally, this technique enables the creation of cell environments with regions of different stiffness, as well as post-gelation modifications.

Poster #: 35

Muscle fiber type transformation through optogenetic exercise

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Native muscle comprises distinct fiber types that determine the dynamics of power output and long-term fatigue resistance. Slow-twitch oxidative muscle fibers are built to maintain posture, power walking, and maintain functionality for most of an animal's waking hours. Fast-twitch glycolytic fibers enable muscle's high-power motions for sprinting and lifting heavy weights. The varied physical tasks in which animals participate is thus reflected in their muscle fiber profiles, and even single fibers can have changing metabolic schemes along their length. Tissue engineered models of skeletal muscle have largely neglected characterizing fiber type distribution and plasticity, highlighting a significant gap in our ability to make muscle that represents native tissue. By leveraging our previously optimized protocols for differentiating optogenetic contractile tissue from C2C12 myoblasts, and a custom platform for high-throughput light stimulation of muscles in a multi-well plate format, we tested the response of muscle cultures to 15-minute exercise regimens of computer-controlled LED pulses at 1, 4, and 10 Hz. We monitored muscle contraction daily and leveraged custom computational frameworks to extract performance metrics of each tissue in response to stimulation (peak force generated, time to peak force, time at peak force, relaxation time) and precisely distinguish performance differences mediated by exercise. After 10 days of training, we extracted RNA from cell lysate and performed qPCR to determine relative distributions of the myosin heavy chain proteins, indicative of fiber type. We anticipate that our studies will help develop more relevant in vitro models for diseases that impact specific fiber types, such as Duchenne Muscular dystrophy (affects fast-twitch fibers) and myotonic dystrophy type 1 (affects slow-twitch fibers). Further, we believe that future biohybrid robots will use training schemes like this one to adapt their musculature for their specific tasks.

Poster #: 36

3D Printed Magnetically Actuated Matrices for Mechanical Stimulation of Engineered Muscle Tissues

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Skeletal muscle, a highly abundant tissue in the human body, plays a critical role in vital physiological functions and organ movements, serving as the primary source of bodily actuation. Despite its impressive regenerative capacity, volumetric muscle loss (VML), occurring when the loss surpasses 15-20% of the total volume, impairs the regenerative response to the extent that functional regeneration becomes unattainable. Our group has previously demonstrated that culturing 2D muscle tissue on magnetically actuated matrices (MagMA) with incorporated magnetic microparticles, actuated via an external magnet, yields promising outcomes. This approach mechanically programs the anisotropic alignment of muscle fibers and enhances coordinated muscle contraction. In conjunction with electrical stimulation, this approach holds significant promise for fabricating engineered grafts that can promote muscle regeneration after injury. We have developed a method to adapt MagMA to the 3D environment by leveraging extrusion bioprinting, a technique that enables precise fabrication of intricately organized functional structures, providing spatial control over cell, biomaterial, and magnetic microparticle deposition to replicate the sophisticated organization of muscle tissue. This study investigates the biofabrication of magnetic muscle tissue through the extrusion of fibrinogen/C2C12 myoblast/magnetic microparticle bioinks within a laponite nanoclay support bath. The biofabricated structure is cultured in differentiation media supplemented with insulin-like growth factor (IGF-1) and is electrically stimulated to promote the maturation of the tissue. This novel fabrication methodology will enable us to investigate how noninvasive and spatiotemporally precise mechanical stimulation of 3D muscle tissue impacts muscle fiber alignment, maturation, and force generation capacity in vitro. Our long-term goal is to leverage 3D bioprinted MagMA muscle tissues to mechanically program regeneration after traumatic injury in vivo.

Poster #: 37

A Sprayable Hydrogel-Based Wound Dressing for Burn Treatment

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Second- and third-degree burns cause severe skin damage which cannot be regenerated without medical attention. The treatment of these wounds is challenging since the irregular size and depth, location, inflammation, reactive oxygen species production, microbial invasion, and ischemic condition can hinder the healing. The limited oxygen supply and infection in the wound area are critical for the wound-healing process. Hydrogel-based dressings are promising for these types of wounds by presenting multiple functions for the treatment. A hydrogel-based wound dressing containing hyaluronic acid and gelatin was developed to maintain homeostasis in the wound microenvironment and promote regeneration by enhancing cell migration and proliferation. HAMA and GelMA enable controllable photocrosslinking and form a durable hydrogel on the wound surface. A sprayable formulation was assessed for their easy administration in situ. GelMA precursor solution within a range of 5-15 % (w/v) was evaluated to have a sprayable formulation using different types of spray bottles. An ex vivo pig skin model was used to demonstrate sprayability of precursor solution and formation of stable hydrogel after administration. HAMA (1% w/v) was added into the precursor solution to provide elasticity and structural strength to the dressing. Calcium peroxide was also added to provide self-oxygenation and antibacterial properties. Various concentrations of CaO2 were added to the determined precursor solution. The composite hydrogels were characterized based on their mechanical, swelling, oxygen-releasing, and antibacterial properties. A composite hydrogel with 10 % GelMA and 1 % HAMA was found as a sprayable formulation. Mechanical and swelling behaviors exhibited typical characteristics of hydrogel-based wound dressings, aiding in maintaining wound area homeostasis and absorbing exudates. CaO2 decomposition provided oxygen, particularly at concentrations of at least 12 mg/mL for up to two weeks in vitro. The released oxygen relieved metabolic stress in fibroblasts and reduced cell death under hypoxia. Furthermore, the zone of inhibition test showed that the composite hydrogel-based dressing exhibited antibacterial properties against common wound infectious bacteria including P.aeruginosa and S.aureus. The effective activity changed depending on the concentration of CaO2. Overall, developed multifunctional sprayable hydrogel is self-oxygenating and antibacterial, enhancing wound healing and skin regeneration for second- and third-degree burns.

Poster #: 38

Hydrogel optimization for 3D co-culture of neural stem cells and brain-specific microvasculature

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INTRODUCTION: The human neurovascular niche (hNVN) is a specialized region of the brain composed of neural stem cells (NSCs) and brain-specific microvasculature which plays a regulatory role in NSC fate; though how is not well understood. Microfluidic models and organoid technologies seek to bridge this gap; however, these models tend to either be optimized towards microvasculature or NSCs in the culture, but rarely both. Glycidyl Methacrylate Hyaluronic Acid (GMHA) is a common biomaterial optimized for neural cell culture since it closely mimics hyaluronic acid which is the most abundant ECM component in the brain, while most microvasculature 3D cell cultures utilize collagen or fibrin as they are part of the endothelial cells' (ECs) natural environment. Our research aims to create a hybrid GMHA:Collagen hydrogel that is optimized for the formation and maintenance of perfusable vasculature as well as the proliferation and/or differentiation of NSCs within a 3D cell co-culture model of the hNVN.

METHODS: Photocrosslinkable GMHA is mixed with the photoinitator LAP and a neutralized Collagen I solution in varying concentrations. The neutralized hydrogel is then combined with ECs, astrocytes (ACs), and pericytes (PCs). The cell-encapsulated hydrogel is then exposed to UV light to crosslink the GMHA and subsequently incubated at 37°C to polymerize the Collagen. Over time, the cells self-organize into blood-brain-barrier-like microvasculature where ECs form a hollow lumen and are supported through direct contact with PCs and ACs.

RESULTS: This work seeks to improve an already established microfluidic model of the hNVN (Winkelman & Dai, 2023) made using fibrin, which is not a native brain ECM component. As a result of this work, we expect for the brain-specific microvasculature formed in our model to maintain a hollow lumen for extended periods of time compared to collagen or fibrin alone due to improved AC function caused by the presence of GMHA which is known to play a role in regulating astrocyte reactivity. Furthermore, we hypothesize that the presence of GMHA in our hybrid hydrogel will support the NSCs in our model better than collagen or fibrin alone and will therefore improve their survival within the co-culture hNVN model.

Poster #: 39

Quantifying Donor-Dependent Morphological and Functional Differences in Primary Human Muscle Cultures

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Engineering in vitro platforms enable high-throughput modeling of living systems and are prevalent in efforts to discover and test therapies for diseases. Many in vitro models of skeletal muscle rely on immortalized murine cell lines that do not always generate accurate models of native tissue. Using primary muscle cells from human donors could thus provide a more accurate method of engineering tissues that model native architecture and function. However, the long-standing issue of inter-patient variability in modeling and treatment of diseases remains an issue for in vitro systems derived from primary muscle cells. There is thus a strong need to first characterize inter-patient differences in the morphology and function of engineered muscle derived from primary cells before attempting to model and/or treat any system or disease. To our knowledge, a detailed description of donor variability contributions to the morphology and function of human skeletal muscle tissue has not been investigated. We have conducted a thorough analysis of diverse donors that span the age and health spectrum to establish how much functional variability can be attributed to normal inter-donor variability versus specific contributions due to pathology. Our database enables us to make quantitative comparisons of muscle fiber type, length, width, fusion index, and frequency-dependent force generation capacity across many individuals. Unlike iPS-derived models, which often lack the maturity of native tissue and are largely suited to modeling diseases of known genetic origin, our database enables quantifying, for the first time, the functional impact of disease with unknown or multivariate causes that affect aging adults. We show, for example, that our database enables benchmarking muscle health and strength for patients with amyotrophic lateral sclerosis (ALS) as compared to an array of healthy age-matched controls. Our research will provide novel methods and open-source databases that enable benchmarking human muscle cultures for mechanistic studies of human disease and high-throughput drug testing.

Poster #: 40

A High-Throughput Microfluidic Gut-On-A-Chip for Disease Modeling

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Inflammatory bowel disease (IBD), which affects about 2.4 million adults in the United States, is a family of debilitating diseases with pervasive effects on the lives of affected individuals, such as a significant decrease in the quality of life, high hospitalization rates, and expensive treatments [1]. The pathophysiology of IBD is largely unknown, making it difficult to treat the disease at the source. However, the emergence of organs-on-a-chip (OOC), has offered improvement in modeling diseases, such as IBD, by mimicking the structure and function of tissue in vitro.

Although OOC have shown promise, current models are low-throughput, requiring extensive time and resources. To address current limitations, we have developed a high-throughput microfluidic gut-on-a-chip (GOC) that is capable of culturing eight independent samples simultaneously. The GOC features a semi-permeable membrane that supports the cell population and forms distinct apical and basolateral compartments for fluid flow, supplying physiologically relevant shear stress. Additionally, our device distributes fluid flow from a single inlet to all eight culture wells, significantly reducing external tubing and pumping requirements. Caco-2 epithelial monolayers were grown and matured on-chip to confirm inter-well consistency. Assessment of cell viability by fluorescence-based live-dead assay indicates high cell viability with limited cell death. Immunostaining of Caco-2 monolayers shows strong expression of tight junction protein ZO-1, a marker of epithelial maturity, in all eight culture wells.

In summary, our work demonstrates a novel approach to high-throughput culture in OOC, enabling simultaneous culture of eight independent ‘gut samples’ in a single GOC device. Specifically, we applied our device to successfully culture Caco-2 cells on-chip,

differentiating to mature epithelial monolayers in all eight culture wells. Ongoing work is focused on applying our high-throughput OOC platform to an in vitro model of IBD, as well as additional organ-chip models.

[1] Lewis, J. D., et al. (2023). "Incidence, Prevalence, and Racial and Ethnic Distribution of Inflammatory Bowel Disease in the United States." Gastroenterology 165(5): 1197-1205 e1192.

Poster #: 41

Comparison between rodent and human heart chips' response to dosed pharmaceuticals

Nolan Burson, Northeastern University

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Cardiovascular disease is the leading cause of death in the Western world, with 931 578 deaths in the US during 2021. Furthermore, pharmaceuticals designed to manage cardiovascular disease have the lowest likelihood of FDA approval based on leading indications during Phase 1 testing. There is a need for cardiovascular pharmaceuticals; however, based on the Phase 1 data, there are issues with the animal models used to develop these pharmaceuticals. An alternative model is a microfluidic platform known as Organs-on-a-chip (OOC). This in vitro platform leverages a three-dimensional culture space, heterogeneous cell population, and biophysical stimuli to mimic in vivo conditions and features. We have previously developed and validated a heart chip of isolated rat cardiomyocytes and sympathetic neurons. Recent work has led to creating a humanized model consisting of human stem cell-derived cardiomyocytes. The rodent and human heart chip was used to screen the effects of epinephrine, milirone, and isoproterenol and determine the adverse effects of the listed compounds. We used video edge capture to determine the cardiomyocytes' beat rate, contractility force, and synchronicity before and after dosing with the pharmaceuticals. The results show that each system behaves similarly to the reported effects of each compound. Further work towards an innervated human heart chip and more complex pharmaceuticals can highlight the species differences in the two systems. Success in a human in vitro heart model can aid in the development of novel cardiovascular drugs, leading to a reduction in death and an increase in approved medications.

Poster #: 42

Injectable Synthetic Platelet-Based Therapy Enhances Healing Potential in Stem Cells Delivered for Joint Injuries

Melika Osareh, North Carolina State University

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Dr. Ashley Brown - Joint Department of Biomedical Engineering, North Carolina State University/The University of North Carolina – Chapel Hill, Raleigh, NC ; Comparative Medicine Institute, North Carolina State University - Raleigh, NC; aecarso2@ncsu.edu

Anterior cruciate ligament (ACL) injuries can occur due to sports or other activities and constitute a major clinical problem worldwide. While extra-articular injuries of the joint are able to heal, intra-articular injuries, such as ACL injuries, have a limited healing capacity. This is believed to be due, in part, to the proteolytic environment of the synovial fluid (SF) with a high clearance rate surrounding the ligament and the low presence of necessary clotting factors such as fibrinogen and thrombin, which hinders the healing process by not allowing fibrin scaffolds to form. For the same reason, therapeutics aiming to use mesenchymal stem cells (MSCs) for promoting intraarticular healing and joint cartilage regeneration, for example in the case of early term osteoarthritis, suffer from lack of cell persistence in the area. In previous studies, platelet-like-particles (PLPs), designed to mimic activated platelets, have successfully established stable formation of a fibrin matrix in synovial fluid from injured joints. A PLP-enhanced fibrin clot could be an effective delivery system for MSCs to the joint area. This study tests MSC activity within PLP-enhanced fibrin clots. Activity was determined by incorporating bovine fetal MSCs within fibrin clots +/- PLPs and

assessing cell viability, cell migration, and exosome release and uptake. The presence of exosomes is believed to enhance healing through delivery of healing cytokines, proteins, and by promoting signals for recruitment of further healing factors. PLP-enhanced clots induced higher cell viability and retention, and significantly higher concentrations of released exosomes relative to non-PLP-driven exosomes. These findings underscore the potential of PLPs in promoting further stages of healing beyond clot stability, to allow enhanced MSC activity for remodeling of tissue. The study provides valuable insights into the application of PLP-enhanced fibrin-based injectable therapeutics for successful delivery of MSCs to intra-articular injuries and early term osteoarthritis.

Poster #: 43

Photocurable citrate-based elastomers for tissue engineering

Ni Chen, Worcester Polytechnic Institute

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Yonghui Ding, Worcester Polytechnic Institute, yding7@wpi.edu

Biodegradable citrate-based polymers (CBPs) have emerged as a distinctive biomaterial platform with significant potential for various biomedical applications, including regenerative engineering. The highly versatile mechanical properties, which can be tailored to match surrounding tissues, are crucial for their use in implantable biomedical devices, ensuring mechanical stability, structural integrity, and tissue integration. A photocurable methacrylate CBP (mCBP) has been developed to enable the high-resolution and high-speed fabrication of biodegradable scaffolds via a light-projection-based 3D printing technique. However, current mCBP inks often produce rigid and brittle polymers due to a high degree of crosslinking. In this study, we aim to modify the crosslinking network of mCBPs through the introduction of reactive diluents and/or chain transfer agents into the mCBP ink and thereby modulate the mechanical properties of photocured polymers. It was found that the addition of isobornyl acrylate (IBOA) resulted in remarkably tougher polymers. Specifically, it increased the Young's modulus by 2 folds (56 MPa vs. 29 MPa) and enhanced the ductility, measured by maximum elongation at fracture, by 2 folds (34% vs. 16%). Further addition of 3,6-dioxa-1,8-octanedithiol (DOD) produced a more ductile polymer, with ductility increasing to 56% compared to 16% for the pristine polymer, while the Young's modulus decreased to 9.2 MPa. Moreover, the modified inks could be successfully 3D printed into scaffolds with complex structures. In summary, this work demonstrates that by introducing reactive diluents and/or chain transfer agents into the mCBP inks, a wide range of mechanical behaviors of degradable polymers are readily achieved while retaining the same 3D printability to produce scaffolds with anatomically accurate structures.

Poster #: 44

3D-Printing of Bioresorbable, Micropatterned Vascular Grafts for Arterial Regeneration

Rao Fu, Worcester Polytechnic Institute

Yonghui Ding, Worcester Polytechnic Institute, yding7@wpi.edu

Tissue-engineered vascular grafts (TEVGs) have emerged as promising alternatives to autologous grafts for small-diameter blood vessel replacement during bypass surgery. Yet none of these TEVGs have reached bedside for patient care. The primary obstacle lies in the incapability of regenerating functional arterial tissues on synthetic graft materials. Evidence showed that the spatial orientations of endothelial cells (ECs) and smooth muscle cells (SMCs) are critical for maintaining mechanical and biological functions of the natural artery. However, it is difficult to precisely control orientations of repopulated cells in TEVGs, particularly in scenarios that require the coordination of multiple cell types. One potential solution is using surface topographic patterns to guide cell orientation and other behaviors. Nevertheless, creating micro-/nano-scale patterns on curved surfaces of 3D scaffolds remains challenging. To address this challenge, we developed a customized 3D printing technology, named continuous liquid interface production (CLIP), to enable the in-process fabrication of surface micro-scale patterns (or micropatterns) on a 3D structured scaffold. In this study, a biodegradable citrate-based polymer (CBP) was 3D-printed to produce a vascular graft with dual micropatterns, i.e. axially aligned grooves on the internal surface and circumferentially aligned rings on the external surface. The CBP exhibited tunable degradation behavior and mechanical properties comparable to the ePTFE grafts currently used in clinical practice. Co-culturing endothelial cells (ECs) and smooth muscle cells (SMCs) on the 3D-printed grafts resulted in the formation of axially oriented endothelium on the internal surface and circumferentially oriented smooth muscle tissue on the external surface, replicating the spatial organization of natural arteries. These findings suggest that bioresorbable, micropatterned vascular grafts hold great promise for facilitating arterial regeneration and maintaining long-term patency.

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2024 REGIONAL SYMPOSIA

September 19–20, 2024

FINAL PROGRAM

MIDWEST SYMPOSIUM:

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2024 Regional Symposia

Midwest Symposium: Case Western Reserve University

September 19 - 20, 2024

Program Agenda

Thursday, September 19, 2024

8:00 AM - 8:30 AM	Registration & Breakfast	Hallway, Ballroom A/B
8:30 AM - 8:45 AM	Welcome from Site Chairs	Ballroom A/B
8:45 AM - 10:00 AM	Concurrent Session I: Engineering Cells & Their Microenvironments *Invited Speaker:* ***Dr. Abhinav Acharya, Associate Professor, Case Western Reserve University***	Ballroom A
8:45 AM - 10:00 AM	Concurrent Session II: Tissue Engineering *Invited Speaker:* ***Dr. Adam Feinberg, Professor, Carnegie Mellon University***	Ballroom B
10:00 AM - 10:15 AM	Coffee Break	All/Any
10:15 AM - 11:30 AM	Concurrent Session III: BioInterfaces *Invited Speaker:* ***Dr. Andrew Shoffstall, Associate Professor, Case Western Reserve Univeristy***	Ballrooom A
10:15 AM - 11:30 AM	Concurrent Session IV: Nanonmaterials *Invited Speaker:* ***Dr. Daniel Gallego-Perez, Associate Professor, Case Western Reserve University***	Ballroom B

Program Agenda

Thursday, September 19, 2024 *(continued)*

11:30 AM - 12:45 PM	Plenary Session I: ***Dr. Shana Kelley, Northwestern University*** ***Dr. Joel Collier, Duke University*** *(2024 Clemson Award for Basic Research Recipient)*	Ballroom A/B
12:45 PM - 1:45 PM	Lunch	Ballroom A/B
1:45 PM - 3:00 PM	Concurrent Session V: Drug Delivery *Invited Speaker:* ***Dr. Katelyn Swindle-Reilly, Associate Professor, The Ohio State University***	Ballroom A
3:00 PM - 4:15 PM	Concurrent Session VI: Engineering Cells and Their Microenvironments *Invited Speaker:* ***Dr. Brendon Baker, Associate Professor, University of Michigan***	Ballroom B
3:00 PM - 4:00 PM	Industry Panel	Ballroom A/B
4:00 PM - 4:15 PM	Coffee Break	All/Any
4:15 PM - 5:30 PM	Plenary Session II: ***Dr. Sarah Stabenfeldt, Arizona State University*** ***Dr. Danielle Benoit, University of Oregon***	Ballroom A/B

Program Agenda

Friday, September 20, 2024

8:15 PM - 8:45 PM	Registration & Breakfast	Hallway Ballroom A or B
8:45 AM - 10:00 AM	Concurrent Session VII: Drug Delivery *Invited Speaker:* ***Dr. Tom Dziubla, Professor, University of Kentucky***	Ballroom A
8:45 AM - 10:00 AM	Concurrent Session VIII: Cardiovascular Biomaterials *Invited Speaker:* ***Dr. William Wagner, Professor, University of Pittsburgh***	Ballroom B
10:00 AM - 10:15 AM	Coffee Break	Any/All
10:15 AM - 11:30 AM	Concurrent Session IX: BioInterfaces and Tissue Interactions *Invited Speaker:* ***Dr. Steven Eppell, Associate Professor, Case Western Reserve University***	Ballroom A
10:15 AM - 11:30 AM	Concurrent Session X: Tissue Engineering *Invited Speaker:* ***Dr. Sam Senyo, Associate Professor, Case Western Reserve University***	Ballroom B

Program Agenda

Friday, September 20, 2024 *(continued)*

11:30 AM - 12:45 PM	Plenary Session III: ***Dr. Elazer Edelman, Massachusettes Institute of Technology*** *(2024 Founders Award Recipient)* ***Dr. Cynthia Reinhart-King, Rice University***	Ballroom A/B
12:45 PM - 1:45 PM	Lunch	Ballroom A/B
1:45 PM - 3:00 PM	Concurrent Session XI: Engineering Cells and Their Microenvironments *Invited Speaker:* ***Dr. John Martin, Assistant Professor, University of Cincinnati***	Ballroom A
1:45 PM - 3:00 PM	Concurrent Session XII: Nanomaterials and Biomaterials Commercialization *Invited Speaker:* ***Dr. Anirban Sen Gupta, Professor, Case Western Reserve University***	Ballroom B
3:00 PM - 4:30 PM	Poster Session	Ballroom C
4:30 PM - 5:00 PM	Coffee Break	All/Any
5:00 PM - 5:30 PM	Closing Remarks	Ballroom A/B

2024 Regional Symposia Chair:

Anita Shukla, PhD, Brown University

Midwest Regional Symposium Co-Chairs:

Steven Eppell, PhD, Case Western Reserve University
Anirban Sen Gupta, PhD, Case Western Reserve University

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Adam Feinberg, PhD, Carnegie Mellon University
Daniel Gallego Perez, PhD, Ohio State University
Brittany E. Givens Rassoolkhani, PhD, University of Kentucky
Brendan Harley, PhD, University of Illinois
John Martin, PhD, University of Cincinnati
Ariella Shikanov, PhD, University of Michigan
Katelyn Swindle Reilly, PhD, Ohio State University
Yang Yun, PhD, University of Akron

CASE WESTERN RESERVE UNIVERSITY

Midwest Biomaterials Symposium 2024

- **Venue:** Case Western Reserve University, Tinkham Veale University Center 11038 Bellflower Rd., Cleveland, OH 44106
- **Date:** September 19-20, 2024

Plenary Speaker

Dr. Shana O. Kelley
Professor, Northwestern University
President, Chan Zuckerberg BioHub, Chicago

Program Committee:

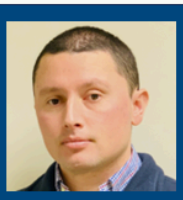
Anirban Sen Gupta, PhD, CWRU (Chair); Steven Eppell, PhD, CWRU (Co-chair); Abhinav Acharya, PhD, CWRU; Yang Yun, PhD, University of Akron; Adam Feinberg, PhD, CMU; Daniel Gallego Perez, PhD, OSU; Katelyn Swindle Reilly, PhD, OSU; John Martin, PhD, U of Cincinnati; Ariella Shikanov, PhD, U of Michigan; Brittany Givens Rassoolkhani, PhD, U of Kentucky

Please scan to Register:

Dr. Adam Feinberg
Professor, Carnegie Mellon University

Dr. Abhinav Acharya
Associate Professor, Case Western Reserve University

Dr. Andrew Shoffstall
Associate Professor, Case Western Reserve University



Dr. Daniel Gallego-Perez
Professor, The Ohio State University

Dr. Katelyn Swindle-Reilly
Associate Professor, The Ohio State University

Dr. Brendon Baker
Associate Professor, University of Michigan

Dr. Tom Dziubla
Professor, University of Kentucky

Dr. William Wagner
Professor, University of Pittsburgh

Dr. Sam Senyo
Associate Professor, Case Western Reserve University

Dr. Steven Eppell
Associate Professor, Case Western Reserve University

Dr. John Martin
Assistant Professor, University of Cincinnati

Dr. Anirban Sen Gupta
Professor, Case Western Reserve University

PLENARY SESSION I
Thursday, September 19

Dr. Shana Kelley
Northwestern University

PLENARY SESSION I
Thursday, September 19

Dr. Joel Collier
Duke University
(2024 Clemson Award for Basic Research)

PLENARY SESSION II
Thursday, September 19

Dr. Sarah Stabenfeldt
Arizona State University

PLENARY SESSION II
Thursday, September 19

Dr. Danielle Benoit
University of Oregon

PLENARY SESSION III
Friday, September 20

Dr. Elazer Edelman
Massachusetts Institute of Technology
(2024 Founders Award)

PLENARY SESSION III
Friday, September 20

Dr. Cynthia Reinhart-King
Rice University

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2024 Regional Symposia

September 19th - 20th

- Northeast: Northeastern University
- Midwest: Case Western Reserve University
- Southeast: Georgia Institute of Technology
- Southwest: University of Texas at Austin
- Western: University of Colorado, Denver | Anschutz Medical Campus
- Northwest: University of Washington

CONCURRENT SESSION I: ENGINEERING CELLS AND THEIR MICROENVIRONMENTS

8:45 AM - 9:10 AM **Invited Speaker: Abhi Acharya, Case Western Reserve University**

9:10 AM - 9:25 AM

Biofabrication of an Advanced Microphysiological System Mimicking Phenotypical Heterogeneity and Drug Resistance in Human Glioblastoma

Sirjana Pun, University of Cincinnati

1. Sirjana Pun, Department of Biomedical Engineering, University of Cincinnati, Cincinnati, USA, punsa@mail.uc.edu

2. Giuseppe Sciumè, Institute of Mechanics and Engineering-12M, University of Bordeaux, France, giuseppe.sciume@u-bordeaux.fr

3. Daniel Pomeranz Krummel, Department of Neurosurgery, University of North Carolina, Chapel Hill, USA, krummel@email.unc.edu

4. Soma Sengupta, Department of Neurosurgery, University of North Carolina, Chapel Hill, USA, Department of Neurology, University of North Carolina, Chapel Hill, USA, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, USA, Email: ssengup@email.unc.edu

5. Riccardo Barrile, Department of Biomedical Engineering, University of Cincinnati, Cincinnati, USA, Center for Stem Cells and Organoid Medicine (CuSTOM), Cincinnati Children's Hospital Medical Center, Cincinnati, USA, barrilro@ucmail.uc.edu

Over the past decade, significant progress in 3D printing and bioprinting has transformed the landscape of in vitro human tissue models, particularly Microphysiological systems (MPSs). While conventional techniques like soft lithography and polydimethylsiloxane (PDMS) pioneered the work, their limitations necessitate innovation for accelerated bio-fabrication [1]. PDMS, despite its affordability, is unsuitable for testing small therapeutic groups targeting brain diseases due to its time-dependent absorption properties. Additionally, soft lithography's reliance on clean rooms, expertise, and mold patterns hampers throughput and personalization. Here we present an advanced MPS system integrating 3D printing and bioprinting approaches. The scaffold was 3D printed using resin material while bioprinting was employed to construct a vascular-like structure lined with primary human brain microvascular endothelial cells (HBMECs). We developed a robust protocol to remove the resin's cytotoxicity, enabling the culture of HBMECs and glial cells including astrocytes and cancer cells. Resin showed low absorption of small molecules such as temozolomide, crucial for effective drug testing. The porous hydrogel was used to accurately replicate physiological conditions such as interstitial fluid flow (IFF), facilitating the modeling of biomechanical forces in the MPS platform. Encouraged by these outcomes, we adapted our system to investigate glioblastoma (GBM), a primary human brain cancer. Co-culturing cancer cells with endothelial cells and astrocytes resulted in a compromised vascular barrier and elevated pro-inflammatory cytokines, including IL8, MCP1, and IL6—key to GBM tumorigenesis. Moreover, our study highlights the influence of IFF in modulating the expression of cancer stem cell markers such as CD133. Notably, perivascular stem-like cells exhibit heightened drug resistance compared to peripheral cancer cells, supporting the hypothesis

that the perivascular microenvironment contributes to GBM drug resistance. In summary, our findings underscore the potential of this model in replicating the intricate and heterogeneous nature of GBM. Its versatility, scalability, and high throughput nature of design position it as a promising tool for drug efficacy and toxicity testing, as well as the development of personalized medicine.

9:25 AM - 9:40 AM

Grafted Poly(N-isopropylacrylamide) on Hyaluronic Acid as an Injectable Hydrogel for Localized Mitochondria Delivery

Jamie Ahmed, University of Kentucky

A. Jamie Ahmed 1 , Jason E. DeRouchey 2 , Patrick G. Sullivan 3,4 , Samir P. Patel 5,6 , Alexander G. Rabchevsky 3,4 , Thomas D. Dziubla 1*

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Mitochondria transplantation (MT) presents a promising approach for treating diseases associated with mitochondrial dysfunction, including neurodegenerative diseases, metabolic disorders, and conditions requiring tissue regeneration like spinal cord injuries (SCI). The ability to augment or replace damaged mitochondria with functional ones offers a novel therapeutic pathway, potentially circumventing the limitations of traditional treatments that merely alleviate symptoms without addressing underlying cellular energetics and dysfunction. Nonetheless, challenges such as maintaining mitochondrial viability in adverse environments and ensuring efficient cellular uptake hinder its efficacy. Our research addresses these obstacles by investigating the potential of Poly(N-isopropylacrylamide) grafted onto Hyaluronic Acid (HA-PNIPAm) hydrogels as a novel delivery system for MT therapeutics. PNIPAm is known for its exceptional lower critical solution temperature (LCST) that is close to human body temperature. On the other hand, HA is recognized for its unique biocompatibility and anti-inflammatory properties, existing in many parts of biological systems across the human body including central nervous system. This combination of co-polymer constitutes an ideal pairing to facilitate sustained and controlled mitochondrial delivery. We synthesized various compositions of grafted hydrogels, evaluating its phase transition and gelation properties using ultraviolet-visible spectroscopy and dynamic scanning calorimetry. Hydrogel erosion and mitochondrial release over time were studied using a closed system drug dissolution module and a fluorescence microplate reader. Lastly, seahorse assay was used to study released mitochondria respiration and viability after incubation in HA-PNIPAm hydrogel.

9:40 AM - 9:55 AM

Synthetic Matrix Fibers Promote Vascular Assembly in Dense Fibrin Hydrogels

Firaol Midekssa, University of Michigan

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Introduction: Vasculogenesis is the de novo formation of vascular networks during embryonic development, organogenesis, and adult neovascularization. This process can be harnessed to vascularize engineered tissues. Most successful in vitro approaches to guide and stabilize vessel-like networks in 3D hydrogels require the admixing of stromal cells such as fibroblasts. However, the inclusion of these support cells often leads to undesirable outcomes such as tissue contraction and stiffening. In our prior work using synthetic fibrous matrices, we found that EC self-assembly is facilitated by cell force-mediated matrix reorganization and mechanical intercellular communication via force transmission along fibers. Here, we hypothesized that the inclusion of synthetic fibers in a fiber-reinforced hydrogel composite (FRHC) can provide mechanical and topographical cues that enhance vasculogenic assembly and vascular integration in vivo.

Methods: Dextran vinyl sulfone (DexVS) electrospun fibers functionalized with cell-adhesive or non-adhesive peptides were embedded in 5 mg ml-1 fibrin hydrogel at 2% v/v along with HUVECs (6M/ml). Network morphometric analysis was performed to assess vascular network formation in vitro. To assess transcriptional activity during vascular assembly, tissue lysates were bulk RNA-sequenced and Gene Ontology terms were analyzed. Additionally, to investigate if FRHCs support vascular integration in vivo, FRHCs supporting vascular assembly were pre-cultured for 5 days and implanted in the omental fat pad of NSG mice for 7 days. Implant integration with host vasculature was assessed by histology and immunostaining.

Results: FRHCs enhanced vascular network assembly compared to pure fibrin hydrogels lacking fiber-reinforcement. Intriguingly, the inclusion of non-adhesive fibers led to the greatest network assembly, as determined by total network length. Additionally, non-adhesive fibers enabled lumen formation by 3 days of culture. Examining the transcriptional response of ECs to non-adhesive and cell-adhesive fibers, we noted enhanced expressions of DEGs and GO terms associated with vasculogenic assembly in non-adhesive FRHCs. Following implantation, FRHCs enhanced host cell infiltration as indicated by H&E staining. Furthermore, mouse erythroid marker Ter-119 immunostaining showed that only non-adhesive FRHCs supported vascular integration and blood perfusion from the host.

Conclusion: Altogether, we demonstrated that fibrin hydrogel reinforcement with non-adhesive synthetic fibers promotes vascular assembly and integration of these networks in vivo.

9:55 AM - 10:00 AM

Delivery of nucleic acids using engineer extracellular vesicles as a therapeutic strategy for neurofibromatosis type 1.

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Natalia Higuita-Castro, Department of Materials Science and Engineering, higuitacastro.1@osu.edu.

Neurofibromatosis type 1 (NF1) is a genetic disorder caused by mutations in the NF1 gene, which leads to the loss of function of the neurofibromin protein. The loss of function of Neurofibromin results in the over-activation of RAS protein, leading to uncontrolled cellular proliferation and tumor development. Patients with Neurofibromatosis have a higher incidence of developing malignant peripheral nerve sheath tumors (MPNST), which are aggressive and invasive cancers with a low rate of survival. Gene therapies for NF1 using viral delivery systems are limited due to the size of the NF1 gene. To address this limitation, our research focuses on the use of engineered EVs extracellular vesicles (eEVs) as non-viral carriers for delivering NF1 cargo (such as mRNA and NF1 plasmid) to restore neurofibromin protein function. This approach holds promise as a novel therapeutic strategy for treating Neurofibromatosis type 1.

Isolated human dermal fibroblast cells (HDFs) were transfected with a plasmid encoding for human NF1. Then eEVs were isolated from the culture media using size exclusion chromatography. Packing of the NF1 genetic material was confirmed using RT-PCR, western blot, and conventional PCRs. The therapeutic effect of NF1 loaded eEVs was assessed in vitro using malignant tumor cells isolated from NF1 patients, and in vivo using a NF1 patient-derived xenografts (PDX) model.

EVs characterization showed effective loading of plasmid and mRNA content of NF1 into the eEVs. NF1 eEVs were effectively captured and incorporated by the malignant tumor patient cells after eEVs exposure. RT-PCRs confirmed that the recipient cells were successfully transfected with the NF1 cargo and showed sustained overexpression of NF1. Additionally, treated cells showed a reduction in phosphorylation and proliferation pathways. In vivo experiments showed a significant reduction of tumor volume and proliferation over time for mice treated with NF1-loaded eEVs.

Overall, these preliminary findings showed the potential of NF1 loaded eEVs as a gene therapy strategy for the rescue of neurofibromin protein function, which can alleviate the burden of neurofibromatosis type 1 disorder and other types of cancer associated with NF1 mutations, such as glioblastoma and melanoma.

CONCURRENT SESSION II: TISSUE ENGINEERING

8:45 AM - 9:10 AM **Invited Speaker: Adam Feinberg, Carnegie Melon University**

9:10 AM - 9:25 AM

Dynamic Granular Hydrogels for Studying Pancreatic Cancer Growth in the Developing Tumor Microenvironment

Ellen Frahm, Purdue University

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Granular hydrogel scaffolds are increasingly developed for tissue engineering applications as the large void space in the 3D granular hydrogels allows cells to proliferate and migrate with less spatial restriction. Granular hydrogels/microgels are commonly prepared from microfluidic droplet generators, which is laborious if one wishes to create an array of microgels with different mechanical or bioactive properties. To address this challenge, we employed gelatin-norbornene-carbohydrazide (GelNB-CH), a dually modified gelatin amenable for photocrosslinking (via thiol-norbornene photo-click reaction) and dynamic stiffening (via hydrazone bonding). In addition, these dynamic microgels can be further annealed into microporous annealing particle (MAP) scaffolds with a third click chemistry – inverse electron-demand Diels-Alder (iEDDA) click reaction. The scope of this work was to develop a heterogeneous MAP scaffold using GelNB-CH microgels of various stiffness and to observe endothelial cell growth and network formation within the granular hydrogel scaffold. A precursor solution of GelNB-CH and PEG-tetra-thiol are mixed in a droplet generator chip with Novec 7500 with 2% Pico Surf at 18 and 90 microliters per minute for the aqueous and oil phase, respectively. The droplets were photocrosslinked into microgels and stiffened with oxidized dextran (oDex) for 24 hours. The microgels were annealed into the MAP scaffold using PEG-tetra-tetrazine for 2 hours. The precursor solution and the pumping phase of the microfluidic system were optimized to create microgels with an initial diameter around 200 microns. Stiffening with greater concentrations of oDex created microgels with continuously decreasing diameters, reflecting a void space comprising around 35% of the MAP scaffold. Endothelial cell (HUVEC) spheroids are encapsulated within a heterogeneous mixture of soft and stiff microgels labeled with fluorescent PEG-thiol. HUVEC spheroids demonstrated spreading around the stiffened microgels over a 48-hour culture period. Current work focuses on post-formation tethering bioactive motifs within the MAP scaffold to compare the contributions of matrix stiffness and bioactivity on endothelial cell migration.

9:25 AM - 9:40 AM

Tuning the Anisotropy of Granular Hydrogels to Promote Lymphatic Tube Sprouting

Daniel Montes Pinzon, University of Notre Dame

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Granular gels, comprising microgel subunits, present compelling prospects in biomedical applications, as they mimic the extracellular matrix and foster a conducive microenvironment for tissue regeneration.

Their significance in regenerative medicine lies in enabling enhanced cell invasion crucial for promoting tissue growth. This study investigates the influence of diverse composite granular gel morphologies on early-stage of lymphatic tube formation. Using norbornene-modified hyaluronic acid (NorHA), microgels were fabricated through pipetting and vortexing techniques, subsequently forming granular gels via centrifugation. Evaluation of microgel generation methods showcased distinct granular hydrogel morphologies. Vortexing-produced gels exhibited higher porosity but wider microgel size distribution, leading to tighter packing compared to pipetting, resulting in smaller pores. This morphology yielded vortexing gels with a higher storage modulus and wider linear viscoelastic region (LVR) ranges due to increased interparticle contact. Morphological variances significantly affected lymphatic sprout formation. Pipetting gels facilitated linear-like sprouts, potentially forming lumen-like tubes. Conversely, vortexing gel's tight packing enhanced microparticle coverage. Quantitative RT-PCR data supported these observations, indicating a four-fold upregulation of LYVE-1 and PDPN markers in pipetting gels during early-stage lymphatic sprout formation. Furthermore, altering interstitial matrix composition affected sprout morphology, mirrored in gene expression levels. These findings emphasize the need for synchronized morphological and compositional tuning in granular hydrogel design to enhance lymphatic sprouting in composite granular hydrogels. Overall, this study provides new insights into 3D in vitro lymphatic tube formation, which is beneficial for basic lymphatic biology, as well as various approaches in lymphatic regeneration.

9:40 AM - 9:55 AM

Bioprinting and Microvascular Assembly within PEGNB Granular Materials

Irene Zhang, University of Michigan

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The development of perfusable and multiscale vascular networks remains one of the largest challenges in tissue engineering. Leveraging the use of colloidal bead suspensions as supportive media for bioprinting, the purpose of this study was to evaluate if clickable PEG microbeads could support bioprinting of mesoscale structures, and whether the beads could be clicked together into a granular hydrogel capable of supporting microvascular self-assembly.

PEG-norbornene (PEGNB) microbeads were formed via microfluidic droplet generation with 0.5% PFPE in NOVEC-7500 and UV photopolymerized. Beads comprised of 10 wt% 8-arm PEGNB, 2 mM LAP, PEG-DT, and 2 mM RGD with a molar ratio of thiol:ene of 0.375:1. Beads were jammed into a slurry via vacuum filtration. Rheological characterizations were performed using a 20 mm diameter parallel plate head. For bioprinting, jammed bead suspensions with additional LAP and PEGDT were packed into a PDMS mold, and then used to support a gelatin bioink extruded with an Allevi-3 bioprinter. After printing, the beads were photocrosslinked together to form a granular construct with the embedded print. To instigate vascular formation, a 1:1 mixture of ECs and normal human lung fibroblasts (LFs) at 2 M/mL was suspended within a 500 L bead/LAP/PEGDT slurry and UV crosslinked to form cell-laden constructs. These were then cultured for 7 days in EGM2 and fixed and stained with UEA (an EC-specific lectin), phalloidin, and DAPI to visualize microvascular networks.

The PEGNB microbead slurry exhibited shear-thinning mechanical behavior suitable for suspended bioprinting, supported suspended printing of sacrificial bioinks, and could be UV crosslinked into a granular construct post-print. Cell-laden PEGNB constructs yielded microvessel-like development in the inter-bead void space, with cells spread along the surface of the nondegradable RGD-modified microbeads. UEA-positive tubule formation was observed, with F-actin staining showing LFs supporting branching morphogenesis in a pericyte-like manner.

Our results show that PEGNB microbeads support suspended bioprinting, secondary photocrosslinking into granular constructs, and self-assembly of microvasculature. Ongoing studies are focused on optimizing sacrificial ink evacuation of printed structures and printing into cell-laden bead baths. Future work will be focused on evaluating vascular inosculation across length scales towards perfusion of an engineered vascular tree.

9:55 AM – 10:00 AM

Tissue Nanotransfection (TNT) Of Pro-vasculogenic And Pro-neuronal Factors For The Treatment Of Peripheral Neuropathies

Jon Stranan[1], Ana Salazar-Puerta[1], Hallie Harris[1], Nada Khattab[1], Mana Saffari[1], Sara Kheirkhah[1], Will Lawrence[1], Devleena Das[1], Jordan Moore[1], Neil Ott[1], Dave Arnold[2], Amy Moore[1], Daniel Gallego-Perez[1].

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Introduction: Peripheral nerve injuries (PNIs) can result in sensory/motor deficits and muscle loss if not treated immediately. In this study, we describe the use of a novel technology, Tissue Nano-Transfection (TNT), that can deliver gene therapies to nerve/muscle aimed at improving the rate of peripheral nerve regeneration and healthy maintenance of injured nerve tissue and denervated muscle through neurogenic and vasculogenic reprogramming.

Methods: Vasculogenic reprogramming factors Etv2, Fli1, and Foxc2 (EFF) were delivered into an injured mouse (C57BL/6) sciatic nerve using TNT to reprogram native fibroblast/Schwann cells into functioning endothelial cells; with the intent of increasing vascularity which should lead to axonal preservation. Varying concentrations of these three EFF factors were assessed in their effectiveness of increasing vascularity in the sciatic nerve by comparing laser speckle imaging (LSI) perfusion in the nerves between all concentration groups and a control (pCMV6) 7 and 14 days after treatment. Histology was also used for comparing the amount of blood vessels (CD31) and axons (NF) formed in the nerves between each group. Another set of experiments were done using TNT delivery of pro-neuronal factors Ascl1, Brn2, and Myt1l (ABM) to help reinnervate leg muscle in cases of sciatic nerve transection. Again, optimization of the concentration ratios is being done currently to determine the most efficient combination of factors for conversion of native myoblasts to induced neurons.

Results: At days 7 and 14 post-treatment using TNT of the EFF vasculogenic factors in crushed nerve tissue, there was a significant increase in blood perfusion in the sciatic nerve measured by LSI. Histology also showed an increase in the number of sciatic nerve blood vessels in the EFF cocktail group where Foxc2 was doubled in concentration (1:1:2) at both 7 and 14 days post treatment. The conversion of muscle to induced neurons using ABM is still an ongoing study.

CONCURRENT SESSION III: BIOINTERFACES

10:15 AM - 10:40 AM Invited Speaker: Andrew Shoffstall, Case Western Reserve University

10:40 AM - 10:55 AM

Understanding and Mitigating Effects of Bacteria at Neural Microelectrode Implant Site

Grace Burkhart, Case Western Reserve University

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Brain-machine interface performance is largely affected by the neuroinflammatory responses resulting partly from blood-brain barrier (BBB) damage following intracortical microelectrode implantation. Recent findings strongly suggest that certain gut bacterial constituents penetrate the BBB and are resident in various brain regions of rodents and humans. Therefore, we hypothesized that damage to the BBB caused by microelectrode implantation could amplify dysregulation of the microbiome-gut-brain axis. We found that bacteria, including those commonly found in the gut, are present in the brain of mice previously implanted with single-shank silicon microelectrodes, but the microelectrodes were sterile at time of implantation. Systemic antibiotic treatment of mice implanted with microelectrodes to suppress bacteria resulted in differential expression of bacteria in the brain tissue and a reduced acute inflammatory response compared to untreated controls, correlating with temporary improvements in microelectrode recording performance. Further research concerns using microelectrodes coated with antimicrobial Titania Nanotube Arrays (TNA) to investigate the effect of the coating on the neuroinflammatory response at the implant site. These arrays serve as a novel platform for loading microelectrodes with drugs to reduce neuroinflammatory damage after microelectrode implantation. Five different drugs were loaded into the TNA-coated microelectrodes and implanted into mice to investigate the drug loading capabilities of the TNA coating in vivo. Such a delivery platform could provide an effective means of localized and sustained delivery directly to the implant site to impact neuroinflammatory damage and control the bacterial response.

10:55 PM - 11:10 PM

Degree of Hyaluronic Acid Polymer Modification Guides Nascent Protein Secretion and Cell Fate Independent of Hydrogel Mechanics

Yu-Chung Liu, University of Michigan
Yu-Chung Liu1, Eleanor M. Plaster1, Claudia Loebel12
1Department of Biomedical Engineering, University of Michigan
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Statement of Purpose
Synthetic hydrogels allow us to mimic many properties of the extracellular matrix (ECM) and assess their impact on cell function. Our recent studies showed that upon embedding into 3D hydrogels, cells actively secrete and remodel the hydrogel-cell interface by depositing nascent ECM. While hydrogel mechanics direct the amount of nascent ECM deposition1, there is limited understanding of how other hydrogel properties, such as ligand exposure and wettability, guide the dynamic interplay between cells, their nascent ECM, and the hydrogel. To address this, we engineered hyaluronic acid (HA) hydrogels of matched mechanics but different degrees of modifications. We then leveraged metabolic labeling to investigate the spatiotemporal dynamics of nascent ECM in response to HA modifications.

Materials and Methods
Chondrocytes were isolated from juvenile bovine joints and passaged once prior embedding into norbornene-modified HA with varying degrees of substitution (DS%): 9.5%, 23%, and 43%, quantified by 1H NMR2. Crosslinking with dithiols was adjusted to obtain moduli of 5 kPa. Embedded chondrocytes were cultured for 7 days in azidohomoalanine media, and nascent ECM was visualized with dibenzocyclooctyne-amine-488 or dibenzocyclooctyne-biotin mediated click chemistry for mass spectrometry. Total ECM protein production was quantified with Bradford Protein Assay and dsDNA quantification, and cell proliferation was assessed by EdU incorporation.

Results and Discussion
Staining of nascent ECM after 7 days showed increased thickness as a function of DS%, ranging from 1.21±0.54 μm (DS9.5%) up to 2.72±1.23 um (DS43%). Quantitative bioassays confirmed increased ECM production for DS43% with 4.43 μg ECM protein/μg DNA compared to DS9.5% with 2.74 μg/μg ECM protein/μg DNA. Additionally, at day 7, EdU incorporation showed a 6-fold increase in dividing cells in DS43% (76.3%±7.5%) compared to DS9.5% hydrogels (12.6%±4.6%). These results suggest that higher HA modifications enhance cell proliferation and nascent ECM production.

Conclusion
This study established a functional link between HA modifications and ECM production and cell proliferation independent of mechanics. Ongoing studies are using pulse labeling and proteomic analysis towards gaining mechanistic insight into the contributions of hydrogel modification to nascent ECM production in 3D hydrogels.

Ref
1Loebel+2020 Adv Func Mater 30,1909802
2Plaster+2023 Carb Polym Technol Appl 6,100360

11:10 AM - 11:25 AM

Hydrogel viscoelasticity modulates cell nascent extracellular matrix deposition

Matthew Tan, University of Michigan

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Statement of Purpose: The extracellular matrix (ECM) is an integral component of a cell's microenvironment that provides structural support and biophysical cues. In addition, cells continuously remodel this ECM through the deposition of newly-synthesized (nascent) ECM, which in turn alters its biophysical properties[1]. Previous studies showed that viscoelasticity is a critical biophysical cue in guiding cell behavior. However, there is a limited understanding of how viscoelasticity directs nascent ECM deposition and remodeling. Here, we utilize an interpenetrating polymer network (IPN) to modulate hydrogel viscoelasticity to probe its role in nascent ECM deposition and how these changes modulate cell function.

Methods: IPN hydrogels were fabricated from simultaneous crosslinking of norbornene-modified hyaluronic acid (NorHA, 5wt%) with (1.75-17.5mM) thiolated adamantane (Ad) and β-cyclodextrin (CD) and methacrylate-modified hyaluronic acid (0.15wt%) with free-radical crosslinking[2]. Shear-rheology was used to measure the elastic (G') and viscous (G'') properties and additional dithiothreitol added to increase elasticity of NorHA networks. Human mesenchymal stromal cell (hMSC) spreading was assessed using fluorescent labeling of the actin cytoskeleton, and nascent ECM visualized through click-labeling of incorporated azidohomoalanine and DBCO-488 and co-immunostained with fibronectin.

Results and Discussion: Addition of varying amounts of Ad-Cd crosslinkers enabled fabrication of IPN hydrogels ranging from low (G''=50 Pa) to high (G''=540 Pa) viscosities while elasticity (G'=2000±163.7 Pa) was held constant by adjusting the amount of dithiothreitol (0-1.30mM). hMSCs seeded on top of high viscosity IPNs showed significant decrease in cell spread area (3977±2560μm2) when compared to low viscosity IPN hydrogels (4865±2424μm2). Interestingly, hMSCs on high-viscosity IPNs showed enhanced nascent ECM and a 1.3-fold increase in fibronectin staining relative to low-viscosity hydrogels, indicating that higher viscosity support fibronectin deposition either via increasing its secretion or retention in IPNs.

Conclusion: IPNs based on simultaneous formation of guest-host and free-radical crosslinks enabled facile and independent tuning of viscous and elastic properties. hMSC spreading and nascent ECM deposition was dependent on hydrogel viscosity, suggesting a feedback mechanism between viscoelasticity and nascent ECM deposition and remodeling. Ongoing studies are measuring the contributions of nascent ECM secretion versus retention and mechanisms of cell mechanosignaling.

References: [1]Loebel+ Nat. Mater. 2019, [2]Abhishek+ Adv. Mater. 2022

11:25 AM – 11:30 AM

Investigation of Polyproline II Peptides as Antifouling Biomaterials

Rebecca Ahn, Case Western Reserve University

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Biomaterials derived from polyproline (PPII) peptide sequences offer significant promise due to their diverse applications, including as antifouling agents for biomaterials and as anchors that encourage high density of other peptide sequences. However, the precise mechanisms underlying their antifouling behavior is not fully understood. This study comprehensively investigates antifouling properties of PPII peptides using bovine serum albumin (BSA) as a model foulant while varying guest amino acids within the PPII sequence. By varying the sequence and controlling their adsoprtion, aim to investigate multiple properties, including secondary structure, hydrophobicity, rate constant of rearrangement, and surface coverage, to elucidate their impact on these on antifouling mechanisms. Our findings indicate that surface coverage and incorporation of proline in the sequence has a significant positive impact on antifouling properties. Moreover, this research marks the first exploration of human mesenchymal cells (hMSCs) as foulants on PPII-functionalized surfaces, revealing successful minimization of cell adhesion that correlates with the model foulant results. In conclusion, our research underscores the potential of PPII design and application as an antifouling biomaterial, paving the way for future advancements in this field including use of these sequences as way to control hMSC spreading and attachment.

CONCURRENT SESSION IV: NANOMATERIALS

10:15 AM - 10:40 AM Invited Speaker: Daniel Gallego Perez, The Ohio State University

10:40 AM - 10:55 AM

Hemostatic Performance Analysis of Biosynthetic Whole Blood Analogue in an in vitro Human Massive Transfusion Model

Sonali Rohiwal, Case Western Reserve University

Sonali Rohiwal1, Dante Disharoon1, Susan Shea2, Selvin Hernandez1, Kevin Tobin3, Ji Li3,

Mathangi Gopalakrishnan3, Joga Gobburu3, Norman Luc1, Hanyang Wang1, Shruti Raghunathan1, Bipin Paruchuri1, William McGhee4, Baylee Traylor5, Emma Quill5, Ujjal Sekhon5, Christa Pawlowski5, Michael Bruckman5, Andre F Palmer6, Griffin Beyer6, Tanmay Salvi6, Asim Mohd. Khan6, Dipanjan Pan7, Stephen Penegor8, James Murto8, Matthew D. Neal2, Philip C. Spinella2, Ashlee Conway3, Allan Doctor*,3, Anirban Sen Gupta*,1

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Introduction: To improve access to blood for hemostatic resuscitation in austere settings, DARPA initiated FSHARP program to develop shelf stable, biosynthetic, lyophilized whole blood analogue (WBA) comprising an O2 carrier, platelet mimetic, and plasma. The objective for WBA development is to meet progressively restrictive non-inferiority (NI) margins (40% in Y1, advancing to 10% in Y4), in comparison to target product profile (TPP defined as sWB performance at median storage duration for in-theater use 26d). WBA incorporates a nanoparticle-based RBC mimic (Erythromer, EM) and/or a conformation-selective, size-restricted polymerized hemoglobin (polyHb), nanoparticle-based platelet mimic

(SynthoPlate, SP) and freeze-dried plasma (FDP). We evaluate WBA's hemostatic mechanism and efficacy in human massive transfusion (HMT) model, using viscoelastometry (ROTEM), fluorescence image-assisted microfluidics (Bioflux), thrombin kinetics, and electrical impedance assessment of platelet aggregation.

Materials and Methods: HMT simulation involved mixing fresh WB (fWB) & sWB (1:1 & 1:3 ratios; fWB:normal saline (NS) dilutions were negative controls) and WBA with EM or polyHb, SP, and FDP, comparing to sWB. Effect size (ES) determined therapeutic performance relative to negative control for NI. ROTEM-MCF assessed as key performance metric for NI; Bioflux measured fibrin lag time for safety metric; other assays provided mechanistic insight.

Results & Conclusions: FDP dilution tolerance was established, impacting WBA performance in static more than flow-based assays. Plasma protein functionality was moderately preserved, while platelet number/function decreased in fWB:sWB mixtures, with sWB superior to normal saline (NS) in restoring hemostasis. WBA formulations effectively restored ROTEM-MCF compared to sWB, meeting NI criteria. Ongoing optimization aims to meet increasingly stringent NI criteria.

Learning objectives:

i. Outline blood product challenges in far-forward battlefield settings.

ii. Describe advantages of field-deployable lyophilizable WBA for hemostatic resuscitation.

iii. Understand functional design and mechanistic evaluation of WBA.

Abstract Disclaimer: This research received funding from DARPA HR001121S0027-FSHARP-FP-001. Research-grade FDP from Vascular Solutions LLC (Teleflex, Inc. subsidiary) not FDA-approved or for sale.ASG is co-founder and CTO and MN is CMO of Haima Therapeutics.AD, DP and PS are co-founders and CSO, CTO and CMO for KaloCyte. The views expressed in this document are those of the authors and do not represent official U.S. Government policies.

10:55 AM - 11:10 AM

Novel EDB-FN Targeted Nanobubble Ultrasound Contrast Agents for Pancreatic Cancer Diagnosis

Theresa Kosmides, Case Western Reserve University

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It is estimated that there will be 66,440 new pancreatic ductal adenocarcinoma (PDAC) cases in 2024 in the US with nearly 80% presenting with non-resectable disease, resulting in a dismal five-year survival rate of 10% [1,2]. To increase treatment options, early PDAC detection tools are needed. Nanobubbles

(NB) are a submicron ultrasound (US) contrast agent which extravasate through permeable vasculature, increasing tumor accumulation [3]. Addition of a PDAC targeting moiety increases contrast agent retention in tumors. Of many biomarkers, extra domain B-fibronectin (EDB-FN) is overexpressed in PDAC [4]. We propose the addition of ZD2, an EDB-FN targeting molecule, to increase PDAC lesion signal intensity, thus improving diagnosis.

Nanobubbles (NB) were prepared as previously reported [3]. ZD2-NBs were prepared by adding ZD2 solution to nanobubble precursor lipid solution. The stability of ZD2-NBs was confirmed with US in an agarose phantom (18MHz fc, 4% power, 1fps). NB diameter was measured using dynamic light scattering. ZD2-NBs' cell uptake was determined by co-incubating rhodamine tagged ZD2-NBs with human PDAC cell lines (Capan-1, BxPC3) and measuring fluorescence intensity. NBs targetability to PDAC was examined using a flank tumor model in immunocompromised mice inoculated with Capan-1 cells. NBs were administered via I.V. injection, and tumor and kidney US signal was monitored in B and NLC mode for 20 minutes post injection. Signal intensity was analyzed using MATLAB. Tissues were preserved after the final time point for histological analysis.

We successfully incorporated an EDB-FN targeting peptide, ZD2, into our ultrasound contrast agents. The addition of ZD2 did not significantly alter NB size, charge, or US stability. ZD2-NBs had comparable US contrast signal to untargeted NBs and peptide addition did not negatively affect signal intensity or stability for 300s. ZD2-NBs had approximately 2-fold higher fluorescence signal compared to untargeted NBs in BxPC3 cells. ZD2-NBs had 2.86-fold higher tumor NLC signal 16 minutes after injection compared to untargeted NBs. Experiments to assess biodistribution and in vivo targeting are ongoing.

References: [1] American Cancer Society. Facts & Figures 2024. [2] Park W et al., JAMA. 2021. [3] De Leon et al., Nanoscale 2019. [4] Han et al., Bioconjugate Chemistry. 2015.

11:10 AM - 11:25 AM

Exploring Origin-Dependent Exosome Uptake and Cargo Release using Fluorescent Chiral Quantum Dots

Gaeun Kim, University of Notre Dame

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Presenter: Gaeun Kim

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Exosomes have come into the spotlight as promising nanocarriers for drug delivery. However, the clinical application of exosomes remains a challenge due to the limited understanding of their physiological properties. While exosomes typically undergo cellular uptake through two potential routes, 1) endocytosis and 2) membrane fusion, the intricate mechanisms involved in their origin-dependent uptake and

subsequent cargo release are not fully explored. Herein, we unravel the mechanisms governing exosome entry into recipient cells and subsequent intracellular release of their cargo. In this study, we utilized chiral graphene quantum dots as representative exosomal cargo, taking advantage of their effective lipid membrane permeability, as well as their unique optical properties for tracking. To investigate the cell-of-origin specific cellular entry mechanism and the following intracellular cargo fate of exosomes, we used confocal laser scanning microscopy imaging analysis, mass spectrometry-based proteomics, Western blot, and fluorometry. We observed that the preferential cellular uptake of exosomes derived from the same cell-of-origin (intraspecies exosomes) is 1.4~3.2 times higher than that of exosomes derived from different cell-of-origin (cross-species exosomes). This uptake enhancement was attributed to the receptor-ligand interaction-mediated endocytosis, as we confirmed a 1.6~2.1 times higher lysosomal uptake of intraspecies exosomes and identified the expression of specific ligands on exosomes that favorably interact with their parental cells. On the other hand, we found that the uptake of cross-species exosomes primarily occurred through membrane fusion (colocalization correlation value of PCC > 0.7), followed by direct cargo release, whereas intraspecies exosomes exhibited a lower correlation value (PCC = 0.3~0.5). Overall, our study envisions valuable insights into the cellular uptake of exosomes depending on the origin of cells, which could drive future advancements in effective drug delivery utilizing exosomes.

11:25 AM – 11:30 AM

Iterative Design-Build-Test-Learn Non-Viral Delivery Vehicle Discovery Platform Rapidly and Efficiently Identifies Lead Candidates

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The ability to rapidly and efficiently identify novel safe and effective non-viral delivery vehicles for use in gene therapy and drug delivery remains a challenge. To overcome this challenge, we have created a design-build-test-learn (DBTL) polymer nanoparticle (PNP) discovery platform capable of rapidly producing and efficiently identifying PNP designs with clear utility for a variety of applications. During each DBTL cycle by this platform, hundreds to thousands of diverse PNP designs are systematically and efficiently screened in vitro and in vivo within months using standardized, reproducible methodology. To date, we have synthesized, characterized, and assessed more than 6,000 novel polymers produced via reversible addition–fragmentation chain transfer (RAFT) polymerization. Each polymer underwent high-throughput purification and characterization, including measurements of molecular weight, monomer conversion, composition, size, polydispersity, zeta potential, loading efficiency, cytotoxicity, and transfection efficiency in vitro. In addition, PNP biodistribution performance was evaluated in C57BL/6 wild type mice. Early proof-of-concept work has yielded multiple examples where this DBTL approach has proven successful in as few as 2 or 3 iterative DBTL cycles. Indeed, we have identified lead PNP delivery candidates that distribute to multiple desired tissue types following intravenous injection in mice. Moreover, we have identified lead PNP candidates capable of distributing specifically to murine sciatic nerve and/or brain tissue following intrathecal injection. Finally, we have identified multiple lead PNP candidates that leveraged specific design parameters, such as controlled molecular weights and monomer composition percentages, to maintain certain physicochemical characteristics (e.g., size, zeta potential, etc.) upon loading with nucleic acid cargo. Altogether, the empirical and modeling insights provided by data collected during each DBTL cycle has enabled us to identify clear patterns in PNP design parameters with significant utility for specific gene and drug delivery applications. The results from these studies have demonstrated the capability of our DBTL platform to rapidly and efficiently identify lead PNP delivery candidates that warrant further investigation. Therefore, this iterative high-throughput synthesis, characterization, and assessment approach to non-viral delivery vehicle discovery is well-positioned to soon offer potentially paradigm-shifting capabilities to the gene therapy and drug delivery fields.

11:30 AM – 12:30 PM Plenary Session 1

Invited Speaker: Shana Kelley, PhD, Northeastern University
(LIVE from Midwest)

Invited Speaker: Joel H. Collier, PhD, Duke University, *Supramolecular Immunomodulators for Inflammatory Diseases*, Clemson Award for Basic Research
(LIVE from Southeast)

CONCURRENT SESSION V: DRUG DELIVERY

2:10 PM - 2:25 PM

Dexamethasone Sodium Phosphate Loaded Platelet-Inspired Nanoparticles Improve Intracortical Microelectrode Performance

Longshun Li, Case Western Reserve University

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Introduction: Brain machine interfacing (BMI) has a significant influence on alleviating the burden of patients with paralysis and limb loss. Current applications of this technology are severely limited by the main signal acquisition device, intracortical microelectrodes (IMEs), that do not have long-term stability and functionality with respect to signal recording quality. The decline in performance is, in part, due to blood-brain-barrier (BBB) disruption resulting in neuron death and infiltration of pro-inflammatory blood cells and proteins. While avoiding vascular trauma is nearly impossible, we may be able to leverage the vascular damage response to interrupt the secondary injury/inflammatory cascade. We have found that platelets are chronically present at the site of injury, despite BBB reformation after 8 weeks. Platelet-inspired liposomal nanoparticles (PINs) that selectively bind to damaged endothelium and activated platelets amplify the natural hemostatic mechanism while simultaneously adhering and aggregating at the site of vascular injuries. Once localized at the site of injury, PINs can deliver a payload of anti-inflammatory drugs like dexamethasone sodium phosphate (DEXSP), which can hasten recovery after IME implantation, reducing neuroinflammation and improving recording performance. In this study, we aimed to 1) assess the feasibility of PINs as a vehicle for targeted drug delivery at the IME site and 2) investigate the therapeutic effects of systematically administered DEXSP-loaded PINs in inhibiting neuroinflammation and enhancing neural recording performance.

Methods:

a. Ex vivo imaging on essential organs to determine PIN biodistributions.

b. Endpoint histology to analyze potential colocalization of PINs with neuroinflammation, platelets and leaky BBB markers at the IME injury site.

c. Extracellular neural signal recording to evaluate the therapeutic effects of treatment of DEXSP-loaded PINs.

Results and Conclusions:

a. Platelet-inspired nanoparticles were locally delivered near the implant site interface over the course of days to weeks, demonstrating the potential to serve as a vehicle for targeted drug delivery to mitigate IMEs-induced neuroinflammation and improve IMEs recording performance.

b. Systematic administration of dexamethasone sodium phosphate loaded nanoparticles attenuated the decline in neural recording performance from IMEs over an 8-week period.

2:25 PM – 2:40 PM

Carbopol-Mediated Electrospun Fiber Membranes as pH-Responsive Biomaterials

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Fibers formed by electrospinning are versatile materials with significant potential for bio/chem/medical applications. Electrospun fiber membranes display very high surface area and porosity, which greatly enhances interaction with ambient environments and response to external stimuli. Electrospun fibers can be formed in complex shapes, in homogenous and multi-layer (core-sheath) structures, and can be embedded with various functional agents released in a controlled manner [1]. Our group has previously reported electrospun membranes for diverse biomedical applications, including dural repair [2], transdermal drug release [3], controlled drug release for brain tumor therapy [4].

One important consideration is the surrounding media pH, as pH plays a major role in many biological systems. Materials capable of sensing and responding to pH changes can be used to provide various treatment modalities and behaviors. For example, fiber membranes have been developed [5] containing different types of pH sensitive Eudragit polymers that dissolve in specific pH conditions and release drug payloads in targeted locations of the gastrointestinal system. Carbopol is an exciting excipient in electrospun membranes due to its ability to modulate viscosity as a function of pH [6].

We report the electrospinning of Carbopol (C974P NF) dispersed in a PVP solution. Carbopol/PVP fiber membranes are designed to rapidly hydrate into a hydrogel. Carbopol becomes more responsive at higher pH (>6), increasing uptake volume of buffer solution - 5x of dry mass at pH 4 and 20x at pH 8 in 15 min. Spreadability measurements of the gel-phase indicate that higher pH and Carbopol concentration result in a more viscous network of hydrated fiber membranes. The pH responsive nature of Carbopol-containing electrospun membranes has multiple potential biomedical uses, including wound healing, drug delivery, and contraceptives.

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[2] B. Campbell, et al., Applied Biomaterials 110(3) (2022) 660-667.
[3] S. Tort, D. Han, E. Frantz, A.J. Steckl, Surface and Coatings Technology 409 (2021) 126831.
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2:40 PM - 2:55 PM

Engineering of Degradable Linkers to Improve Oxidative Sensitivity of Thioketal Based Biomaterials

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Bioresponsive materials that leverage biological stimuli such as reactive oxygen species (ROS) have been of increasing interest in regenerative medicine applications. ROS are essential mediators in numerous biological pathways and are selectively generated by cellular activity. Biomaterials containing oxidation-sensitive thioketal (TK) linkers have shown promise in regenerative medicine applications. However, some TK-based systems demonstrate insufficient sensitivity to physiological doses of ROS. As the conventional TK bond is relatively hydrophobic, we hypothesize that engineering TK linkers to contain more hydrophilic pendant groups will yield biomaterial formulations that are more sensitive to degradation via ROS.

TK linkers were synthesized via an acid catalyzed reaction with several different ketone containing compounds with various levels of hydrophilicity. 1H NMR was used to confirm structure and LogP was determined experimentally to determine hydrophilicity. Samples were incubated in varying concentrations of hydrogen peroxide (H2O2) and evaluated using NMR over two weeks. TK linker peaks were integrated in reference to an internal standard and compared over time. These studies showed that all TK linkers were stable in aqueous conditions and more hydrophilic formulations were more degraded in lower concentrations of ROS.

To evaluate TK linker's performance in a biomaterial's platform, TK linkers were crosslinked with 4-arm PEG maleimide to form hydrogels. Hydrogels were incubated in varying concentrations of H2O2, and the swelling ratio was evaluated over 10 days. Hydrogels constructed with more hydrophilic TK linkers degraded faster than traditional TK linker hydrogels.

Similarly, TK linkers were crosslinked with trimethylolpropane triglycidyl ether to form scaffolds. Scaffolds were incubated in various concentrations of H2O2, and mass loss was tracked over 30 days. Scaffolds containing more hydrophilic TK formulations were degraded more in all concentrations of H2O2 and degraded at an accelerated rate. All scaffolds constructed with more hydrophilic TK linker degraded fully in 50 and 100 mM H2O2 by day 30.

These data sets support the initial hypothesis that more hydrophilic TK formulations yield materials that are more sensitive to degradation via ROS. These studies demonstrate the potential for numerous TK formulations to be synthesized and employed in different biomaterials platforms for a wide range of regenerative medicine applications.

2:55 PM – 3:00 PM

Formulation for Controlled Self-Assembly of Polymer Nanoparticles

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Non-viral vehicles, such as polymer nanoparticles show promise as biomedical tools for the future of gene delivery. Polymer chemistry affords the ability to determine features of the self-assembled nanoparticle by tuning the monomeric sequence of the molecular backbone. Battelle has developed >6,000 novel polymers, and characterized their structure and function as gene delivery vehicles. Unlike lipids, many of these polymers can be directly dispersed in water, without the need for a solvent dilution processing step. However, exploration of formulation for these polymers still provides useful parameters for tuning the characteristics of the PNP. Here, we describe formulation techniques that enable further control over the characteristics and performance of these PNPs as gene delivery vehicles. We selected five polymers from our libraries synthesized via reversible addition-fragmentation chain transfer polymerization. We explored the self-assembly of these polymers into PNP using a direct hydration method as well as nanoprecipitation methods with and without genetic cargo. We measured the particle size via dynamic and static light scattering, exploring the effects of polymer concentration, rate of solvent removal, and payload loading on the nanostructures obtained. We demonstrated the use of nanoprecipitation to produce reproducible, stable PNPs, dispersed in water, that were not possible by directly hydrating the polymers. The mean particle size for the nucleic acid loaded PNPs obtained by nanoprecipition was 280 ± 7 nm for triplicate formulations. We extended this process to four other polymers, demonstrating applicability to a greater collection of our polymer library, and we showed the ability of the process to effectively load nucleic acids into these PNPs. This work demonstrates the capability nanoprecipitation to open the design space of Battelle's platform to produce and characterize libraries of PNPs with greater diversity and potential as gene delivery vehicles. We are able to use these methods to formulate PNPs loaded with genetic cargos that are not otherwise able to be studied, obtaining in vitro transfection and in vivo biodistribution data. These results lay a foundation to accelerate the development of non-viral gene delivery and new genetic therapies for untreatable and devastating diseases.

CONCURRENT SESSION VI: ENGINEERING CELLS AND THEIR MICROENVIRONMENTS

1:45 PM – 2:10 PM Invited Speaker: Brendon Baker, University of Michigan

2:10 PM - 2:25 PM

Lamellipodia-mediated osteoblast haptotaxis guided by fibronectin ligand concentrations on a multiplex chip

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Skull morphogenesis is a complex, dynamic process involving two different germ layers and progressing to the coordinated, directional growth of individual bones. The mechanisms underlying directional growth toward the apex are not completely understood. Here, we utilize a microfluidic chip-based approach to test whether calvarial osteoblasts undergo haptotaxis on a gradient of Fibronectin1 (FN1) via lamellipodia. Mimicking the embryonic cranial mesenchyme's FN1 pattern, we establish FN1 gradients in the chip using computer modeling and fluorescent labeling. Primary mouse calvarial osteoblast progenitors are plated in the chip along an array of segmented gradients of adsorbed FN1. We perform single-cell tracking and measure protrusive activity. Haptotaxis is observed in an intermediate FN1 concentration, with a

directional migration index (yFMI) of 0.08 ± 0.023 (mean ± standard error), exhibiting a sevenfold increase compared to controls. An increase in protrusive activity is observed during haptotaxis on the chip. Haptotaxis is an Arp2/3-dependent lamellipodia-mediated process, and calvarial osteoblasts treated with the Arp2/3 inhibitor, CK666, show diminished haptotaxis (yFMI 0.03 ± 0.016). Together, these results demonstrate haptotaxis on an FN1 gradient as a new mechanism in the apical expansion of calvarial osteoblasts in development and shed light on the etiology of calvarial defects.

2:25 PM - 2:40 PM

Modifiable, Biomimetic Polyethylene Glycol (PEG) Hydrogel Enables Follicle Aggregation in Co-Culture of Early-Stage Ovarian Follicles

Taylor Schissel, University of Michigan

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Introduction. Prepubertal patients undergoing lifesaving yet gonadotoxic treatments have limited options for fertility preservation, especially those with bloodborne or metastatic cancers. Currently, the only available method is cryopreservation and auto-transplantation of ovarian tissue, which carries the risk of reintroducing malignant cells. In vitro culture of ovarian follicles can mitigate this problem; however, traditional materials like alginate prevent large-scale volumetric expansion and have limited functionality. In this report, we utilized a proteolytically degradable polyethylene glycol (PEG)-based hydrogel modified with bioactive, extracellular matrix (ECM) sequestering peptides. This design created a 3D system that can be locally remodeled as a follicle expands and that promotes follicle-follicle and follicle-matrix interactions. Methodology. Ovaries from 10–12-days old mice were enzymatically digested using Liberase to obtain primary stage follicles that were encapsulated in groups of ten. Hydrogels were formed by pre-reacting 5% wt 8-arm PEG-VS with ECM-sequestering (BMB) peptides followed with crosslinking with plasmin-degradable peptides. Hydrogels were cultured in 150uL of alpha-MEM based media supplemented with fetuin, insulin, transferrin, selenium, bovine serum albumin, and follicle stimulating hormone (FSH) for 12 days, with brightfield images taken and half of the media (75uL) refreshed every two days. At the end of culture, follicles were either fixed in 4% paraformaldehyde for sectioning and staining or incubated in maturation media (alpha-MEM supplemented with fetal bovine serum, epidermal growth factor, human chorionic gonadotropin, and FSH) for in vitro maturation. Results. During culture, murine follicles formed multi-follicle, organoid-like aggregates that exhibited granulosa cell proliferation and antrum formation. In vitro maturation of oocytes collected from follicle aggregates resulted in meiotic resumption to the mature meiosis II stage, and immunofluorescent staining showed intact gap junctions between granulosa cells, suggesting that follicles maintained cell-cell connections critical for successful folliculogenesis and maturation. Conclusion. Co-encapsulation of multiple murine follicles in a biomimetic hydrogel promoted folliculogenesis in vitro. Further development of this platform has the potential to

move follicle culture towards a more translatable method for fertility preservation and improved patient quality of life.

vinyl sulfone (VS); basement membrane binder peptide (BMB)

2:40 PM - 2:55 PM

In Situ Native Matrix Crosslinking Directs Local Lung Stem Cell Function and Differentiation

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Statement of Purpose: Within most tissues, the extracellular matrix serves as a dynamic signaling hub, offering mechanical and biochemical cues that guide cellular function. In particular, the deposition and crosslinking of matrix components, such as collagen proteins, play crucial roles in tissue development, regeneration, and repair[1]. Although engineered hydrogel platforms have been developed to recreate aspects of matrix crosslinking, there remains a limited understanding of how the matrix directs stem cell function within tissues like the lung, where alveolar stem cell differentiation is still unclear. To address this, we developed a matrix crosslinking technique based on photo-inducible dityrosine-bonds within ex vivo murine and human lung tissue. Using this strategy, we probe the mechanisms of matrix-induced lung stem cell function based on cytoskeletal tension and cell-matrix interactions in native tissue.

Methods: Lung tissue slices were prepared from freshly harvested human donor and mouse lungs (surfactant protein C (Sftpc)CreERT2;mTmG mice to lineage trace alveolar epithelial type 2 (AT2) stem cell progeny), vibratome sectioned, and cultured for 5 days. Local matrix crosslinking was induced upon blue light and photosensitizer-initiated dityrosine-bond formation of matrix-containing tyrosyl residues[2]. Cytoskeletal tension and cell-matrix interactions were pharmacologically modulated to analyze their roles in stem cell differentiation and matrix remodeling in response to matrix crosslinking.

Results: Matrix crosslinking led to a 2-fold increase in matrix stiffness as measured by nanoindentation. Within photo-stiffened regions, immunostaining showed an increase in AT2 cell spreading and differentiation into squamous alveolar type 1 (AT1) cells that was accompanied with an increase in fibronectin deposition. Chemically perturbing cytoskeletal tension or cell-matrix binding reduced epithelial cell differentiation and matrix deposition. However, blocking fibronectin binding sites rescued matrix crosslinking-induced stem cell differentiation, suggesting that integrin signaling regulates cell fate.

Conclusions: Our engineered ex vivo platform enables the study of epithelial crosstalk with the mesenchyme. The ability to locally crosslink native ECM holds great promise in investigating the role of matrix remodeling in epithelial differentiation. Our observations suggest that matrix deposition is integrin-dependent, and that matrix remodeling drives AT1 differentiation, highlighting the importance of matrix crosslinking in informing epithelial phenotype.

References: [1]Díaz-de-la-Loza+ C&D (2024), [2]Bjork+ Biomaterials (2011)

2:55 PM – 3:00 PM

Non-viral Co-transfection of Plasticity-inducing and β Cell Patterning Transcription Factors Mediates Pro-β Cell Reprogramming in Fibroblast Cultures

Andrew Gotschall, Natalia Areiza Mazo, Luke Lemmerman, Devleena Das, Natalia Higuita-Castro, Daniel Gallego-Perez

The Ohio State University

Introduction: Previously, we have shown that plasmid DNA that encodes transcription factors for β cell patterning (βC) (e.g., Pdx1, Ngn3, Mafa) can drive the direct reprogramming of dermal fibroblasts (DFs) into induced β cells (iβCs) with potential to become an alternative method to treat type I diabetes. Recently, our objective has been to investigate whether plasmid DNA that encodes transcription factors for skin plasticity (SP) (e.g., Tcf3, Sox9, Trp63) can increase DFs multipotency and, therefore, β cell reprogramming efficiency.

Materials and Methods: Combinations of plasmid DNA encoding for 3SP+7βC factors or pCMV6 (control plasmid DNA) were delivered to mouse DFs in vitro and in vivo using electroporation. Gene and protein expressions were analyzed using qRT-PCR or histology as appropriate.

Results: The ability of 3SP to open the chromatin landscape was significant 7 days post-electroporation. DFs transfected with 3SP+7βC transcription factors significantly increased gene expression for both insulin 1 and insulin 2 14 days post-electroporation. This increased insulin 1/2 expression correlated with a small population (~1%) of transfected DFs expressing insulin protein 14 days post-electroporation.

Conclusion: Our findings suggest that DFs transfected with plasmid DNA encoding for 3SP factors exhibit increased multipotency. Furthermore, DFs transfected with DNA encoding for 3SP+7βC factors have the potential to reprogram into iβCs. Current experimentation includes exploration of βC micro-environmental factors and other βC induction methods. Future development of this alternative cell source to treat and potentially cure type I diabetes could greatly improve quality of life for diabetic patients worldwide.

3:00 PM – 4:00 PM **INDUSTRY PANEL DISCUSSION**

4:00 PM – 4:15 PM **COFFEE BREAK**

4:15 PM – 5:30 PM **Pleanary Session 2**

Invited Speaker: Sarah Stabenfeldt, PhD, Arizona State University, *Using biomaterials systems for addressing complex neurotrauma pathology*
(LIVE from Western)

Invited Speaker: Danielle Benoit, PhD, University of Oregon, *Engineering Biomaterials for Precision Medicine in Musculoskeletal Applications*
(LIVE from Northwest)

CONCURRENT SESSION VII: DRUG DELIVERY

8:45 AM – 9:10 AM **Invited Speaker: Tom Dziubla, University of Kentucky**

9:10 AM – 9:25 AM **Invited Speaker: Dhariyat Menendez-Lustri, Case Western Reserve University**

9:25 AM - 9:40 AM

Adjuvant Particle for Increased Heart-Targeting of Gene Therapy

Lauren Switala, Case Western Reserve University

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Gene therapy is not yet clinically approved for cardiac applications because of the dangerously high dose required to reach efficacy. We have developed an adjuvant particle, “enhancer polymer (ePL),” which increases heart uptake and transduction, thereby enabling cardiac gene therapy. ePL is a cargo-less, spherical particle composed of poly (lactic-co-glycolic acid) (PLGA). Screening a series of particles resulted in the following key characteristics: a diameter of 200 nm, a viscosity of 0.6 dL/g, a 65/35 lactic-to-glycolic acid ratio, and 100% of lactic acid being the L enantiomer of the monomer. The particles are synthesized by nanoprecipitation using poly (vinyl acetate) (PVA) as a surfactant. ePL is not a component of any gene therapy particle and does not alter existing gene therapy technology; rather, it is a separate, adjuvant particle that increases heart delivery and decreases liver delivery.

ePL improves heart-targeting of adeno-associated virus (AAV). We measured the uptake of serotypes AAV1 and AAVrh74 carrying the transgene for common biomarkers with and without ePL in vivo. ePL increased heart-targeting of both serotypes, shown by PCR and Western blot (AAVrh74: 14-fold change, AAV1: 1.5-fold change). Further, ePL decreased the amount of dose delivered to the liver. This encompasses the overall effect of ePL: AAV is redirected from the liver to the heart. The mechanism by which ePL does this is by temporarily overwhelming the liver, thereby blocking its uptake of AAV. This increases the circulation half-life of AAV, allowing it to be taken up by the heart. Further, our data shows that ePL induces the release of cell-signaling factors that directly increase uptake of AAV in the heart. Overall, ePL has the potential to enable cardiac gene therapy by improving delivery and reducing harmful off-targeting.

9:40 AM - 9:55 AM

Liposomal Nanoparticle-based Drug Delivery to Overcome Fibrinolytic Resistance In Vitro

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Thrombosis is the pathology underlying several leading causes of death. The nonsurgical standard of care for thrombosis is tissue plasminogen activator (tPA), a fibrinolytic drug. Unfortunately, tPA is associated with an increased hemorrhagic risk and limited efficacy, failing to clear 40% of occlusive thrombi. Clot recalcitrance to tPA therapy has been attributed in part to neutrophil extracellular traps (NETs), protein-rich extrusions of DNA that generate in blood clots following neutrophil recruitment and activation. However, the mechanism by which NETs contribute to tPA resistance remains unexplored. Several microfluidic models for NET-rich blood clots to investigate NET-dependent tPA recalcitrance in vitro were developed. In these models, NET-rich blood clots were characterized by a core-shell structure, with NETs most tightly intercalated in the shell. Correspondingly, only the shell exhibited significant resistance to tPA therapy in fibrinolysis assays. Therapeutic concentrations of tPA took 12.3 ± 2.7 min to breach the tPA-resistant shell, which is significantly longer than the in vivo half life of tPA (5 minutes). Deoxyribonuclease I (DNAse), an enzyme that degrades NETs, restored tPA efficacy, allowing breach of the shell within 2.5 ± 1.2 min (n=3). Building on these findings, we hypothesized that localized co-delivery of tPA and DNAse could remediate clot recalcitrance while minimizing iatrogenic effects.

tPA-loaded nanoparticles (tPA-NPs) decorated with a peptide targeted to fibrin, and DNAse-loaded nanoparticles (DNAse-NPs) decorated with a peptide targeted to neutrophil elastase (NE) were engineered. A thrombin-cleavable substrate was incorporated into tPA-NPs such that they degraded upon exposure to thrombin, an enzyme upregulated in blood clots. Similarly, a NE-cleavable substrate was incorporated into DNAse-NPs such that they degraded upon exposure to NE, the primary enzymatic component of NETs.

In functional assays, DNAse-NPs alone did not induce clot lysis of fibrin- and NET-rich blood clots. tPA-NP induced lysis within 30 min, but with a prolonged lag time and a slow fibrinolysis rate. The combination of DNAse-NPs and tPA-NPs triggered complete clot dissolution within 12 min (n=4, $p<0.05$). In conclusion, the functionalized drug delivery system achieved localized co-delivery of tPA and DNAse, resulting in complete lysis of clots that are traditionally recalcitrant to traditional therapies.

9:55 AM – 10:00 AM

Copper Oxide Nanoparticles Induce DNA Damage in Endometrial Cancer Cells

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Chemotherapeutic resistance across many types of cancer is one of the greatest complications to cancer therapy and results in few treatment options for patients plagued with this disease. The vital demand for innovative anti-cancer drugs and drug delivery systems arises from these issues, revealing the importance that research on anti-cancerous compounds has on effective care for patients. Endometrial cancer is the most common gynecological cancer worldwide and is commonly treated using carboplatin, a platinum-based drug. Carboplatin resistance and unequitable access to treatment for endometrial cancer patients validates the need for a non-invasive form of endometrial cancer therapy. It is proposed that copper oxide nanoparticles can be used as an alternative to carboplatin when encapsulated in a polymeric nanoparticle for use as a drug delivery system. Copper oxide nanoparticles pose the potential as an anti-neoplastic compound due to Cu2+ ions demonstrating the ability to induce reactive oxygen species leading to double stranded DNA breaks ultimately resulting in apoptosis. Through viability assays, endometrial cell lines all showed sensitivity to copper oxide nanoparticles; however, trends were not identified across all cell lines that were tested. Using this information, Western Blot analysis was used to identify specific expression levels of apoptotic proteins, DNA repair proteins, and protein markers when DNA is damaged. Western Blots involved the treatment of cells in 24-well plates with varying concentrations of copper oxide nanoparticles for 24 hours. Gel electrophoresis separates proteins and primary antibody complexes. The differences in the expression levels of these proteins after cells are exposed to copper oxide nanoparticles can indicate the mechanism of toxicity within the different cell lines, ultimately explaining the differences in viability.

CONCURRENT SESSION VIII: CARDIOVASCULAR BIOMATERIALS

8:45 AM - 9:10 AM Invited Speaker: Dr. William Wagner, University of Pittsburgh

9:10 AM - 9:25 AM

Tunable Methacrylated Decellularized Heart Matrix: A Versatile Scaffold for Cardiac Tissue Engineering

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Decellularized heart matrix (DHM) therapy is a tissue engineering

strategy to repair cell death, reduce fibrosis, and preserve heart function that follows ischemic heart injury. Natural biomaterials such as DHM exhibit weak

mechanical integrity. To address this limitation, we designed crosslinkable DHM with tunable mechanical properties for soft lithography patterning to organize donor cells. The formulations were achieved by chemically modifying DHM with methacryloyl functional groups (DHMMA) to enable crosslinking via UV. We characterized DHMMA's degree of methacrylation, crosslinking, viscoelastic properties, and micropatterning integrity. In addition to varying the degree of functionalization, UV crosslinking time and protein concentration were altered to achieve a wide range of mechanical properties. Subsequently, the swelling ratio and protein release kinetics (active and passive) were determined for different formulations. The porosity of crosslinked DHMMA was analyzed by measuring pore diameters from SEM imaging. To assess biocompatibility, cardiomyocyte cell viability on crosslinked DHMMA was measured. We show that the stiffness and durability of DHMMA can be modulated by varying the degree of methacrylation, UV crosslinking time, and protein concentration. Furthermore, we demonstrate DHMMA utilization for soft lithography by micropatterning grooves on the surface of the crosslinked substrate. In addition, cardiomyocytes cultured on crosslinked DHMMA attach and remain viable. The current evidence demonstrates that minimum topographical features can be lowered with higher aspect ratios dependent on crosslinking density.

9:25 AM - 9:40 AM

Examining the Role of Mechanotransduction in Vascular Smooth Muscle Cell Calcification

Jessica Amaya, Mississippi State University

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Cardiovascular complications are one of the leading causes of death in patients with diabetes or kidney disease. Among these complications is vascular calcification, defined as an active process where vascular smooth muscle cells (VSMCs) will phenotypically switch to osteoblast-like cells. This results in hydroxyapatite crystal deposition into arterial tissue which leads to hypertension, atherosclerotic plaque burden, and decreased elastance. Previously our group has shown high levels of phosphate in human aortic vascular smooth muscle cells (HAVSMCs) cultured in calcification media that lead to the over-expression of osteogenic markers such as the runt-related transcription factor 2 (RUNX2) in VSMCs. This was done using an in vitro model using HAVSMCs 3 mmol inorganic phosphate to induce the calcification. Once calcified, the cells showed a decrease in α-smooth muscle actin activity and an increase in RUNX2 that shows the potential of an osteogenic switch. In this previous study, Sclerostin (a potent WNT antagonist) was used to prevent Frizzled and LRP 5/6 co-receptors from attaching to the WNT proteins and thus preventing the up-regulation of calcification. Sclerostin showed potential to regulate vascular calcification and down-regulate RUNX2. RUNX2 is directly linked to the wingless/integrated (WNT) signaling pathway that activates the regulation of bone production. Current experiments seek to examine the role of mechanotransduction in WNT activation for VSMC calcification. Using a FlexCell BioFlex tension system, we are simulating hypertension in VSMCs and identifying any biomarkers related to WNT activation and the osteogenic switch of VSMCs.

9:40 AM - 9:55 AM

Probing cellular response to dynamic curvature

Avinava Roy, University of Michigan

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Introduction: Atherosclerosis is a major risk-factor of myocardial infarction (MI), a disease that annually affects more than 500,000 people worldwide. Patients with arterial anomalies like tortuosity show higher incidences of atherosclerosis and thus higher risk for MI. Buckling of previously straight to tortuous

arteries results in new stress distributions prompting wall remodeling, increased endothelial cell (EC) proliferation, and extracellular matrix remodeling. This disrupts intercellular junctions, leading to higher vascular permeability and atherosclerotic plaque build-up. However, the cellular mechanisms linking arterial buckling to atherosclerosis remain unclear due to limited access to culture platforms that replicate arterial curvatures in vitro. To address this, we engineered a hydrogel platform with user-controlled buckling to assess EC function.

Methods: Hydrogels films (10±4kPa) were fabricated via simultaneous crosslinking of acrylamide (12wt.%) and alginate (2wt.%), attached onto silicone substrates, that were then laterally compressed to buckle. Resulting curvatures were characterized using 'tortuosity index (TI)' - the ratio of buckled to shortest length between artery endpoints. Human Umbilical Vein ECs were seeded atop flat hydrogels (0.5x106/cm2) and cultured for 3 days prior inducing curvature. EC function at day 5 was assessed by fluorescent labeling of VE-Cadherin junctions, actin cytoskeleton and YAP/TAZ with or without perturbation of cytoskeletal assembly (Y27).

Results and Discussion: Increasing stretch enabled fabrication of hydrogel curvatures mimicking low (TI~1.03) to high tortuosity (TI~1.70). Atop flat hydrogels, hUVECs formed a monolayer as with a continuous VE-Cadherin network and cortical actin. Upon inducing curvature, ECs showed increase in VE-Cadherin network heterogeneity, formation of actin stress fibers and a 30% increase in nuclear YAP/TAZ, suggesting curvature-induced EC mechanosensing. Y27 treatment of ECs on flat hydrogels induced discontinuity in VE-Cadherin and a 6% decrease in nuclear YAP/TAZ without significant changes for ECs cultured on curved hydrogels. These findings suggest that curvature strengthens the EC actin cytoskeleton.

Conclusion: Hydrogels with inducible curvatures provide a suitable platform to mimic varying degree of arterial tortuosity in vitro and holds promise to probe the mechanisms of increased stress distributions on EC function. Ongoing work is to assess changes in extracellular matrix remodeling towards studying the mechanism of curvature-induced EC dysfunction in atherosclerosis.

9:55 AM – 10:00 AM

Impact of Hyperglycemia on Vascular Smooth Muscle Cells and Implications for Type 2 Diabetes -Induced Cardiovascular Disease Treatment

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Type 2 diabetes mellitus (T2DM) is a major public health concern with significant cardiovascular complications (CVD). Despite extensive epidemiological data, the molecular mechanisms relating hyperglycemia to CVD remain incompletely understood. Since this has huge implications in tissue engineering and regenerative medicine approaches, we here investigate the impact of chronic hyperglycemia on SMC phenotype and function using human aortic smooth muscle cells (HASMCs)

cultured under varying glucose conditions in vitro, mimicking normal (5 mM/L), pre-diabetic (10 mM/L), and diabetic (20 mM/L) conditions, respectively. Patient-derived T2DM-SMCs were included for comparative analysis cultured for up to 21 days.

Results showed distinct morphological changes with significant increases in cell area, perimeter and F-actin expression in SMCs treated at higher glucose levels. Cell shape index was 0.23 ± 0.01 in SMCs exposed to 10 and 20 mM/L glucose, and 0.48 ± 0.03 for T2DM cells and normal SMCs (5 mM/L) ($p < 0.001$). AFM analysis showed significant reduction in the Young's modulus, membrane tether forces, membrane tension, and surface adhesive forces in SMCs with increasing glucose levels. In all these cases, T2DM cells exhibited levels noted in 20 mM/L glucose conditioned cells. A 5 to 6 -fold increase in cell proliferation was observed in SMCs treated with 20 mM/L glucose and in T2DM-SMCs, at day 7, compared to their original seeding density. T2DM-SMCs exhibited elevated levels of pro-inflammatory markers such as IL-6, IL-8, and MCP-1 compared to glucose-treated SMCs. Conversely, growth factors like FGF-2 and TGF-β were higher in SMCs exposed to moderate glucose but lower in T2DM-SMCs. Pathway enrichment analysis showed a significant increase in the expression of inflammatory cytokine-associated pathways, especially involving IL-10, IL-4 and IL-13 signaling in genes that are upregulated at higher glucose levels. Differentially regulated gene (DGE) analysis showed that, compared to SMCs cultured with normal glucose, 513 genes were upregulated, and 590 genes were downregulated in T2DM-SMCs; however, fewer genes (16 – 23) were differentially expressed in SMCs receiving 10 or 20 mM/L glucose. We identified the altered genes involved in ECM organization, elastic fiber synthesis and formation, and ECM proteoglycans, highlighting the role of hyperglycemia in CVD progression.

10:00 AM – 10:15 AM COFFEE BREAK

CONCURRENT SESSION IX: BIOINTERFACES & TISSUE INTERACTIONS

10:15 AM – 10:40 AM

Invited Speaker: Steve Eppell, PhD, Case Western Reserve University

10:40 AM - 10:55 AM

Cell-instructive peptide-graphene oxide conjugates for improved bone regrowth and vascularization

Michelle Wolf, Carnegie Mellon University

Bone fractures are the most frequent large organ, traumatic injury in humans, and some known as

critical size bone defects are too large to heal on their own, requiring intervention. Current research in bone regeneration favors the development of biocompatible, biodegradable scaffolds over traditional metal implants and natural bone grafts. The ideal scaffold should be mechanically similar to bone, nontoxic, cell-instructive, porous, and should slowly degrade over time to make room for native bone ingrowth. Cell instruction should encourage cell recruitment and adhesion, and should stimulate new bone tissue growth and healing of the vascular network. Graphene oxide (GO) is a promising scaffold material as it is easily and cheaply synthesized, strong, cytocompatible, osteoinductive, has a well-characterized aqueous degradation pathway, and has tailorable porosity. It is also a great substrate for functionalization with biomolecules that can aid in the bone regeneration process. Covalent chemical functionalization in particular offers sustained bioactivity. The present work discusses three different covalently-linked peptide-graphene oxide conjugate materials featuring short peptides that enhance cell adhesion, osteogenesis, and angiogenesis. Safety is established with cytocompatibility testing, and efficacy is demonstrated with in vitro assays investigating spreading of fibroblasts on the material surface, osteogenesis of mesenchymal stem cells, and angiogenesis of vascular endothelial cells. The additional bioactivity given to graphene oxide through functionalization with these peptides makes it a more promising candidate as a biodegradable bone scaffold material.

10:55 AM - 11:10 AM

Optimizing Granular Hydrogel Composites for Vasculogenic Assembly

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Background

Tissue vascularization remains a major tissue engineering challenge. While natural hydrogels are conducive to vasculogenic assembly, they are not suitable for producing permanent tissue grafts as they rapidly resorb after implantation. Synthetic hydrogels are more readily modifiable to better suit tissue

grafting, but they typically slow or inhibit vasculogenic assembly due to their nanoporous nature. Here, we explored the potential for vascular network formation in granular hydrogel composites (GHCs), a method of scaffold fabrication that incorporates microscale porosity orthogonal to hydrogel stiffness and degradability.

Methods

Dextran vinyl sulfone (DexVS) microgels (60 µm diameter) were fabricated via microfluidics and suspended with human lung microvascular endothelial cells (LMVECs) (6M/mL) in EGM2-MV or fibrinogen solution (5mg/mL) to form microporous annealed particle (MAP) or GHC constructs, respectively. Microgels were packed via centrifugation and annealed via poly(ethylene glycol) dithiol (15 mM) with/without inclusion of fibrinogen/thrombin (1 U/mL) to fill void spaces. Samples were gelled at 37 ºC, cultured with EGM2-MV supplemented with FBS, VEGF, and phorbol 12-myristate 13-acetate (PMA), and assessed by nuclear/F-actin staining and immunostaining.

Results

Our studies suggest that commonly utilized MAP hydrogels do not support vascular network assembly. LMVECs in MAP gels adopted a monolayer-like morphology, while LMVECs in GHCs formed cord-like structures comparable to those seen in pure fibrin hydrogels. This was supported by morphometric analysis of formed endothelial structures, indicating that LMVECs in MAP gels had larger average cell surface areas and thus were more spread out and monolayer-like than LMVECs in GHC and pure fibrin gel conditions. This is non-ideal as monolayer formation opposes vascular network formation. LMVECs typically adopt a monolayer-like morphology when adhering to 2D surfaces, suggesting that LMVECs in MAP gels mainly adhered to microgel surfaces. In contrast, the formation of cord-like structures in GHCs suggests the fibrin encapsulating LMVECs enabled 3D matrix interactions. These structures also appeared to be lumenized, with podocalyxin staining confirming proper apical-basal polarization of their constituent LMVECs.

Conclusions

Taken together, these studies demonstrate the potential of GHCs to support the formation of microvasculature. These findings will guide future research directions towards integrating capillary networks in GHCs with host vasculature.

11:10 AM - 11:25 AM

Magnetoactive hammocks to probe lung epithelial cell function

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Magnetoactive hammocks to probe lung epithelial cell function

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Statement of purpose: Mechanical forces provide critical biological signals to cells. Within the distal lung, tensile forces act across the basement membrane and epithelial cells atop. Stretching devices have supported studies of mechanical forces in distal lung epithelium to gain mechanistic insights into pulmonary diseases1,2. However, the integration of curvature into devices applying mechanical forces onto lung epithelial cell monolayers has remained challenging. To address this, we developed a hammock-shaped platform that offers desired curvature and mechanical forces to lung epithelial monolayers.

Methods: We developed hammocks using polyethylene terephthalate (PET)-based membranes and magnetic particle modified silicone elastomer films within a 48-well plate that mimic the alveolar curvature and tensile forces during breathing. These hammocks were engineered and characterized for mechanical and cell-adhesive properties to facilitate cell culture. Using human small airway epithelial cells (SAECs), we measured monolayer formation and mechanosensing using F-Actin staining and immunofluorescence for cytokeratin to visualize intermediate filaments.

Results: We demonstrate a multi-functional design that facilitates a range of curvatures along with the incorporation of magnetic elements for dynamic actuation to induce mechanical forces. Using this system, we then showed that SAECs remain viable, proliferate, and form an epithelial cell monolayer across the hammock. By further applying mechanical stimulation via magnetic actuation, we observed an increase in proliferation and strengthening of the cytoskeleton, suggesting an increase in mechanosensing3.

Conclusion: This hammock strategy provides an easily accessible and tunable cell culture platform for mimicking distal lung mechanical forces in vitro. We anticipate the promise of this culture platform for mechanistic studies, multi-modal stimulation, and drug or small molecule testing, extendable to other cell types and organ systems.

References: 1Tschumperlin+ Am J Cell Mol Phys (1998), 2Davidovich+ Am J Respir Cell Mol Biol (2013), 3Laly+ Sci Adv (2021)

11:25 AM – 11:30 AM

COLLASLING: EVALUATION OF A PURE-COLLAGEN STRESS URINARY INCONTINENCE SLING IN A LONG-TERM OVINE MODEL

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In previous work, we developed a composite midurethral sling (MUS) from bovine electrocompacted collagen (ELAC) thread to address stress urinary incontinence (SUI). An ovine feasibility study showed the sling was biocompatible. Currently, we have braided ELAC thread into pure-collagen, macroporous slings, CollaSlings, and implanted them in sheep. We hypothesized the CollaSling would produce a lasting, ligamentous tissue band around the midurethra. The objective was to determine the tissue response to and mechanical properties of a novel pure collagen sling in an ovine model prior to human clinical trials. CollaSlings were fabricated by braiding ELAC threads into a 12mm wide ribbon, segmenting into 50cm sections, and crosslinking with genipin. Ten adult Dorset ewes were implanted with the CollaSling using a standard retropubic approach. Ewes were sacrificed at 3 months (n=3), 6 months (n=3), 9 months (n=4). Lengths of CollaSling passing through the space of Retzius were mechanically tested for ultimate tensile strength (UTS), strain, and modulus. Histology sections were taken in the midurethral and muscle regions and scored according to ISO10993-6. No urinary retention or other adverse effects were observed. Grossly, the CollaSling integrated with the surrounding tissues at harvest with no fibrous encapsulation at any site. The sling formed a ligament that was distinct and palpable through the vagina, which felt supple at harvest. Histologically, there was no acute inflammatory response. There was minimal-mild dense fibrosis with low clusters of infiltrating lymphocytes, plasma cells, macrophages, and multi-nucleate giant cells, and evidence of neovascularization for all time points, and a trend towards slightly greater fibrosis present within the skeletal muscle site as compared to the midurethral site across all time points. Explants were significantly stronger at all time points compared to hydrated, naïve CollaSling due to tissue ingrowth

and de novo collagen deposition. Strain and modulus were consistent over time, with values similar to native tissue. CollaSling is a pure collagen MUS deployable using a standard retropubic procedure. The sling is biocompatible and promotes new fibrous tissue deposition. Mechanical properties indicate that the sling can support the midurethra. Clinical studies will be run to determine efficacy in treating SUI.

CONCURRENT SESSION X: TISSUE ENGINEERING

10:15 AM - 10:40 AM Invited Speaker: Sam Senyo, Case Western Reserve University

10:40 AM - 10:55 AM

Photo-Click Thiol-Norbornene Hydrogels for Sacrificial Bioprinting

Jonathan Bryan, Weldon School of Biomedical Engineering, Purdue University

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Purpose:

3D bioprinting is an emerging technique to fabricate physiologically relevant tissue constructs for disease modeling and regenerative medicine applications. One area of 3D bioprinting is to create dissolvable templates for fabricating hollow structures (e.g., blood vessels, voids and lumen structures, etc.). These methods utilize sacrificial bioinks which support surrounding features during fabrication and are subsequently removed. Alginate and gelatin are the most commonly used sacrificial bioinks. However, the dissolution of these bioinks requires enzymatic reactions or metal ion chelator. Here, we report the development of a novel photocrosslinkable and rapidly hydrolyzable bioink - Poly(ethylene glycol)-Norbornene-Tyramine (PEGNB-T). PEGNB-T can be readily crosslinked into hydrogels with different stiffness and shapes via thiol-norbornene photocrosslinking. The crosslinked hydrogels are readily dissolved in aqueous solution via rapid but tunable hydrolysis.

Materials and Methods:

PEGNB-T was synthesized from 8-arm PEGOH (20kDa) via a two-step process. First, PEGOH was reacted with carbic anhydride to produce PEGNBCA. Tyramine was then conjugated to the additional carboxylic acid moiety through standard carbodiimide chemistry. PEGNB-T and dithiothreitol (DTT) were photocrosslinked into hydrogels using visible light (405 nm) based digital light processing (DLP) bioprinter with lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as the photoinitiator.

Results and Discussion:

PEGNB-T hydrogels showed tunable stiffness but equally rapid degradation time. For example, hydrogels printed with initial G' of 50-7000 Pa showed similar degradation profiles and almost identical degradation time. Gels with G' >1000 Pa degraded completely in just over 5 hours at room temperature and in less than 2 hours at 37 ℃. Gels made with a mixture of PEGNB-T and PEGNBCA showed delayed degradation (days). The printed PEGNB-T hydrogels could serve as a negative mold with complex shapes for non-printable materials, creating channels and networks after rapid hydrolysis of the printed PEGNB-T hydrogels. Current work focuses on the optimization of casting and embedding for fabrication of cell culture models.

10:55 AM - 11:10 AM

Soft Matter-based Engineered High-yield Organoid Culture Platform for High-content High-throughput Drug Screening

Hyunsu Jeon, University of Notre Dame

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The evolving drug discovery landscape requires efficient methods for evaluating candidate drugs, especially in high-content and high-throughput screening (HCHTS). Traditional HCHTS based on 2D cell culture may face challenges in faithfully reproducing in-vivo conditions, leading to limited accuracy in predicting therapeutic and side effects. Here, we introduce a hydrogel-based scaffold with an inverted colloidal crystal (ICC) geometry designed for uniform and high-yield organoid culture. This scaffold is fabricated from an assembly of uniform alginate microgels within an agarose-based bulk backbone, exhibiting a hexagonal crystalline packing (HCP) structure with consistent void spaces (e.g., ~250 μm in diameter) and interconnecting channels (e.g., <50 μm). The ICC scaffold, a versatile platform for high-yield organoid culture, achieves remarkable yields of over 3,000 organoids per well in a standard 96-well plate and facilitates efficient transport of suspension cells, nutrients, waste, and drugs through interconnected channels, ensuring cost-effectiveness and time-saving. With high biocompatibility, customizable features, and the ability to replicate complex cell-to-cell interactions, this platform is well-suited for creating HCHTS models mimicking tissue and disease in vitro. Moreover, the unique HCP-arranged geometry and the transparency of the hydrogel materials seamlessly integrate with in-situ imaging and automated data processing, significantly enhancing our understanding of drug therapeutics through high-content assessments (e.g., >100 organoids·analysis-1; >300 times faster), thereby accelerating therapeutic discovery and drug development. Eventually, this biomimetic ICC scaffold will represent a significant advancement in 3D cell culture as a high-yield organoid culture platform, offering enhanced biological relevance in tissue engineering, disease modeling, drug development, and biomedical research.

11:10 AM - 11:25 AM

Biofunctionalized, Blend-Electrospun Scaffolds Direct Biochemical Signaling to Promote Nerve Repair

Andrew Bryan, University of Cincinnati

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Introduction: While current intervention strategies for traumatic peripheral nerve injuries involve direct repairs, nerve grafts, or nerve transfers, these surgeries are only effective in limited circumstances. To overcome this hurdle, conduits meant to guide damaged axons across injury gaps are being lab-manufactured with customizable properties; however, no single design has managed to possess the physical, electrical, and biochemical signaling capabilities needed to promote a full functional recovery. To mitigate these inadequacies, this study formulates a piezoelectric biomaterial with a structure capable

of directing cell growth while possessing endogenous electrical signaling and the ability to biochemically communicate with its environment.

Methods: PVDF-TrFE scaffolds were constructed and biofunctionalized from a polymer solution containing PVDF-TrFE powder dissolved in DMF-acetone incorporated with cell-derived extracellular matrix that had been decellularized (dECM). Prior to electrospinning, dECM was lyophilized, digested, and mixed into the precursor solution to form an entirely homogeneous mixture. Four total scaffold groups each containing different w:v of dECM to PVDF-TrFE electrospun: 0%, 0.2%, 0.3%, and 0.4% dECM. Scaffolds were characterized with scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) to confirm dECM presence.

Results/Discussion: Primary Schwann cells cultured on biofunctionalized scaffolds were assayed to measure metabolic activity. At 3 h, scaffolds containing 0.3% and 0.4% dECM presented higher activity rates than 0% and 0.2% scaffolds ($p < 0.05$), suggesting greater dECM content promotes metabolic activity. To examine morphological and growth differences, primary Schwann cells were visualized for F-actin at 3 h and 24 h. At 3 h, cells on 0% and 0.4% dECM scaffolds displayed elongated and aligned cell shapes compared to 0.2% and 0.3% scaffolds, which possessed fanned cell morphologies and less aligned growth patterns ($p < 0.05$). Cells on 0% and 0.4% scaffolds maintained highly aligned structures at 24 h, 0.4% presented with greater alignment. Contrary to phenotypes exhibited at 3 h, cells on 0.2% and 0.3% scaffolds showed a high degree of alignment at 24 h; however, statistical differences between these intermediary scaffolds and 0.4% dECM suggests scaffolds with the most bioactive content can efficiently direct cell behavior in a manner that promotes cell alignment in nerve regeneration.

11:25 AM – 11:30 AM

Vasculogenic cell therapies drive improved memory and reduced neuropathological burden in a mouse model of Alzheimer's Disease

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Numerous studies indicate a strong correlation between cerebrovascular impairment and the development of Alzheimer's Disease (AD). These cerebrovascular alterations precede the formation of amyloid plaques, tangles, and the onset of cognitive decline, suggesting that impaired cerebrovascular function plays a key role in the onset and/or progression of AD neuropathology. In consequence, therapeutic interventions aiming to restore cerebrovascular function constitute crucial strategies to attenuate the progression of the disease. Cell-based therapies constitute promising strategies to address vascular deficiencies in the brain. To implement safe and efficient cell therapies in AD, we used electroporation, to deliver 3 pro-vasculogenic transcription factors to reprogram mouse primary embryonic fibroblasts (pMEFs) into induced endothelial cells (iECs). To evaluate their therapeutic potential, pMEFs pre-labeled with 5-bromo-2'-deoxyuridine (BrdU) and transfected with Etv2, Foxc2, Fli1 (EFF) or a control empty plasmid were delivered with 3 intracranial injections into the lateral ventricles (LV) of females from the triple transgenic murine model of AD (3xTg-AD) or Wild-Type Controls. Within each cage mice with the same genotype were randomly assigned to either vasculogenic or control cell injections. Two weeks after the last injection spatial memory was analyzed with the Barnes Maze. Subsequently, brain tissue was processed for immunostaining and biochemical analysis. Our results indicate that pMEFs pre-programmed into vasculogenic iECs and delivered to the LVs induce an increase in global cerebral blood flow (CBF) as early as 7 days post-injection. Vasculogenic cells also lead to a reduction of spatial memory deficits in the 3xTg-AD mice. Histological analysis shows that the injected cells were able to migrate to multiple brain regions and survive for at least 4 weeks in close contact with brain blood vessels. Notably, animals injected with vasculogenic cells show an increase in the total vascular area in the cortex and a reduced amyloid-beta load. Together our results indicate that the development of electroporation-based cell therapies by direct reprogramming constitutes a promising approach to treat AD and AD-related dementias.

11:30 AM – 12:45 PM Plenary Session 3

Invited Speaker: Elazer Edelman, MD, PhD, Massachusetts Institute of Technology - *MATERIALS, MEDICINE, HEALTH: Integrated Co-Development and Impact*, SFB 2024 Founders Award Recipient
(LIVE from Northeast)

Invited Speaker: Cynthia Reinhart-King, PhD, Rice University
(LIVE from Southwest)

12:45 PM – 1:45 PM LUNCH

CONCURRENT SESSION XI: ENGINEERING CELLS AND THEIR MICROENVIRONMENTS

1:45 PM - 2:10 PM **Invited Speaker: John Martin, PhD, University of Cincinnati**

2:10 PM - 2:25 PM

Hyaluronic Acid-Based Hydrogel Platform to Explore Ovarian Follicle-Extracellular Matrix Spatio-temporal Interactions

Emily Thomas, University of Michigan

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Purpose: Work towards the in vitro culture of ovarian follicles aims to expand fertility preservation options. A key consideration for developing biomaterials for follicle culture is to recreate the extracellular matrix (ECM) in vitro which can provide key biological and structural cues [1]. Materials can be modified with biomimetic peptides to sequester ECM allowing the follicle to recreate its own microenvironment. Here we show the development of a hyaluronic acid-vinyl sulfone (HA-VS) culture platform which provides increased capacity for modification with ECM sequestering peptides than existing materials used in follicle culture which may permit more intensive investigation of the role of nascent ECM in follicle development.

Methods: HA-VS was synthesized by dissolving 88 kDa sodium hyaluronate in 0.1 M NaOH and adding divinyl sulfone in excess at a molar ratio of 1.25:1. The reaction was quenched with 6 M HCl, and the product was dialyzed and lyophilized. Secondary ovarian follicles were harvested from D12 C57Bl6/CBA F1 mice. Isolated follicles were encapsulated in 2 wt% unmodified HA-VS hydrogels were used to culture secondary follicles for 10 days (n=36).

Results: NMR analysis of the HA-VS confirmed that 68% of the hyaluronic acid disaccharide repeat units were successfully functionalized with VS offering an over 5-fold increase of reactive easily modifiable functional groups over polyethylene glycol-vinyl sulfone hydrogels previously used for follicle culture with ECM sequestering peptides [2]. Follicles cultured in HA-VS increased in size by 46% on average growing from 137.0 ± 4.4 μm to 200.9 ± 79.4 μm. Follicle antrum formation was observed indicating that the material was conducive to follicle maturation. Future work will investigate HA-VS modification and characterize ECM retention across folliculogenesis.

2:25 PM - 2:40 PM

A Bioprintable Model of Glioblastoma for Dissecting Cellular Mechanisms of Tumor Invasion and Drug Resistance

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Glioblastoma multiforme (GBM), the most lethal form of primary brain cancer, employs various receptors to infiltrate the human brain and establish biological niches and resist chemotherapy. Despite the significance of CD44 and integrins in tumor invasion and drug resistance, prevailing in vitro GBM studies

predominantly employ suspended tumor spheres or tumoroids without any scaffold. While this approach aids cancer stem cell maintenance, it constrains our comprehension of the extracellular matrix's role in tumorigenesis and the essential biochemical and biomechanical cues governing tumor progression and treatment resistance. Our study introduces a novel design that incorporates a bioprintable hydrogel consisting of Gelatin and hyaluronic acid. This carefully tailored composition enables the growth of cancer spheroids either in isolation or in co-culture with other cell types, including brain endothelial cells. Furthermore, the viscosity of the hydrogel has been optimized to maintain the spheroids well segregated during the bioprinting process, ensuring accurate positioning within the constructs. The hydrogel can be easily mixed with 3D spheroids and crosslinked through a brief UV-light treatment. The presented biofabrication approach seamlessly integrates with a high-throughput imaging-based approach, providing spatial insights into cancer progression in response to drug treatment. Our innovative hydrogel design offers a versatile and customizable platform with applications in cancer research and tissue engineering. Our results showcase the effectiveness of our bioprinting approach in preserving GBM spheroids without damage and generating complex microtissues including different ECM components and cell types including brain endothelial cells and astrocytes. Microscopic characterization of our biofabricated model reveals the retention of cancer stem cell markers within this 3D structure, a phenomenon typically lost in traditional Collagen I-based scaffolds. This advancement holds promise for unraveling intricate mechanisms governing GBM behavior, providing a more physiologically relevant platform for drug testing and furthering our understanding of tumor biology.

2:40 PM - 2:55 PM

Cargo-less Nano-Immunometabolic Therapy for Chronic Cardiac Fibrosis in Heart Failure

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Cargo-less nano-immunometabolic modulators (NIMMs) to target splenic macrophages were developed and optimized to relieve chronic heart fibrosis utilizing high throughput screening. 144 formulations of poly(L-lactide-co-glycolide) (PLLGA)-based NIMMs were synthesized by nanoprecipitation, oil-in-water emulsion, and emulsion-diffusion with various PLGA polymer molecular weights, L-to-G ratios, and choices

of surfactants. In vitro macrophage uptake and arginase upregulation was examined and the lead formulation, NIMM22, was identified. A multiple linear regression model was established, using the screening data, which indicated a statistically significant parameter to improve macrophage uptake and arginase upregulation was the use of “eat-me” signal phospholipids as surfactants.

Pathological heart fibrosis, as a major cause of heart failure, results from infiltration of hyper-activated T cells due to unresolved inflammation. Yet there is no therapeutic strategy targeting this immunological pathway. NIMM22 which upregulates arginase in macrophages can be therapeutically beneficial because it’s linked to T cell suppression via arginine starvation. Primary murine macrophages received treatment of the leading NIMM22 formulation (diameter 102.8±33.9 nm), showed a dose-dependent T cell suppression in vitro. This effect was lost when the arginase inhibitor was given to macrophages synergistically with NIMM22. Intravenous administration of NIMM22 to mice showed at least 3.6-fold higher accumulation in the spleen, which is the key organ driving chronic heart fibrosis and source of T cell infiltration. Furthermore, NIMM22 was tested in various mouse heart injury models. NIMM22 restored the levels of splenic effector and regulatory T cells in injured mice to non-injured levels, while reducing cardiac fibrosis and restoring ejection fraction.

Collectively, this study presents NIMM22, a PLLGA-based nanoparticle formulation selected through high throughput screening and statistical modeling. NIMM22 targets splenic macrophages, reduces heart fibrosis and restores cardiac function, addressing the gap of fibrosis treatment aiming at upstream immunological pathways.

CONCURRENT SESSION XII: NANOMATERIALS & BIOMATERIALS COMMERCIALIZATION

1:45 PM - 2:10 PM **Invited Speaker: Anirban Sen Gupta, Case Western Reserve University**

2:10 PM - 2:25 PM

Altering the Biochemical Properties of Medical Gauze Utilized in Vaginal Seeding in order to Optimize Newborn Microbiome Restoration

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Introduction: A c-section delivered newborn bypasses the vaginal canal and receives minimal exposure to maternal bacteria, predominately lactobacillus. This is associated with an increased risk for infections, allergic disorders, and childhood obesity in newborns. Vaginal seeding, a technique utilizing sterile gauze to extract vaginal fluid for maternal bacterial transfer to their newborn, can partially restore a newborn's microbiome. We suspect that altering the gauze's chemical properties could enhance bacterial transference and microbiome restoration. Given lactobacillus' preference for healthy acidic vaginal pH and biofilm affinity, we hypothesize that acidic and hydrophilic conditions will yield the highest transference.

Methods: Five conditions were represented by crosslinked gauze in the following solutions: ionic, hydrophobic, hydrophilic, acidic, and basic. Each condition was then inoculated with lactobacillus for 0, 2, and 6 hours and transferred to another agar plate for 24 hour growth. Relative transference percentage was calculated to determine which conditions demonstrated optimal transference.

Results: After 0 and 2 hours of incubation, hydrophilic (61.9%, 11.4%), ionic (59.4%, 12.8%), and basic (41.2%, 7.4%) conditions showed enhanced lactobacillus transference compared to control gauze. Meanwhile, acidic (38.5%, -53.3%) treated gauze showed enhanced transference at 0 hours but negative transference at 2 hours. All other conditions showed negative transference.

Conclusion: These findings suggest that modifying gauze properties holds promise for improving bacterial transfer. Particularly, hydrophilic, basic, and ionic conditions showed the greatest bacterial transference, aligning with existing literature. It is hypothesized that charge and hydrophilicity play a factor in bacterial adherence due to the polar nature of the bacterial cell surface, which would result in attractive forces. Though lactobacillus thrives in acidic conditions, our results showed lower than expected bacterial transference in acidic conditions. This may be due to the chemotoxicity of lower than vital pH levels in this experiment's acidic condition as a pH meter miscalibration was discovered mid-experiment. Considering

that a healthy vaginal canal has an approximate pH of 4, the proposed material may show optimal results in a more weakly acidic environment. To augment the confidence of these results, testing other bacterial species' adherence and other specific conditions is warranted.

2:25 PM - 2:40 PM

Development of a printable gel polymer electrolyte (GPE) for use in electrolyte-assisted electrospinning (ELES)

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This study combines gap electrospinning and electrolyte-assisted electrospinning techniques to develop a method for creating nanofiber membranes with controlled size and shape. The method utilizes syringe extrusion printing of a gel polymer electrolyte (GPE) solution to accurately focus electrospun nanofibers (ESNF). The printable GPE ink is formulated to ensure it possesses the necessary conductivity, shear-thinning, and thixotropic properties, in addition to being biocompatible for potential applications involving human cells.

We have developed a gelatin-based GPE ink, enhanced with Laponite to improve shear-thinning properties and salts to increase conductivity. The ink's rheology and conductivity were thoroughly examined, with the latter measured using a custom developed four-point probe system for gels. These properties support the ink's use in precise ESNF membrane patterning with specific geometries on dielectric surfaces.

A key part of our research is the assessment of the ink's biocompatibility, especially when in contact with human cells, using the Cell Counting Kit-8 (CCK-8) assay. This evaluation is crucial for confirming the material's suitability for biomedical applications. The CCK-8 assay results indicate that the GPE ink is compatible with cell viability, underscoring its potential use in areas such as regenerative medicine and tissue engineering.

In summary, our work introduces a novel approach for the controlled preparation of ESNF membranes, supported by the development of a specialized GPE ink. The study's findings, particularly regarding biocompatibility, suggest that the ink could be a valuable material for biomedical engineering applications, although further research is needed to explore its full potential.

2:40 PM - 2:55 PM

SanguiStop: Intravenous Nanomedicine for Injury-Targeted Direct Delivery of Thrombin for Hemorrhage Control.

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Background: Traumatic hemorrhage and coagulopathies remain a primary cause of preventable mortality in the battlefield and civilian settings. Hemostatic resuscitation early on can save lives. To this end, robust efforts are being focused on improving the prehospital availability of blood products. Additionally, research is ongoing to develop intravenous biosynthetic therapeutics that are donor-independent and can provide prehospital hemostatic resuscitation capabilities when blood products are of limited availability. In this framework, we are developing a liposomal nanotherapeutic technology, SanguiStop, that can localize at the injury site via specific platelet-mimetic biomolecular interactions and deliver thrombin to rapidly generate fibrin for hemostasis.

Methods: Platelets rapidly anchor to the bleeding injury site, via simultaneous binding to von Willebrand Factor (vWF) and collagen. Mimicking this on a liposomal nanoparticle (LNP) via heteromultivalent surface-decoration with collagen-binding peptide and vWF-binding peptide, enabled active anchorage of the particles to injury-simulating surfaces, as confirmed by fluorescence-assisted microfluidic studies. Payload encapsulation and its release triggered by injury site-specific enzyme phospholipase A2 was first studied using a model payload 5(6)-Carboxyfluorescein (CF). Subsequently, thrombin was used as a payload and enzyme-triggered release of thrombin was evaluated using ELISA, and fibrin generation assay in high shear microfluidics to mimic the traumatic injury site.

Results: SanguiStop LNPs exhibited vesicular morphology in electron microscopy studies. Microfluidic studies confirmed that the dual peptide-decorated LNPs had significantly enhanced binding to injury-simulating surfaces compared to single peptide-decorated particles. CF-loaded LNPs showed significantly enhanced release of the payload triggered by the enzyme, compared to diffusive release. Thrombin loading and release from these LNPs improved fibrin kinetics in coagulopathy-relevant platelet-depleted and platelet-dysfunctional plasma.

Conclusions: SanguiStop is a liposomal system that binds to the injury site via platelet-mimetic mechanisms and delivers thrombin directly to generate fibrin. In trauma-associated coagulopathies like platelet defects and hypocoagulation, this system can potentially restore fibrin status for hemostasis. Future studies will focus on evaluating the optimized SanguiStop system in vivo in appropriate models of trauma.

POSTERS

Poster Number: 1

Delivery of nucleic acids using engineer extracellular vesicles as a therapeutic strategy for neurofibromatosis type 1.

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Neurofibromatosis type 1 (NF1) is a genetic disorder caused by mutations in the NF1 gene, which leads to the loss of function of the neurofibromin protein. The loss of function of Neurofibromin results in the over-activation of RAS protein, leading to uncontrolled cellular proliferation and tumor development. Patients with Neurofibromatosis have a higher incidence of developing malignant peripheral nerve sheath tumors (MPNST), which are aggressive and invasive cancers with a low rate of survival. Gene therapies for NF1 using viral delivery systems are limited due to the size of the NF1 gene. To address this limitation, our research focuses on the use of engineered EVs extracellular vesicles (eEVs) as non-viral carriers for delivering NF1 cargo (such as mRNA and NF1 plasmid) to restore neurofibromin protein function. This approach holds promise as a novel therapeutic strategy for treating Neurofibromatosis type 1.

Isolated human dermal fibroblast cells (HDFs) were transfected with a plasmid encoding for human NF1. Then eEVs were isolated from the culture media using size exclusion chromatography. Packing of the NF1 genetic material was confirmed using RT-PCR, western blot, and conventional PCRs. The therapeutic effect of NF1 loaded eEVs was assessed in vitro using malignant tumor cells isolated from NF1 patients, and in vivo using a NF1 patient-derived xenografts (PDX) model.

EVs characterization showed effective loading of plasmid and mRNA content of NF1 into the eEVs. NF1 eEVs were effectively captured and incorporated by the malignant tumor patient cells after eEVs exposure. RT-PCRs confirmed that the recipient cells were successfully transfected with the NF1 cargo and showed sustained overexpression of NF1. Additionally, treated cells showed a reduction in phosphorylation and proliferation pathways. In vivo experiments showed a significant reduction of tumor volume and proliferation over time for mice treated with NF1-loaded eEVs.

Overall, these preliminary findings showed the potential of NF1 loaded eEVs as a gene therapy strategy for the rescue of neurofibromin protein function, which can alleviate the burden of neurofibromatosis type 1 disorder and other types of cancer associated with NF1 mutations, such as glioblastoma and melanoma.

Poster Number: 2

Development of Structurally-Diverse Aromatic Peptides for Overdose Treatment

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Synthetic peptides have garnered significant research interest due to their potential in biotechnology and medicine. These materials have versatile applications in bioimaging, tissue engineering, and antimicrobials due to the chemical diversity of amino acid residues and their ability to adopt secondary structures. Despite also being employed in a variety of drug delivery applications, there have been no reports that evaluate the use of synthetic peptides as adsorbent materials. Traditionally, activated charcoal has been administered as an adsorbent to achieve gastrointestinal decontamination following drug overdose. Although it is effective, activated charcoal is associated with several post-treatment complications such as lung edema formation and pulmonary compromise resulting from aspiration of the material during administration. Recent studies reveal that peptide adsorbents are a biocompatible alternative which may circumvent the drawbacks of treatment using activated charcoal. This work reports the preparation, characterization, kinetics, adsorption capacity, and biocompatibility studies regarding several novel classes of structurally diverse aromatic peptides. In coordination with the Division Chief of Medical Toxicology at UT Southwestern, several compounds of clinical relevance were selected to test against the synthetic peptide adsorbents. Adsorption capacities were rationalized based on physical and chemical structures of the adsorbent and adsorbate to identify important features to optimize during peptide synthesis. These investigations provide evidence that synthetic peptides can adsorb high concentrations of small organic molecules in vitro. Further, data shows that these peptide materials adsorb compounds as rapidly as activated charcoal--with comparable or higher adsorption capacity--in a pH-dependent manner. While these compounds hold promise in the context of drug adsorption, comprehensive pharmacokinetic profiling is still necessary to understand the viability of synthetic peptides in mainstream medicine.

Poster Number: 3

Investigation of Polyproline II Peptides as Antifouling Biomaterials

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Biomaterials derived from polyproline (PPII) peptide sequences offer significant promise due to their diverse applications, including as antifouling agents for biomaterials and as anchors that encourage high density of other peptide sequences. However, the precise mechanisms underlying their antifouling behavior is not fully understood. This study comprehensively investigates antifouling properties of PPII peptides using bovine serum albumin (BSA) as a model foulant while varying guest amino acids within the PPII sequence. By varying the sequence and controlling their adsoprtion, aim to investigate multiple properties, including secondary structure, hydrophobicity, rate constant of rearrangement, and surface coverage, to elucidate their impact on these on antifouling mechanisms. Our findings indicate that surface coverage and incorporation of proline in the sequence has a significant positive impact on antifouling properties. Moreover, this research marks the first exploration of human mesenchymal cells (hMSCs) as foulants on PPII-functionalized surfaces, revealing successful minimization of cell adhesion that correlates with the model foulant results. In conclusion, our research underscores the potential of PPII design and application as an antifouling biomaterial, paving the way for future advancements in this field including use of these sequences as way to control hMSC spreading and attachment.

Poster Number: 4

Engineering Synthetic Cartilages: Harnessing Bioactive Polymers for Improved Repair

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Osteoarthritis affects approximately 32.5 million Americans and significantly reduces mobility and quality of life due to severe joint pain. This condition, caused by the degradation of articular cartilage often due to repetitive movements, is exacerbated by the limited ability of cartilage to regenerate. Current treatments involve invasive procedures with extended recovery times or the use of injectable hydrogels, which also require considerable healing periods.

In this study, we propose an approach involving the development of injectable hydrogels that conform to the shape of the joint and mimic the properties of cartilage providing a matrix for cartilage regeneration. We investigate a combination of chondroitin sulfate A and collagen mimetic peptides which mimic the natural composition of cartilage, to create hydrogels with mechanical properties like cartilage. By tuning the crosslinking mechanism and weight percentages of chondroitin sulfate and collagen mimicking peptides, we can tailor the hydrogels and assess their ability to bear loads and withstand stretching.

The successful identification of hydrogels with mechanical properties comparable to articular cartilage could transform the treatment of osteoarthritis, providing a minimally invasive and more effective alternative.

Poster Number: 6

Biomaterial-Mediated Polyamine Delivery: A Novel Approach for Modulating Immunometabolism in Alzheimer's Disease Pathogenesis

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Alzheimer's disease (AD), characterized by amyloid beta (Aβ) accumulation and neuropsychiatric symptoms, is the leading cause of late-onset dementia affecting a large population of our aging society. Polyamines (e.g., spermine, spermidine, putrescine) delivered via biomaterials can play a significant role in AD pathogenesis by modulating neuro immune metabolism. Specifically, biomaterial-facilitated polyamine delivery acts as a substrate for hypusination of a lysine residue in eukaryotic initiation factor 5A (eIF5A). Since eIF5A2 in the brain regulates microglial cell metabolism, delivering polyamines through biomaterials may initiate anti-inflammatory response. Triggering receptor expressed on myeloid cells-2 (TREM2) is an innate immune receptor highly expressed on microglia, essential for mediating the phagocytic clearance of neuronal debris. However, delivering polyamines to the brain poses challenges. Therefore, the aim of this project is to develop biomaterials capable of delivering immunometabolism-modulating polyamines near the brain (using blood-brain-barrier (BBB) non-permeable technology) to mitigate neuroinflammation in AD.

Our hypothesis suggests that sustained local delivery of spermine or spermidine (with receptors at the BBB) via biomaterials will regulate mitochondrial function, the TCA cycle, and mitochondrial DNA (mtDNA) homeostasis in the brain, thereby ameliorating mitochondrial deficiencies, neuroinflammation, and neurodegenerative AD phenotypes.

Poster Number: 7

WITHDRAWN

Poster Number: 8

Application of electrochemistry to detect the transition behavior of elastin-like polypeptides attached to a gold surface

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Elastin-like polypeptides (ELPs) represent a fascinating area of study due to their soluble-to-insoluble transition in response to various stimuli, including ionic strength, temperature, pH, and concentration. This transition involves conformational changes from extended to collapsed states. Tailoring ELP hydrophobicity through sequence adjustments allows precise modulation of this transition, making them valuable for diverse applications such as switchable interfaces, tissue engineering, cell culture, and drug delivery. While the transition behavior of amino acids is well-characterized in solution, our understanding of surface-tethered ELPs remains limited, impeding crucial surface-based applications in sensing, cell sheet engineering, and targeted drug release.

In this study, we utilized novel electrochemical approaches to characterize the transition behavior of surface-bound ELPs and investigated the effect of different stimuli, including salt concentration and temperature. By comparing these surface-based behaviors with solution-phase counterparts, we aim to provide insights into ELP behavior on solid surfaces. Our results demonstrated electrochemistry as a reliable technique for quantifying the surface-bound transition, offering a pathway for developing predictive models and enhancing understanding of surface-based elastin biotechnology.

Poster Number: 9

Non-viral Co-transfection of Plasticity-inducing and β Cell Patterning Transcription Factors Mediates Pro-β Cell Reprogramming in Fibroblast Cultures

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Introduction: Previously, we have shown that plasmid DNA that encodes transcription factors for β cell patterning (βC) (e.g., Pdx1, Ngn3, Mafa) can drive the direct reprogramming of dermal fibroblasts (DFs) into induced β cells (iβCs) with potential to become an alternative method to treat type I diabetes. Recently, our objective has been to investigate whether plasmid DNA that encodes transcription factors for skin plasticity (SP) (e.g., Tcf3, Sox9, Trp63) can increase DFs multipotency and, therefore, β cell reprogramming efficiency.

Materials and Methods: Combinations of plasmid DNA encoding for 3SP+7βC factors or pCMV6 (control plasmid DNA) were delivered to mouse DFs in vitro and in vivo using electroporation. Gene and protein expressions were analyzed using qRT-PCR or histology as appropriate.

Results: The ability of 3SP to open the chromatin landscape was significant 7 days post-electroporation. DFs transfected with 3SP+7βC transcription factors significantly increased gene expression for both insulin 1 and insulin 2 14 days post-electroporation. This increased insulin 1/2 expression correlated with a small population (~1%) of transfected DFs expressing insulin protein 14 days post-electroporation.

Conclusion: Our findings suggest that DFs transfected with plasmid DNA encoding for 3SP factors exhibit increased multipotency. Furthermore, DFs transfected with DNA encoding for 3SP+7βC factors have the potential to reprogram into iβCs. Current experimentation includes exploration of βC micro-environmental factors and other βC induction methods. Future development of this alternative cell source to treat and potentially cure type I diabetes could greatly improve quality of life for diabetic patients worldwide.

Poster Number: 10

Electrosprayed Polymerized Cyclodextrin for Extended Release of Minocycline to Prevent Implant Associated Infections

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Health-care associated infections (HAIs) in the United States cost $28.4 to $33.8 billion a year and 25.6% of HAIs come from the use of medical devices. There is a need for a strategy to decrease infection rates to prevent further surgeries in patients. An electrosprayed polymerized cyclodextrin (pCD) coating for stainless steel presents a new way to prevent implant associated infections. The fabrication of spherical droplets of pCD was shown to be successful in multiple different formulations through SEM analysis and ATR-FTIR confirmed the success of crosslinking. However, the drug loading and release study did not have the expected results. Through further analysis it was shown that the coating was not strongly adhered to the stainless steel (SS). Therefore, future work is proposed on surface modification and functionalization to create a stronger bond between the polymer coating and the SS surface.

Poster Number: 11

Vasculogenic cell therapies drive improved memory and reduced neuropathological burden in a mouse model of Alzheimer's Disease

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Numerous studies indicate a strong correlation between cerebrovascular impairment and the development of Alzheimer's Disease (AD). These cerebrovascular alterations precede the formation of amyloid plaques, tangles, and the onset of cognitive decline, suggesting that impaired cerebrovascular function plays a key role in the onset and/or progression of AD neuropathology. In consequence, therapeutic interventions aiming to restore cerebrovascular function constitute crucial strategies to attenuate the progression of the disease. Cell-based therapies constitute promising strategies to address vascular deficiencies in the brain. To implement safe and efficient cell therapies in AD, we used electroporation, to deliver 3 pro-vasculogenic transcription factors to reprogram mouse primary embryonic fibroblasts (pMEFs) into induced endothelial cells (iECs). To evaluate their therapeutic potential, pMEFs pre-labeled with 5-bromo-2'-deoxyuridine (BrdU) and transfected with Etv2, Foxc2, Fli1 (EFF) or a control empty plasmid were delivered with 3 intracranial injections into the lateral ventricles (LV) of females from the triple transgenic murine model of AD (3xTg-AD) or Wild-Type Controls. Within each cage mice with the same genotype were randomly assigned to either vasculogenic or control cell injections. Two weeks after the last injection spatial memory was analyzed with the Barnes Maze. Subsequently, brain tissue was processed for immunostaining and biochemical analysis. Our results indicate that pMEFs pre-programmed into vasculogenic iECs and delivered to the LVs induce an increase in global cerebral blood flow (CBF) as early as 7 days post-injection. Vasculogenic cells also lead to a reduction of spatial memory deficits in the 3xTg-AD mice. Histological analysis shows that the injected cells were able to migrate to multiple brain regions and survive for at least 4 weeks in close contact with brain blood vessels. Notably, animals injected with vasculogenic cells show an increase in the total vascular area in the cortex and a reduced amyloid-beta load. Together our results indicate that the development of electroporation-based cell therapies by direct reprogramming constitutes a promising approach to treat AD and AD-related dementias.

Poster Number: 12

Testing for the Presence and Characteristics of Collagen Fibrils via Mechanical Means

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Collagen fibrils projecting perpendicularly from a surface would mimic naturally occurring tissue structures, like those found at the musculotendinous and osteotendinous junctions. While electron microscopy has offered some success, most imagery depicts fibrils lying parallel to the surface rather than projecting upright. Our research aims to investigate projecting fibrils under aqueous conditions. To do this, we have developed a technique to measure the force needed to shear two coverslips both decorated with fibrils that have been crosslinked to each other. To assemble the sample, nucleation sites for fibril growth were created by attaching ketone-collagen monomers onto a glass coverslip surface decorated with oxyamine-terminated alkane silane linkers. Using a custom fabricated titanium preparation vessel, we exposed the coverslip surfaces to bovine dermal type-I collagen monomer (Cohesion Corp.) using a phosphate buffered saline solution (PBS, 120mM NaCl, 2.7mM KCl and 10mM phosphate) (Sigma) at pH 7.4 and allowed fibrils to grow for 24 hours. To crosslink, coverslips containing fibrils were submerged into a crosslinking solution containing 10x PBS, 2.5% glutaraldehyde, and 0.4%w/v 10µm polystyrene beads for 12 hours. Polystyrene beads ensure a gap between the coverslips that could only be bridged if collagen fibrils were projecting many micrometers above the surface. The coverslips were subsequently sheared by hand and compared to five controls with a rating of 1 or 2: 1 representing minimal force and 2 representing notable force. The coverslip sandwich was also sheared in a mechanical testing machine at a rate of 0.5mm/min, where it was found that the force increased as the displacement of the coverslips with respect to each other increased, with a maximum force ranging from 2-5N. The controls indicate that polymeric coatings, polystyrene beads, and fibril entanglement do not cause a significant interaction. The perpendicularly oriented fibrils being present is supported by the quantitative force data, allowing us to estimate the number of fibrils present, their density, and their characteristics. In the future, we plan to use this method to measure desired increases in the linear density of fibrils and gain a more accurate understanding of the fibrils' interaction on larger scales.

Poster Number: 13

Detection of Hemostatic Impairment Caused by Platelet Number and Function Defects using Platelet-specific Dielectric Coagulometry

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Background: Platelets play a central role in hemostasis. Deficiencies in platelet number or function are associated with significant bleeding risk. Platelet transfusions are used to mitigate such risks, but current transfusion is guided only by platelet count, with no correlation to hemostatic function. Methods like aggregometry can only assess platelet aggregation capability, but is not predictive of hemostasis. Viscoelastometry can characterize overall clot kinetics and stability, but has low sensitivity to platelet-specific defects. Technologies that can provide distinct correlation of hemostatic status associated with platelet defects can be clinically important.

Aims: We investigated a dielectric coagulometer with sensitivity to platelet number and function defects that is predictive of compromised hemostasis.

Methods: We manufactured microfluidic coagulometers with gold electrodes to characterize whole blood coagulability using dielectric spectroscopy. For platelet-sensitivity, the electrodes were coated with thrombin receptor activating peptide 6 (TRAP-6). Healthy donor blood was modified in vitro to recapitulate platelet number defects (by depleting platelets) or platelet function defects (using platelet inhibitors), and tested on TRAP-6-coated electrodes to establish coagulometry readout signatures. Finally, clinical samples obtained through an IRB-approved protocol were used to validate the platelet-relevant signatures.

Results: The coagulometer provides two parameters, Tpeak (clotting kinetics), and Δεr,max (clot firmness). Compared to healthy control, Tpeak was prolonged in blood with <50,000 platelets/μL, and Δεr,max was reduced at 100,000 platelets/μL. Δεr,max was also compromised with platelet GPIIb/IIIa inhibition and Tpeak was impaired with platelet PAR-1 inhibition. In patient samples, coagulometry showed impairment in both Tpeak and Δεr,max in severe thrombocytopenia, but only Δεr,max was significantly affected in moderate thrombocytopenia.

Conclusion: We developed a dielectric coagulometry assay sensitive to platelet number and function defects that can inform on functional hemostatic status. Translational advancement of this technology could provide a unique diagnostic modality to guide platelet transfusions.

Poster Number: 14

Characterization of a Nanocomposite Sweat Lactate Sensor for Use in High-Level Athletes

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Currently, no known method exists to noninvasively and continuously monitor lactate levels during exercise. Lactate build-up due to the anaerobic metabolism of glucose during moderate to intense exercise can cause cramping, fatigue, and overall decreased performance in athletes. The purpose of this research was to create a fabric-based nanocomposite sensor for the determination of sweat lactate levels during exercise. This fabrication process of the sensor included creating a base of non-woven nylon fabric that was treated with an aqueous dispersion of multi-walled carbon nanotubes (MWCNT) that have a carboxylic acid functional group. The functional layer of the sensor was synthesized using pyrrole and lactate oxidase. Characterization of the dispersion quality and average particle size of the MWCNT dispersions was completed using UV-vis spectroscopy and Zetasizer analysis. To analyze the electrochemical properties of the sensor, cyclic voltammograms of the sensor in response to synthetic sweat solutions containing lactate were measured. Additionally, further testing of the response of the sensor was completed using various spiked and synthetic sweat solutions containing common interferents like glucose, potassium, calcium, and sodium. Overall, initial benchtop testing confirmed that the sweat lactate nanocomposite sensor was able to detect physiologically relevant levels of sweat lactate. Future testing of this sensor will include evaluation of the accuracy of the sweat lactate sensor during on body cycling trials.

Poster Number: 15

Nanocomposite Sensor for Measuring Sweat Potassium Ion Concentration

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The focus of the present research is to determine the commercialization potential of a nanocomposite sensor to detect sweat potassium levels. This sensor will be modeled after a previously patented sweat sodium ion sensor. This potassium ion sensor will be made of a nonwoven, electro spun nylon-6 mat treated with multi-walled carbon nanotubes (MWCNT) and functionalized with calix[n]arene. For the first prototype of this sensor, the dispersion of MWCNTs will be analyzed through sonicating with a bath sonicator and a probe sonicator in Triton X-100. To quantitatively characterize the dispersion of the MWCNTs, particle size and absorbance will be taken from a Zetasizer and a UV-Vis Spectrophotometer respectively. To characterize the response of the fully assembled potassium ion sensor, it will undergo calibrations in the laboratory with synthetic and spiked solutions that contain various potassium and sodium ion concentrations within the physiological range of these ions in sweat. Potassium ions are larger than sodium ions, so any calix[n]arene molecules that bind to potassium ions will also bind to sodium ions on the sensor. The objective is to effectively distinguish between sodium and potassium values. Upon successful testing, there is a high probability for commercialization of this nanocomposite sensor to detect sweat potassium levels.

Poster Number: 16

COLLASLING: EVALUATION OF A PURE-COLLAGEN STRESS URINARY INCONTINENCE SLING IN A LONG-TERM OVINE MODEL

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In previous work, we developed a composite midurethral sling (MUS) from bovine electrocompacted collagen (ELAC) thread to address stress urinary incontinence (SUI). An ovine feasibility study showed the sling was biocompatible. Currently, we have braided ELAC thread into pure-collagen, macroporous slings, CollaSlings, and implanted them in sheep. We hypothesized the CollaSling would produce a lasting, ligamentous tissue band around the midurethra. The objective was to determine the tissue response to and mechanical properties of a novel pure collagen sling in an ovine model prior to human clinical trials. CollaSlings were fabricated by braiding ELAC threads into a 12mm wide ribbon, segmenting into 50cm sections, and crosslinking with genipin. Ten adult Dorset ewes were implanted with the CollaSling using a standard retropubic approach. Ewes were sacrificed at 3 months (n=3), 6 months (n=3), 9 months (n=4). Lengths of CollaSling passing through the space of Retzius were mechanically tested for ultimate tensile strength (UTS), strain, and modulus. Histology sections were taken in the midurethral and muscle regions and scored according to ISO10993-6. No urinary retention or other adverse effects were observed. Grossly, the CollaSling integrated with the surrounding tissues at harvest with no fibrous encapsulation at any site. The sling formed a ligament that was distinct and palpable through the vagina, which felt supple at harvest. Histologically, there was no acute inflammatory response. There was minimal-mild dense fibrosis with low clusters of infiltrating lymphocytes, plasma cells, macrophages, and multi-nucleate giant cells, and evidence of neovascularization for all time points, and a trend towards slightly greater fibrosis present within the skeletal muscle site as compared to the midurethral site across all time points. Explants were significantly stronger at all time points compared to hydrated, naïve CollaSling due to tissue ingrowth and de novo collagen deposition. Strain and modulus were consistent over time, with values similar to native tissue. CollaSling is a pure collagen MUS deployable using a standard retropubic procedure. The sling is biocompatible and promotes new fibrous tissue deposition. Mechanical properties indicate that the sling can support the midurethra. Clinical studies will be run to determine efficacy in treating SUI.

Poster Number: 18

Biocompatibility and Differentiated Performance in Medical Grade Polymers

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Thermoplastic polyurethanes (TPUs) were discovered in 1959 and originally used for non-medical applications. In the 1960s and 70s medical device designers discovered polyurethanes' properties (mechanical strength, tunable flexibility, versatile processability, and relative biostability/biocompatibility) made them uniquely suited for use in medical devices; TPUs are now common in healthcare and are used in many life-saving devices. While original equipment manufacturers (OEMs) must consider the efficacy of a device, its safety is equally important. The selection of component materials for the medical device must be carefully considered to ensure its safety. Evolving global regulatory standards must be considered alongside manufacturability, biocompatibility, chemical characterization, quality control and change management. Lubrizol's innovation team is dedicated to advancing medical polymer research and commercializing TPUs with differentiated performance while supporting our customers regulatory filings with robust and well documented safety profiles to deliver safe and effective performance. Biocompatibility testing (ISO 10993), industry challenges, and future direction of safety testing will be presented in the context of polymers for medical applications.

Poster Number: 19

Initiator-free photocrosslinking of dynamic gelatin-based hydrogels

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The extracellular matrix (ECM) dynamically influences cell fate via continuous matrix remodeling. However, traditional static 3D models lack the ability to replicate this dynamism, emphasizing the necessity for culture systems mimicking spatiotemporal changes in the ECM. Hydrogels prepared from photopolymerization are ideal for mimicking the dynamic changes of ECM properties, but a photoinitiator is often required to initiate the crosslinking of conventional hydrogels. Here, we introduce an initiator-free photocrosslinking of gelatin-based hydrogels to serve as a dynamic culture platform. This was achieved by combining reversible dithiolane-mediated ring opening polymerization with irreversible thiol-

ene click reaction. We synthesized 8-arm poly(ethylene glycol)-lipoic acid (PEG-8LA) and gelatin norbornene (GelNB) for their initiator-free photocrosslinking (365nm, 20mW/cm2) into hydrogels with dynamically tunable properties. Varying initial irradiation light irradiation time (2 to 10 minutes) led to a wide range of stiffness (G' ~ 1.5kPa to 10kPa). Uniquely, GelNB/PEG-8LA hydrogels can be dynamically stiffened via secondary light exposure without additional macromers. The incorporation of irreversible thiol-ene bonds did not impact dithiolane-thiol exchange for matrix stiffening. Interestingly, GelNB/PEG-8LA hydrogels exhibited fast stress relaxation, with relaxation halftime ranging from 100 to 250 seconds for pure PEG-8LA and GelNB/PEG-8LA hydrogels. The cytocompatibility of the GelNB/PEG8LA hydrogels (G' ~ 1.5kPa to 5kPa) were tested using NIH/3T3 fibroblasts over 7 days. By day 3 of culture, significant cell spreading, characterized by a flattened and elongated morphology was observed. This spreading is attributed to the secretion of matrix metalloproteinases by fibroblasts. These enzymes degrade gelatin macromers, remodel GelNB/PEG-8LA hydrogels, and promote cell motility. In conclusion, we have developed dynamic photocrosslinkable gelatin-based hydrogels without the use of photoinitiator. Ongoing research focuses on further optimizing the hydrogel crosslinking conditions and utilizing this hydrogel system for dynamic culture of stem cells.

Poster Number: 20

ENHANCING THE TARGETING EFFICACY OF ENDOTHELIAL COLONY FORMING CELLS FOR RENAL REGENERATION VIA KIDNEY-TARGETED LIPOSOMAL NANOPARTICLES

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Stem cell engineering has been one of the most promising approaches for precision health nowadays. The Endothelial Colony Forming Cells (ECFCs) have been demonstrated to exhibit a high proliferative potential and have the unique ability to create de novo blood vessels in-vivo, indicating a high angiogenic potential. ECFCs have shown promising therapeutic future for various vascular disorders [1, 2, 3]. Acute renal failure, also known as AKI (Acute Kidney Injury), is characterized by a sudden decline in renal function primarily caused by ischemia/reperfusion (I/R) injury. This type of injury results in significant vascular damage,

leading to capillary loss. Considering these challenges, researchers have explored the administration of exogenous

endothelial cells as a potential strategy for treating AKI [4, 5]. Upon immediate injection, ECFCs were detected predominantly in the lungs, with an occasional presence in the kidney, liver, spleen,

and heart. However, after 1 hour, ECFC density decreased, and their presence in other tissues became scarce [6]. This highlights the need to engineer a solution that can enhance the retention of ECFCs in the kidney, thereby improving their performance in promoting kidney regeneration. In this project, our goal is to utilize Liposome Nanoparticles (LNPs) not as conventional drug carriers but as vehicles to facilitate cell homing and create what is referred to as a "backpack molecule". To achieve this, a lipid formulation containing thiol-reactive maleimide headgroups is employed. This reaction is made possible by the presence of free thiol groups on the surface of ECFCs, which readily interact with the maleimide groups in the LNPs [7]. However, it is important to note that this interaction offers specificity.

While LNPs have demonstrated specificity, they lack selectivity, apart from their size, which is a crucial factor in cross-organ barriers. The Kidney Targeting Peptide (KTP) has demonstrated an enhanced binding and high accumulation in proximal tubules and vascular endothelium of the kidney. These findings highlight the exceptional renal selectivity of KTP [8]. Therefore, our general hypothesis is that our unique backpack molecule KTP-nanoparticle design will enhance the targeting efficacy of the ECFCs leading to vascular renal regeneration.

Poster Number: 21

Sustained heme-albumin release as a potential therapeutic for age-related macular degeneration

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One of the leading causes of vision loss worldwide is age-related macular degeneration (AMD), affecting over 200 million individuals. This neurodegenerative disease is a burden to the healthcare system, coming in at $250 billion in global cost. AMD risk factors include progressive age, genetics, obesity, smoking, and race. Early-stage pathogenesis of AMD is the accumulation of reactive oxygen species (ROS) in aging tissue leading to retinal inflammation, known as dry AMD (dAMD). While infrequent, dAMD may progress to wet AMD (wAMD), which is choroidal neovascularization resulting in blood and fluid leakage, furthering permanent vision loss. Currently, there is no cure for AMD and treatment options predominantly target wAMD through invasive and frequent intravitreal injections. Only recently have potential new therapeutics for dAMD emerged, targeting the complement system, but these also require monthly intravitreal injections. To target dAMD and mitigate disease progression, a novel anti-inflammatory heme-bound human serum albumin (heme-albumin) complex was investigated. We hypothesize that heme-albumin will induce heme oxygenase-1 (HO-1) in retinal pigment epithelium (RPE) cells by catabolizing heme into carbon monoxide (CO) and biliverdin for antioxidant and anti-inflammatory properties, respectively. This protein complex has been encapsulated in polydopamine nanoparticles (PDA NPs) for sustained release as well as ROS scavenging. Heme-albumin demonstrated no significant cytotoxicity in human RPE cells, ARPE-19, and reduced oxidative stress in inflammatory and ROS models in vitro. PDA NPs loading heme-albumin showed sustained release for up to 6 months with a reduction in oxidative

stress and minimal cytotoxicity. Successful cellular uptake of heme-albumin and loaded PDA NPs through flow cytometry was demonstrated alongside significant HO-1 expression in ARPE-19 cells. Through in vitro verification, we validate that heme-albumin loaded PDA NPs reduce oxidative stress and inflammation and has potential to serve as a sustained therapeutic for AMD.

Poster Number: 22

Tunable Injectable Bi-layered Nanofiber Capsules for Sustained Release of Therapeutics

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Age-related macular degeneration (AMD) is a progressive ocular condition that affects the retina and results in impairment or loss of central vision. AMD is the fourth most common cause of blindness in the world. Currently, available treatments for dry and wet AMD typically require intravitreal injections up to monthly. Treatments for wet AMD incorporate anti-VEGF therapy to prevent angiogenesis over a patient's lifetime. This current treatment approach is associated with several limitations including invasiveness, inflammation, retinal detachment, as well as patient discomfort, need for frequent visits, and therapeutic waste. Therefore, there is a need for an improved therapeutic approach to reduce injection frequency for AMD patients. In this study, we modulated injectable bi-layered capsules for the sustained release of therapeutics. In particular, the influence of changing porogens on tuning nano porosity was investigated in this study.

We previously investigated the sustained release of anti-VEGF therapeutics from bi-layered capsules containing chitosan. In these studies, a hollow cylinder structure with poly(caprolactone) (PCL) outer-shell and poly(L-lactide-co-ε-caprolactone) (PLLACL) inner-layer was designed and fabricated with tunable porosity to modulate drug release. The production process is a combination of materials processing technologies, including electrospinning, sintering, and salt leaching to create microcapsules. Capsules with different mass ratios of PCL to HEPES sodium salt (98:2, 97:3, 96:4; 95:5; 90:10) were prepared to assess the impact of salt concentration on porosity, and their structures were analyzed before and after salt leaching by scanning electron microscopy (SEM). In vitro cytotoxicity and cell viability of capsules were evaluated by MTS assay conducted with human retinal pigment epithelial (ARPE-19) cells.

The effects of different PCL/salt concentrations on capsule structure and in vitro drug release were investigated in this study and it was observed that the pore size of capsules could be tuned by changing the concentration of salt. MTS assays showed retinal cell viability of more than 90% with capsules prepared with the new polymers.

The injectable bi-layered capsules could be prepared and tuned by modifying both the porogen and inner polymer layer with the goal of extending therapeutic release for up to one year.

Poster Number: 24

Engineering Extracellular Vesicles for Targeted Delivery of Neurofibromatosis Type 1 Gene Therapy

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder that affects the peripheral nervous system, characterized by the development of tumors. It has an estimated prevalence of approximately 1 in 3000 individuals. NF1 manifests through the presence of cutaneous neurofibromas and various abnormalities in the nervous and skeletal systems. The primary cause of NF1 is the loss of function of the Neurofibromin 1 (NF1) gene (350 kb), and the subsequent absence or non-functionality of its encoded protein, neurofibromin, during Schwann cell development. Neurofibromin functions as a tumor suppressor protein, primarily expressed in neurons and glial cells, where it regulates cellular proliferation and differentiation by downregulating the RAS/MAPK pathway and other signaling pathways.

Treatment options for NF1 are currently under development, with one major challenge being the delivery of the NF1 gene for gene therapy due to its large size. In this project, we propose a novel approach utilizing engineered extracellular vesicles (EVs) as a targeted delivery systems to Schwann cells. We achieved this by transfecting plasmids encoding SC-targeting transmembrane proteins into donor cells, leading to the production of engineered EVs. We evaluated the properties of the EVs at the gene and protein expression levels and assessed their tropism through uptake experiments in vitro and the biodistribution in vivo. Additionally, we successfully loaded the NF1 gene into these EVs by co-transfecting donor cells with appropriate ligands and NF1 plasmids, demonstrating the feasibility of delivering the therapeutic gene to NF1-deficient cells. Moving forward, we aim to optimize the engineered EV formulation and advance our therapeutic approach by employing tissue nanotransfection (TNT) to induce the production of Schwann cell-targeting designer EVs within the epidermis of NF1 model mice. This innovative strategy allows for systemic delivery of therapeutic cargo, potentially offering a promising avenue for the treatment of cutaneous neurofibromas and other manifestations of NF1.

Poster Number: 25

In vivo transfection to produce Insulin as an alternative therapy in Diabetes.

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Cell reprogramming is a promising strategy for the treatment of various diseases involving the loss of cell function, such as diabetes mellitus (DM). DM is a chronic metabolic disease with an increasingly high incidence that is projected to double in the next 30 years (ONG et al., 2023). Therefore, there is a need to explore novel, large-scale, and reliable methods for insulin production to address the growing demand for insulin required to treat DM. The capacity to create cells that produce insulin by utilizing modern reprogramming techniques offers a reliable and innovative solution for insulin production and treatment of DM. However, current approaches to cellular reprogramming face various limitations including difficulty with obtaining pluripotent cells, randomness of the process, and the lack of specificity in cellular transformation. Tissue nanotransfection (TNT) is an in vivo non-viral cell reprogramming technology that uses a nanochannelled device and has the potential to overcome the limitations of cell-based therapies by allowing for the use of more accessible cellular sources. Here we explore the use of TNT to transfect the skin of transgenic INS-GFP mice to facilitate the delivery of a cocktail of transcription factors associated with insulin production in beta cells that had previously been shown to significantly increase insulin gene expression in vitro in primary mouse embryonic fibroblasts (PMEFs). At 7 days post-transfection, “in vivo imaging system” (IVIS) was used to measure the presence of green fluorescent signal associated with GFP and insulin expression in the skin of transfected mice. GFP expression was present in the skin of mice where TNT was performed using plasmids loaded with the aforementioned transcription factors that were not observed in control mice. These results indicate that TNT shows promise as a strategy for gene delivery for the purposes of cell reprogramming. The utilization of this technology paves the way for the development of new sources of insulin production, allowing for new therapeutic strategies that address the current limitations associated with treating DM.

Poster Number: 26

Copper Oxide Nanoparticles Induce DNA Damage in Endometrial Cancer Cells

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Chemotherapeutic resistance across many types of cancer is one of the greatest complications to cancer therapy and results in few treatment options for patients plagued with this disease. The vital demand for innovative anti-cancer drugs and drug delivery systems arises from these issues, revealing the importance that research on anti-cancerous compounds has on effective care for patients. Endometrial cancer is the most common gynecological cancer worldwide and is commonly treated using carboplatin, a platinum-

based drug. Carboplatin resistance and unequitable access to treatment for endometrial cancer patients validates the need for a non-invasive form of endometrial cancer therapy. It is proposed that copper oxide nanoparticles can be used as an alternative to carboplatin when encapsulated in a polymeric nanoparticle for use as a drug delivery system. Copper oxide nanoparticles pose the potential as an anti-neoplastic compound due to Cu2+ ions demonstrating the ability to induce reactive oxygen species leading to double stranded DNA breaks ultimately resulting in apoptosis. Through viability assays, endometrial cell lines all showed sensitivity to copper oxide nanoparticles; however, trends were not identified across all cell lines that were tested. Using this information, Western Blot analysis was used to identify specific expression levels of apoptotic proteins, DNA repair proteins, and protein markers when DNA is damaged. Western Blots involved the treatment of cells in 24-well plates with varying concentrations of copper oxide nanoparticles for 24 hours. Gel electrophoresis separates proteins and primary antibody complexes. The differences in the expression levels of these proteins after cells are exposed to copper oxide nanoparticles can indicate the mechanism of toxicity within the different cell lines, ultimately explaining the differences in viability.

Poster Number: 27

Experimental Determination of 3D – Extrusion Based Bioprinting Parameters for Biomaterial Processing in Corneal Tissue Fabrication

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Extrusion based 3D bioprinting is a mechanism used in biomaterial processing, including tissue engineering and drug delivery. One such implication is the development of corneal tissue grafts using transparent and compatible base inks for partial-thickness corneal replacement. One of the challenges of using bioprinting is the difficulty of controlling the printing parameters to get a scaffold with optimized properties. Bioinks are composed of liquids with different viscoelastic properties. Using experimental testing of two bioinks, SilMA (methacrylated silk fibroin) and GelMA (methacrylated gelatin), we propose to develop standard printing parameters reflective of the components of this bioink. These bioinks were chosen due to the robust mechanical properties provided by silk fibroin-based inks and the greater cell adhesion provided by gelatin-based inks. Methacrylation enables further crosslinking of the materials, promoting mechanical stability. A 1:1 mix of these components was evaluated for absorbance and rheological properties, demonstrating sufficient transmittance (80-90%) and shear thinning behavior. Producing an ideal range of testing parameters based on these materials can offer more uniform deposition, allowing for greater control of the printed structure. This is especially significant for tissue

scaffolds that require greater structural specificity, like corneal tissue fabrication. Parameters that are significant to printing include pressure, nozzle size, print speed, print-head temperature, print bed temperature, crosslinking, etc. The printer used was a CELLINK BioX 3D bioprinter. Characterization of the printed scaffolds and the base inks was done with optical and mechanical testing, such as light transmission and shear rheology. Future works would involve the development of a mathematical model that would allow for the prediction of the appropriate printing parameters using the viscoelastic properties of various composite bioinks. Ultimately, with an improved mechanism of determining the relationship of the printing parameters and character of the bioinks, the use of 3D-extrusion based bioprinting can be simplified for a vast range of fields looking for the appropriate conditions within the diverse ecosystems of the human body.

Poster Number: 28

Development of a cell-degradable photopolymerizable resin for additive manufacturing

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Within the field of tissue engineering, additive manufacturing (AM) techniques allow for the design, creation, and implementation of tissue engineered constructs (TEC) with complex morphologies. SLA is an AM technique which leverages UV induced polymerization of a liquid resin by irradiation of light in a layer-by-layer manner to create 3D morphologies matching that of native tissue. Furthermore, SLA allows for precise control of TEC internal structure allowing for precise pore sizes, and pore geometries allowing greater cellular infiltration. Thioketal (TK) containing polymers are an emerging class of materials with exciting potential applications within the biomedical domain. TKs are used as synthetic precursor monomers that specifically degrade by oxidative molecules such as the cell-produced signal reactive oxygen species (ROS). Current dogma suggests that ROS are implicated in tissue regeneration mechanisms and are involved in immunologic cell signaling. TK chemistries have yet to be utilized in a 3D printable manner. Therefore, we have created TK-based resins which undergo photo-crosslinking on a commercial SLA printer. Additionally, we developed multiple thiol-ene resins for variation in mechanical properties and degradation kinetics all possessing selective ROS-triggered degradation. The culmination of this work will result in novel photopolymerizable, biocompatible, cell-degradable resins for SLA 3D printing.

Poster Number: 29

Tissue Nanotransfection (TNT) Of Pro-vasculogenic And Pro-neuronal Factors For The Treatment Of Peripheral Neuropathies

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Introduction: Peripheral nerve injuries (PNIs) can result in sensory/motor deficits and muscle loss if not treated immediately. In this study, we describe the use of a novel technology, Tissue Nano-Transfection (TNT), that can deliver gene therapies to nerve/muscle aimed at improving the rate of peripheral nerve regeneration and healthy maintenance of injured nerve tissue and denervated muscle through neurogenic and vasculogenic reprogramming.

Methods: Vasculogenic reprogramming factors Etv2, Fli1, and Foxc2 (EFF) were delivered into an injured mouse (C57BL/6) sciatic nerve using TNT to reprogram native fibroblast/Schwann cells into functioning endothelial cells; with the intent of increasing vascularity which should lead to axonal preservation. Varying concentrations of these three EFF factors were assessed in their effectiveness of increasing vascularity in the sciatic nerve by comparing laser speckle imaging (LSI) perfusion in the nerves between all concentration groups and a control (pCMV6) 7 and 14 days after treatment. Histology was also used for comparing the amount of blood vessels (CD31) and axons (NF) formed in the nerves between each group. Another set of experiments were done using TNT delivery of pro-neuronal factors Ascl1, Brn2, and Myt1l (ABM) to help reinnervate leg muscle in cases of sciatic nerve transection. Again, optimization of the concentration ratios is being done currently to determine the most efficient combination of factors for conversion of native myoblasts to induced neurons.

Results: At days 7 and 14 post-treatment using TNT of the EFF vasculogenic factors in crushed nerve tissue, there was a significant increase in blood perfusion in the sciatic nerve measured by LSI. Histology also showed an increase in the number of sciatic nerve blood vessels in the EFF cocktail group where Foxc2 was doubled in concentration (1:1:2) at both 7 and 14 days post treatment. The conversion of muscle to induced neurons using ABM is still an ongoing study.

Poster Number: 30

Creation of dual-stage, ROS-responsive microparticles for drug delivery to critically-sized bone defects

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Segmental bone defects of greater than 2 cm are unlikely to heal spontaneously following skeletal stabilization and are thus deemed “critically” sized. Regenerating these bone injuries or defects in the clinic remains particularly challenging. Critically-sized defects require planned reconstruction, and have many technical and biological challenges in creating a fully functional vascular network. This work outlines an injectable, environmentally-responsive, dual-stage drug release system to controllably deliver multiple therapeutics to promote remodeling and healing of critically sized bone defects. This utilizes a two-compartment system with polymers that selectively respond to cell-generated reactive oxygen species (ROS). Inner drug-loaded microparticles have been formed with ROS-responsive poly(propylene sulfide) (PPS) and Benzene based thioketal (BTK) polymers, and non-responsive, hydrolytically degradable, poly(lactic-co-glycolic) acid (PLGA). The drug coating has been formed with ROS-responsive poly(thioketal) (PTK) polymers and non-responsive, hydrolytically degradable, poly(β-amino ester) (PBAE) polymer. We

hypothesize that this dual-stage system will sequentially deliver multiple therapeutics upon triggering by cell-generated ROS.

Microparticles loaded with fluorescently tagged model proteins were formed via an oil/water/oil emulsion technique. To analyze particle stability, agglomeration, and average size, particles were imaged under confocal microscopy and scanning electron microscopy (SEM). To further assess stability microparticles were coated with a secondary drug depot. Particles were sequentially incubated using an adapted layer-by-layer (LbL) technique with, PTK and PBAE polycations. These dual-loaded particles were examined with fluorescent microscopy to examine dual-drug payloads and layer stability.

Microparticles made of PPS were consistently less stable in aqueous media compared to PLGA and BTK particles. To maximize oxidation-responsive drug release and maintain particle stability in aqueous conditions, BTK particles were chosen as the lead candidate formulation. Based on SEM imaging, the base particles were determined to be within 5-10 µm. The LbL-coated PPS microparticles feature fluorescent signal from both compartments. These images confirm this material system's capability to independently load multiple drug payloads and the stability of the system.

Preliminary data have shown that the formation of stable, ROS-sensitive, dual-stage microparticles is feasible. Future work will establish release kinetics, of single and multi drug payloads, and evaluate this system with therapeutic growth factors for in vivo bone regeneration.

Poster Number: 31

Optimization of fabrication and coating of ROS-responsive microparticles for critically-sized bone defects

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Introduction: A challenge in the clinic is the healing of critically-sized bone defects. A critically-sized bone defect is classified as a bone defect, usually greater than two centimeters in size, that will not spontaneously heal on its own without planned skeletal stabilization.1 Most current technologies that locally deliver the therapeutics utilize traditional drug delivery systems which are passive, have limited drug payload, and have inevitable drug leakage.3,4 Our work involves an injectable, cell signaling responsive, and dual-stage drug release to deliver multiple therapeutics to promote the healing of critical-sized bone defects. Our current focus is to optimize the particle retention during LbL coating along with the coating characteristics.

Methods and Materials: The dual-stage drug release microparticles are made with a oil/water/oil emulsion that has been adapted from a previous protocol.4 The microparticles are then coated using the LbL (layer by layer) process using poly-thioketal cation/polyacrylic acid anion/tetrarhodamineisocyothionate cation/polyacylic acid anion. To analyze the optimal protocol for the LbL process to minimize the amount of particles lost, different parameters of the process were altered.

This includes centrifuge time (two, four, six, and eight minutes), incubation conditions (still, shaker table, vortex, and rotisserie), centrifuge speed (16.1 rcf and 25 rcf), and vessel size (2mL and 15mL).

Results and Discussion: We expect to see differences in the amount of particles retained based on the parameters that were altered. We also expect to see differences in the coating uniformity, quality, and agglomeration based on the parameters that were altered.

Conclusion: Preliminary data suggests that there may be measurable differences in particle retention and coating uniformity, quality, and agglomeration.

Poster Number: 33

Microporous Immune Isolating Capsule with Improved Diffusion for Restoration of Ovarian Endocrine Function and Immune Protection

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Live saving chemotherapy treatments are toxic to the ovaries leading to premature ovarian insufficiency (POI) in adolescent girls. Young women with POI are treated with hormone replacement therapy (HRT) which is unable to reproduce the dynamic, pulsatile and reciprocal interactions of a functioning ovary with the rest of the body. The lack of physiological ovarian endocrine function leads to elevated risks of various endocrine, cardiovascular and musculoskeletal disorders. We have developed an immune isolating polyethylene glycol (PEG) hydrogel capsule that protects an ovarian tissue allograft while facilitating the exchange of nutrients and hormones. The capsule consists of two layers: an inner biodegradable layer that interfaces with the tissue and promotes growth, and the outer non-degradable layer that prevents rejection. To improve the diffusion across the hydrogel, we incorporated sacrificial gelatin microgels into the outer shell of the capsules. When heated to body temperature, the gelatin becomes soluble, leaving disconnected micropores measuring 30 m in diameter in the bulk nanoporous hydrogel. We analyzed PEG hydrogels loaded with 1%, 5% and 10% micropores (PEGG+) using fluorescence microscopy and confirmed the microgels were homogeneously distributed throughout the bulk gel. Rheological characterization of the mechanical properties of the 10% PEGG+ gels indicated a 4-fold increase in shear yeild strain when compared to bulk PEG gels with a 56% increase in elasticity and little change viscosity of the material. Next, we measured the diffusion of 4, 40 and 150kDa dextran. The diffusion of 40 kDa dextran increased from 16% in the control nanoporous bulk gels to 26% after 96 hours. The diffusion of 150 kDa dextran only slightly increased from 10% in the bulk gels to 11% in PEGG+ gels reassuring that the microporous shell could still prevent infiltration of antibodies and cells. No difference in the diffusion of 4 kDa dextran was observed for all gel conditions. Future in vivo testing will investigate whether the capsule prolongs the survival of the human ovarian xenografts when implanted subcutaneously in ovariectomized mice for

2, 10 and 20 weeks. In conclusion, incorporation of sacrificial micropores in a nanoporous bulk hydrogel improves diffusion without compromising its mechanical properties.

Poster Number: 34

Development of polyacrylamide-methacrylated hyaluronic acid hydrogels to study responses of astrocytes in an inflammatory environment

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Established protocols for fabricating various elastic stiffness ranges of polyacrylamide (PA) hydrogels – substrates commonly used in cell culture – have been developed; however, there is a need to improve the biological relevance of this substrate toward biomimetic cultures. Hyaluronic acid (HA), a linear glycosaminoglycan found in the extracellular matrix (ECM) of the central nervous system (CNS) tissues offers exceptional biocompatibility, non-toxicity and chemical inertness, often requiring modification to enhance its mechanical durability. PA gels have not been modeled to study the stiffness ranges found in healthy and diseased CNS tissues. Previous research has shown that injured CNS tissue is significantly softer as its ECM is unorganized and the cell architecture is unstructured which contrasts with a slightly stiffer, orderly arrangement of cells in healthy CNS tissue. This disorganized arrangement is partially attributed to the post injury responses including the establishment of the 'glial scar' in the injured region. The glial scar consists of astrocytes that secrete and assemble chondroitin sulfate proteoglycans (CSPGs), which are highly inhibitory for axonal regeneration. As such, the presence of these proteoglycans presents a significant barrier for regeneration in SCI. Moreover, CSPGs not only inhibit axonal growth but they also influence cell migration, differentiation and the overall inflammatory response. The focus of this research is to study how matrix stiffness affects glial cell responses in vitro on a polyacrylamide-methacrylated hyaluronic acid hydrogels mimicking injured and healthy spinal cord tissues. I hypothesize that the presence of an inflammatory environment will exhibit altered cellular responses, increased proliferation and upregulated expression of inflammatory markers as well as CSPGs as compared to astrocytes in non-inflammatory conditions. To test this hypothesis polyacrylamide-methacrylated hyaluronic acid hydrogels were created at various stiffness spanning chronically injured to healthy CNS tissue (EY range = 150 and 480 Pa respectively). Astrocytes are seeded on them, and exposed to TNFα and IL-1a to simulate inflammatory signaling and resulting cellular responses, studied using microscopy (brightfield and IHC) and biochemical assays Thus, this research will provide a foundation for further understanding how CSPGs affect the cellular environment in vitro by more faithfully replicating the environment of SCI.

Poster Number: 35

Nano Lipid Carrier Synthesis for Encapsulation of Dastinib

Anna-Kate Miedler, The Ohio State University

I would like to be considered for a Rapid Fire Oral Presentation

Proliferative vitreoretinopathy is a complication of retinal detachment where contractile cellular membranes form on both sides of the retina and within the vitreous. Current treatment requires multiple surgical procedures that do mostly result in retinal reattachment but cause the eye to lose some of its visual function due to fibrosis. Nanostructured lipid carriers (NLCs) have attracted attention as an ocular drug delivery vehicle due to their ability to sustain drug release and their enhanced biocompatibility [1]. Current research studying the encapsulation of Dasatinib within NLCs is limited. In this work, a protocol for nanostructured lipid carrier synthesis using melt emulsification was adapted for Dasatinib encapsulation.

Dynamic light scattering was used to determine the hydrodynamic diameter and polydispersity index at seven-day intervals for twenty-one days. The zeta potential was measured by a zetasizer. Drug efficiency and loading were determined using UV-VS spectrophotometry at 323 nm.

Preliminary data has found the unloaded NLCs have an average size of 96 nanometers with a polydispersity index of 0.26 and an average zeta potential of –6.88 while the Dasatinib loaded NLCs have an average size of 114 nanometers with a polydispersity index of 0.31 and a zeta potential of -0.10. Over time, these values stayed roughly constant. The drug efficiency and loading values were 76.78% and 3.97% respectively.

Future work will include visualizing the morphology of the NLCs using transmission electron microscopy and analyzing drug release with high performance liquid chromatography.

Poster Number: 36

WITHDRAWN

Poster Number: 37

Investigating Neuron Axonal Alignment in 3D-Bioprinted Hyaluronic Acid and Gelatin Scaffolds

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Spinal cord injuries (SCI) represent a critical health concern, with approximately 18,000 new cases each year in the USA, excluding fatalities at the incident site, according to the National Spinal Cord Injury Statistical Center. SCI leads to neuronal necrosis and an environment that results in no axonal regeneration from surviving neurons distant to the primary injury site. Without axonal regeneration and

lack of synaptic plasticity, SCI repair is not possible. Tissue engineering has emerged as a promising therapeutic approach to overcome this barrier.

In this study, we developed 3D-printed scaffolds composed of methacrylated versions of hyaluronic acid and gelatin, materials that are biocompatible and native to the spinal cord. I hypothesize that a 3D bioprinted scaffold integrating native ECM and structural features found in the spinal cord will allow for improved neuronal viability while encouraging axonal outgrowth and alignment because of the presented hepatotactic cues as compared to control scaffolds. To achieve this, we have designed a 3D printed scaffold, where the scaffold geometry mimics the architecture of the native spinal cord in a way that allows the axons to extend through an aligned construct. Methacrylated hyaluronic acid and methacrylated gelatin are prepared by adding methacrylic anhydride to hyaluronic acid and gelatin, respectively. After purification and lyophilization the resulting product was obtained and used for 3D bioprinting. Neurons are seeded onto these hydrogels as compared to printed (encapsulated) neurons. The integration and viability of neurons with the scaffolds are assessed to determine the efficacy of the biomaterials. Axonal alignment and neuronal viability within the scaffolds are primarily analyzed via immunohistochemistry (IHC). NeuN is used as a marker for mature neurons, while axonal outgrowth is examined using GAP43. Assessments aim to verify the potential of these biocompatible scaffolds in promoting neuronal alignment, viability, and axonal regeneration before proceeding to potential in vivo studies. The findings of this study could provide critical insights into the development of effective tissue-engineered solutions for SCI repair, ultimately contributing to improved functional recovery and quality of life for SCI patients.

Poster Number: 38

3D cell culture-on-chip platform for oxygen zonated study of liver microtissues

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A 3D cell culture-on-a-chip provides technology with tantalizing potential to revolutionize drug screening and personalized medicine approaches. Concerningly, 50% of drugs fail to predict severe human hepatotoxicity. While 3D cell culture-on-a-chip technologies provide the favorable dynamic conditions provided to assemble and cultivate cells in a liver phenotypic manner, current approaches do not capture the full response range of liver metabolic responses, possibly leading to false estimations of drug-induced liver injury (DILI). To address this, we propose a novel bioreactor design integrating our unique biomaterials approaches for the cultivation of oxygen-zonated hepatocytes with precise control of oxygen levels inside 3D cultured tissues. Our design aims to address these limitations by ensuring oxygen availability, important for cell/tissue viability, and the liver's important zonal architecture and hepatocyte molecular phenotypes and functions. A cornerstone of our approach uses our chitosan-based oxygenating microparticles (MPs), which enhance oxygen tensions via inclusion of conjugated perfluorocarbon moieties, facilitate native liver cell adhesion via conjugated ECM ligands to improve tissue assembly. We have shown in static culture that this approach boosts cell survival and characteristic liver functions including improved secretion of albumin and urea ($p \leq 0.0001$), and an increase (~1.5-fold) in enzymatic

activity of the important human drug metabolism enzyme cytochrome P450 3A4 (CYP3A4) as compared to control groups without MPs ($p \leq 0.0001$). We hypothesize that our approach integrated with a perfused 3D culture bioreactor, will create an oxygen zonated stepwise arrangement (spanning 65-30 mmHg PO2) that enables improved studies of hepatotoxicity responses over extended periods, essential for modeling DILI which may take weeks or months to manifest. Key biological/metabolic activity readouts such as oxygen tensions, protein (albumin/urea) production, and metabolic enzyme (i.e., CYP3A4) activity will be monitored over 2-3 weeks using standard approaches. This study addresses a withstanding challenge in the field, offering a more reliable platform for drug screening and advancing the prospects of personalized medicine for life-threatening diseases, including liver cancers.

Poster Number: 39

Iterative Design-Build-Test-Learn Non-Viral Delivery Vehicle Discovery Platform Rapidly and Efficiently Identifies Lead Candidates

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The ability to rapidly and efficiently identify novel safe and effective non-viral delivery vehicles for use in gene therapy and drug delivery remains a challenge. To overcome this challenge, we have created a design-build-test-learn (DBTL) polymer nanoparticle (PNP) discovery platform capable of rapidly producing and efficiently identifying PNP designs with clear utility for a variety of applications. During each

DBTL cycle by this platform, hundreds to thousands of diverse PNP designs are systematically and efficiently screened in vitro and in vivo within months using standardized, reproducible methodology. To date, we have synthesized, characterized, and assessed more than 6,000 novel polymers produced via reversible addition–fragmentation chain transfer (RAFT) polymerization. Each polymer underwent high-throughput purification and characterization, including measurements of molecular weight, monomer conversion, composition, size, polydispersity, zeta potential, loading efficiency, cytotoxicity, and transfection efficiency in vitro. In addition, PNP biodistribution performance was evaluated in C57BL/6 wild type mice. Early proof-of-concept work has yielded multiple examples where this DBTL approach has proven successful in as few as 2 or 3 iterative DBTL cycles. Indeed, we have identified lead PNP delivery candidates that distribute to multiple desired tissue types following intravenous injection in mice. Moreover, we have identified lead PNP candidates capable of distributing specifically to murine sciatic nerve and/or brain tissue following intrathecal injection. Finally, we have identified multiple lead PNP candidates that leveraged specific design parameters, such as controlled molecular weights and monomer composition percentages, to maintain certain physicochemical characteristics (e.g., size, zeta potential, etc.) upon loading with nucleic acid cargo. Altogether, the empirical and modeling insights provided by data collected during each DBTL cycle has enabled us to identify clear patterns in PNP design parameters with significant utility for specific gene and drug delivery applications. The results from these studies have demonstrated the capability of our DBTL platform to rapidly and efficiently identify lead PNP delivery candidates that warrant further investigation. Therefore, this iterative high-throughput synthesis, characterization, and assessment approach to non-viral delivery vehicle discovery is well-positioned to soon offer potentially paradigm-shifting capabilities to the gene therapy and drug delivery fields.

Poster Number: 40

Development of Liquid Core Ultrasound Contrast Agents for Enhanced Cellular Tracking in Medical Imaging

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Medical ultrasound imaging is a widely used diagnostic technique for visualizing internal organs and vasculature. Ultrasound contrast agents (USCAs) are nanospheres with either a gas or a liquid core, commonly employed in vascular imaging. USCAs improve image quality by enhancing the contrast by increasing echogenicity. Interestingly, USCAs can also be used for cell-tracking applications. This application calls for the development of a novel class of USCAs that can be safely internalized by cells, enabling advanced cellular tracking within live organisms.

This study focuses on the liquid-core USCAs due to their enhanced long-term stability, compared to the gas-core alternatives. The performances of different perfluorocarbon liquid cores, Perfluoro-n-pentane (PFP), tetradecafluoro-n-hexane (TDFH), and perfluorononane (PFN), were evaluated for the proposed cell tracking USCAs. These core materials were encapsulated using a lipid shell (DPPC, DPPE, and DPPA).

The stability of the USCA was evaluated by monitoring its size and echogenicity outside the cells for up to 7 weeks. Adipose-derived stem cells (ASCs) were used to assess cellular uptake by exposing them to USCAs at different dilution factors. The cytotoxicity of the USCAs and the echogenicity of the cells after USCAs absorption were evaluated to determine the USCAs formulation yielding the highest cellular viability and echogenicity. The echogenic ASCs were further assessed to determine signal loss in subsequent generations. The results indicate that USCAs with the PFN core are toxic to the cells, and UCAs with the PFP core could be used to create viable echogenic cells. This study demonstrates the potential that lipid-encapsulated perfluorocarbon core USCA can be safely internalized by ASCs and can provide improved contrast and cellular tracking capabilities for advanced medical ultrasound imaging applications.

Poster Number: 41

Collagen scaffold to investigate contraction, remodeling, and immunomodulatory events in hypermobile Ehlers Danlos Syndrome

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Ehlers Danlos Syndrome (EDS) is a group of hereditary connective tissue disorders that clinically manifest as joint hypermobility, skin hyperelasticity, atrophic scarring, and blood vessel fragility. There is currently no cure for EDS, only symptom management, and it is underdiagnosed. The most common subtype of EDS, hypermobile EDS (hEDS), is estimated to affect 1 in 500 to 1 in 5000 people and is the only clinical EDS subtype for which the genetic basis and primary affected protein is unknown. The lack of an identified genetic defect suggests a need to identify alternative hEDS phenotypes. We are developing a non-mineralized collagen scaffold with dermal characteristics for ex vivo fibroblast culture. Fibroblasts contribute to the maintenance of connective tissues through secretions of collagen proteins, creating structural support and connecting tissues or organs in the body. Fibroblasts have previously been identified as cells of note in EDS. Here we describe the development of a collagen scaffold assay to assess dermal fibroblast samples from patients with and without hEDS. Two crosslinking methods were employed to modify the stiffness of the scaffolds without altering the physical structure: a chemical, carbodiimide-based (EDAC) method and a dehydrothermal-based (DHT) method. Chemical crosslinking took place at either a 5:2:1 or 1:1:5 ratio of EDAC:NHS:COOH while DHT was performed at 105oC for 24 hours. Scaffolds were seeded at either 75,000 or 150,000 Normal Human Lung Fibroblast (NHLF) cells per scaffold. The 1:1:5 scaffolds for both seeding conditions were found to contract 4x more than their

counterparts for either seeding density. Metabolic activity increased for all scaffold conditions, with the DHT and the 5:2:1 condition showing the greatest fold change in metabolic activity by day 10 for the 150,000 and 75,000 cell conditions respectively. Further, cell contraction was significantly increased in the 1:1:5 collagen scaffold. Together, these studies suggest DHT and 1:1:5 EDC crosslinking allow for extended analysis of dermal contraction. Ongoing efforts are defining scaffold mechanical properties as well as shifts in genomic and secretome signatures of seeded cells over 14-day culture as well as immunohistochemical assessment of alpha-smooth muscle actin (α-SMA) expression to compare cell groups.

Poster Number: 42

Release of Doxycycline from Cyclodextrin Loaded Collagen Hydrogels

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Limb prosthesis osseointegration directly into bone is desirable. However, percutaneous metal implants invite infection and inflammation. A proposed solution is a local reloadable controlled drug release system attached at the skin/prosthesis junction. We present a two-component drug release system that can be robustly adhered to an implant surface. One component is cyclodextrin oligomers (CD) allowing for affinity-based loading and release of drugs. The other component is a collagen hydrogel attached to the prosthesis surface with a novel covalent/self-assembled process1.

Three sample groups were prepared: polymerized CD, collagen hydrogel, and CD loaded collagen hydrogel. The hydrogels were prepared from type I collagen placed in fibril forming solution (tris buffered saline with phosphate, pH 7.2) and incubated at 37°C for 24 hr. For drug release studies, the hydrogels were dehydrated then crosslinked with genipin. To incorporate CD into the hydrogel, powdered CD oligomers were dissolved in fibril forming solution prior to addition of collagen monomers. For drug loading, samples were submerged in 1 ml of 1X phosphate buffered saline containing 40 mg/ml. Aliquots were taken periodically for 11 days. Doxycycline concentration was determined using absorbance at 345nm. Cumulative doxycycline release was plotted against time and mathematically modeled to exponential functions with curve fitting on MATLAB.

CD loaded gels released 220% more drug compared to collagen alone. All three groups had qualitatively similar release plots: initial burst release within 24 hrs. followed by small nonzero releases. Polymerized CD and pure collagen hydrogel were fit to a single-exponent: $f(t) = 1 – \exp(-B*t)$. The fitted exponential coefficients show a substantially slower release from CD than from collagen alone: BCD = 0.26, Bcoll = 1.31. CD loaded hydrogels were fit to a double-exponential equation: $f(t) = 1 – ACD*\exp(-0.26*t) – Acoll*\exp(-1.31*t)$. Where the A coefficients, obtained by fitting, describe the relative amounts of drug bound to CD and collagen respectively. ACD= 0.56, Acoll=0.43.

CD+collagen increases drug loading and slows subsequent release. The CD loaded collagen gel showed a 220% increase in drug loading compared to collagen alone. The ratio of fitted coefficients ACD/Acoll shows 56% of the release comes from CD while 43% comes from the collagen gel.

Poster Number: 44

Formulation for Controlled Self-Assembly of Polymer Nanoparticles

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Non-viral vehicles, such as polymer nanoparticles show promise as biomedical tools for the future of gene delivery. Polymer chemistry affords the ability to determine features of the self-assembled nanoparticle by tuning the monomeric sequence of the molecular backbone. Battelle has developed >6,000 novel polymers, and characterized their structure and function as gene delivery vehicles. Unlike lipids, many of these polymers can be directly dispersed in water, without the need for a solvent dilution processing step. However, exploration of formulation for these polymers still provides useful parameters for tuning the characteristics of the PNP. Here, we describe formulation techniques that enable further control over the characteristics and performance of these PNPs as gene delivery vehicles. We selected five polymers from our libraries synthesized via reversible addition-fragmentation chain transfer polymerization. We explored the self-assembly of these polymers into PNP using a direct hydration method as well as nanoprecipitation methods with and without genetic cargo. We measured the particle size via dynamic and static light scattering, exploring the effects of polymer concentration, rate of solvent removal, and payload loading on the nanostructures obtained. We demonstrated the use of nanoprecipitation to produce reproducible, stable PNPs, dispersed in water, that were not possible by directly hydrating the polymers. The mean particle size for the nucleic acid loaded PNPs obtained by nanoprecipition was 280 ± 7 nm for triplicate formulations. We extended this process to four other polymers, demonstrating applicability to a greater collection of our polymer library, and we showed the ability of the process to effectively load nucleic acids into these PNPs. This work demonstrates the capability nanoprecipitation to open the design space of Battelle's platform to produce and characterize libraries of PNPs with greater diversity and potential as gene delivery vehicles. We are able to use these methods to formulate PNPs loaded with genetic cargos that are not otherwise able to be studied, obtaining in vitro transfection and in vivo biodistribution data. These results lay a foundation to accelerate the development of non-viral gene delivery and new genetic therapies for untreatable and devastating diseases.

Poster Number: 45

Functionalized Engineered Extracellular Vesicles for targeted delivery to Intervertebral Disc Cells

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Introduction

Chronic low back pain is a leading cause of disability worldwide and often results from intervertebral disc (IVD) degeneration1. Our innovative approach involves using engineered extracellular vesicles (eEVs) loaded with transcription factors (TFs) and functionalized with cell specific transmembrane ligands to target nucleus pulposus cells (NP) and annulus fibrosus cell (AF) within the diseased IVD. This strategy uses eEVs as a drug delivery biomaterial to treat IVD degeneration.

Materials and Methods

In vitro, eEVs loaded with key developmental TFs and functionalized with Netrin-1 to target NP cells or with CD11a to target AF cell, were derived from adult human dermal fibroblasts after electroporation with expression plasmids for each TF and ligand2-4. Characterization involved nanoparticle tracking analysis, Western Blot, super resolution microscopy, and qRT-PCR to confirm eEV presence, and therapeutic TF and ligand packing. Fluorescently labeled eEVs were used to assess preferential uptake by human degenerate IVD cells (IRB 2015H038). Therapeutic efficacy of the functionalized TF loaded eEVs was evaluated via qRT-PCR analysis of catabolic, inflammatory, and pain markers in degenerate cells before and after exposure to eEVs loaded with TFs.

Results

Our research emphasizes the potential of using eEVs loaded with developmental TFs and functionalized with ligands as a biomaterial to target delivery to NP or AF. Characterization validated successful loading of developmental TFs and ligands. Confocal microscopy reveals that functionalized eEVs may be preferentially internalized by NP or AF cells. In contrast, non-functionalized EVs were captured nonspecifically. Additionally, our findings demonstrated successful reprogramming of NP and AF cells towards a healthier, pro-anabolic phenotype following exposure to eEVs, as confirmed by qRT-PCR and enhanced collagen production.

Conclusions

Our research demonstrates the potential of functionalized eEVs for efficient and selective delivery of developmental TFs to degenerate cells within the IVD, resulting in reprogramming of both cell types towards a healthier phenotype. These findings underscore the importance of further exploring eEV-based nanocarriers for targeting specific cell types. This research opens possibilities for tailored interventions in regenerative medicine.

Poster Number: 46

Development of a Physiologically Relevant Me-HA Hydrogels Incorporating Pluripotent-derived Neural Stem Cells for Spinal Cord Injuries

Katherine Bradshaw, University of Akron

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Spinal cord injuries (SCI) are traumatic events resulting in less than 1% of patients regaining neurological recovery. Common therapeutic models introduce neural stem cells (NSCs) into the injury area to regenerate damaged tissue. Previous work has shown poor NSC survival when introduced into an SCI without extracellular matrix like support. Methacrylated hyaluronic acid (Me–HA) hydrogels were fabricated and analyzed for compressive stiffness via Young's Elastic Modulus (EY, targeted in the range of 0.7 – 0.9 kPa to encourage neural differentiation and axonal growth) via Atomic Force Microscopy (AFM) indentation testing. RGD (arginine-glycine-aspartic acid) was integrated into Me-HA hydrogels by acrylate modified RGD peptides (GCGYGRGDSPG with N–terminal acryl addition) to Me-HA. Dual SMAD inhibition patterning was used to create neural progenitor cells (NPCs), NSCs, and spinal motor neurons (SMNs) from human induced pluripotent stem cells (hiPSCs). Me-HA hydrogels were successfully created and tested, and EY determined for all compositions. The RGD peptide was confirmed to be homogenously

incorporated throughout the Me-HA hydrogel. NPCs, NSCs, and SMNs, were successfully generated from hiPSCs, and verified via cyto-immunohistochemistry (IHC) for neural specific markers indicating the targeted populations were present. NPCs were positive for Nestin, SOX2, and Vimentin by Day 3. NSCs were positive for Nestin, PAX6, and SOX1 by Day 5. SMNs were generated by Day 20 and were positive for β–III Tubulin, Islet–1, and OLIG2. This initial research demonstrates the ability to create Me-HA hydrogels that are physiologically relevant to the CNS and for encouraging the differentiation of distinct neural cell populations to provide future therapeutic approaches for SCI. Further research is working towards combining the NPCs, NSCs, and SMNs, with the Me-HA hydrogels to investigate how the hydrogel impacts neurogenesis, differentiation, and axonal outgrowth.

Poster Number: 47

Investigation of the Spatiotemporal Changes in the Biomechanics of Injured Spinal Cord Using Atomic Force Microscopy

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Spinal cord injury (SCI) results in severe complications, including paralysis and secondary issues, primarily due to the formation of glial and fibrotic scars that create barriers to neuronal regeneration. This study aims to assess the mechanical properties of scar lesions after SCI using atomic force microscopy (AFM) and to understand how changes in tissue stiffness (Young's modulus, EY) impact cellular behavior. Cryo-sectioned spinal cord tissues from both injured and uninjured rats were analyzed at four time points: one day, one week, one month, and six months post-injury. The mechanical properties were measured under various conditions using a tipless AFM cantilever with a 20 μm bead. The study found that the grey matter's EY was approximately three times higher than that of white matter. In injured tissues, the average EY of grey and white matter one-month post-injury were 228.1 ± 255.6 Pa and 76.95 ± 118.8 Pa, respectively, with scar tissue exhibiting higher stiffness. Viscoelastic properties were also assessed, revealing increased initial stiffness in scar tissues due to denser fibrotic formations and altered viscoelastic behavior compared to uninjured tissues.

The spatial distribution of stiffness was mapped to visualize differences across injury sites. The results indicated that the stiffness of spinal cord glial scars decreased radially outward from the injury core. Stress-relaxation and oscillatory deformation tests provided insights into the time-dependent viscoelastic properties of the tissues. Injured tissues displayed higher instantaneous EY, reflecting increased stiffness and changes in storage and loss moduli. These findings underscore significant differences in the spatiotemporal Young's moduli between SCI scar tissues and controls, highlighting the importance of

biomechanical characterization in understanding tissue pathology. The study's results pave the way for developing therapeutic strategies focused on mechanical manipulation to repair injured tissues, presenting new opportunities for treatment advancements.

Poster Number: 48

Impact of Hyperglycemia on Vascular Smooth Muscle Cells and Implications for Type 2 Diabetes -Induced Cardiovascular Disease Treatment

Smriti Bohara, Cleveland State University

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Type 2 diabetes mellitus (T2DM) is a major public health concern with significant cardiovascular complications (CVD). Despite extensive epidemiological data, the molecular mechanisms relating hyperglycemia to CVD remain incompletely understood. Since this has huge implications in tissue engineering and regenerative medicine approaches, we here investigate the impact of chronic hyperglycemia on SMC phenotype and function using human aortic smooth muscle cells (HASMCs) cultured under varying glucose conditions in vitro, mimicking normal (5 mM/L), pre-diabetic (10 mM/L), and diabetic (20 mM/L) conditions, respectively. Patient-derived T2DM-SMCs were included for comparative analysis cultured for up to 21 days.

Results showed distinct morphological changes with significant increases in cell area, perimeter and F-actin expression in SMCs treated at higher glucose levels. Cell shape index was 0.23 ± 0.01 in SMCs exposed to 10 and 20 mM/L glucose, and 0.48 ± 0.03 for T2DM cells and normal SMCs (5 mM/L) ($p < 0.001$). AFM analysis showed significant reduction in the Young's modulus, membrane tether forces, membrane tension, and surface adhesive forces in SMCs with increasing glucose levels. In all these cases, T2DM cells exhibited levels noted in 20 mM/L glucose conditioned cells. A 5 to 6 -fold increase in cell proliferation was observed in SMCs treated with 20 mM/L glucose and in T2DM-SMCs, at day 7, compared to their original seeding density. T2DM-SMCs exhibited elevated levels of pro-inflammatory markers such as IL-6, IL-8, and MCP-1 compared to glucose-treated SMCs. Conversely, growth factors like FGF-2 and TGF-β were higher in SMCs exposed to moderate glucose but lower in T2DM-SMCs. Pathway enrichment analysis showed a significant increase in the expression of inflammatory cytokine-associated pathways, especially involving IL-10, IL-4 and IL-13 signaling in genes that are upregulated at higher glucose levels. Differentially regulated gene (DGE) analysis showed that, compared to SMCs cultured with normal glucose, 513 genes were upregulated, and 590 genes were downregulated in T2DM-SMCs; however, fewer genes (16 – 23) were differentially expressed in SMCs receiving 10 or 20 mM/L glucose. We identified the altered genes involved in ECM organization, elastic fiber synthesis and formation, and ECM proteoglycans, highlighting the role of hyperglycemia in CVD progression.

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2024 REGIONAL SYMPOSIA

September 19–20, 2024

FINAL PROGRAM

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2024 Regional Symposia

Southeast Symposium: Georgia Institute of Technology

September 19 - 20, 2024

Program Agenda

Thursday, September 19, 2024

7:30 AM - 8:30 AM	Registration and Continental Breakfast
8:30 AM - 8:45 AM	Welcome by Site Chairs
8:45 AM - 10:00 AM	Session I: Nanomaterials ***Invited Speaker: Evan Scott, University of Virginia***
10:00 AM - 10:15 AM	Coffee Break
10:15 AM - 11:30 AM	Session II: Biomaterial-Tissue Interaction ***Invited Speaker:*** ***Andrés J. García, Georgia Institute of Technology***
11:30 AM - 12:45 PM	Plenary Session I: ***Shana Kelley, Northwestern University*** ***Joel Collier, Duke University*** *(2024 Clemson Award for Basic Research Recipient)*
12:45 PM - 1:45 PM	Lunch and Poster Session (#1 - 48)
1:45 PM - 3:00 PM	Session III: Immune Engineering 1 ***Invited Speaker: Jennifer Elisseeff, Johns Hopkins Univeristy***

Program Agenda

Thursday, September 19, 2024 *(continued)*

3:00 PM - 4:15 PM	Session IV: Drug Delivery 1 ***Invited Speaker: Thomas Werfel, University of Mississippi***
4:15 PM - 5:30 PM	Plenary Session II: ***Sarah Stabenfeldt, Arizona State University*** ***Danielle Benoit, University of Oregon***
5:30 PM - 6:45 PM	Session V: Tissue Engineering 1 ***Invited Speaker: Cherie Stabler, University of Florida***
7:00 PM - 9:30 PM	Dinner Reception

Program Agenda

Friday, September 20, 2024

8:15 AM - 8:45 AM	Registration and Continental Breakfast
8:45 AM - 10:00 AM	Session VI: Engineering Cells and Their Microenvironments
10:00 AM - 10:15 AM	Coffee Break
10:15 AM - 11:30 AM	Session VII: Immune Engineering 2 ***Invited Speaker: Marian Ackun-Farmmer, University of Maryland/Georgia Institute of Technology***
11:30 AM - 12:45 PM	Plenary Session III: ***Elazer Edelman, Massachusettes Institute of Technology*** *(2024 Founders Award Recipient)* ***Cynthia Reinhart-King, Rice University***
12:45 PM - 1:45 PM	Lunch and Poster Session (#49 - 91)
1:45 PM - 3:00 PM	Session VIII: BioInterfaces ***Invited Speaker:*** ***Juhi Samal, University of Alabama at Birmingham***
3:00 PM - 4:15 PM	Session IX: Drug Delivery 2 ***Invited Speaker: Jarrod Call, University of Georgia***

Program Agenda

Friday, September 20, 2024 *(continued)*

4:15 PM - 5:30 PM	Session X: Tissue Engineering 2 ***Invited Speaker: Daniel Abebayehu, University of Virginia***
5:30 PM - 6:45 PM	Concurrent Session XI: "POSTAR" Award Rapid Fire Talks

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Anita Shukla, PhD, Brown University

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John Wilson, PhD, Vanderbilt University

2024 Society for Biomaterials (SFB) Southeast Symposia, Georgia Tech, Atlanta, GA

Plenary Speaker

Dr. Joel Collier
Duke University
(Clemson Award for Basic Research)

Invited Speaker

Dr. Jennifer Elisseeff
Johns Hopkins University

Invited Speaker

Dr. Cherie Stabler
University of Florida

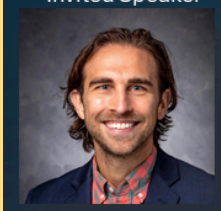
Invited Speaker

Dr. Andrés J. García
Georgia Institute of Technology

Invited Speaker

Dr. Evan Scott
University of Virginia

Invited Speaker



Dr. Jarrod Call
University of Georgia

Invited Speaker

Dr. Daniel Abebayehu
University of Virginia

Invited Speaker

Dr. Thomas Werfel
University of Mississippi

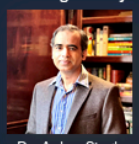
Invited Speaker

Dr. Juhi Samal
University of Alabama at Birmingham

Invited Speaker

Dr. Marian Ackun-Farmmer
Georgia Institute of Technology

Regional Symposia Co-Chairs



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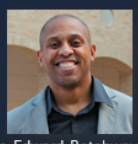

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PLENARY SESSION I
Thursday, September 19

Dr. Shana Kelley
Northwestern University

PLENARY SESSION I
Thursday, September 19

Dr. Joel Collier
Duke University
(2024 Clemson Award for Basic Research)

PLENARY SESSION II
Thursday, September 19

Dr. Sarah Stabenfeldt
Arizona State University

PLENARY SESSION II
Thursday, September 19

Dr. Danielle Benoit
University of Oregon

PLENARY SESSION III
Friday, September 20

Dr. Elazer Edelman
Massachusetts Institute of Technology
(2024 Founders Award)

PLENARY SESSION III
Friday, September 20

Dr. Cynthia Reinhart-King
Rice University

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2024 Regional Symposia

September 19th - 20th

- **Northeast: Northeastern University**
- **Midwest: Case Western Reserve University**
- **Southeast: Georgia Institute of Technology**
- **Southwest: University of Texas at Austin**
- **Western: University of Colorado, Denver | Anschutz Medical Campus**
- **Northwest: University of Washington**

SESSION I: NANOMATERIALS

8:45 AM - 9:10 AM **Invited Speaker: Evan Scott, UVA/NorthWestern**

9:10 AM - 9:22 AM

Ingestible Molecular Probes for Breath-Based Detection of Gastrointestinal Disease

Vishal Manickam, Georgia Institute of Technology

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Amy Kang, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA, akang60@gatech.edu

Leslie W. Chan, Ph.D., Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA, Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, 30332, USA, leslie.chan@gatech.edu

Endoscopic imaging is the gold standard for the detection and clinical assessment of many gastrointestinal (GI) diseases including GI cancers, ulcers, and inflammatory bowel disease. However, patient discomfort, potential complications with bowel perforation, cost, and length of the procedure deter individuals from recommended testing. The invasiveness of endoscopy also makes it an unsuitable tool for routine monitoring. Breath tests are non-invasive alternatives that are quick and can be completed with ease at great frequency and in a variety of settings for monitoring applications. Therefore, we have established a diagnostic platform that can induce exhalation of synthetic breath biomarkers that reflect disease activity in the GI tract.

Our platform is comprised of a new class of ingestible molecular probes that are metabolized by aberrant intestinal glycosidase activities into volatile (i.e gaseous) reporters that are exhaled in breath. Glycosidases are enzymes that hydrolyze glycosidic bonds in carbohydrates, and are essential for digestion, host immunity, and ECM remodeling. During GI disease, activities of certain host and microbiome glycosidases are altered. Therefore, we have developed ingestible sugar-based probes that transit the GI tract and release volatile reporters upon breakdown by specific intestinal glycosidases. Liberated reporters diffuse across the intestinal epithelium to enter systemic circulation and are detectable in breath after pulmonary gas exchange. Reporter trafficking to breath occurs in minutes, providing near real-time readout for probe activity.

Probes have been synthesized for glycosidases with altered activity in colorectal cancer (CRC). Probes were incubated with recombinantly-expressed glycosidases in cleavage assays, which confirmed that (1) probes are specifically cleaved by targeted glycosidases and (2) release volatile reporters, which are detectable via mass spectrometry. Cleavage assays using mouse tissue homogenates also confirmed

probe stability in the GI tract. In mouse studies, breath was collected 0-1.5h and 1.5-3h after oral probe delivery, the time intervals during which probes transit the small and large intestine, respectively. Exhaled reporter levels accurately reflected glycosidase activities in each GI segment. Preliminary studies demonstrate differential breath signals in healthy versus CRC mouse models. Altogether, we have developed ingestible probes that have the potential for broad utility in breath-based detection of GI diseases.

9:22 AM - 9:34 AM

Engineering protein self-assemblies through sequence-level tuning of thermal hysteresis

Kai Littlejohn, Georgia Institute of Technology-Emory University

Felipe Quiroz, Ph.D., Coulter Department of Biomedical Engineering, Georgia Institute of Technology-Emory University, felipe.quiroz@emory.edu

Intrinsically disordered proteins (IDPs) diverge from the traditional sequence-3D structure paradigm. Various IDPs self-assemble through stimulus-triggered phase separation, facilitating the formation of intracellular and extracellular structures. Recent advances in IDP engineering suggest the potential for fine-tuning IDP sequence and composition to control the dynamics and mechanical properties of IDP-assemblies. Recently, our group discovered that IDPs can be genetically engineered to access a range of nonequilibrium phase behaviors, such as thermal hysteresis, demonstrating its utility to program the stability of IDP nano-assemblies. However, the tunability of these hysteretic behaviors remains incipient. Here, we report the first observations of a sequence-level molecular dial of thermal hysteresis. To study this phenomenon, we exploit the repetitive architecture of IDP polymers (IDPPs). Previously, we developed thermoresponsive elastin-like IDPPs characterized by their degree of thermal hysteresis. Surprisingly, adding a polar residue (threonine) to a hydrophobic elastin-like motif led to IDPPs with the greatest hysteresis. Informed by a nanoscopic view of rehydration dynamics in hysteretic IDPPs, we posited that varying hydrogen bonding capability, by substituting threonine in the repeat unit with other polar residues (serine, asparagine and glutamine), would modulate hysteresis. To determine the length dependence of this behavior, we engineered, purified and characterized 40- and 80-mer IDPPs for each motif. UV–visible spectrophotometry revealed that increasingly polar IDPPs showed upward shifts in the phase transition temperature upon heating, enlarging the observable range of thermal hysteresis upon cooling. For large IDPPs, overall length was a weak modulator of hysteresis. Unexpectedly, near the phase transition temperature, controlled cooling of hysteretic IDPPs triggered the rapid growth of IDPP assemblies that also exhibited thermal hysteresis, exposing a novel self-assembly route that was inaccessible for conventional elastin-like IDPPs. Next, we applied our hysteretic dial to program the stability of IDP nano-assemblies. We engineered di-block IDPPs fusing a corona-forming IDPP encoding negligible hysteresis and hysteretic core IDPPs encoding large hysteresis. Our preliminary data suggest that tuning hydrogen bonding in hysteretic IDPPs enables potent stabilization of the cores of monodisperse IDPP nano-assemblies. Our findings demonstrate sequence heuristics to program thermal hysteresis in IDPPs, priming their use in bottom-up design of out-of-equilibrium IDP assemblies.

9:34 AM - 9:46 AM

Hysteretic phase separation dynamics as new variable for highly stable protein nanoparticle design

Alexa Regina Avecilla, Wallace H. Coulter Department of Biomedical Engineering at Emory University and Georgia Tech

Alexa Regina Chua Avecilla (alexa.avecilla@emory.edu; Wallace H. Coulter Department of Biomedical Engineering at Emory University and Georgia Tech), Felipe Garcia Quiroz (felipe.quiroz@emory.edu; Wallace H. Coulter Department of Biomedical Engineering at Emory University and Georgia Tech)

Phase-separation-driven assembly has enabled the engineering of protein-based nanostructures for drug delivery. Driving this progress are intrinsically disordered protein polymers (IDPPs) that reversibly phase transition above tunable critical temperatures. By engineering hydrophobic IDPPs fused to hydrophilic IDPPs, in a core-corona diblock architecture, IDPP nano-assembly can be guaranteed at body temperature. However, because phase-separated nano-cores exist in equilibrium, IDPP nanoparticles favor disassembly upon dilution —unavoidable for injectables. The recent discovery of non-equilibrium hysteretic phase transitions suggests a new variable to stabilize IDPP nanoparticles. Hysteretic IDPPs undergo phase separation upon heating above a critical temperature but require significant cooling below this temperature to regain solubility. Here, by recombinantly fusing IDPPs with divergent hysteretic behaviors to a common hydrophilic (non-hysteretic) IDPP, we engineered novel self-assembling hysteretic IDPP nanoparticles and characterized their nanomorphology and stability against conventional IDPP nanoparticles under physiologically-relevant conditions. Following temperature-dependent IDPP self-assembly with cryo-electron microscopy (Cryo-TEM) and UV-vis absorbance measurements, we captured two distinct assembly regimes: (i) nanoparticles, upon heating past the critical transition temperature of the core-forming IDPP (Tc1) and (ii) bulk phase separation, upon heating above the critical point of the corona-forming IDPP (Tc2, where Tc2>Tc1). Hysteresis determined the extent of sustained nanoparticle assembly below the initial Tc1~45°C, with moderately-hysteretic and highly-hysteretic nanoparticles respectively disassembling at 20 and 30°C below Tc1. This enhanced stability was also evident as a function of time at temperatures well below Tc1, resulting in fully stable nanoparticles at 37°C over at least 15 hours. Notably, hysteresis sustained nanoparticle stability as samples were diluted 25-fold to 1 μM. In contrast, non-hysteretic IDPPs (Tc1=30 °C) that readily accessed nanoparticle regimes at body temperature showed expected progressive shifts in Tc1 upon dilution. After a 25-fold dilution at 37°C, these non-hysteretic nanoparticles bordered on complete disassembly. Cryo-TEM also captured differences in nanoparticle stability under cryogenic conditions, with hysteretic IDPP diblocks consistently yielding high-contrast nanoparticles. As expected for the relatively slow process of vitrification (~ 1 ms), non-hysteretic diblocks failed to sustain nanoparticle assembly. Overall, our new findings demonstrate thermal hysteresis as a new engineering variable, separate from hydrophobicity, for the engineering of highly stable protein-based nanomaterials.

9:46 AM - 9:58 AM

Neonatal Fibrin Based Nanoparticles for Wound Healing

Sanika Pandit, Joint Department of Biomedical Engineering at UNC Chapel Hill and NC State

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Ashley Brown, aecarso2@ncsu.edu, Joint Department of Biomedical Engineering, North Carolina State University and the University of North Carolina at Chapel-Hill, Raleigh, NC; Comparative Medicine Institute, NC State University, Raleigh, NC

Chronic non-healing wounds pose a significant clinical problem due to clotting deficiency and persistent inflammation. This deficiency in clotting results in a poorly formed fibrin matrix, which can contribute to the formation of non-healing wounds. In such cases, it is important to speed up the wound healing process. We have previously developed colloidal fibrin-based nanoparticles constructed from adult fibrinogen (aFBNs) that are pre-polymerized and use physiologically relevant thrombin and fibrin concentrations, unlike traditional fibrin glues typically used to treat non-healing wounds. The working time of the FBNs can be tuned by particle density, and the particles can be lyophilized and stored at room temperature. Previous work has shown that bulk neonatal fibrin scaffolds enhance in vitro wound healing.

To enhance wound healing and promote cell activity, we developed and evaluated colloidal neonatal fibrin-based nanoparticles (nFBNs) for wound healing applications. nFBNs can successfully be synthesized to comparable sizes of aFBNs. As compared to aFBNs, nFBNs are more effective in increasing fiber density within a fibrin clot. When incorporated into fibrin clots, nFBNs significantly impacted overall clot structure, resulting in decreased pore area and increased number of overall pores. Incorporating nFBNs into fibrin clots did not significantly impact the stiffness of the clots. However, nFBNs did slow clotting time as compared to aFBNs. Overall, aFBNs clotted faster and showed a dose dependent increase in clotting speed; no such relationship was seen with the nFBNs. nFBNs also promote cell attachment in a fibrin clot. Additionally, nFBNs enhance cell spreading and migration within a fibrin clot, as compared to aFBNs. To assess the wound healing capabilities of the nFBNs in vivo, full-thickness dermal wounds were created on diabetic mice and assessed over nine days. nFBNs increased the rate of wound healing and led to better healing outcomes, which were confirmed by transcriptomic analysis. Overall, nFBNs have immense potential as a wound healing therapeutic.

SESSION II: BIOMATERIAL-TISSUE INTERACTION

10:15 AM – 10:40 AM Invited Speaker: Andrés J. García, Georgia Institute of Technology

10:40 AM – 10:52 AM

Invited Speaker: Juan Mendenhall, Morehouse College, *Using Additive Manufacturing to Engineer Synthetic Tissues with Functional Gradients at Various Length Scales*

10:52 AM - 11:04 AM

Comparative proteomics analysis of wounds treated with microporous annealed particle scaffolds

Alejandra Suarez Arnedo, Duke University

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Microporous annealed particles (MAP) scaffolds have been shown to promote a unique regenerative response in skin wound healing. Specifically, MAP comprised of D-chiral crosslinkers (D-MAP) impart a wound healing response mediated by an adaptive immune response. However, the mechanism of action is yet to be elucidated. Using proteomic analysis based on LC-mass spectrometry, we identified 5,000 proteins common across different wound treatment groups, namely L-chiral MAP (L-MAP), D-MAP, and WounDres (a commercial control). In subsequent gene ontology analysis. we identified genetic pathways that are upregulated in different treatment groups. At early stage (Day 4), D-MAP-treated wounds showed an enrichment of multiple immune response processes, such as the adaptive immune response, complement activation, and lymphocyte development. In contrast, L-MAP promoted processes linked to keratinization and skin development. At Day 14, muscle contraction associated processes and keratinization were highly enriched in D-MAP, while L-MAP showed over presentation of functions associated with collagen organization and other extracellular matrix components. As the wounds progressed toward resolution (Day 21), we observed high enriched in keratinization, muscle contraction and low enrichment in immune specific terms when compared both D-MAP and L-MAP against WounDres. When we looked at the actual wound healing outcomes, D-MAP-treated wounds presented more appendages like structures, while L-MAP-treated wounds have extracellular matrix than resembles the architecture of normal skin. These results further elucidated the differences in regenerative characteristics between L- and D-MAP, as we characterized genetic pathway regulation as one of the key factors that contribute to a divergent wound healing response. Multiple temporal specific processes (e.g., adaptive immune response) contribute to the resolution of the MAP-treated wound and that functions are context in material specific when we compared MAP based on crosslinkers of different chirality. This study also showcased the power of proteomic analysis, with which we were able to uncover the relevance of the timing and the strength of which biological functions are enriched.

11:04 AM - 11:16 AM

Effects of Heparin Hydrogel Sulfation Level on TSG-6 Release and Tissue Response After Supraspinatus Muscle Injury

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Tumor necrosis factor stimulated gene 6 (TSG-6) is a broadly anti-inflammatory protein that can help polarize macrophages towards anti-inflammatory phenotypes (M2). Its actions can be altered by complexing with glycosaminoglycans. In particular, fully sulfated heparin (Hep) potentiates the anti-plasmin effects of TSG-6 compared to desulfated heparin (Hep-). Rotator cuff tears can cause chronic muscle inflammation and degeneration that increases over time. We hypothesized that Hep + TSG-6 would promote muscle regeneration spatiotemporally after rotator cuff tear.

First, poly(ethylene glycol) (PEG) was conjugated with an MMP-cleavable peptide. Heparin sodium salt (Hep) and desulfated heparin derivative (Hep-) were prepared . The conjugated PEG and heparin gels were formed by free radical polymerization, fragmented to form particles, then injected into the rat supraspinatus muscle after rotator cuff injury. In vivo imaging evaluated TSG-6 release from Hep and Hep- systems for 21 days (n=6). In vivo inflammatory cell response was assessed through flow cytometry (n=8, days 3 and 7) and myogenic response through immunohistochemistry (n=5, days 7 and 14) for three groups: Saline injection, Hep+TSG-6 (Hep) and Hep-+TSG-6 (Hep-).

In vivo, similar rates of controlled release of TSG-6 were found from Hep and Hep- for 14+ days. Hep showed higher overall and type 2a, anti-inflammatory macrophages on day 7, (Hep: 2 +/- 1.3% cells, Hep-: 0.65 +/- 0.34%, Saline 0.54 +/- 0.3%) and increased embryonic myosin heavy chain (eMHC) positive fibers on day 7 compared to saline (Hep: 125 +/- 14 cm-2, Hep-: 69 +/- 80 cm-2, Saline: 5 +/- 15 cm-2). On day 14, Hep demonstrated significantly increased centrally located nuclei (CLN) compared to all groups (Hep: 560 +/- 205 cm-2, Hep-: 210 +/- 40 cm-2, Saline: 120 +/- 80 cm-2). Additionally, asymmetrical muscle regeneration was observed: samples in the myotendinous junction or near intramuscular tendon showed more eMHC staining on day 7 and more CLN on day 14 compared to samples from the muscle belly or far from intramuscular tendon.

Together, these results demonstrated that TSG-6 release from fully sulfated heparin resulted in increased overall anti-inflammatory cellular response and enhanced early muscle regeneration particularly in particular regions of this muscle injury model.

11:16 AM - 11:28 AM

Aligned, 3D, and electrically conductive multicompartment collagen-glycosaminoglycan scaffolds for musculotendinous tissue engineering in volumetric muscle loss

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Skeletal muscle, composed of 3D, highly aligned, and electrically excitable muscle fibers, possesses an endogenous regeneration ability that aids in minor injury repair. However, this inherent regenerative limit is exceeded in the case of a volumetric muscle loss (VML) injury, due to the traumatic loss and damage of large amounts of muscle tissue. Tissue engineered scaffolds with combination of cells and conductive polymer particles are emerging due to the lack of effective treatment methods for VML injuries. However, fabricating biomaterials combining 3D structural alignment and electrical conductivity found in native skeletal muscle is challenging. Additionally, VML injuries are often associated with polytraumatic and accompanied by damage to the musculotendinous junction, adding complexity to the necessary treatment. We developed collagen-glycosaminoglycan scaffolds with an aligned microporous structure using a directional freeze-drying approach. 'Muscle' and 'tendon' compartments with a smooth interface mimicking the native interface are made by layering a type I collagen suspension with and without conductive polymer particles respectively. Polypyrrole (PPy) particles were synthesized and incorporated in type I collagen suspension to make the 'muscle' compartment of the scaffold electrically conductive. Scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS) techniques showed the successful formation of scaffolds with longitudinally aligned micropores due to directional freeze-drying and uniform distribution of conductive PPy particles respectively. C2C12 myoblasts and 3T3 fibroblasts cultured on muscle and tendon compartments respectively showed significantly increased metabolic activity (measured non-destructively using an alamarBlue assay) over 11 days of culture in growth and differentiation media. Both the compartments demonstrated enhanced cell infiltration, with the 'muscle' compartment showing positive myosin heavy chain (MHC) expression, indicative of myogenic differentiation. Our current research focuses on synthesizing nanoscale polypyrrole particles to enhance conductivity and promote phagocytosis/clearance in vivo. Furthermore, we are investigating the mechanical properties of multicompartment scaffolds and their ability to support differentiation of multiple cell types.

SESSION III: IMMUNE ENGINEERING 1

1:45 PM – 2:10 PM Invited Speaker: Jennifer Elisseeff, JHU

2:10 PM - 2:22 PM

PEG-4MAL Synthetic Immune Tissues for In Vitro Recapitulation of Adaptive Immune Response to E. Coli Bacterial Lysate

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Along the intestine, secondary lymphoid organs named Peyer's Patches (PPs) house adaptive immune cells which play an important role in human health and disease. B and T cells specifically help to orchestrate immune responses in the PP that generate memory and responses to bacteria in the gut, which lead to secretion of IgA. Here, we report synthetic immune organoids for evaluation of immunological changes when exposed to Escherichia coli (E. Coli), a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium commonly found in the intestine. The lymphoid organoid is generated culturing isolated human tonsil mononuclear cells within PEG-4MAL hydrogels with tissue specific bio adhesive cues, like fibronectin and collagen peptide mimics, RGD and GFOGER. Using this platform, we have evaluated increases in inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12p70, IFN-α2, IFN-β, IFN-λ1, IFN-λ2/3, IFN-γ, TNF-α, IP-10, GM-CSF), secreted immunoglobulin isotypes (IgG1, IgG2, IgG3, IgE, IgG4, IgA, IgM, IgD) as well as flow cytometry markers of the germinal center response (CD45, CD19, CD38, CD27, CD138, BCL-6). From these outputs, we have observed that by Day 10 we are able to obtain a significantly higher expression of sIgA levels in collected media in comparison to naïve lymphoid organoids (One Way ANOVA, $p = 0.0416$) as well as E. Coli lysate treated lymphoid organoids at day 4 (One Way ANOVA, $p=0.0190$). Flow cytometry analysis of isolated lymphoid cells indicates that cells retain high cell viability and increase in mature germinal center and plasma cell phenotypes, CD19+ CD27+ CD38+ and CD19-CD138+. We also see significant increases in inflammatory cytokines related to infection, especially with IL-6, IL-8, and GM-CSF. Lymphoid organoids fixed and imaged for CD19 and AID, a marker of germinal center response, show increase in AID. These results preliminarily demonstrate the efficacy of our system in recreating immune responses to bacterial inflammatory processes in vitro and hold promise in screening the effects of bacterium on adaptive immunity.

2:22 PM - 2:34 PM

RIG-I Activating Nanoparticles for Treatment of Glioblastoma

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Introduction: Glioblastoma (GBM) is a rare form of brain cancer with a dismal outlook for patients who face a mean survival time of 12-18 months post-diagnosis. Higher levels of the pattern recognition receptor retinoic acid-inducible gene-I receptor (RIG-I) are associated with improved survival outcomes in GBM, but RIG-I immunotherapy has not yet been explored for GBM. The clinical utility of 3pRNA RIG-I agonists is currently limited by significant drug delivery barriers, which we will seek to overcome using RIG-I activating nanoparticles (RANs).

Methods and Materials: Di-block polymer was formed with reversible addition–fragmentation chain transfer (polymerization using a first block of 10K polyethylene glycol and a second block of poly[(DMAEMA-c-butyl methacrylate. The di-block copolymer was dissolved in ethanol, diluted with sterile-filtered citrate buffer (pH=4.2), and complexed with RNA for 45 minutes to form micelles. Mouse Glioma261 (GL261) or CT2A cells were treated with RANs, then 24 hours later, supernatants were collected for ELISA, and cells were stained for flow cytometry. Mice were inoculated with 1,000,000 GL261 or CT2A cells on the right flank and treated intratumorally with RANS at a tumor volume of ~50 mm3 and on days 3 and 6 post treatment initiation. Alternatively, mice were engrafted with 200,000 GL261 cells in the right striatum at the following coordinates: +1.00 mm anterior, 2.0 mm lateral, and 3.00 mm deep.

Results, Discussion, and Conclusions: RANs were ~90 nm in diameter. We show that GL261 and CT2A cells treated in vitro with RANs produced significantly more interferon beta (IFN-β) and had increased MHC I expression following RAN treatment compared to cells treated with control RNA-loaded RANs (cRANs). In addition to cancer cells, RANs also activate antigen presenting cells, with THP1-Dual (monocytes) and RAW-Dual (macrophages) producing more type one IFN in response to RAN treatment. Following intratumoral treatment of GL261 and CT2A flank tumors with RANs, mice had prolonged survival with cRANs providing no benefit compared to vehicle control. We have successfully engrafted GL261 tumors and immunophenotyped the tumor and surrounding brain tissue. We demonstrate that RANs can activate multiple cell types and show efficacy against flank tumors.

2:34 PM - 2:46 PM

Improved transplant survival of human stem cell derived beta-cells overexpressing PD-L1 in a human HLA mouse model of autoimmune diabetes.

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Type 1 diabetes (T1D) is an autoimmune disease characterized by T-cell mediated destruction of pancreatic beta-cells. Human stem-cell derived beta cells (sBCs) provide an abundant and renewable cell source for the replacement of these destroyed beta-cells. However, there is a need for localized immune tolerance to counter autoimmunity without the complications involved with systemic immunosuppression. We investigated whether sBCs engineered to overexpress the immune checkpoint molecule PD-L1 or that are knockout for HLA class I molecules could achieve localized protection against autoimmunity. We tested these engineered sBCs in a human HLA mouse model of autoimmune diabetes. NOD-HDD mice develop spontaneous diabetes, but express human HLA class I A2 instead of mouse MHC class I, such that islet-reactive mouse CD8+ T cells can mount an autoimmune attack in vivo compatible with human class I HLA. We have further developed this model by isolating T cells from spleens of diabetic NOD-HHD mice and adoptively transferring them into immune deficient NSG-HHD mice that also harbor human HLA Class I, thus transferring diabetes and challenging sBCs grafted under the kidney capsule. In the adoptively transferred NSG-HHD mice, there was improved survival of the sBCs overexpressing PD-L1 but eventual rejection of all sBC grafts by 3 weeks. As our strategy was primarily designed to control CD8+ T cells, we attributed the lack of long-lasting tolerance to graft infiltration by both CD4+ and CD8+ T-cells, indicating a need for a synergistic tolerance strategy that can also regulate the CD4+ T-cells.

From here, we developed an approach to extend presentation of T cell modulators (e.g. TRAIL) into the graft microenvironment using biomaterials engineering. To this end, we developed a granular hydrogel based on 4-arm polyethylene glycol (PEG) maleimide microparticles. Briefly, PEG-4MAL microgels with PEG-dithiol crosslinker were generated using a single-emulsion approach resulting in microspheres with an average diameter of 10-20 µm. These particles were further surface functionalized with immunomodulators using click chemistry approaches. We are currently conducting studies to investigate the synergistic ability of the biomaterials combined with engineered sBCs to control graft autoimmune rejection in the human HLA diabetic mice.

2:46 PM - 2:58 PM

STING Activating Polymer Drug Conjugates for Cancer Immunotherapy

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Cancer remains the second leading cause of death in the United States. Immune checkpoint blockade (ICB) is revolutionizing treatment of diverse cancer types; however, these treatments only benefit a minority of cancer patients. Low response rates are correlated to immunogenically “cold” tumors, meaning they lack sufficient tumor antigen-specific CD8+ T-cell infiltration and other proinflammatory cell types. Therefore, there is a need for therapeutic systems that will shift tumors towards an immunogenically “hot,” environment, increasing antitumor innate immunity and enhancing responses to immunotherapy. This may be acheived through targeting the stimulator of interferon genes (STING) pathway. STING activation triggers a type I interferon (IFN-I)-driven inflammatory response, which stimulates dendritic cell cross-presentation of tumor antigens, leading to mobilization of tumor-specific CD8+ T cells, which reduce tumor progression and can lead to immunological memory.

STING agonists are being explored as next generation cancer therapeutics but are limited due to poor drug-like properties such as low bioavailability, high renal clearance, and off target toxicities. A dimeric amidobenzimidazole (diABZI) STING agonist developed by GlaxoSmithKline demonstrates promising antitumor effects through systemic administration; however, diABZI lacks tumor specificity and activates STING indiscriminately. Therefore, we have developed SAPCon, a STING activating polymer-drug conjugate, for the delivery of a chemically-modified diABZI that allows for enhanced circulation, tumor-targeting and environmentally-responsive drug release. diABZI-V/C-DBCO was designed for chemical-conjugation to the platform and intracellular drug release through cathepsin cleavage. The polymeric carrier platforms were synthesized through reversible addition- fragmentation chain-transfer (RAFT) polymerization and were conjugated to the STING agonist through copper-free click chemistry. Conjugation of the drug to various molecular weight polymers significantly improves pharmacokinetics and biodistribution, resulting in diminished tumor burden and prolonged survival in murine breast cancer models. These treatments successful shift the tumor microenvironment to a proinflammatory phenotype and synergize with approved ICB, opening an exciting door for the next generation breast cancer treatment.

SESSION IV: DRUG DELIVERY 1

3:00 PM - 3:25 PM

Glycopolymers enrich less immunogenic protein coronas and improve tumor delivery compared to PEG

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PEGylated nanoparticles remain the most widely employed vehicles for delivering cargo such as chemotherapeutics, targeted therapies, or nucleic acids to tumors. However, it is being increasingly appreciated that PEG causes immunogenic reactions and is susceptible to rapid clearance via the binding of anti-PEG antibodies. Therefore, as an alternative to nanoparticle PEGylation, we engineered nanoparticles with a glycopolymer-based corona. The particles are composed of a diblock copolymer, poly(2-(diisopropylamino)ethyl methacrylate)-b-poly(methacrylamidoglucopyranose) [PDPA-b-PMAG], that is synthesized via reversible addition-fragmentation chain transfer (RAFT) controlled radical polymerization. In this study, we compared the physicochemical characteristics, biocompatibility, cell uptake, protein adsorption, and protein corona composition of PDPA-b-PMAG and PDPA-b-PEG NPs. We found that PDPA-b-PMAG and PDPA-b-PEG NPs could be produced with similar physicochemical characteristics (e.g., size, surface charge, and morphology) and cellular biocompatibility. However, these NPs exhibited vastly different biological interactions which were dependent upon the presence of the NP protein corona. Compared to PDPA-b-PEG NPs, PDPA-b-PMAG NPs exhibited roughly 5-fold higher uptake in MDA-MB-231 breast cancer cells in cell culture. Moreover, the PDPA-b-PMAG NPs also exhibited significantly less uptake in RAW 264.7 macrophages than PDPA-b-PEG NPs. These differences were exacerbated further in the presence of human serum because pretreatment of the NPs with human serum further increased the uptake of PDPA-b-PEG NPs in RAW 264.7 macrophages while PDPA-b-PMAG NP uptake in the macrophages reduced further. Upon analysis of the protein coronas of each NP formulation, it became clear that PDPA-b-PMAG NPs adsorb less immunogenic proteins than PDPA-b-PEG. First, SDS-PAGE and BCA experiments showed that PDPA-b-PMAG NPs adsorb significantly less protein than PDPA-b-PEG NPs. Moreover, the abundance of IgG-class and complement proteins was reduced ~5-fold and ~10-fold, respectively, on the surface of PDPA-b-PMAG NPs compared to PDPA-b-PEG NPs. These early results suggest that glycopolymers such as PMAG have great promise as less-immunogenic alternatives to PEG in nanomedicine. Our future studies will assess the in vivo delivery of PMAG-based NPs to confirm their utility as improved delivery vehicles for cancer and other diseases.

3:25 PM - 3:37 PM

Controlled release of rhBMP2 from porous tissue engineering graft promote bone regeneration in a rat spinal fusion model

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With a notable 118% surge in the last two decades, incidences of lumbar spinal fusion procedures in the United States are experiencing a remarkable upward trend. However, currently available FDA-approved collagen-based Infuse™ spinal fusion bone graft has been associated with serious complications including heterotopic ossification, paralysis, bowel/bladder dysfunction and local inflammation due to the presence of higher doses of recombinant human bone morphogenetic protein (rhBMP2), a potent osteogenic growth factor. These challenges have heightened the urgent need for novel spinal fusion bone grafts that offer greater fusion rate and superior clinical outcomes without the risk of adverse side effects. To address this, we have developed a novel porous biomimetic bone graft combining a pro-regenerative porcine small intestinal submucosa derived extracellular matrix (SIS-ECM) with an osteoinductive biphasic mixture of calcium phosphates (20% hydroxyapatite and 80% beta-tricalcium phosphate) in a 1:2 organic and inorganic ratio, closely resembling the molecular composition of native bone. Furthermore, this graft incorporates rhBMP2 encapsulated in a biodegradable polyelectrolyte polymer nanoparticle for controlled release of the growth factor over an extended period. This porous graft exhibits enhanced cellular attachment, proliferation and upregulation of osteogenic differentiation markers compared to the conventional collagen sponges. When implanted in a clinically translatable rat posterolateral lumbar spinal fusion model, the spinal graft accelerates bone formation and remodeling leading to early bony union after 8 weeks as shown by microcomputed tomography (μCT), histological analysis, and mechanical testing. Notably, the graft exhibits higher total and percentage bone volume, trabecular number as well as smaller trabecular separation compared to the control collagen sponge infused with 10-fold higher concentration of rhBMP2. Histological evidence by H&E and Masson's trichrome further confirms continuous bridging of bone this biomimetic bone graft whereas fibrotic tissue with nonunion could be seen for collagen sponges with the same amount of rhBMP2. Immunohistochemical staining and flow cytometry analysis of the post-operative tissue did not reveal any significant adverse immune reaction against the novel biomimetic graft. These findings underscore the promising potential of our novel biomimetic spinal fusion scaffold in advancing spinal fusion surgeries by promoting faster fusion rates and accelerated bone regeneration.

3:37 PM - 3:49 PM

Enhanced Delivery and Gene-silencing in Brain Border-Associated Macrophages using Lipid-siRNA Conjugates

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Despite the worldwide increase in the incidence of Alzheimer's Disease (AD), robust therapeutics to treat its underlying causes are lacking. There is increasing evidence that neuro-immune crosstalk plays a pivotal role in the development of neurodegenerative diseases such as AD. In particular, brain border-associated macrophages (BAMs), which are situated in the meninges, choroid plexus, and perivascular space, are emerging as critical effectors in these diseases. However, current strategies to therapeutically target BAMs do not exist. Nucleic acid therapeutics, such as short interfering RNA (siRNA), can induce potent and durable knockdown in specific gene targets. These treatments are commonly administered via the cerebrospinal fluid (CSF). We have developed a lipid-siRNA conjugate (termed EG18), which exhibits improved transport through the CSF into the perivascular space, choroid plexus, and parenchyma, and induces potent gene silencing across the CNS, including BAMs. Following injection into the lateral ventricles, EG18 distributes along CSF flow pathways, including perivascular spaces. The perivascular space is a fluid filled space which surrounds arterioles and venules in the brain, is involved in CSF fluid flow, and contains disease-relevant cells such as perivascular macrophages. Through immunofluorescence staining, we identified the localization of dye-labeled EG18 in the perivascular space. Additionally, to

assess EG18's delivery into BAMs, including specific disease-relevant subsets, we performed immunofluorescence staining 48 hours after injection. Furthermore, we found that EG18 induces robust BAM delivery even at lower doses (0.5 nmol), while mitigating off-target delivery to the spleen and liver. The effectiveness of gene silencing by EG18 in specific cell types, including BAMs, was evaluated using single-cell RNA sequencing. Owing to its ability to access perivascular spaces, our conjugate achieves widespread distribution throughout the CNS. Notably, we observed particularly robust delivery into BAMs, including the canonical Lyve1pos and MHCIIpos subsets, as evidenced by immunofluorescence staining. We found that EG18 potentiates gene silencing in a wide range of cell types including BAMs, compared to a non-targeting control.

In sum, EG18 represents a promising advancement in siRNA delivery technology within the CNS, effectively targeting key immune cells. This approach holds potential for developing treatments in AD through inhibition of disease-driving genes.

3:49 PM - 4:01 PM

Microfluidic Assembly of Mitochondria-Loaded Microparticles for On-Demand Delivery

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With the discovery that mitochondria can be actively transferred from one cell type to another and promote wound healing, new therapeutic approaches have been developed to harness the benefits of this phenomena. Nevertheless, promoting sufficient levels of exogenous mitochondria transfer to observe tissue level changes can be both variable and difficult to predict. As such, the goal of this work is to (1) increase mitochondrial uptake through polymer-based mitochondrial surface modification and (2) enable targeted mitochondria release through the encapsulation in matrix metalloproteinase (MMP) cleavable hydrogel microparticles. Using this system, we hypothesized that increased mesenchymal stem cell (MSC) derived mitochondria transfer to smooth muscle cells and osteoprogenitor cells would improve in vitro myogenesis and in vitro osteogenesis respectively. To facilitate free-mitochondria uptake, TAT-peptides (QPRRRQRRKKKRG) and triphenol phosphate (TPP) were conjugated to dextran via carbodiimide chemistry (TAT-dextran-TPP). The isolated mitochondria were incubated with 2.5wt% TAT-dextran-TPP for 12 hours and subsequently encapsulated in MMP-cleavable hydrogels (10µg/mL total mitochondria protein) using droplet microfluidics. MSC-derived mitochondria encapsulated in ~90µm diameter MMP-cleavable hydrogel microparticles were actively released over 48 hours when in the presence of 1mg/mL collagenase. No release was observed in a collagenase-free solution, illustrating selective release in the presence of MMPs. Furthermore, we observed a >6-fold increase in mitochondria uptake by C2C12 and MC3T3-E1 cells following 24-hour incubation with mitochondria decorated with TAT-dextran-TPP polymer and released from hydrogels. After 5 days, we observed greater differentiation of myoblasts into myotubes for conditions with increased mitochondria uptake. Similarly, we observed increased

osteogenic differentiation of precursor osteoblasts post mitochondria delivery marked by an increase in calcium deposition. From this study, we are able to illustrate that mitochondria can be isolated from MSC culture, loaded into MMP-cleavable hydrogel microparticles and released, all while preserving their function in vitro. Moreover, mitochondria surface modification with TAT-dextran-TPP increases internalization by C2C12 and MC3T3-E1 cells, promoting a larger degree of myogenic and osteogenic differentiation respectively. We note that while these results are most directly applicable for muscle and bone regeneration, on-demand mitochondrial release for localized cellular uptake has applications in a wide range of regenerative medicine applications.

4:01 PM - 4:12 PM

Supramolecular Peptide Hydrogel for Delivery of Locally Sustained Enzyme Therapeutics

Madeline Fuchs, University of Florida

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Functional biomaterials fabricated from supramolecular self-assembly are of interest for many biomedical applications. However, to be useful for applications such as catalysis, whole protein must be installed to provide the biomaterial with this functionality. This can be challenging because covalently grafting proteins onto post-assembled biomaterials can alter protein bioactivity and is often non-specific and highly variable. Instead, the protein can be installed via recombinant covalent fusion to an assembly peptide in the pre-assembled state. The CATCH (Co-Assembly Tags based on CHarge complementarity) biomaterial system consists of a pair of oppositely charged variants of the synthetic -sheet fibrillizing peptide, Q11. Separately, the CATCH(4K+) and CATCH(6E-) peptides do not self-assemble due to electrostatic repulsion, but once combined, they co-assemble into nanofiber-based hydrogels. CATCH peptides recombinantly fused to folded proteins can be incorporated into the nanofibers during assembly, endowing the hydrogels with functionality. Immobilizing therapeutic enzymes to CATCH nanofiber-based hydrogels can locally prolong availability and functional efficiency of enzyme therapeutics. Localized delivery can decrease the rate of renal clearance and improve therapeutic outcomes, while also reducing off-target effects and administered dose.

The overall goal of this work is to show the versatility of this biomaterial system to be utilized in localized and sustained delivery of enzyme-based therapeutics. We show the functional incorporation of four different CATCH-fusion proteins. Using NanoLuc as a reporter enzyme, we observed that the CATCH vehicle can prolong retention of active enzyme in vivo for 20 days. Uricase, a clinically used therapeutic enzyme, reduces inflammation in a mouse model of gout when delivered locally in the CATCH vehicle. And finally, Indolamine-2,3-dioxygenase and Adensosine synthase A provide localized immunomodulation in response to LPS-induced inflammation when delivered in the CATCH vehicle. These results, and the modular design of the CATCH system, suggest that CATCH(4K+/6E-) could be a broadly useful vehicle for improving the local pharmacokinetics of existing and emerging enzyme therapeutics.

Session V: Tissue Engineering 1

5:30 PM – 5:55 PM **Invited Speaker: Cherie Stabler, PhD, University of Florida**

5:55 PM - 6:07 PM

A 3D bioprinted perfusable model of neuroblastoma to study the impact of tumor microenvironment on therapy response

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Introduction

Despite multimodal therapies, survival rate for patients with high-risk neuroblastoma (NB) remains <50%. Combining current therapies with monoclonal antibodies targeting disialoganglioside (GD2) on NB cells has improved survival. However, primary tumors in the soft tissue often remain unaffected. The tumor microenvironment (TME) and extracellular matrix (ECM) parameters can promote cancer cell survival and prevent anti-tumor immune cell infiltration. Understanding of these mechanisms is limited due to lack of robust in vitro models. Based on our previous models, this study employs 3D bioprinting and perfusion bioreactor technologies to develop a tunable in vitro model of NB, paving the way for studying the impact of ECM and TME properties on NB growth, aggression, and therapy response.

Methods

Models were designed to include vascularization channels, and a chamber for NB spheroid. Methacrylated gelatin (GelMA) was synthesized and bioprinted using a BioX bioprinter, with gelatin microparticles as support bath. Print fidelity, chemical and mechanical properties of the constructs were evaluated. Human umbilical vein endothelial cells (HUVECs) were seeded into the bioprinted vascular channels. Human derived NB cell line, IMR-5, were grown as neurospheres in neurobasal media and seeded in the housing, along with peripheral blood mononuclear cells (PBMCs) isolated from healthy donors, and cultured under static and dynamic perfusion conditions. The cells were then analyzed using brightfield microscopy, Live/Dead imaging, flow cytometry and immunohistochemistry (IHC).

Results and Discussion

The constructs were bioprinted at a high fidelity, accurately representing the 3D design for vascular channels, and the NB/PBMC chamber. Seeded HUVECs displayed a high viability and proliferation in three days, forming a uniform endothelial layer in both static and dynamic culture conditions. NB spheroids and PBMCs demonstrated adequate viability, as assessed via Live/Dead imaging. IHC and flow cytometry analyses demonstrated an optimal population of PBMC and NB cells following culture, confirming the ability of 3D engineered TME in maintaining viable and functional multicellular components. Compared to previous model, the novel perfused 3D bioprinted models developed and optimized here were shown to accurately replicate different cellular/ECM elements of TME, demonstrating potential for use as a research enabling platform for studying NB aggression and treatment strategies.

6:07 PM - 6:19 PM

Sialic-acid Modified Adult Fibrinogen to Promote Neonatal-like Wound Healing in Adults

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Chronic wounds are a worldwide problem impacting 10.5 million people in the United States alone. Chronic wounds do not follow a timely repair which can cause infection leading to amputation and death. Fibrinogen plays a major role in wound healing as it undergoes proteolytic cleavage; thereby, turning into a crosslinked fibrin network that acts as a provisional matrix for platelets, growth factors, and other cell types to assist in hemostasis. However, if this matrix is insufficient, non-healing wounds can occur. Therefore, topical therapies that expedite the wound healing process and avoid infection should be explored. Neonates exhibit faster wound closure than adults. Fetal fibrinogen, a molecular fibrinogen variant, is integral to accelerated healing. Fetal fibrinogen has different subunit molecular weights and higher sialylation levels; it also exhibits greater cell attachment and accelerated healing. Given ethical concerns and lack of availability of fetal fibrinogen, we propose modifying adult fibrinogen to be more fetal-like to enhance wound healing. Because fetal fibrinogen has higher sialic acid levels than adult fibrinogen and sialic acid has been shown to alter clot properties, we hypothesize that increasing sialic acid on adult fibrinogen can achieve neonatal-like wound healing. To create adult fibrinogen with varying sialic acid concentrations, adult fibrinogen was mixed with α-2,3-sialyltransferase and cytidine-5'-monophospho-N-acetylneuraminic acid sodium, incubated for 24-48 hours, and quantified via NANA assay. Polymerization kinetics were evaluated by measuring clot turbidity over time. Clot structure was evaluated by adding 488-labeled fibrinogen to clots and imaging on confocal microscope. Cellular interactions were evaluated by seeding fibroblasts onto clots and incubating for 24 hours. Cell nuclei and membranes were stained to measure cell count and area. Results show adult fibrinogen with varying sialic acid levels can be created, and sialic acid level has an inverse relationship with clot turbidity. Furthermore, increased fibroblast attachment and spreading was seen on clots created from fetal fibrinogen and adult fibrinogen with high sialic acid. In conclusion, sialylation can be modified on adult fibrinogen to create neonatal-like clots that promote cell attachment and spreading. Future experiments will be conducted to evaluate cell activation, clot mechanics, and in vivo wound healing outcomes.

6:19 PM - 6:31 PM

Anisotropic granular fiber hydrogels for skeletal muscle tissue engineering

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Volumetric muscle loss (VML) injury results in permanent deficit of skeletal muscle function and drastic reduction in quality of life. Granular materials promote de novo myogenesis by enabling cell infiltration from surrounding tissue, pro-regenerative immunomodulation, and promotion of angiogenesis. However, these materials composed of discrete spherical particles prevent functional recovery due to their tortuous, isotropic pore structure that inhibits myofiber alignment. Therefore, development of a macroporous granular scaffold with anisotropic structural cues would be useful for VML repair. To address this need, we have developed a microfiber-based granular scaffold comprised of both sacrificial (gelatin) and non-sacrificial (gelatin-methacryloyl or GelMA) fibers, providing space for myogenesis and aligned structural cues respectively. Fibers were fabricated using a bulk fragmentation approach. Warm solutions of either gelatin or GelMA were drawn into a tipless syringe and incubated at 4ºC until solid. The gel was extruded through a cell strainer, yielding microfibers of the same geometry as the cell strainer pores. Pore apertures of 20, 40, or 80 µm were used as they cover a wide range of myofiber diameters. The mean diameter of the 80 µm fibers was found to be 88 ± 20 µm based on confocal images of fluorophore-labeled fibers. To determine if the GelMA fibers exhibit properties necessary for 3D printing, photo-crosslinked fibers underwent a cyclic strain rheometric test. Similarly to other granular systems, this material exhibited recovery within 30 seconds of strain cessation. Extrusion-induced alignment of fibers was visualized with fluorescent confocal imaging. Quantitative analysis showed fiber orientation along 0° in the aligned group, while orientation was uniformly distributed in the unaligned control. Preliminary cell culture experiments suggest high cell motility and proliferation in the granular fiber hydrogel compared to a nanoporous group. In summary, we developed an anisotropic granular material for use as a skeletal muscle tissue scaffold, and we are working to characterize the effect of pore size and alignment on in vitro myogenesis.

6:31 PM - 6:43 PM

Application of Tissue Penetrating Hyaluronic Acid Hydrogel Augments The Chondrocyte Microenvironment Reducing Catabolic Activity

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Joint injuries are increasingly common and initiate a degenerative cascade in the articular cartilage. Chondrocytes experience both intra- and extra-cellular changes during the initial phases of this process, starting a degenerative process that progresses towards osteoarthritis (OA). Thus, restoration of chondrocyte homeostasis and maintenance of its microenvironment may be critical towards slowing, or even preventing, OA. Previously, we developed a tissue-penetrating hyaluronic acid (tp-HA) system that reinforced damaged cartilage and prevented its breakdown; the objective of this study was to explore the micro-scale implications of tp-HA treatment. In particular, we 1) characterized the integration and localization of tp-HA, 2) established its ability to reinforce the chondrocyte microenvironment, and 3) determined how HA augmentation of the chondrocyte microenvironment influenced cellular morphology and catabolic activation.

To characterize the diffusion of tp-HA, bovine cartilage explants were harvested. tp-HA solution (4 wt%, fluorescently tagged) was applied to the cartilage superficial layer , allowed to diffuse for 5 minutes, and photocrosslinked for 5 min to gel within the cartilage. We demonstrated that tp-HA integrated into the tissue approximately 150μm. Furthermore, when stained for type VI collagen to visualize the PCM, we observed increased tp-HA (via fluorescence) in the PCM, indicating its integration into the chondrocyte microenvironment. To determine how the tp-HA system restored cartilage micromechanics, explants were digested in collagenase for 30 min (to mimic OA) and reinforced with tp-HA. Explant cryosections (5μm) were stained for type VI collagen, and nanoindentation was used to map non-reinforced and reinforced regions, with tp-HA demonstrating restoration of PCM mechanics to near-healthy values. Finally, to investigate how HA hydrogel impacts chondrocyte behavior, bovine chondrocytes were encapsulated within gelatin hydrogels (5% methacrylated gelatin; GelMA) or gelatin-HA hydrogels (4% GelMA, 1% MeHA), followed by IL-1β application (10ng/mL) for 2 hours. Gels were stained for NF-κB (catabolic activation) and phalloidin (F-Actin). Chondrocytes in GelMA demonstrated sharp increases in cell perimeter and NF-κB intensity, both of which were almost completely mitigated in GelMA-MeHA gels.

In summary, our tp-HA hydrogel integrated with the chondrocyte microenvironment and restored its mechanical properties; incorporation of HA into the microenvironment early in OA may help prevent its progression.

SESSION VI: ENGINEERING CELLS AND THEIR MICROENVIRONMENTS

8:45 AM - 8:57 AM

Guest-host interlinked microporous annealed particle hydrogels as an injectable microenvironment for islet transplantation

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Microporous annealed particle (MAP) hydrogels are composed of closely packed microgels that form interstitial spaces to create porosity. These microgels can slide past one another to form a shear-thinning and injectable material for cell and tissue engineering. We use guest-host interactions to interlink polyethylene glycol maleimide (PEG-MAL) microgels (MAP-PEG) for increased stability and self-healing of the material, which we characterized through rheology. Clinical islet transplantation has been used as a therapy for type 1 diabetes to replace insulin-producing beta cells in the islets of Langerhans. This therapy has been successful in controlling blood glucose, but due to alloimmunity and reoccurrence of autoimmunity, the transplants can be rejected. We investigated MAP-PEG as a tunable and injectable microenvironment for the protection of transplanted islets in vivo. Syngeneic mouse islets were co-delivered with MAP-PEG under the kidney capsule of C57BL/6 mouse to determine viability and engraftment. After 14 days, microgels remained well-compacted and did not cause an inflammatory foreign body response in vivo. We found that the addition of guest-host interactions did not negatively affect the viability and engraftment of the islets compared to unfunctionalized microgels and islet-only implants. Mason's trichrome straining showed little to no fibrotic encapsulation of MAP-PEG transplants but substantial fibrotic collagen deposition around degradable bulk hydrogels and islet only controls. The MAP-PEG gels were permissive to islet graft revascularization at 14 days without additional growth factors. Gels without islets had significantly lower vessel density, suggesting that islets within the MAP-PEG released sufficient angiogenic signals to vascularize the material. The advantage of MAP-PEG is that it provides the ability to control the cellular signaling environment near the islet graft. We have successfully conjugated immunomodulatory biomolecules to the surface of MAP-PEG hydrogels. Ongoing in vivo studies are characterizing a panel of immunomodulatory proteins and drugs presented on MAP-PEG co-transplanted with beta cells to prevent autoimmune rejection in a humanized mouse model of type 1 diabetes.

8:57 AM - 9:09 AM

Biomaterials-based human lung with integrated mucosal and humoral immune responses

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The need for lung mucosal and lymph node systemic immunity is crucial for comprehensive protection against respiratory pathogens. The balance of mucosal and systemic immunity is critical in determining the outcome of severe, lethal influenza. An effective immune response can help to control the virus and prevent severe complications, while an overactive or dysregulated response can contribute to the severity of the illness. Influenza research often relies on animal models for pre-clinical studies, but these models do not accurately replicate human immune responses or the immunological diversity of the human system. To address this limitation, we have developed a fibrin-collagen-based immune-competent lung-on-chip system that mimics mucosal responses to influenza infection in a vascularized, perfusable lung epithelium. The immune competency here corresponds to the presence of all immune cells, and using single-cell RNA sequencing we demonstrate a strong immune-stroma crosstalk in infected lungs (manuscript in Review). While this model represents a step toward a more complete human-based system, it does not fully capture the physiological complexity needed to understand the impact of pulmonary infection on secondary lymphoid organs, where most humoral immune responses are generated. To overcome this critical gap, we have further developed a maleimide-functionalized polyethylene glycol hydrogel-based lymphoid organoids and integrated it with a fibrin-collagen-based immune-competent lung on the chip. The chip design allowed for molecular transport from lung to lymphoid by establishing interstitial flow promoting antigen transfer from lung to lymphoid organoid. We demonstrate the lung-lymphoid immune crosstalk and generation of adaptive immunity in response to influenza infection in the lung. We noted that infection of the lung epithelium with live virus triggered the activation of immune cells in the lung interstitial space, while also stimulating antibody-secreting cell maturation in lymphoid organoids.

9:09 AM - 9:21 AM

Matrix stiffness influences response to chemo and targeted therapy in brain metastatic breast cancer cells

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Breast cancer is the most common malignancy accounting for 12.5% of all newly diagnosed cancer cases across the globe. Breast cancer cells are known to metastasize to distant organs (i.e., the brain), wherein they can exhibit a dormant phenotype for extended time periods. These dormant cancer cells exhibit reduced proliferation and resistance to therapy. However, the mechanisms by which the dormant cancer cells exhibit resistance to therapy, in the context of brain metastatic breast cancer (BMBC), is not well understood. In this study, we employed hyaluronic acid (HA) hydrogels with varying stiffnesses (i.e., ~0.4 kPa vs. ~4.5 kPa) to study drug responsiveness in dormant vs. proliferative BMBC cells. We found that cells cultured on soft HA hydrogels (~0.4 kPa) that showed a non-proliferative (dormant) phenotype exhibited resistance to Paclitaxel or Lapatinib. In contrast, cells cultured on stiff HA hydrogels (~4.5 kPa) that showed a proliferative phenotype exhibited responsiveness to Paclitaxel or Lapatinib. Moreover, dormancy-associated resistance was found to be due to upregulation of serum/glucocorticoid regulated kinase 1 (SGK1) gene which was mediated, in part, by the p38 mitogen-activated protein kinase (MAPK) signaling pathway. Finally, we inhibited the expression of SGK1 using a SGK inhibitor (GSK650394) in BMBC cells cultured on soft HA hydrogels, which resulted in a dormant-to-proliferative switch and response to therapy. Overall, our study demonstrates that matrix stiffness influences dormancy-associated therapy response and that the p38/SGK1 axis, in part, mediates therapy response in our HA hydrogel platform.

9:21 AM - 9:33 AM

Quantitative Effects of Extracellular Matrix Composition on Breast Cancer Phenotype

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Detection of metastatic breast cancer (BC) will not occur until after the cancer has spread, resulting in delayed treatment. Therefore, it is critical to understand the mechanisms underlying metastasis for better detection of metastatic cancer. The role that extracellular matrix (ECM) composition plays on BC aggressiveness, separate from stiffness, is not fully understood. In the present work, eight mammary cell

lines were cultured and evaluated using an impedance-based assay system to elucidate the relationship between ECM composition, cell phenotype, and 2D migration. The long-term goal of this work is to improve current cancer assessment methods and determine a correlation between quantifiable impedance-based characteristics, 2D cell morphology, and cancer subtype.

Selected cells included two non-cancerous cell lines and six BC cell lines, five of which are triple-negative (ER-/PR-/HER2-), a highly aggressive and often metastatic subtype. Cells were cultured using a Maestro Tray-Z (Axion Biosystems). The plates were thinly coated in ECM protein (collagen I, collagen IV, fibronectin, laminin, Matrigel) prior to cell seeding and cell proliferation was monitored by impedance. At confluency, cell mediums were switched to a nutrient-reduced formula. Wells were scratched to create a wound and cell migration was monitored for 36 hours. A distinct difference in impedance was observed across all cell lines that can be linked to single-cell morphology and growth patterns. Three of the triple-negative BC cells showed wound closure under multiple ECM conditions, confirming cancer aggressiveness based on reported cell line characterization. ECM was also shown to affect cell-cell adhesion for five cancer cell lines. ECM composition was not shown to affect wound closure for any cell line evaluated, suggesting that the specific processes underpinning breast cancer outgrowth and epithelial-to-mesenchymal-transition are found outside of the cell-ECM protein relationship.

Our hypothesis that ECM composition affects cell behavior was supported, although ECM proteins did not affect 2D migration specifically. These findings demonstrate the feasibility of using an impedance-based assay to quantitatively characterize cancer cell migration, proliferation, and response to microenvironment. Overall, this work creates a foundation for our long-term goal of improving current assessment methods to yield a more detailed and objective evaluation of cancer metastatic behavior.

9:33 AM - 9:45 AM

Epigenetic modulation of malignant B cells by T cells in lymphoid cancers

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Activated B cell-like diffuse large B-cell lymphoma (ABC-DLBCL) is an aggressive subtype of non-Hodgkin's lymphoma associated with poor prognosis. Despite new pharmacological targets identified though molecular profiling of ABC-DLBCL, clinical trials using target therapy have not benefited these patients. To improve therapeutic strategies, immune-competent tissue models are needed to understand how DLBCL cells evade or resist treatment. This study employed synthetic hydrogel-based lymphoma organoids to illustrate how cues from the lymphoid tumor microenvironment (Ly-TME) can impact B cell receptor (BCR) signaling and tri-methylation of histone 3 at lysine 9 (H3K9me3) to dampen the effects of BCR inhibition. Using imaging techniques, we showed T cells directly increased DNA methyltransferase 3A expression and cytoskeleton formation in neighboring ABC-DLCBL cells that was regulated by H3K9me3 expression. Using expansion microscopy on lymphoma organoids captured T cell-mediated increase in the size and quantity of spatially segregated H3K9me3 clusters in proximal ABC-DLBCL cells, suggesting restructuring of high-order chromatin structures that may be associated with novel transcriptional states. Treating ABC-DLBCL cells with an inhibitor of G9α histone methyltransferase prior to inhibition of the BCR pathway protein MALT1 reversed T cell-induced H3K9me3 upregulation and mitigated T cell-mediated the dampened treatment response to BCR pathway inhibition. This study underscores the need for biologically relevant tissue models to understand how Ly-TME signals can alter DLBCL progression, spatially and temporally, and suggests targeting both aberrant signaling pathways and epigenetic cross-talk could enhance treatment efficacy for high-risk patients.

9:45 AM - 9:57 AM

Effect of 3D Viscoelasticity on Endothelial-to-Mesenchymal Transition in Atherosclerosis

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Endothelial-mesenchymal transition (EndoMT) is a biological process in which vascular endothelial cells (ECs) acquire a mesenchymal identity that give rise to lineages like fibroblasts and smooth muscle-like cells. Pathologically, EndoMT is involved in the progression of numerous cardiovascular diseases such as atherosclerosis, in which ECs give rise to smooth muscle-like cells within the plaque. However, there is essentially a dearth of knowledge of how the biochemical and biomechanical cues from the extracellular matrix (ECM) milieu directly influence the progression of EndoMT. To address the limited knowledge of ECM effects on EndoMT, we propose to develop a 3D tissue engineered model with independently tunable biochemical and biomechanical cues, including stiffness, stress relaxation rate and ECM composition. The direct effects of biomechanical properties on EndoMT are difficult to study in vivo, owing to complex

microenvironmental factors of soft tissues. In contrast, in vitro platforms allow the study of biomechanical and associated mechanosignaling pathways in a well-controlled environment. The main research objective was to encapsulate primary human aortic endothelial cells into tunable viscoelastic 3D alginate hydrogels and study EndoMT progression in 3D. To study this, we optimized alginate hydrogels properties to have modular mechanical and viscoelastic properties. Primary human coronary artery endothelial cells were co-cultured with human fibroblasts to get a blood vessel network in the 3D alginate hydrogels. Additionally, we observed viscoelasticity dependent migration of ECs into the alginate hydrogel. Lastly, we quantified higher SM22 expression (EndoMT) in ECs in fast relaxing hydrogels, pointing towards viscoelasticity dependent progression of EndoMT. These results underscore the importance of mechanical factors such viscoelasticity in addition to stiffness in progression of diseases like atherosclerosis. Future work involves performing transcriptomics analysis to uncover the mechanism behind viscoelasticity dependent EndoMT progression.

SESSION VII: IMMUNE ENGINEERING 2

10:15 AM - 10:40 AM

Incorporating covalent crosslinks into immune constructs to enable co-delivery of distinct immunomodulatory cues

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Multiple sclerosis occurs when autoreactive lymphocytes attack the protective myelin sheath surrounding neurons in the central nervous system. Current MS treatments are non-curative and cannot distinguish between healthy and diseased immune cells, motivating the need for new strategies to treat the disease. Toll-like receptors (TLRs), which traditionally recognize pathogen-associated molecular patterns (PAMPs), are upregulated in MS patients. Thus, an emerging therapeutic strategy is to deliver TLR antagonists and self-antigens, to achieve immune tolerance without broad immunosuppression. Herein, we developed covalently cross-linked carriers built entirely from immune cues – termed immune polyelectrolyte multilayers (iPEMs) to co-deliver self-antigen - myelin oligodendrocyte glycoprotein (MOG) and TLR9 antagonist - GpG. We pursued the co-delivery of Rapamycin (Rapa), a small molecule drug well-documented to expand TREGS, simultaneously to target innate and adaptive immune cells involved in MS. Specifically, we hypothesized that Rapa loading could be achieved in iPEMs, and covalent cross-links could be used to stabilize MOG and GpG cargo without disrupting cargo loading and impeding Rapa loading.

iPEMs synthesized using 3-bilayer bilayer depositions of MOG and GpG were incubated with varying glutaraldehyde (GA) cross-linking solutions to achieve covalent cross-links prior to removal of the sacrificial template. We found that cross-linking density could be modulated by adjusting GA concentration and incubation times; without impacting the functional capabilities of iPEMs. We importantly show comparable loading of small molecule Rapamycin between non-cross-linked iPEMs. Using an adoptive transfer approach, we show that myelin-specific T cells respond to cross-linked and non-crosslinked designs comparatively. Altogether, this work shows a proof-of-concept design to simultaneously engage innate and adaptive immune cells to drive robust immune tolerance in the context of MS and can be generally adapted to the autoimmune diseases.

10:40 AM - 10:52 AM

Albumin hitchhiking nanobody-STING agonist conjugates to improve immunotherapy

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While immunotherapy has revolutionized cancer treatment, the tumor microenvironment (TME) limits the efficacy of therapies CAR-T cells especially in solid tumors. To combat the immunosuppressive nature of the TME, we focused on targeting the stimulator of interferon genes (STING) pathway. We developed an “albumin hitchhiking” strategy that leverages an anti-albumin nanobody to which drugs can be conjugated and delivered.

We first characterized the anti-albumin, anti-B7H3, and anti-albumin/anti-B7H3 fusion nanobody using mass spectrometry. Next, the ability to conjugate payloads to nanobodies was tested by conjugating Cy5, a fluorescent molecule, to each nanobody and confirmed using SDS-PAGE as well as mass spectrometry. For initial studies, we selected only the anti-albumin nanobody conjugated to Cy5 (a fluorescent dye) to assess pharmacokinetics and biodistribution. We observed an increased half-life of approximately twentyfold compared to a control nanobody against GFP administered alone. Further, the protein conjugate was highly localized to the tumor compared to other internal organs. The activity of the protein conjugates when linked to diABZI was tested in vitro on A549D and THP1D (IFN-ß reporter lines), which showed increased expression of interferon-beta (IFN-ß), an inflammatory marker. Furthermore, in an in vivo breast cancer model, bulks tumors were processed analyzed via PCR to show an increase in inflammatory markers that promote anti-tumor responses. Flow cytometry analysis in this same model showed an increase in CD8+ T cells and within that population an increase in CD69 expression. An in vivo survival study in a subcutaneous breast cancer mouse model showed a decrease in tumor burden. In a less immune responsive model of neuroblastoma, tumor growth was similarly attenuated when treated with anti-albumin nanobody drug conjugates as well as the fusion anti-albumin and ant-B7H3 conjugate compared to controls. Current studies are underway to determine the effect of combining our treatment with CAR-T cell therapy in an immunosuppressive neuroblastoma model. Further, we will perform flow cytometry to determine the extent of immune reprogramming in the tumor post-treatment and identify changes in CAR T phenotype when administered with a STING agonist. Taken together, this data indicates potential to improve efficacy of already existing therapies for immunosuppressive solid tumors.

10:52 AM - 11:04 AM

Controlling Naïve T Cell Fate with miRNAs using Cationic Polymer-Functionalized Nanowires

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T cell-based therapies leverage the phenotypic plasticity of T cells and responsiveness to microenvironmental cues. Manufacturing T cells from naïve or stem-like memory T lymphocytes can enhance antitumor and anti-viral responses while reducing cytokine release syndrome. However, it is

extremely difficult to deliver genetic material to naïve T cells and direct differentiation towards beneficial phenotypes such as stem-like memory (TSCM) and effector (TEFF)) during ex vivo T cell engineering processes. Consequently, conventional processes hinder long-term therapeutic efficacy and can result in early-onset T cell exhaustion. Here, we present a cationic polymer-functionalized nanowire technology, with 20 nm tip size, for efficiently delivering T cell fate-determining miRNAs to naïve mouse and human T cells, therefore bypassing the need for prior activation of naïve T cells using T cell receptor (TCR)-stimulating antibodies or cytokines. Covalent conjugation of cationic polymer Polyethyleneimine (PEI) to a silicon nanowire surface enabled the adsorption of miR-23a, miR-28, and miR-130, which modulate T cell functions, and pre-program phenotypic and functional trajectory during ex vivo differentiation. After genetic pre-programming, primary naïve human or murine T cells were activated with TCR-stimulating antibodies. Flow cytometry revealed that the nanowire platform delivered miR-29-mimic to naive murine and human T cells with >90% efficiency and viability, surpassing lentiviral, nucleofection, and lipofectamine approaches. A single delivery of 6-FAM-miR-29-mimic downregulated transcriptional targets T-bet and Eomes while increasing CD62L expression, maintaining less-differentiated T cell phenotypes. Additionally, we have demonstrated codelivery of 6-FAM-miR-29-ASO and APC-miR-130-mimic to non-activated murine T cells, resulting in additional increases in CD45RA+CD62L-CCR7- TEFF cell yield and proinflammatory cytokine expression (i.e., perforin, granzyme A and B, IFNγ, and IL-2). Interestingly, delivery of a distinct modulator of T cell exhaustion, miR-23a, biased T cell fate towards a significantly higher % CD45RA+ CD62L+ TSCM and induced rapid ex-vivo expansion. Our findings demonstrate a unique ability of perturbing multiple regulatory pathways in naïve T cells using a biomaterials-based nanoscale technology to modulate T cell phenotype and function for therapeutic T cell production.

11:04 AM - 11:16 AM

Macromolecular Polymeric STING Agonists for Cancer Immunotherapy Applications

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Cancer immunotherapies have revolutionized the treatment of many cancer types, offering patients hope for complete tumor regression and life-long immunological memory to prevent disease recurrence. One promising cancer immunotherapy target is the Stimulator of Interferon Genes (STING) pathway, as it is a crucial link between innate and adaptive anti-tumor immunity. Activation of the STING protein triggers a type-I interferon (IFN-I) inflammatory response which provides the proper immunological context to activate antigen presenting cells (APCs), T cells, and natural killer cells, leading to potent anti-tumor responses. Unfortunately, local and systemic administration of endogenous STING agonists (e.g., 2’3’-cGAMP) are limited by poor stability and cytosolic delivery, which has prompted the development of non-nucleotide, synthetic STING agonists. Of the non-nucleotide STING agonists in use, recently described diamidobenzimidazole (diABZI) compounds have been shown to facilitate potent STING activation and anti-tumor activity but they pose significant formulation and administration challenges due to their hydrophobicity and short half-life, respectively. Consequently, we have developed reversible addition-fragmentation chain transfer (RAFT) polymerization-compatible diABZI-based chain transfer agents (CTAs)

and have leveraged these to create macromolecular polymeric STING agonists for systemic cancer immunotherapy and neoantigen peptide cancer vaccine applications. Herein, we have created a library of N,N-dimethylacrylamide (DMA)-based polymeric constructs using diABZI-CTAs with cleavable and non-cleavable linkers. We see that STING agonism from the polymeric constructs is independent of molecular weight, but dependent on linker cleavability, as polymers synthesized with cleavable diABZI-CTAs demonstrate robust STING activation and antitumor activity in murine cancer models. Additionally, we created a polymeric neoantigen peptide vaccine platform with the diABZI-CTA system through the copolymerization of DMA and pyridyl disulfide ethyl acrylamide (PDSMA), which enables peptides containing an N-terminal cysteine to be conjugated to the polymer via a disulfide exchange reaction. We synthesized vaccine constructs containing synthetic long peptide variants of MHC-I and MHC-II-restricted ovalbumin model neoantigens and found these constructs were able to significantly outperform soluble mix controls in antigen presentation and T cell priming both in vitro and in vivo.

11:16 AM - 11:28 AM

PEGylation of Indoleamine 2,3-Dioxygenase for Systemic Immune Regulation

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Indoleamine 2,3, dioxygenase (IDO), the rate-limiting enzyme of tryptophan catabolism, is anti-inflammatory in many inflammatory and autoimmune diseases. Exogenously delivered IDO is immunosuppressive, allowing dendritic cells to maintain an immature state. These properties make IDO an attractive protein therapy candidate. However, it faces quick clearance in vivo. Coupling poly(ethylene glycol) (PEG) with IDO creates a modified protein-polymer complex that persists in circulation while maintaining its enzymatic function, resulting in systemic immunosuppression. To conjugate the PEG polymer to the protein, PEG chains with a single maleimide reactive group were combined with IDO. Size of the PEG-IDO product was varied by increasing the length of the PEG chain. To determine circulation time, PEG-IDO was injected intraperitoneally; IDO in blood was analyzed via blood draws and quantified via Western blot, using an exponential decay curve to determine circulation half-life. A mouse model for psoriasis was used to determine therapeutic effects of systemically delivered PEG-IDO. Disease state was assessed via clinical scoring of erythema, scaling, and skin thickening. Mice were treated on day 3 and day 8 as needed. PEG-IDO had a greater half-life than IDO alone, and increasing the PEG chain length increased the half-life – 10kDa PEG-IDO persisted to 5 days in circulation and 30kDa PEG-IDO showed no drop in circulation levels out to 7 days, while WT IDO was barely detectable even 2 hours post injection. Mice treated with two doses of 10kDa PEG-IDO showed lower cumulative scores from day 5 onwards as compared to saline treated mice, while mice treated with 30kDa PEG-IDO only required one dose to achieve similar decrease in scores. This showed that treatment with longer persisting PEG-IDO formulation is more effective than the same treatment with a shorter persisting formulation

SESSION VIII: BIOINTERFACES

1:45 PM – 2:10 PM Invited Speaker: Juhi Samal, University of Alabama ant Birmingham

2:10 PM - 2:22 PM

Copper coated nanotextured stainless steel for antibacterial application

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Bacterial adhesion to commonly shared surfaces can lead to severe infections, posing a risk of mortality and significant healthcare costs. Antibiotics have addressed this concern to a certain extent, but continuous usage of antibacterial agents can lead to development of drug-resistant bacteria which caused approximately 1.27 million deaths worldwide in 2021. Various studies have explored non-chemical methods of killing Gram-negative and Gram-positive bacteria. However, the outer membrane of Gram-negative bacteria makes them particularly resilient. Hence, there is a significant need to develop antibacterial surfaces effective for both Gram-positive and Gram-negative bacteria. Some of the conventional approaches include antifouling materials, which often contain toxic compounds as coating such as tributyltin and dichlofluanid. Additionally, nanotextured polymers, metals, and metal oxides have been explored for their potential in antibacterial surfaces. While these materials have shown efficacy against Gram-positive bacteria, they fall short against Gram-negative strains. Hence, there is a need to create an affordable antibacterial surface for shared environments which can kill Gram-negative and Gram-positive bacteria without promoting drug resistance.

Stainless steel 316L (SS316L) is widely used in public settings, including sinks, toilets, surgical tools, and cardiovascular and orthopedic implants, owing to its favorable mechanical strength, corrosion resistance, and biocompatibility. In this study, we have demonstrated nanotextured stainless steel (nSS) fabrication followed by Cu coating using an electrochemical technique and its potential as an antibiotic-free biocidal surface against Gram-positive and negative bacteria. The synergistic effects of nSS and Cu provide a promising avenue for combating bacterial infections without contributing to drug resistance. The dual antibacterial activity rises due to the small grooves and ridges on the nanotextured surface, which make it difficult for bacteria to attach and spread, and release of copper ions that kill or inhibit the growth of bacteria. Our method involves the application of a copper coating on nanotextured stainless steel, resulting in significant antibacterial activity within 30 minutes. Cu-coated nSS demonstrated a remarkable reduction of 97% in Gram-negative and 99% in Gram-positive bacteria. Overall, our material holds promise for developing effective, scalable, and sustainable solutions to mitigate bacterial infections stemming from surface contamination, all without exacerbating drug resistance issues.

2:22 PM - 2:34 PM

The Impact of Porous Scaffold Geometry on Host Responses In Vivo

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Cell-based therapies, such as 3D porous scaffolds, are a promising treatment for many diseases, whereby the scaffold mechanically supports and distributes donor cells while facilitating host engraftment. The optimal scaffold geometry to fulfill these key objectives has yet to be identified. Published studies have found correlations between global pore size and foreign body responses, yet the results are contradictory and confounded by material degradation in vivo. To overcome this, we have created a porous 3D-printed, biostable polydimethylsiloxane (PDMS) scaffold, which can provide control over implant geometry to clearly interrogate the impact of geometrical features on host responses to the implant. These scaffolds are fabricated using a reverse cast method that involves integrating PDMS into a 3D-printed sacrificial mold, curing overnight, and removing the mold to leave a flexible scaffold. Three distinct prototypes were modeled with PDMS rung thicknesses of (300, 200, or 150 µm in x-y; all 100 µm in z) and uniform pore size (300x300x100 µm; x-y-z). Actual feature sizes were validated using brightfield imaging and ImageJ. These scaffolds were transplanted into the epididymal fat pad of C57BL/6 mice before explantation; intra-device vascularization was visualized with tomato lectin staining and host engraftment with trichrome staining. Prototype images showed significant differences in scaffold rung thickness ($p < 0.0001$) with uniform pore sizes ($p = 0.082$). In vivo, scaffolds with thicker rungs exhibited increased collagen deposition and fibrotic features than scaffolds comprised of thinner rungs. Promising scaffolds (150x150x100 µm rung thickness) were then loaded with allogeneic islets within a fibrin hydrogel and transplanted into the omentum of diabetic Lewis rats. Optimized 3D printed scaffolds were compared to PDMS scaffolds fabricated using traditional particulate leaching methods. In our diabetic model, both scaffold fabrication methods reversed diabetes; however, the time to normoglycemia ($p = 0.0189$) and risk of rejection ($p = 0.0351$) was significantly lower with the 3D-printed scaffold, demonstrating the impact of geometric features on transplant efficacy. In conclusion, geometric scales can shift host responses from favorable to deleterious and impact transplant outcomes. Future work will identify which cells are driving these variable responses using digital spatial profiling.

2:34 PM - 2:46 PM

Engineering Nitric Oxide-releasing Antimicrobial Dental Coating for Targeted Gingival Therapy

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Bacterial biofilms play a central role in the development and progression of periodontitis, a chronic inflammatory condition affecting the oral cavity. One solution to current treatment constraints is using nitric oxide (NO) – with inherent antimicrobial properties. In this study, an antimicrobial coating is developed from the NO donor S-nitroso-N-acetylpenicillamine (SNAP) embedded within polyethylene glycol (PEG) to prevent periodontitis. The SNAP-PEG coating design enabled controlled NO release, achieving tunable NO levels for more than 24 h. Testing the SNAP-PEG composite on dental floss showed its effectiveness as a uniform and bioactive coating. The coating exhibited antibacterial properties against Streptococcus mutans and Escherichia coli, with inhibition zones experiments. Furthermore, SNAP-PEG coating materials were found stable when stored at room temperature, with 93.65% of SNAP remaining after 28 d. The coatings were biocompatible against HGF and hFOB 1.19 cells through a 24 h controlled release study. This study presents a facile method to utilize controlled NO release with dental coatings comprising SNAP-PEG. This coating can be easily applied to various substrates, providing a user-friendly approach for targeted self-care in managing gingival infections associated with periodontitis.

2:46 PM - 2:58 PM

Shape-shifting protein nanostructures driven by molecular juxtaposition of opposing phase-separation grammars

Jeremy Hannon, Emory University

Jeremy Hannon, Maria Camila Giraldo Castano, Alexa Regina Chua Avecilla, Felipe Garcia Quiroz.

Intrinsically-disordered proteins (IDPs) exhibit environmentally-sensitive conformational dynamics and phase transitions that drive the formation and dynamics of protein assemblies across biological systems. Temperature-responsiveness distinguishes phase transitions above a lower critical solution temperature (LCST) or below an upper critical solution temperature (UCST). IDP polymers (IDPs) with these opposing behaviors are known to have distinct sequence grammars. However, the interplay of these opposing grammars in multi-domain IDPs and IDPPs is poorly understood. We and others previously showed that UCST-LCST diblock IDPPs can be programmed as nanoparticles that undergo disassembly upon heating. Unexpectedly, the phase behavior of the UCST-IDPP domain was strongly influenced by molecular juxtaposition of the LCST-IDPP, as evidenced by dramatic shifts in the expected critical temperature for nano-assembly. This behavior contrasted with the highly predictable critical temperature for assembly of LCST-LCST diblock IDPPs. To understand how opposing phase-separation grammars interact to dictate the

overall phase behavior, here we recombinantly synthesized and characterized a library of novel UCST-LCST diblock IDPPs. These designs explored the molecular-level fusion of three UCST IDPPs of progressive hydrophobicity but fixed length, and two LCST IDPPs of increasing hydrophilicity and length. Excitingly, temperature-dependent UV-visible absorbance measurements showed that UCST-LCST diblocks in our library often exhibited a rarely-seen band-pass phase behavior marked by dual UCST and LCST transitions (LCST>UCST). Select diblock IDPPs transitioned from nanoparticles into micron-sized assemblies exclusively in a narrow temperature range (~10-15°C), before dispersing into single-chain IDPPs at higher temperatures. Across our designs, the hydrophobicity of UCST-IDPP domains tuned the LCST-type transition and set the width of the band-pass regime. Surprisingly, UCST-LCST IDPPs with short LCST blocks formed nanoparticles at low temperatures that rapidly reconfigured through an LCST-type transition within physiologically relevant conditions. At higher temperatures, these diblock IDPPs also showed band-pass behavior. The shape-shifting regime was highly reversible and Cryo-TEM images showed that the LCST-triggered shape-shifts involved monodisperse nanoparticles. Unlike folded proteins with well-insulated modules, our findings suggest that the phase behavior of multi-domain IDPs results from protein-level integration of the juxtaposed phase-separation grammars. The discovery of shape-shifting UCST-LCST nanoparticles will stimulate the integration of complex phase separation grammars in IDP engineering and nanotechnology.

SESSION IX: DRUG DELIVERY 2

3:00 PM - 3:25 PM

Advancing β-adrenoreceptor agonism for recovery after volumetric muscle loss through biomaterial delivery

Jarrod Call, University of Georgia

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Volumetric muscle loss (VML) injury results in the unrecoverable loss of muscle mass and strength. The β2-adrenergic receptor agonist formoterol produces a modest recovery of muscle mass and strength when delivered orally in VML-injured mice. The objective of this study was to determine if a regenerative medicine paradigm could enhance the recovery of VML-injured muscle. Regenerative medicine involved direct delivery of formoterol to VML-injured muscle using a nonbiodegradable poly(ethylene glycol) biomaterial. Preliminary biomaterial characterization included batch-to-batch size optimization, in vitro formoterol release analysis, visual and histological evaluation of the biomaterial residence within the muscular compartment. Based on the preliminary findings, VML-injured mice were randomized to receive a single intramuscular biomaterial injection at 1-month post-injury with either empty or with formoterol. Muscle mass, contractile function, metabolic function, and histological evaluations were used to determine if the regenerative medicine approach was effective at 2-months post-injury. The regenerative medicine model produced greater permeabilized muscle fiber mitochondrial respiration and electron conductance through the electron transport system compared to untreated VML-injured mice; however, the non-biodegradable biomaterial was associated with lower muscle quality (i.e., lower muscle mass-normalized contractility) and fewer total muscle fibers. The conclusions reached from this study are: i) regenerative medicine strategies utilizing formoterol require further optimization (e.g., biodegradable optimization) but showed promising outcomes; and ii) in general, β-adrenergic receptor agonism continues to be a physiologically-supportive intervention to improve muscle metabolic function after VML injury.

3:25 PM - 3:37 PM

Sustained Release of siRNA from Antioxidant Polymer Microparticles for the Treatment of Osteoarthritis

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When reactive oxygen species (ROS) levels exceed the body's natural antioxidant abilities, cells experience oxidative stress, which has been attributed to the progression of degenerative diseases, such as osteoarthritis (OA). One factor attributed to the cause of OA, age, triggers cells to become senescent, a state in which replication is ceased but the cell does not die. Senescent cells secrete the SASP (senescence-associated secretory phenotype) which includes inflammatory cytokines, proteases, and growth factors that influence the behavior of nearby cells, contributing to OA progression. A common senescence target, Cdkn2a, encodes for p16 and p19, two cell cycle inhibitor proteins. We hypothesized that knockdown of Cdkn2a could inhibit cells from becoming senescent, decreasing SASP factors, and reducing joint damage caused by post traumatic osteoarthritis (PTOA).

Previous delivery systems have utilized PLGA microparticles (MPs), however these have been shown to cause negative immune responses in vivo. Instead, poly(Propylene sulfide) (PPS) has been shown to be inherently antioxidant. Due to its amorphous nature, MP formation with PPS yielded particles of small size (~2um). To yield MPs of larger size, PPS was copolymerized with ethylene sulfide (PPSES), a more crystalline polymer. PPSES was synthesized via an anionic ring opening polymerization with molar ratios of the two monomers ranging from 0-50% ES. Polymers with greater than 40% ES exhibited enough crystallinity to be formulated into MPs. Specifically, 50:50 PPSES formed large, stable MPs, making it an ideal candidate for sustained intra-articular drug release in OA.

Chemically-stabilized siRNA lipid conjugates were loaded into 50:50 PPSES MPs via solid/oil/water emulsion. A cyclic repeat loading model was used to induce PTOA in mice via bilateral loading. On day zero, particles were injected intravenously into mice at 1 mg PPSES/5.5 ug siRNA. After four weeks, tissues of interest were harvested for qPCR. Free siRNA conjugates failed to have significant knockdown of Cdkn2a. In contrast, PPSES-siCdkn2a MPs achieved ~68% knockdown compared to a control sequence in joint tissues, indicating sustained efficacy with MP delivery. Future work will investigate phenotypic joint changes with sustained Cdkn2a knockdown to further characterize the effect of siRNA-MPs in PTOA.

3:37 PM - 3:49 PM

Mucosa-targeted Inflammatory Bowel Disease Therapeutics for Localized Intestinal Immunosuppression

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Background: Crohn's disease (CD) is an inflammatory bowel disease mainly affecting the small intestine. Immunosuppressive drugs, including TNF-α-neutralizing antibodies and small molecule immunomodulators, are the cornerstone of CD treatment and are taken for months to years. However, widespread drug biodistribution after intravenous (IV) administration leads to systemic immunosuppression and increased risk for severe infections. Targeted delivery of CD drugs to the inflamed intestinal mucosa is needed to improve drug efficacy and localize immunosuppression to minimize off-target effects. Additionally, sustained release formulations are needed to improve medication compliance. To address these needs, we have developed a new delivery platform for sustained and targeted CD drug delivery to the intestinal mucosa that couples two components: (1) mucosa-homing bile acid (BA)-drug conjugates and (2) biodegradable microspheres for drug depot formation after intramuscular (IM) injection.

Methods: BAs are produced in the liver and released into the small intestine for lipid absorption. BA transporters facilitate efficient BA recycling, enabling reabsorption from the small intestine into blood circulation and uptake of circulating BAs back into the liver. To determine if hepatobilliary BA transporters could be leveraged for intestinal delivery, a model cargo (fluorescein) was conjugated to cholic acid (CA), a primary BA. CA-fluorescein biodistribution was evaluated in mice via IVIS imaging after IV and IM injection. To assess utility of biodegradable microspheres for sustained intestinal delivery, oil-in-water emulsion evaporation methods were used to encapsulate CA-fluorescein in poly(lactic-co-glycolic) acid microspheres for biodistribution studies.

Results: CA-fluorescein conjugates homed to the small intestine within minutes after IV/IM injection. Fluorescence signal was strongest in the small intestine with signal also appearing in the gallbladder, the organ that stores BAs before their release in the small intestine. Altogether, this supports the proposed mechanism of circulating conjugate uptake via hepatobiliary BA transporters for subsequent intestinal delivery. Small intestine signal was absent by 4h. For prolonged delivery, CA-fluorescein was successfully encapsulated in 30-µm diameter microspheres. Biodistribution studies are underway and formulations will be optimized for up to 2-week long release. CA conjugates with the small molecule immunomodulator, methotrexate, have been synthesized more recently to assess this delivery system using a CD drug cargo.

3:49 PM - 4:01 PM

Emulsion-Induced Polymersomes Taming Tetrodotoxin for Prolonged Duration Local Anesthesia

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Injectable local anesthetics that can provide a continuous nerve block approximating the duration of a pain state would be a life-changing solution for patients experiencing post-operative pain or chronic pain. Tetrodotoxin (TTX) is a site 1 sodium channel blocker that is extremely potent compared to clinically used local anesthetics. Challengingly, TTX doses are limited by its associated systemic toxicity, thus shortening the achievable duration of nerve blocks. Here, emulsion-induced polymersomes (EIP) are explored as a drug delivery system to safely use TTX for local anesthesia. By emulsifying hyperbranched polyglycerol-poly (propylene glycol)-hyperbranched polyglycerol (HPG-PPG-HPG) in TTX aqueous solution, HPG-PPG-HPG self-assembles into micrometer-sized polymersomes within seconds. The formed polymersomes have microscopically visible internal aqueous pockets that encapsulate TTX with an encapsulation efficiency of up to 94%. Moreover, the polymersomes are structurally stable, enabling sustained TTX release. In vivo, the freshly prepared EIP/TTX formulation can be directly injected and increase the tolerated dose of TTX in Sprague–Dawley rats to 11.5 μg without causing any TTX-related systemic toxicity. In the presence of the chemical penetration enhancer sodium octyl sulfate (SOS), a single perineural injection of EIP/TTX/SOS formulation produces a reliable sciatic nerve block for 22 days with minimal local toxicity.

4:01 PM - 4:12 PM

Albumin-binding nanobody-antigen fusions enhance antigen presentation and improve vaccine responses through pharmacokinetic modulation

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Peptide vaccines hold the potential to treat many diseases. These therapies utilize the exact antigen epitopes (~2 kDa) that are presented by major histocompatibility complexes (MHCs). Unfortunately, the efficacy of peptide-based vaccines has been limited due to inefficient trafficking to the draining lymph

node (dLN), rapid renal clearance, susceptibility to proteolysis, and presentation in an inappropriate context without the spatiotemporal co-delivery of an adjuvant. To address this, we have developed a platform for peptide antigen delivery using a nanobody (Nb) targeting mouse serum albumin (MSA). Nbs are alpaca-derived single-domain antibodies of small size (~12-15 kDa) and high target specificity. About ten times smaller than conventional antibodies (Abs), Nbs exhibit efficient tissue penetration and clear rapidly when unbound to their target, making them effective delivery vehicles for conjugated therapeutic cargos. We hypothesized that the fusion of peptide epitopes with a Nb targeting MSA (69 kDa), termed nAlb, would enhance antigen draining to the lymphatics by surpassing the renal clearance cutoff (~40 kDa). In this work, we synthesized fusions of nAlb and relevant peptide antigens. For stimulatory vaccines, we adjuvanted using a conjugate of nAlb and the STING agonist diamidobenzimidazole (diABZI). For pharmacokinetic and biodistribution studies, an nAlb-Cy5 conjugate was generated. Conjugates were generated using a combination of sortase and SPAAC reactions. Serum half-life was determined by treating mice with 2 nmol equivalent Cy5, collecting blood at indicated timepoints, and quantifying the Cy5 concentration via fluorescence spectroscopy. Ex vivo biodistribution, demonstrating accumulation in the draining lymph node (dLN), was determined by sacrificing mice 24 h post Cy5 injection and imaging the dLNs and major clearance organs using IVIS. Cy5 uptake by cell type in the spleen and dLN was determined using flow cytometry. Treatment with nAlb-OVA251-270 and nAlb-diABZI generated robust antigen specific immunity, quantified using a tetramer stain, that improved therapeutic responses in both prophylactic and therapeutic vaccination models of B16.F10 melanoma. Additionally, as robust antigen presentation absent co-stimulation drives tolerance, treatment of mice with nAlb-MOG35-55 inhibited the clinical onset of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS).

SESSION X: TISSUE ENGINEEREING 2

4:15 PM – 4:40 PM **Invited Speaker: Daniel Abebayehu, UVA**

4:40 PM - 4:52 PM

Guiding Early Cell Mechanoresponse and Precise Matrix Deposition for Anisotropic Meniscus Tissue Engineering

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To replicate the aligned nature of the meniscus via tissue engineering, advances have been made in fiber-reinforcement for recapitulation of its circumferentially-oriented architecture. However, most studies focus on macro-scale aspects, neglecting to consider the micro-scale cell-biomaterial interactions that govern matrix deposition/assembly towards organized neo-tissue. During development of the meniscus, cell alignment precedes aligned tissue deposition, highlighting the need to consider early response. Since the spatial response of cells within a fiber-reinforced microenvironment likely influences architecture of tissue, our objective was to elucidate patterns of cell response within these microenvironments. We utilized cell shape and mechanoresponse parameters, applying machine-learning strategies to better uncover these trends. To simulate a fiber-reinforced microenvironment, suture fragments were embedded within fibrin gels containing marrow-derived cells. Constructs were cultured for 3 days supplemented with TGF-β3 and varying concentrations of aprotinin (anti-fibrinolytic to tune remodeling). Cell and nuclear shape parameters, YAP nuclear ratio, and distance from suture were measured for individual cells following culture. Principal component analysis (PCA) and Agglomerative Hierarchical Clustering (AHC) was applied to cluster cells into 3 groups based on 23 cell parameters. By analyzing cell clusters identified by PCA-AHC, significant differences in cellular conformity and YAP nuclear localization were revealed, yielding clusters of High Response (HR), Medium Response (MR), and Low Response (LR) cells. Spatial patterns between clusters were evident, with a general trend of decreasing response with increasing distance from fiber. Modulation of matrix stiffness showed that stiffness influenced HR cell mechanoresponse, with softer environments allowing for greater response. Reduction of fibrin remodeling by aprotinin caused HR cells to localize closer to the suture, showing that the range of cell sensing can be influenced by remodeling of surrounding matrix. Together, this provides evidence that, in a fiber-reinforced microenvironment, matrix stiffness mediates cell response (“how”), while remodeling mediates localization of responding cells (“where”). This study demonstrated patterns of cell responsivity in fiber-reinforced microenvironments, which can be utilized to organize precise matrix deposition in scaffolds for meniscus replacement. Ongoing work aims to correlate these cell mechanoresponses to

composition of aligned matrix to improve design of meniscus scaffolds at the micro-scale, optimizing macro-scale tissue organization.

4:52 PM - 5:04 PM

Engineering Synthetic Human Immune Organoids to Recapitulate Humoral Immune Response

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In response to viral infection and vaccination, naïve B cells are activated and begin to proliferate with the aid of cytokines and co-stimulatory signals from helper T cells, and these cells terminally differentiate into plasma cells and memory B cells within the specialized areas of lymphoid tissues known as germinal centers (GCs). A robust B cell maturation is crucial for producing high-quality antibodies and establishing enduring humoral immunity. Unfortunately, reproducing the human B cell maturation process ex vivo is difficult, mainly because the intricate biological activities and signals within the lymphoid microenvironment that direct B cell maturation are not fully understood. Despite advances in technologies that focus on human secondary lymphoid organs, no ex vivo systems have demonstrated the influence of lymphoid tissue microenvironment. Here, we created synthetic hydrogels that recapitulate the lymphoid tissue environment and promote the formation of germinal centers using B cells from tonsils and peripheral blood mononuclear cells (PBMCs). Our findings show that poly(ethylene glycol) hydrogels with maleimide functionality (PEG-4MAL) maintain the life of human B cells and generate responses to both live and inactive influenza viruses, as well as to commercial vaccines. When comparing organoids derived from human tonsils and PBMCs, we found that the PBMC-derived organoids sustained the production of germinal center B cells and plasma cells over 24 days, resulting in the formation of light zones and dark zones and differential responses to adjuvanted H1N1 vaccines. Investigating the T cell populations within these organoids, we detected the engagement between follicular helper T cells and B cells, suggesting the T cells' supportive role in B cell maturation. Flow cytometry confirmed the longevity of follicular T cells in PBMC-derived organoids for up to 16 days. Moreover, bulk RNA sequencing showed that the origin of the cell (tonsil versus PBMC) regulated the longevity of B cell maturation in biomaterials-based immune organoids.

5:04 PM - 5:16 PM

The Impact of Provisional Matrix Remodeling on Cartilage Repair Microenvironments

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Focal cartilage injuries are a common musculoskeletal malady, which is a cause of concern due to its lack of ability to regenerate on its own. Repair methods like microfracture (MFx) rely on marrow recruitment to the site of injury, forming a provisional clot that is remodeled. Unfortunately, the inferior fibrocartilage that forms often leads to poor long-term outcomes. Our objective was to investigate early provisional matrix remodeling and its impact on MFx environments and their functional regeneration.

To understand in vivo contraction and fibrosis, full thickness trochlear defects were created in 3 rabbits, followed by microfracture. At one week, defects demonstrated considerable contraction (~50% defect fill) and expression of fibrotic markers (alpha smooth muscle actin, Collagen type I). To study this further, marrow-derived cells (MDCs) were isolated from juvenile bovine femoral condyles and encapsulated within fibrin gels. Microgels (10L) were fabricated in an 8-well chamber slide and cultured for 3 days with our without aprotinin (fibrinolysis inhibitor; 100 KIU/mL) and/or TGF-β3 (chondrogenic agent; 10ng/ml), fixed/stained (Phalloidin, a-SMA, YAP/TAZ, fibronectin), and imaged with confocal microscopy. TGF-β3 increased micro-scale contraction of the fibrin network and promoted fibrotic gene expression. Aprotinin mitigated both this fibrin remodeling and fibronectin deposition. Interestingly, aprotinin and TGF-β3 both increased YAP/TAZ nuclear localization in MDCs, with an additive effect when both were applied. Finally, macrogels (100L) were cultured for 7 days in chemically-defined media (control, aprotinin, TGF-β3, aprotinin+TGF-β3) and imaged at the terminal point to measure contraction. Terminal gene expression of urokinase-type plasminogen activator (uPa), a-SMA, and plasminogen-activator inhibitor (PAI-1) were performed. Aprotinin partially reduced TGF-β3-mediated contraction; however, aprotinin and TGF-β3 both increased pro-fibrotic expression, a result that was additive in combination.

In summary, microfracture exhibited contraction and fibrosis as early as one week, and the addition of TGF-β3 led to increased fibrinolysis, contraction, and early fibrosis. Aprotinin reduced fibrin remodeling and fibronectin deposition, but surprisingly, both aprotinin and TGF-β3 synergistically increased YAP/TAZ activity, contrary to our expectations. Thus, antifibrinolytics may prevent clot contraction but exacerbate MDC fibrosis. Further studies of an alternative method that combat both contraction and fibrosis (PL) are currently being investigated.

POSTERS

Poster #: 1

Bacteroides Species-Mediated Enzymatic Degradation of Extracellular Matrix Components

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The extracellular matrix (ECM) is a structure composed of natural biomaterials as organized arrays of macromolecules. The ECM plays an active role in the severity and exacerbation of disease, while its dynamic remodeling is also controlled by diseased microenvironments and mechanisms through enzymatic degradation. Within the context of gastrointestinal environments, the interactions at the microbe-ECM biointerface between members of the gut microbiome and native colonic ECM are poorly understood. Bacterium within the Bacteroides genus are opportunistic pathogens of high abundance in the gut that encode an assortment of enzymes. An increased prevalence of specific Bacteroides species has been associated with the exacerbation and progression of diseases characterized by ECM remodeling, such as inflammatory bowel disease and colorectal cancer. Thus, we hypothesized that members of the Bacteroides genus can alter their microenvironment through enzymatic ECM degradation. The present work aimed to characterize degradation of individual natural ECM biomaterials by bacterial proteases and carbohydrate-active enzymes (CAZymes). We found that strains of B. ovatus and B. thetaiotaomicron degraded glycosaminoglycansusing hyaluronic acid and chondroitin sulfate substrates, with species-level differences. In contrast, we determined that crude supernatants from four strains of B. fragilis could degrade gelatin, collagen I, collagen IV, and elastin with strain-level differences. Meanwhile, filtered cell-free B. fragilis supernatants could only degrade collagen IV and elastin. When evaluating enzymatic activity in the presence of various protease inhibitors, our results suggest the involvement of cysteine proteases, serine proteases, and matrix metalloproteases. We also sought to test the impact of the growth environment on ECM degradation activity. Fluctuations in pH during culture influenced the extent of elastin and glycosaminoglycans degradation by B. fragilis or B. ovatus and B. theta, respectively. Overall, we concluded that Bacteroides species can degrade ECM components through the inducible production of proteases or CAZymes that include elastin, collagen I, collagen IV, hyaluronic acid, and chondroitin sulfate. Additionally, our results suggest that the synthesis of these enzymes is likely impacted by environmental conditions during bacterial growth. Our work highlights the importance of microbe-ECM biointerface interplay as an overlooked process leading to remodeling of natural biomaterials.

Poster #: 2

Engineering thermoresponsive biointerfaces: the role of polymer grafting architecture on surface wettability

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Thermoresponsive surfaces exhibiting temperature-dependent hydrophobicity allow for control over bioadhesive processes, offering an alternative to traditional enzymatic cell harvest techniques. Enzymatic harvest degrades cell surface proteins and extracellular matrix (ECM), negatively impacting cell viability and the regenerative capacity of cell-based therapies. In contrast, the switchable hydrophobicity of thermoresponsive surfaces enables the detachment of intact cell sheets simply by lowering the temperature. At physiological temperature, cells adhere, but detach as the temperature is lowered and the substrate becomes hydrophilic. To leverage thermoresponsive surfaces for effective cell sheet harvest, it is imperative to understand the interplay between thermoresponsive polymer chemistry and resulting surface properties which ultimately influence biological outcomes such as protein adsorption and cell behavior.

We synthesized thermoresponsive copolymers comprised of di(ethylene glycol) methyl ether methacrylate (DEGMA) and methacrylic acid (MAA) as thermoresponsive units and surface anchoring units, respectively. Whilst poly(N-isopropylacrylamide) (pNIPAAm) is often used to develop thermoresponsive surfaces, polymers of DEGMA demonstrate similar functional properties without thermal hysteresis behavior, which is anticipated to yield cleaner cell sheet release. To evaluate the impact of grafting architecture on temperature-dependent surface wettability, copolymers were synthesized using reversible addition fragmentation chain transfer (RAFT) polymerization to install surface anchoring units in block and random configurations with controlled molecular weight. The spatial distribution of tethering points is expected to impact thermoresponsive chain mobility, influencing the extent of surface hydration and thus, substrate wettability, which guides biointerfacial interactions with proteins and cells.

Libraries of random and block copolymers with varying mol% MAA were synthesized and characterized using 1H nuclear magnetic resonance (NMR) spectroscopy to evaluate molecular weight and monomer distribution. Random and block copolymers were grafted to aminated glass surfaces and available surface amines before and after polymer addition were investigated using ninhydrin assays. To evaluate surface wettability, contact angle measurements were conducted at physiological and refrigerator temperatures, with lower contact angles expected below the hydrophilic transition temperature. By advancing understanding of how polymer chemistry impacts wettability, this work informs the design of temperature-responsive biointerfaces to propel cell biomanufacturing.

Poster #: 3

Collagen-like syntax imparts order and closed-loop phase behavior to intrinsically disordered protein polymers

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Intrinsically disordered protein polymers (IDPPs) are an emerging class of repeat proteins that can undergo phase separation in response to biomedically relevant stimuli. IDPPs mimic intrinsically disordered proteins (IDPs), which exhibit large molecular-level conformational fluctuations. Native IDPs often drive phase separation to control the assembly of cellular structures. Their phase behavior comes in two flavors, differentiated by whether the phase transition occurs above a lower critical solution temperature (LCST) or below an upper critical solution temperature (UCST). Collagen and elastin, two prototypical IDPs, represent these two extremes: UCST for collagen and LCST for elastin. These proteins share a disordered Pro- and Gly-rich scaffold but differ in the specific arrangement (syntax) of Pro and Gly residues and overall amino acid composition —with collagens uniquely featuring oppositely charged amino acids. Notably, the IDP domain of collagen drives the assembly of triple-helical domains, undergoing a disorder-to-order transition, a remarkable feature absent in engineered IDPPs. Inspired by these contrasting features, here we recombinantly synthesized IDDPs that incorporate collagen syntax and composition into elastin-like sequences. The resulting library consisted of over twenty IDPPs largely composed of matching elastin/collagen pairs that only differed in the underlying Pro-Gly syntax, ensuring that collagen-like IDPPs encoded perfect GXY repeats —a known syntactic element for triple-helix formation. Excitingly, computational examination of our designs by AlphaFold and Rosetta showed that collagen-like IDPPs uniquely fold into triple helical structures, whose stability is governed by the length of the IDPP and the choice of charged and aromatic residues. Contrasting the phase behavior of purified IDPPs, we discovered that the compositional bias of collagens encoded a novel dual LCST-UCST behavior (where LCST

Poster #: 4

Laminin I mediates resistance to Lapatinib in HER2-positive brain metastatic breast cancer cells in vitro

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The role of extracellular matrix (ECM) prevalent in the brain metastatic breast cancer (BMBC) niche in mediating cancer cell growth, survival, and response to therapeutic agents is not well understood. Emerging evidence suggests a vital role of ECM of the primary breast tumor microenvironment (TME) in tumor progression and survival. Possibly, the BMBC cells are also similarly influenced by the ECM of the metastatic niche; therefore, understanding the effect of the metastatic ECM on BMBC cells is imperative. Herein, we assessed the impact of various ECM components (i.e., Tenascin C, Laminin I, Collagen I, Collagen IV, and Fibronectin) on brain metastatic human epidermal growth factor receptor 2 (HER2)-positive and triple negative breast cancer (TNBC) cell lines in vitro. The highly aggressive TNBC cell line was minimally affected by ECM components exhibiting no remarkable changes in viability and morphology. On the contrary, amongst various ECM components tested, the HER2-positive cell line was significantly affected by Laminin I with higher viability and demonstrated a distinct spread morphology. In addition, HER2-positive BMBC cells exhibited resistance to Lapatinib in presence of Laminin I. Mechanistically, Laminin I-induced resistance to Lapatinib was mediated in part by phosphorylation of Erk 1/2 and elevated levels of Vimentin. Laminin I also significantly enhanced the migratory potential and replicative viability of HER2-positive BMBC cells. In sum, our findings show that presence of Laminin I in the TME of BMBC cells imparts resistance to targeted therapeutic agent Lapatinib, while increasing the possibility of its dispersal and clonogenic survival.

Poster #: 5

The Impact of Natural Extracellular Matrix Proteins on Quiescence Induction in In Vitro Cultured CCD-18 Colonic Fibroblasts

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Intestinal fibrosis, a common complication in Crohn's disease (CD), is associated with the activation of fibroblasts, a key cell population in the intestinal wall. However, studying these cells in their quiescent state in vitro is challenging due to their propensity to undergo phenotype changes in response to the underlying substrate stiffness, like tissue culture polystyrene flasks (TCPS). Thus, conventional culture methods are inadequate for studying fibroblast activation processes. Our research aims to develop a protocol to induce quiescence in CCD-18 colonic fibroblasts in vitro, focusing on the role of natural extracellular matrix (ECM) proteins as cell culture coatings. Both ECM protein coatings and Vitamin D (VD) treatment have been suggested to mitigate pro-fibrotic effects in fibroblasts.

In our experiments, fibroblasts were cultured on different ECM coatings (collagen I, collagen III and laminin) or in VD-containing media with varying concentrations. We also assessed the effects of media supplementation with fibroblast growth factor (FGF). The fibroblast phenotype was evaluated by quantifying cell proliferation, viability, and α-smooth muscle actin (α-SMA) protein expression. Our results showed that treatment with 10 μM VD in conjunction with FGF led to a more pronounced fibrotic phenotype. Interestingly, under the influence of 10 μM VD alone, cells exhibited reduced α-SMA production. The choice of ECM coating on the culture plates also elicited diverse effects on cell activation, with lower concentrations of laminin exhibiting the capability to induce fibroblast quiescence. These findings highlight the important role that natural biomaterials can play in dictating fibroblast phenotype and underscore the challenges of developing a protocol to attain quiescence. Our data demonstrates diverse activity phenotypes in response to different treatments. To address this challenge, we are currently developing an optimal protocol by employing a full-factorial design of experiment (DOE), which considers the synergic impacts of ECM protein coatings, VD, and FGF to induce the maximum transition of fibroblast phenotype to the quiescent state. Our systematic approach will ultimately contribute to our understanding of fibroblast quiescence and yield a protocol that will allow us and other research groups to explore the mechanisms that trigger intestinal fibrosis.

Poster #: 6

Plodia interpunctella silk fibers: protein structure and composition influences on structure and cytocompatibility

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Silk-based biomaterials, primarily derived from the silk fibroin protein of the Bombyx mori (B. mori) silkworm, have been studied for tissue engineering applications due to their advantageous mechanical properties, biocompatibility, and commercial availability. More recent efforts have aimed to expand the range of achievable silk-based biomaterial properties by investigating alternative sources of silk proteins that vary from B. mori silk in sequence and structure. These structural distinctions drive differences in physical and chemical properties of silk fibers, primarily due to the varying degree of crystalline content in the polymers. Crystalline content in silk fibroin is also the primary factor influencing the performance of silk biomaterials, translating to advantageous properties such as high elasticity, increased tensile strength, or enhanced bioactivity. For the development of alternative silk-based materials, we investigate silk from the Plodia interpunctella (P. interpunctella) silkworm. Early investigations into P. interpunctella silk have highlighted differences between the P. interpunctella silk fibroin proteins and B. mori silk fibroin proteins; however, P. interpunctella silks still largely lack development and characterization at the fiber level. This work evaluates the structural, thermal, mechanical, and cell-material properties of P. interpunctella silk as a raw material for biomaterial fabrication. As silk fibroin is the primary constituent in silk-based biomaterials, we explore how isolation of silk fibroin through degumming processes shifts properties and cytocompatibility from the non-degummed material. We utilize atomic force microscopy (AFM), dynamic mechanical analysis (DMA), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR) to analyze material properties in addition to assessing proliferation and metabolic activity of normal human lung fibroblasts (NHLF) cells seeded on silk fiber sheets. Observed properties are used to identify links between silk fibroin protein sequence and fiber function in addition to forming hypotheses in how P. interpunctella silk-based biomaterials will perform in comparison to other natural biopolymers used in biomaterials. Ongoing work aims to develop methods to process P. interpunctella silk into material formats for medical applications in drug delivery and tissue engineering, utilizing the material characteristics determined here as a baseline for shifts in material performance.

Poster #: 7

Biodegradable Metallic nanoparticles incorporated nanofiber mesh: A potent fibrous platform for wound healing application

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I prefer poster presentation only

Metal particles incorporated polymer matrices in various forms and geometry are found attracted materials platform for promoting wound healing and prevention of infection. However, the faith of these metal particles and their degraded products in the tissue environment is still unknown as both can produce cytotoxic effects and promote unwanted wound reactions. In this study, we synthesized and analyzed biodegradable metal particles, magnesium (Mg) and zinc (Zn) incorporated nanofiber mesh scaffolds for potential wound healing application. We first developed the coated metal particles with zein, a soluble protein from corn, and then imbedded those in polycaprolactone (PCL) nanofibers via electrospinning. We performed multi-modal evaluations of properties of the fibrous scaffolds' including physio-chemical properties and in vitro cellular responses. Several physicochemical properties such as fiber morphology, crystallinity, mechanical strength, hydrophilicity, degradation and release of metal ions, so forth were characterized. In vitro cellular response of the scaffolds was evaluated utilizing direct and indirect cytotoxicity assays and immunocytochemistry analysis with human dermal cells, Human umbilical vein endothelial cells. We provide evidence that the integration of metal particles in PCL nanofibrous scaffolds improved its physicochemical and biological functions. The immunocytochemistry analysis confirmed the elevation of vimentin and α-smooth muscle actin with the scaffolds suggesting that the fibroblast cells were highly differentiated into myofibroblasts. These fibrous scaffolds have significant potential in encapsulating and delivering both bio-active components and modulate the cellular activities that are involved in tissue repair and remodeling.

Poster #: 9

Combination therapy of microporous hydrogel scaffolds and supramolecular peptide assemblies to enhance wound healing

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Disruption to the skin's barrier function triggers a coordinated cascade of immune processes aimed at restoring homeostasis. However, dysregulation of these immune responses may result in overactivation of inflammatory pathways and elevated secretion of pro-inflammatory molecules, ultimately resulting in delayed and poor wound healing. Supramolecular peptide nanofibers and Microporous Annealed Particle (MAP) scaffolds are promising strategies to modulate local immune activity for the safe and rapid treatment of inflammation without the need for additional immunostimulatory factors or recurrent administrations. Additionally, MAP scaffolds allow for rapid wound closure and significant regeneration to cutaneous wounds, including augmented regeneration of skin appendages, and increased tissue tensile strength. As a dual-arm strategy to modulate inflammation during wound healing, we have designed an immunotherapy utilizing MAP scaffolds and the supramolecular peptide nanofiber Coil29. Our MAP+Coil29 material elicits robust epitope-specific Th2-biased humoral and cellular immune responses, while also enabling rapid tissue regeneration following a single application at the wound site. Our work aims to modulate inflammatory pathways via local application of MAP+Coil29 scaffolds at the injury site of a murine wound healing excisional model. We hypothesize that in addition to a local Th2-biased response favoring regeneration, materials raising autologous antibody responses against inflammatory signaling pathways will result in further enhanced regeneration of cutaneous wounds, with longer lasting efficacy compared with current passive blockade technologies.

Poster #: 10

Engineered 3D In Vitro Bone Niche for Modeling Breast Cancer Metastasis in Bone

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Introduction: Breast cancer patients often develop metastases in bone, causing severe morbidities. Understanding breast cancer bone metastasis (BCBM) in vivo remains challenging due to uncontrollable factors and complexities. An in vitro platform is crucial to systematically study BCBM mechanisms. This study involved the development of a 3D printed polyester-ceramic composite scaffold to explore interactions between breast cancer cells and the bone niche.1 This approach could offer new insights into preventing and treating BCBM.

Materials and Methods: Polylactide (PL, 45wt%) pellets and nano-β-tricalcium phosphate (TCP, 55wt%) were dissolved in dichloromethane. The solvent was evaporated, and the dried composite was extruded at 165°C into filament. Composite and PL filaments were 3D printed into porous scaffolds (7mm diameter ×5 mm height). The filament diameter, pore geometry, surface roughness, and water contact angle (n ≥3) were measured. Subsequently, 1×105 MCF7 breast cancer cells in 75µL media were seeded onto the scaffolds and adhesion was evaluated.

Results and Discussion: The fabricated scaffolds had pore sizes of 546.5 ± 30µm for both PL and PL/TCP groups, similar to the pore size of native cancellous bone (300-600 µm). The TCP scaffolds had a lower water contact angle (76.77 ± 0.85°) compared to the PL scaffolds (118.77 ± 1.97°), indicating improved hydrophilicity. Similarly, the surface roughness of the TCP scaffolds (10.8 ± 1.36µm) was significantly higher than that of the PL scaffolds (2.51 ± 0.31µm). As anticipated, the number of MCF7 cells adhering to the TCP scaffolds (7.6 ± 0.3 × 104) was higher than that in the PL scaffolds (6.3 ± 0.75 × 104), suggesting that enhanced hydrophilicity and surface roughness positively influenced cell adhesion.

Conclusions: The 3D printed bone scaffolds fabricated from novel filament (PL and 55wt% nTCP) were demonstrated as a first step toward building an in vitro BCBM model. Ongoing studies will study the use of the scaffolds to elucidate the interactions between osteoblasts and MCF7 cells within the niche.

References:

1. Chen W, et al, “Developing an Engineered In Vitro Bone Niche for Rapid Cell Adhesion and Early-Stage Interactions of Osteoblasts and Metastatic Breast Cancer Cells”, 2023 BMES Annual Meeting.

Poster #: 11

Plodia interpunctella silk: a pathway to sustainable and consistent biomaterials

Bryce Shirk, University of Florida

I would like to be considered for a Rapid Fire Oral Presentation

Silk, a naturally derived biopolymer produced by various arthropod species, has been highly valued throughout history for applications in textiles, cosmetics, and biomedical industries. Traditionally, sericulture has focused on the Bombyx mori species to increase manufacturing capacity and enhance silk fiber properties. However, these efforts have led to unintended variations in gene expression and silk protein production due to the cost-effective necessity of outdoor rearing practices. Seasonal changes and global warming concerns, which alter temperature and food source quality, further impact silk fiber consistency, posing a challenge for the commercialization of silk-based biomaterials. To address these issues, we explored the alternative silkworm species Plodia interpunctella, which can be reared indoors, allowing for controllable silk production. This study evaluates the parameter space for rearing P. interpunctella and its impact on silk fiber production and growth, assessing its potential for consistent, commercial-scale silk fiber production. We controlled temperature (24°C, 26°C, and 30°C), resource availability (larvae/gram diet), and population density (larvae/mL) to optimize silk fiber production and homogeneity. Our results indicated that higher temperatures accelerated insect growth, reducing their life cycles. Additionally, population density had the greatest impact on total silk production. Optimal conditions for maximizing silk production were found to be 24°C, with 180 larvae and 18 grams of food per 250 mL container. Under these conditions, only nine standard rearing boxes were needed to produce 5 grams of dry silk fibers in approximately 20 days. These findings suggest that P. interpunctella may be a promising alternative to B. mori due to its ease of laboratory rearing and controlled fiber reproducibility. Future work will investigate how the surface area within the rearing container affects total silk protein production. Additionally, we are genetically modifying the silk-producing organism to generate silk fibroin-like proteins for advanced biomaterial functionality. This controlled indoor rearing capacity allows for ethical and tightly regulated management of genetically altered species, paving the way for innovative biomaterials.

Poster #: 13

Launching Research - Online Delivery of Research Initiation Modules

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Introduction: We previously introduced a slate of research professional skills modules as a starting point for adaptation to a particular setting that can be successfully used in didactic or research settings [SFB 2019]. More recently, we found the need to develop them into an online repository to facilitate asynchronous learning.

My NRMN (my.nrmnet.net) is an online networking/mentoring platform to help connect Mentors and Mentees across the country. It is partially supported by the National Institutes of Health and has a strong biomedical focus.

Materials and Methods: The premise of the “Launching Research Part 1: Laboratory Readiness” was that students would benefit from the content that we have found useful with our own undergraduate and graduate researchers but it would be accessible through the online network and still mentor supported by the mentoring team. That is, students could complete the modules within the context of a conversation with an online mentor. We posed a series of pre and post questions to assess the incoming mindset of the learners and any change produced through the modules.

Results and Discussion: Questions such as Research is: 1) Finding new solutions to problems or to create new devices, 2) Testing and formulating new principles to improve understanding in a field, 3) Critically examining current ideas in your “field”, and 4) All of the above elicited the correct “All of the Above” from most students entering the course. We believe the value in the modules is in the details such as “A good email always starts with a "Hey So-and-So" greeting” 1) True or 2) False which showed improvement pre and post. It was surprising that the participants appeared to came into the course with a fairly sophisticated understanding of research.

Conclusions: Future work will be completed to refine the modules and to add additional content, following a customer discovery process. It appears viable to provide mentor augmented content in an online content.

Acknowledgments: Funding for this work was provided by the Harbor Lights Endowment.

References:

1. Burg TCB and KJL Burg. 2019. “Biomaterials Experiential Learning: Integrating Research and Professional Development”, Trans Society For Biomaterials.

Poster #: 14

Soft implantable printed bioelectronic system for wireless continuous monitoring of restenosis

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Atherosclerosis is the most common underlying condition of cardiovascular disease, which remains the leading cause of death worldwide. This disease, where plaque builds on the inner walls of arteries causing blood vessels to narrow, is generally addressed through angioplasty and stenting. While implanting stents is a common procedure, a frequent complication is in-stent restenosis, where the artery re-narrows due to scar tissue. There are limited clinical options when diagnosing in-stent restenosis, as it can be asymptomatic until a severe blockage causes rapid degradation of a patient's condition.

To address this major clinical gap, we report an implantable vascular electronic device using a newly developed miniaturized capacitive strain sensor. A microneedle and capillary-based printing system is employed to achieve a high-resolution patterning of a soft, capacitive strain sensor. The sensor is made from alternating layers of polyimide and silver nanoparticle ink, then fully encapsulated in PDMS. The sensor is integrated with a wireless vascular stent to offer a battery-free, wireless monitoring system compatible with conventional catheterization procedures. The vascular stent is fabricated with enhanced laser cutting and electroplating settings to ensure low resistance and reliable antenna performance.

This sensor successfully fits within the stent to allow for seamless integration within a catheter, ensuring proper deployment into blood vessels. Restenosis was successfully diagnosed at variable restenosis stages using continuous monitoring of resonant frequency changes through pulsatile testing. Collectively, the arterial implantable bioelectronic system shows the potential for wireless, real-time monitoring of various cardiovascular diseases and stent-integrated sensing and treatments. This research demonstrates a fully printed, low profile strain sensor with high sensitivity to remotely detect restenosis within a stent. Furthermore, the device has clinical implications to provide high-risk patients with real-time monitoring of their health, providing them with more data to customize and continue their care.

Poster #: 15

Optimizing Delivery of Therapeutic Satellite Cells using a Platelet-like-Particle Laded Fibrin Scaffold in a Murine Model of Hind Limb Ischemia

Isabel Wallgren, Emory University

I prefer poster presentation only

Peripheral artery disease (PAD) occurs when atherosclerotic plaque builds up in the limbs, blocking blood flow to the region. While current interventions, like exercise regimens and management of underlying conditions such as diabetes and hypercholesteremia limit disease progression, invasive surgical intervention is often necessary to prevent the advancement of critical limb ischemia. An alternate, less invasive approach is based on promoting angiogenesis and arteriogenesis to strengthen the collateral vessel network, circumventing the blockage. The Hansen lab has demonstrated that satellite cells, skeletal muscle stem cells that repair muscle fibers and release growth factors, can be harnessed as a potential therapeutic for promoting tissue regeneration. The prior delivery method, encapsulating satellite cells in alginate, allowed for the release of angiogenic factors but prevented cells from moving into and repairing damaged muscle. To optimize this therapy in a mouse model of PAD, we hypothesize that a fibrin-based scaffold will allow the cells to more successfully promote angiogenesis and repair ischemic tissue compared to encapsulation in alginate. An in vitro study compared satellite cell viability when contained in fibrin gels of three stiffnesses (0.1, 0.25, 0.4 U/mL thrombin) and determined that while cells maintained high viability through day 14 in all groups, cell population increased as the concentration of thrombin increased. Another study explored the effect of initial seeding density on cell viability using 10k, 50k, and 100k cells per 250 uL clot in 0.1 and 0.4 U/mL thrombin. Preliminary results confirm that higher thrombin concentrations have greater proliferation and demonstrate that seeding 100k cells per clot is a feasible density for in vivo injections. Despite greater proliferation, higher thrombin clots hinder in vivo delivery by clogging the injection needle. We hypothesize that by utilizing the Brown Lab's composite fibrin-colloid scaffold with fibrin-binding platelet-like-particles (PLPs), we can use lower thrombin concentrations while still achieving higher stiffness. A current in vitro study is evaluating cell viability in PLP-laden fibrin clots containing 0.25 mg/mL PLPs with 0.1 or 0.25 U/mL thrombin, which will identify what groups to use for in vivo testing in a murine model of hind limb ischemia.

Poster #: 17

Engineering quiescent valve interstitial cells to explore the role of gut metabolites in valve disease

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Calcified Aortic Valve Disease (CAVD) is associated with diet-related shifts in the gut microbiome. Recent studies suggest that trimethylamine oxide (TMAO), a gut metabolite, may serve as a biomarker for atherosclerosis. We hypothesize that TMAO might also contribute to CAVD by activating valve interstitial cells (VICs), the primary cell type in the aortic valve given the share risk factors and molecular triggers.

To test this hypothesis, VICs isolated from female porcine aortic valves were cultured with 10% FBS on tissue culture plastic. TMAO treatments (25µM to 150µM) were then applied alongside control groups (untreated and transforming growth factor beta - TGF-β, an inducer of VIC activation). TMAO treatment had no significant effect on proliferation or α-SMA expression, markers of VIC activation. Conventional culture conditions spontaneously activate VICs into a myofibroblastic phenotype, complicating trigger identification.

We sought to assess if TMAO activates VICs under physiological conditions. We induced quiescence by our lab engineered protocol using a collagen-the primary biomaterial found in healthy aortic valves. To generate quiescent VICs (qVICs), VICs were cultured on collagen-coated plates (2µg/cm2) supplemented with 2% FBS, insulin, and fibroblast growth factor (FGF) for 10 days. We then treated these qVICs with TMAO (25µM to 150µM) for up to 5 days.TMAO treatment of qVICs increased proliferation and α-SMA expression (markers of activation), reaching levels comparable to activated VICs and TGF-β-treated cells.

TMAO treatment also increased qVIC production of extracellular matrix (ECM) proteins, reactive oxygen species, and angiogenic cytokines, suggesting that TMAO may also induce other hallmarks of CAVD beyond VIC activation. We were able to successfully identify the pathway utilized by this gut metabolite to initiate VIC activation. Inhibition of the Protein Kinase R-like ER kinase (PERK) pathway effectively attenuated the effects of TMAO on qVICs.

These results emphasize the significance of leveraging natural biomaterials to engineer physiologically relevant cell phenotypes. Our collagen-based protocol allowed us to establish the impact of TMAO treatment on healthy quiescent VICs. Overall, these findings suggest that TMAO may play a role in the development and progression of aortic valve disease, highlighting the importance of considering host-microbe interactions in cardiovascular disease progression.

Poster #: 18

Comparison of cartilage pre-digestion techniques for auricular cartilage tissue constructs

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Auricular cartilage has limited regeneration capacity; therefore, researchers have explored reconstruction strategies like chondrocytes in 3D printed scaffolds, minced cartilage embedded in fibrin gels, and isolated chondrocytes encapsulated in hydrogels. As per these examples, different cartilage pre-treatment types can be used, so the goal of this study was to compare different levels of cartilage pre-digestion (isolated chondrocytes (isolated), partially digested minced cartilage (partially digested), minced cartilage (minced)) within poly (ethylene glycol) (PEG)-based hydrogels in terms of neo-cartilage formation within the constructs. Specifically, in this study, we developed a method for a partially digested minced cartilage matrix that we hypothesized can support chondrocyte phenotype maintenance due to the presence of native cartilage extracellular matrix (ECM).

Fresh auricular cartilage was cut into 1mm2 and 2mm2 pieces for the isolated and partially digested groups, respectively, and then treated with a 0.6 wt.% collagenase solution for 4 hours. All cartilage digestion levels were encapsulated in a 15 wt.% PEG-diacrylate (PEGDA) solution and crosslinked via radical polymerization. Cell viability was measured using Live/Dead staining (n=4). Over 4 weeks in vitro, gene expression of collagen 2 (COLII) and aggrecan (ACAN) was measured using PCR (n=4). In addition, hydrogels were stained for H&E and Safranin O and underwent immunohistochemistry (IHC: COLI, COLII, ACAN) (n=4).

All groups remained viable over 7 days. Isolated and partially digested groups showed a significant increase in COLII expression by week 2 (isolated:1018 ± 265, partially digested: 90 ± 56; (fold change over passage 2 chondrocytes)), while the isolated group expressed significantly more COLII (74 ± 16) and ACAN (7 ± 2) by week 4. H&E and Safranin O revealed a homogenous distribution of isolated chondrocytes and glycosaminoglycan deposition in the isolated group. IHC of the isolated group showed intense COLI, COLII, and ACAN staining around nuclei and additional ACAN deposition in the hydrogel matrix at week 4. This novel study provided a head-to-head comparison of different cartilage digestion levels as tissue sources for various cartilage tissue engineering applications.

Poster #: 19

Supramolecular peptide-protein granules for intracellular CRISPR-Cas9 protein delivery

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I would like to be considered for a Rapid Fire Oral Presentation

CRISPR-Cas9 protein has been revolutionary for both gene therapy and generation of engineered cell lines due to its ease of use and versatility. However, because of its large size, charge, and hydrophilicity, it does not readily cross the cell membrane. Current delivery methods either create pores in the cell membrane to allow it to cross or utilize nanomaterials such as lipid nanoparticles to allow for uptake via endocytosis. These methods are limited by toxicity and efficiency respectively. Here, we present a supramolecular peptide-protein delivery vehicle for intracellular protein delivery that seeks to address these challenges. This approach utilizes charge-complementary molecules known as CATCH(+) peptides and CATCH(-) fusion proteins. Alone, these molecules do not self-assemble due to electrostatic repulsion, but when combined form β-sheet fibrils. If a crowder is added such as polyethylene glycol (PEG), Tween-80 micelles, or excess CATCH(+) peptide, nanoscale CATCH(+/-) granules are formed at peptide concentrations ~10-fold lower than the critical fibrillization limit in dilute conditions (~200 mM). Granules are internalized in greater than 90% of treated cells within minutes by various suspension and adherent cell lines including fibroblasts, HEK293, T cells, dendritic cells, monocytes, and neutrophils with minimal cell death. Additionally, CRISPR-Cas9 protein that is endocytosed also needs to escape the endosome and traffic to the nucleus to reach its genomic target. MDA-MB-231 cells engineered to express galectin 8 fused to YFP were utilized to visualize endosomal escape of CRISPR-Cas9 protein granules. PEG, CATCH(+), and CATCH(-) fusion protein did not demonstrate endosomal escape alone. However, granules did show significant endosomal escape within minutes. Next, CRISPR-Cas9 targeting GFP for knockout was delivered to HEK293 cells engineered to express GFP. Granules had ~50% GFP knockout efficiency, which is comparable to other gold standard methods with minimal cytotoxicity. Collectively, these data establish CATCH(+/-) granules as a simple and flexible nanomaterial platform for cell engineering via intracellular protein delivery.

Poster #: 20

Tailoring nanoparticle design and transport by emulsion-mediated PEGylation

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How a drug delivery system (DDS) transports through the body to the intended target helps determine the effectiveness of the bioactive cargo. The DDS properties, like size and incorporation of poly(ethylene glycol) (PEG), are vital to navigating transport barriers within the body. For particles made using emulsion polymerization, like poly(propylene sulfide) nanoparticles (PPS-NP), the surfactant alters the particle properties as it is incorporated into the particle corona. Therefore, PEG-containing copolymer surfactants are used to synthesize and PEGylate PPS-NPs, controlling particle properties and PEGylation during synthesis, which then mediate biological transport, eliminating the need for post-synthesis modulation. PPS-NP were synthesized using a copolymer library at various concentrations. The copolymers differ in the lengths of the PEG and poly(propylene glycol) (PPG) components, yielding different copolymer properties, such as PEG chain length and overall hydrophilicity. NPs were characterized by thermogravimetric analysis (TGA) and dynamic light scattering (DLS) to determine composition, extent of surface PEGylation, and hydrodynamic size. Linear models were constructed to evaluate the correlations between copolymer and NP properties, with and without the influence of copolymer concentration. NPs synthesized using various copolymers exhibited properties dependent on copolymer concentration. After removing the dominating influence of copolymer concentration, NP size was most correlated to the PEG length and percentage of PEG in the copolymer. Meanwhile, the extent of PEGylation was most correlated to the hydrophilicity and PPG length, which controls the distance between PEG chains on the NP surface, demonstrating the influence of copolymer properties on the resulting NP properties. Next, NP diffusivity through collagen, an in vitro model of skin interstitium, was evaluated by fitting the fluorescence of fluorophore-labeled NPs throughout the collagen to a diffusion model. The NP diffusivity depended on copolymer concentration, PEG length, and PPG length. Diffusion is a size-dependent process, agreeing with the correlation to copolymer concentration, which strongly mediates the NP size. Furthermore, the PEG and PPG lengths, which modulate the size and density of the PEG chains on the NP surface, additionally influence diffusivity, mediating the interactions between the NP and collagen. The copolymer properties that influenced changes in NP properties also manifested changes in NP transport.

Poster #: 22

Royal Jelly's 10-Hydroxy-2-decenoic-acid Imparts Potential Antimicrobial Properties to High Density Polyethylene through Immersion

Elizabeth Matlock-Buchanan, University of Memphis

I would like to be considered for a Rapid Fire Oral Presentation

High Density Polyethylene (HDPE) is a common polymer material currently used to fabricate food containers, medical consumables, and implants. Unfortunately, HDPE is prone to contamination by microorganisms through attachment and formation of biofilm colonies. Biofilm formation may result in severe infections, which then may cause implant rejection, repeated surgeries, and may even lead to death. 10-Hydroxy-2-decenoic-acid (10-H2DA) is an antimicrobial unsaturated fatty acid and is the main lipid component found in the honeybee's royal jelly.10-H2DA has been reported to have antitumor, antimicrobial, and other inhibitory properties. Here the therapeutic is investigated as a dispersal signaling molecule, or DSM, to inhibit the spread of microbes and eventual formation of biofilm colonies on the polymer HDPE. HDPE coupons were immersed in a solution of 10 mg of 10-H2DA fatty acid to 1 mL of ETOH and then air dried. Characterization included FTIR, contact angle, and an HPLC analysis of the three-day elution study in phosphate buffered saline, or PBS. The HDPE coupons were immersed in 200 µL of PBS with the entire solution removed and frozen at each time check. Fresh PBS was then added back to each coupon until the next time check with nine time checks total up to 72 hours from initial immersion in therapeutic. Results of FTIR and contact angle analyses confirm that the 10-H2DA therapeutic was adsorbed to the surface without chemical conjugation. Elution studies showed that approximately 75% of the adsorbed fatty acid was released in the first three hours, with an additional 20% eluted within the first six hours. This almost complete initial release happened because the therapeutic only adhered through hydrophobic interactions. With 95% of the original amount of the 10-H2DA therapeutic released within the first six hours. This simple coating strategy could be used to provide early protection from microbes without using traditional antibiotics.

Poster #: 23

Fabrication of antibody-loaded microparticles for sustained-release immunotherapy of ovarian cancer

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Low success rates have been reported in the systemic administration of checkpoint-inhibitor immunotherapy of ovarian cancer. We hypothesize that through the localized administration of an antibody reservoir, treatment can be sustained and concentrated in the tumor region. In this study, we present the design and development of antibody-loaded particles towards sustained-release immunotherapy. Coaxial electrospray (CES) is an emerging technology in the encapsulation of biomolecules because it's a one-step process that can achieve high encapsulation rates. The encapsulation of the fluorescently-labeled human immunoglobulin G (IgG-FITC) antibody was achieved in PLGA microspheres using CES.

Poster #: 24

Engineered Hexameric Coiled-Coil Fusion Protein as Potential Potent COVID-19 Therapeutic and Prophylactic

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After almost 7 million deaths worldwide and its lasting effects on everyday life, COVID-19 is here to stay. One of the most common approaches to neutralize the disease's pathogen, SARS-CoV-2, is through blocking the Receptor Binding Domain (RBD) region of the viral spike protein from associating with Angiotensin Converting Enzyme 2 receptor on human cells, thus preventing cell entry and viral replication. Due to the speed at which SARS-CoV-2 mutates, however, the customary approach of using highly specific monoclonal antibodies (mAbs) to target this region proves to be challenging, due to their size, production complexity and cost of production. To address this problem, we proposed using Designed Ankyrin Repeat Protein (DARPin) – smaller, less complex antibody-mimetic proteins. To achieve multivalency, we genetically fused and displayed these proteins on CC-HEX, a self-assembling hexameric coiled coil nanocarrier. The fusion proteins are modular and were efficiently produced in E.Coli, making them much more adaptable to viral mutations. Here, we demonstrated potent neutralization of SARS-CoV-2, across multiple variants (Wuhan, Delta, Omicron) through pseudovirus assay. Additionally, these fusions proteins conserve their structure and neutralizing ability after aerosolization using a vibrating mesh nebulizer. Through this work, we hope to establish HEX-DARPin proteins as potential prophylactics and therapeutics for COVID-19.

Poster #: 25

Peptide-hydrogel properties influence the development of anti-drug antibody against immobilized biopharmaceuticals

Lucas Melgar, University of Florida

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Biomaterial approaches for biopharmaceutical delivery can directly address first-pass challenges including targeting to disease site and extending half-life. However, prolonged drug exposure time also increases the likelihood of emergence of anti-drug antibodies (ADAs) against the foreign biological macromolecule, compromising its therapeutic effects. We have developed a hydrogel platform for protein immobilization based on pairs of cationic and anionic peptides that co-assemble into supramolecular fibrils known as "CATCH(X+/Y-)". Changing the number (X,Y = 2, 4, or 6) or identity (X = K/R; Y = D/E) leads to changes in CATCH(X+/Y-) stiffness and structure. CATCH(+/-) hydrogels are injectable and retained at subcutaneous injection sites for more than 2 weeks with weak, rapidly resolving inflammation. Using a subcutaneous repeated injection model to deliver a therapeutic enzyme, we show that ADA development depends on CATCH(X+/Y-) material properties and formulation conditions. In contrast with similar peptide fibril systems, increased ADA was not associated with a particular CATCH(X+/Y-) hydrogel charge state; instead, net neutral pairs were more immunogenic than net cationic or anionic pairs. Increased stiffness of CATCH(X+/Y-) hydrogels also correlated to higher antibody titers than did softer gels, irrespective of overall charge. CATCH(X+/Y-) in a free-flowing "sol state" nanofiber formulation resulted in lower antibody titers compared to the hydrogel state, although, this was accompanied by a decreased residence time at ~7 days. CATCH(6K+/6D-) mixtures formed unique spherulitic structures in the sol state (i.e., "microspheres"), which had increased residence time on par with that of the hydrogel state. CATCH(6K+/6D-) microspheres showed the lowest ADA titers even with this increased residence time. Collectively, these data demonstrate that antibody development against an immobilized enzyme is highly dependent on the characteristics of the CATCH(X+/Y-) carrier including its charge, stiffness, residence time, and architecture, which can be ultimately tuned to increase pharmacokinetics of the biopharmaceutical without negative impacts on immunogenicity.

Poster #: 26

Thermosensitive Hydrogel for Local, Sustained Release to Lymphatic Vessels

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Statement of Purpose: Lymphedema is a chronic disease characterized by accumulation of interstitial fluid, impaired lymphatic vessel pumping, and fibrosis, leading to swelling, infections, and limited mobility. It affects over 130 million people worldwide, but despite its prevalence, there is no pharmacological treatment. Microcystic lymphatic malformations, another disease of lymphatic vessels, is characterized by the growth of infiltrative lesions containing lymph or blood which can cause deformation, pain, and organ dysfunction. While rapamycin has been explored as a treatment, long term use of this drug at high enough doses to induce lesion regression may not be feasible given the side effects. Therefore, there is a need in both diseases for a local, sustained release drug delivery platform which delivers immune modulatory drugs directly to lymphatic vessels. In this work, we present a thermosensitive hydrogel (F127-g-gelatin) made from the biocompatible polymers gelatin and Pluronic® F127, which are widely used in humans. This injectable hydrogel degrades into drug-loaded micelles which are optimally sized for lymphatic uptake, enabling locoregional delivery of drugs to lymphatic vessels. A variety of drugs, including rapamycin, can be loaded into F127-g-gelatin by simple mixing.

Methods: In vitro release of rapamycin-loaded F127-g-gelatin was assessed through degradation and drug release studies. A stability study of F127-g-gelatin was conducted on polymer stored dry or dissolved at 4.5 wt% in PBS at several temperatures for up to 6 months. Gelation temperature and degradation of the hydrogel were measured at each time point.

Results & Conclusion: F127-g-gelatin loaded with various concentrations of rapamycin can sustainably release the drug over 8-10 days without influencing the degradation pattern of the hydrogel, potentiating its use for treating lymphatic malformations. Moreover, the degradation and drug release behavior of rapamycin-loaded F127-g-gelatin was unaffected by storage at -20 °C. The stability study revealed that gelation and degradation of F127-g-gelatin remains stable under dry storage at -80 °C, -20 °C, and room temperature and under storage in PBS at -80 °C and -20 °C. The hydrogel's stability when loaded with drugs and stored under various conditions opens opportunities for its use in collaborative projects and heightens its potential for translatability.

Poster #: 27

Pulsatile Drug Delivery Platform for Use in Mental Health and Drug Addiction

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The opioid crisis and treatment-resistant depression (TRD) are pressing public health challenges that demand innovative solutions. Our research focuses on developing a pulsatile drug delivery platform, which holds promise for delivering medications with precision and control. This platform utilizes bioresorbable microdevices that employ a microfluidic "fuse" made from surface-eroding cellulose acetate phthalate (CAP) and Pluronic® F-127 (P) polymer composites. This technology enables automated interval delivery of pharmacotherapies, a critical advancement in the treatment of these conditions.

For TRD, our goal is to demonstrate the capability of our implants to deliver a range of release schedules, including daily, every 72 hours, and weekly doses. We aim to accommodate multiple release schedules within a single device, offering personalized treatment options. The long-term integrity of these microfabricated implants will be thoroughly assessed, both in vitro and in vivo. We will focus on the bioactivity of various antidepressants, such as brexpiprazole, olanzapine, and esketamine, upon their release from the device. This approach addresses the need for consistent and controlled drug delivery, potentially improving patient adherence and treatment outcomes.

In addressing opioid addiction, our platform seeks to provide a fully biodegradable device capable of delivering tapering dosages of fentanyl, alongside withdrawal-symptom-reducing medications like lofexidine and buprenorphine. This innovative approach aims to mitigate the risks associated with opioid treatment, such as abuse and overdose, by ensuring precise and controlled drug delivery. The long-term integrity and bioactivity of synthetic opioids released from these implants will be rigorously evaluated, ensuring their safety and effectiveness.

Preliminary in vitro studies have shown promising results, with our devices demonstrating the ability to release fluorescent model drugs in a controlled manner, closely mimicking the desired release profiles of actual medications. These findings highlight the potential of our drug delivery platform to revolutionize the treatment of TRD and opioid addiction. By providing precise and consistent drug delivery, our technology offers a transformative approach to treatment, enhancing adherence and reducing the potential for abuse.

Poster #: 28

Endosomolytic Polymersomes Enhance Intracellular Delivery of Nucleic Acid Therapeutics for Improved Anticancer Immune Responses

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Cancer immunotherapy has revolutionized the fields of oncology and drug delivery, with immune checkpoint blockade (ICB) demonstrating remarkable potential as an alternative to traditional treatment regimens. Although ICB has demonstrated disease control in certain cancers, it has also indicated limited therapeutic efficacy in poorly immunogenic cancers. Through the activation of specific cellular pattern recognition receptors (PRRs), the immunosuppressive tumor microenvironment (TME) of these cancers can be reprogrammed to a more immunogenic, 'hot' phenotype with larger populations of infiltrating T cells, directly correlating with improved responses to ICB. Indeed, activation of the retinoic acid-inducible gene I (RIG-I) pathway has been found to elicit a downstream signaling cascade resulting in the production of type I interferons which can induce this shift in the TME. Unfortunately, most RIG-I-activating therapeutics are not able to freely cross the cell membrane where they are required to activate this cytosolic PRR. The goal of this work is to optimize the loading of 5'-triphosphorylated RNA (3pRNA), a potent RIG-I agonist, within pH-responsive polymeric nanocarriers via a flash nanoprecipitation (FNP) process and assess intracellular delivery and therapeutic efficacy in a cancer model. To accomplish this, we analyzed the effect of copolymer properties on nanocarrier physical properties, RNA loading, cytotoxicity, endosomolytic activity, and RIG-I activation in vitro. We then validated the ability of the system to mitigate disease progression in a murine cancer model by intratumorally administering our 'lead' formulation and monitoring tumor volume, weight loss, and survival over the course of the experiment. With this FNP platform, we highlight the potential for tailored production of a variety of polymeric nanocarriers for potential translatable use in other drug delivery and immunomodulatory applications.

Poster #: 29

Implantable controlled-release chemotherapeutic drug-eluting mesh for postoperative abdominal cancer tumor eradication

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Hypothermic intraperitoneal chemotherapy (HIPEC) is a potent therapeutic approach for several abdominal cancers, including colorectal, ovarian, gastric and appendiceal cancer as well as peritoneal carcinomatosis. This approach involves surgical tumor removal followed by the localized application of heated chemotherapeutic drugs within the abdominal cavity to eliminate residual cancer cells. Our study focuses on developing an implantable chemotherapeutic drug-releasing mesh that will eradicate any remaining cancer cells during the period of postoperative scar tissue formation.

The mesh has a tubular braided structure fabricated from resorbable poly(lactic acid-co-glycolic acid (PLGA) multifilament yarns on a specialized 16-bobbin braiding machine. Subsequently after heat-setting, the mesh is coated with a doxorubicin-loaded PLGA hydrogel. The braided mesh exhibits a favorable degradation profile over four weeks, ensuring compatibility with the postoperative healing process. ToF-SIMS analysis confirms a uniformly dispersed coating of doxorubicin across the mesh's surface. Moreover, in an in-vivo toxicity

assessment involving the implantation of the drug-free mesh in a mouse model, no significant inflammatory reactions were observed, confirming the mesh's biocompatibility.

Our ongoing work focuses on optimizing the hydrogel-coated mesh to fine-tune the release kinetics of the doxorubicin. We are also assessing the chemical and mechanical properties of the mesh, enabling us to better understand its long-term stability and efficacy. In addition, the drug kinetics of the hydrogel-loaded mesh are being evaluated. To assess the mesh's therapeutic potential, we are conducting in vitro experiments using various cancer cell models and exploring various in vivo models to validate our findings. This research represents a significant step toward enhancing the precision and effectiveness of HIPEC procedures for abdominal cancer treatment. The development of this controlled drug-releasing mesh holds promise for improving patient outcomes by providing a targeted and

sustained delivery system for chemotherapeutic drugs, ultimately contributing to the advancement of abdominal cancer therapy.

Poster #: 30

Glycopolymeric Nanoparticles for Targeted Delivery to Tumor-Associated Macrophages: Biodistribution, Toxicity and Anticancer Efficacy

Veeresh Toragall, The university of Mississippi

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Tumor-Associated Macrophages (TAMs) play crucial roles in tumor progression and immune suppression, making them attractive targets for cancer therapy (promoting an anti-tumor immune response or inhibiting pro-tumorigenic activities). Further, the impact of TAM-mediated efferocytosis as an emerging immune checkpoint on the tumor microenvironment phenotype. Therefore, directing therapies towards TAMs holds greater promise in enhancing the efficacy of cancer treatments while reducing off-target effects. Hence, we fabricated polymeric mannose (PMAM) nanoparticles (GNPs) that target macrophages and show controlled drug release. We also examined for their targeted delivery, toxicity, biodistribution and anticancer efficacy in 4T1 induced tumor mice model. The formulated GNPs showed the ability to release cargo in a pH-dependent manner which makes them ideal for endosomal drug delivery. Further, in vivo biodistribution studies showed the GNPs were capable of internalization by TAMs compared to tumor cells in the TME. Also, toxicity study revealed that the GNPs (< 3 mg/kg BW) did not show any sign toxicity in healthy animals and was safe to use. Finally, in vivo studies showed that GNPs has significantly reduced tumor volume compared to free UNC2025, showing greater therapeutic efficacy in triple negative breast cancer model. We believe that glycopolymer-based nanoparticles, which inhibit efferocytosis, hold potential as a standalone cancer immunotherapy and as a complementary agent to enhance response rates to checkpoint immunotherapy.

Poster #: 31

Programmably Degradable Hydrogels for Optimizing Monoclonal Antibody Release

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Monoclonal antibodies have revolutionized treatment paradigms across a range of diseases, owing to their specificity and efficacy. However, their therapeutic potential is often constrained by conventional delivery methods, leading to suboptimal dosing and increased side effects. This study introduces an approach to control the release dynamics of Immunoglobulin G (IgG) through hydrogel encapsulation and/or tethering, focusing on drug delivery mechanisms that address these challenges. By employing hydrogels for the sustained release of therapeutic agents, this research proposes a promising strategy for improving drug efficacy and patient compliance while reducing adverse effects. We investigate the use of oxanorbornadiene (OND)-modified dextran hydrogels, utilizing the retro-Diels-Alder (rDA) mechanism for programmable gel degradation, which leads to highly tunable release profiles. Our findings suggest that IgG release kinetics can be meticulously controlled by varying the types of OND molecules with different rDA half-lives, the degree of substitution (DS) in OND-dextran complexes, polymer weight percentages, and polymer chain lengths. These insights contribute to the design of drug delivery systems with programmable dosing profiles. Preliminary results indicate that IgG can be released sustainably for up to three weeks, demonstrating both traditional first-order and intriguing non-first-order release kinetics. This work highlights the potential of hydrogel-based platforms in patient-centric therapy and presents a novel method for the programmable delivery of monoclonal antibodies.

Poster #: 32

Biofunctionalized gelatin hydrogels support development and maturation of iPSC-derived cortical organoids

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Human neural organoid models have become an important tool for studying neurobiology. However, improving the representativeness of neural cell populations in such organoids remains a major effort. In this work, we compared Matrigel, a commercially available matrix, to an N-cadherin peptide-functionalized gelatin methacryloyl hydrogel (termed GelMA-Cad) for culturing cortical neural organoids. After robust material characterization by atomic force microscopy, scanning electron microscopy, and NMR, we cultured induced pluripotent stem cells in the material. We then employed full-mount and section immunostaining and single-cell RNA sequencing to characterize the resulting cell fates after cortical organoid differentiation.

We determined that both crosslinking conditions and peptide presentation can tune cell fate and diversity in gelatin-based matrices during differentiation. Of particular note, cortical organoids cultured in GelMA-Cad produce higher numbers of neurogenic and ciliated radial glia, which mapped to developmental human progenitor states. Moreover, in GelMA-Cad, upper layer and deep layer neurons collectively represented 27% of the detected cells at day 120 (15% and 12%, respectively). In contrast, at day 120, upper layer and deep layer neurons comprised <1% cells detected in Matrigel controls. We also observed enrichment of choroid plexus epithelial cells in higher crosslinking conditions, providing compelling evidence that matrix properties can influence neural organoid differentiation. In transcriptomic comparisons, GelMA-Cad embedded organoids had lower signatures related to stress pathways, a known problem with conventional Matrigel techniques. Taken together, outcomes from this work showcase GelMA-Cad as a simple and defined hydrogel alternative to Matrigel for neural organoid culture, marking it as a valuable resource.

Poster #: 33

Aptamer-functionalized Nucleic Acid-Collagen Complexes: An Advanced Bioactive Matrix for Angiogenesis

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I prefer poster presentation only

Introduction

The increasing need for organ regeneration has led researchers to pursue the development of biomaterial technologies to restore native tissue-like structures and provide an extracellular matrix (ECM)-like environment. We seek to facilitate the enhanced promotion of angiogenesis which is crucial for cell survival and tissue functionality.

The term nucleic acid-collagen complexes (NACCs) describes a wholly native biomaterial that potentially offers localized, and targeted bioactivity toward angiogenesis through function-specific DNA aptamers. NACCs are a versatile and fibrous system that arises from the spontaneous self-assembly of single-stranded DNA (ssDNA) with collagen and can be functionalized with any desired oligonucleotide sequence. They have been shown to offer targeted bioactivity that does not rely on the use of growth factor supplementation, and the presence of micro- and nano-fibers within their architecture confers structural support for endothelial cells.

Methods and Results

To evaluate the ability of NACCs to stimulate endothelial cells towards angiogenesis, we used a known DNA aptamer sequence that acts as a vascular endothelial growth factor (VEGF) agonist. This sequence selectively binds to the VEGFR-2 receptor of endothelial cells and activates it. We performed sprouting and tubulogenesis assays to assess the emergence of endothelial cell tubules and sprouts within the biomaterial. Phase contrast and confocal microscopy indicated increased tubular network area and number of sprouts, compared to control NACCs functionalized with a random ssDNA sequence not known to have any bioactivity. Additionally, we used a reverse transcription polymerase chain reaction (rt-PCR) gene array to quantify gene expression of key signal transduction pathway-focused genes involved in modulating the angiogenic process. In aptamer-functionalized NACCs, we saw the enhanced expression of genes such as matrix metalloproteinase 2 (MMP-2), angiopoietin 2 (ANGPT-2), and cadherin 5 (CDH-5).

Conclusion and Significance

Our results give us more reasons to keep focusing our studies on controlling angiogenesis since it can mean a dramatic change in immunotherapies, cancer research and personalized medicine. The significance of our work is that we will keep working to position NACCs as dynamic biomaterials for translational applications.

Poster #: 34

Recombinant Fusion Proteins as Versatile Tools for Protocell Development with Embedded Sensing Functions

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In the pursuit of elucidating the rules of life, one promising avenue is the bottom-up creation of cell-mimetic structures, called 'synthetic cells. These synthetic cells can be engineered to imitate the structure, functions, and properties of natural cells, achievable through various approaches. Among all functions, sensory capabilities are crucial for the operation of living cells, enabling them to communicate and respond to external stimuli by performing appropriate actions. Recombinant protein self-assembled vesicles open new opportunities to incorporate functionally folded, sensory proteins into membranes with high modularity.

Here, we develop self-assembled protein vesicles that can sense and respond to specific signaling molecules by leveraging the ternary complex system involving FKBP-Rapamycin-FRB. The sensory protein domain, either FRB- or FKBP-, was genetically fused to a fluorescent protein-leucine zipper fusion protein (i.e., FRB-mCherry-ZE or FKBP-sfGFP-ZE). These sensory-fluorescent-leucine zipper fusion proteins self-assemble into vesicles with a counter leucine zipper fused with elastin-like polypeptide (ZR-ELP) by forming amphiphilic protein building blocks in water at the temperature above the lower critical solution temperature of ELP. We have successfully created vesicles self-assembled from FRB-mCherry-ZE and FRB-sfGFP-ZE with ZR-ELP. The rapamycin-induced interaction of these vesicles reveals the critical role of signaling molecule concentration in the modulation of vesicle intercommunication, where vesicle aggregation serves as an indicator of sensory activity. To characterize the vesicle's sensory response to rapamycin, we utilized dynamic light scattering to analyze their size and aggregation. At the same time, fluorescence and confocal microscopy techniques offered insights into their spatial distribution and aggregation behavior. Co-localization studies further quantified their interactions, enhancing our understanding of the vesicle's functionality for sensing. This paves the way for further exploration of various potential applications of protein vesicles to recapitulate minimal cellular functions.

Poster #: 35

Comparing the use of brain-derived and synthetic phosphatidylserine in lipid-polymer hybrid particles for modulating macrophage inflammation

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Macrophages are immune cells that respond to inflammatory stimuli throughout the body. Macrophage dysfunction contributes to many aspects of chronic inflammatory disease. Thus, macrophages are an important target for therapies to resolve chronic inflammation. In previous work, we successfully synthesized poly(lactide-co-glycolide) (PLG) particles surface-functionalized with phosphatidylserine (PS). On the surface of cells undergoing apoptosis, PS functions as an “eat me” signal and a cue to resolve inflammation. Accordingly, macrophages bind and take up PS-bearing apoptotic cells through cell-surface receptors, and subsequently initiate an anti-inflammatory response. Likewise, we have shown that PS-presenting PLG (PS:PLG) particles are readily taken up by lipopolysaccharide (LPS)-inflamed bone marrow-derived macrophages (BMDMs) and decrease the section of inflammatory cytokines TNF-a and IL-6 while increasing the release of IL-10, an anti-inflammatory cytokine. However, for those studies, the PS used was derived from porcine brain, which is not recognized as safe by the FDA due to concerns involving the transmission of infectious encephalopathies such as mad cow disease. Hence, in this study, we sought to use 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS), a synthetically derived PS, and compare its efficacy to the porcine brain-derived PS. Using transmission electron microscopy to investigate the organization of PS on the particle surface, we observed that PS:PLG particles made with DOPS (DOPS:PLG) presented a unique flower-like morphology when compared to PS:PLG, which presented a spherical morphology. Despite this difference, DOPS:PLG particles achieved the same loading of PS as measured by NMR. Importantly, we found that DOPS:PLG particles were able to target and modulate LPS-induced inflammation in BMDMs with the same efficacy as particles made with brain-derived PS. These findings advance the use phosphatidylserine-PLG hybrid particles as a potential therapeutic for patients with chronic inflammation. Future studies will investigate the use of these DOPS:PLG particles to deliver small-molecule drugs that can further modulate the anti-inflammatory effects of the particles.

Poster #: 36

Three-Dimensional Printed Piezoelectric Breast Cancer Bone Metastasis Model

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Bone has been identified as the most prevalent site for breast cancer metastasis; however, the cause is not fully understood. The mechanosensitive property of bone cells plays an important role in breast cancer metastasis to bone. In vivo, bone tissue experiences mechanical loading and creates electrical signaling as a result of this piezoelectric effect. Evidence suggests that breast cancer cells undergo increased proliferation and migration in the presence of electrical fields caused by the electrical signaling of bone cells. Three-dimensional (3D) in vitro models are widely used in cancer research; however, current models lack this piezoelectric component. An accurate, biomimetic model that mimics in vivo signaling is necessary to better understand the process of breast cancer metastasis to bone. This may be accomplished by introducing a piezoelectric polymer into the model, that yields an electrical field when mechanical stimulation, such as ultrasound, is applied. This study aimed to evaluate a novel 3D in vitro piezoelectric breast cancer bone metastasis model by observing the interactions between metastatic breast cancer cells and the 3D bone microenvironment. Our model consists of a 3D-printed scaffold made of polycaprolactone (PCL), demineralized bone matrix, and polyvinylidene fluoride (PVDF). The scaffolds are fabricated using 3D pneumatic printing. The high levels of sheer stress caused by pneumatic printing induce the formation of β phase content in the PVDF. Preliminary studies evaluated the piezoelectric properties of PVDF-loaded scaffolds and osteogenic differentiation within the model. Results showed an increase in piezoelectric properties when pneumatic printing was used for scaffold fabrication; osteogenic differentiation increased when cells were cultured within the dynamic model. Ongoing work includes the investigation of triple negative breast cancer cell migration within the bone model, with and without ultrasonic stimulation.

Poster #: 37

Multi-domain disordered proteins drive robust intracellular self-assembly through convergent phase transitions

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I prefer poster presentation only

Intrinsically disordered proteins (IDPs), both synthetic and endogenous, undergo phase transitions to scaffold cellular structures. Sequence-level molecular information distinguishes IDPs that phase separate above a lower critical solution temperature (LCST) or below an upper critical solution temperature (UCST). IDPs with a singular LCST or UCST phase-separation sequence grammar dominate the known landscape of IDP-driven phase transitions in nature and across "smart" biomaterials. We and others recently reported UCST-LCST diblock IDP polymers (IDPPs) with useful in vitro self-assembly, which were engineered by specifying one domain as the sole driver of phase separation. However, the intracellular behavior of multi-domain IDPPs with competing phase-separation grammars remains unexplored. To fill this gap, here we recombinantly synthesized and characterized a library of nine novel multi-domain IDPPs that integrate potent UCST and LCST grammars at either the repeat or the domain levels. Across the library, we used two UCST motifs and one LCST motif, and systemically varied UCST/LCST ratios. We successfully purified these multi-domain IDPPs with good yield (e.g., ~100 mg/L of culture) through a highly scalable phase separation-driven protocol. Surprisingly, temperature-dependent UV-visible absorbance measurements in a physiological buffer revealed that all multi-domain IDPPs converged towards LCST-type phase transitions, even for IDPPs with high UCST/LCST ratios. Moreover, increasing UCST content in multi-domain IDPPs consistently lowered the LCST cloud point temperature well below 37 °C. Fusing these at the gene level to a green fluorescent protein and using live-cell microscopy to probe their intracellular self-assembly, we saw robust formation of liquid-like biomolecular condensates in human cells. IDPPs with convergent LCST cloud points close to 37 °C appeared diffuse in the cytosol or formed small condensates. Multi-domain IDPPs with repeat-level integration of competing UCST-LCST grammars consistently led to prominent formation of multiple micron-sized condensates per cell. Our ongoing experiments are probing the ability of intracellular multi-domain IDPPs to disconnect from their single-grammar IDPP counterparts (either diffuse in the cytosol or phase separated). Because known intracellular IDPs predominantly exhibit UCST phase transitions, our findings suggest that multi-domain IDPPs with convergent LCST phase separation behavior are exciting new building blocks for de novo engineering of organelle-like condensates in human cells.

Poster #: 38

Comparison of alginate and fibrin hydrogels for cell delivery

Jared Albert, North Carolina State University & UNC- Chapel Hill

I prefer poster presentation only

Alginate is a commonly used biomaterial due to its natural origin and low cytotoxicity levels. Alginate is widely used for cell encapsulation due to its favorable gelation properties. Some examples of cell encapsulation utilizing alginate hydrogels include encapsulation of islet cells to avoid immune recognition for treating diabetes and encapsulation of satellite cells to increase angiogenesis in a hind limb ischemia model. However, encapsulation does not allow for cell permeation and engraftment with the surrounding microenvironment, which minimizes cell survival and functionality. Fibrin matrices represent an alternative biomaterial for cell encapsulation and delivery. Fibrin is also naturally derived and is generated by adding thrombin to fibrinogen. Thrombin activates fibrinogen to produce insoluble fibrin fibers which form a fibrillar fibrin matrix. Since fibrin matrices are naturally generated in the body and have been shown to allow cell infiltration and engraftment during wound healing, it has great promise as a biomaterial. We hypothesize that the fibrin matrices will lead to better long-term cell survival compared to alginate hydrogel encapsulation. Human dermal fibroblasts were either encapsulated within a 2% alginate hydrogel or within a fibrin matrix comprised of water, HEPES buffer, 2 mg/mL fibrinogen, and 0.1 or 0.25 U/mL thrombin. 3 hours or 2 days following encapsulation, NucBlue Live and NucGreen Dead ReadyProbe Reagent stains were used to assess cell viability. The hydrogels and matrices were imaged using a Leica MICA WideFocal with 10X lens on confocal settings and the percent of living cells was determined. Results show an increase in cell viability for cells in fibrin matrices created with 0.1 U/mL thrombin compared to fibrin matrices created with 0.25 U/mL thrombin or alginate hydrogels. On-going studies are evaluating how incorporation of a fibrin-specific targeting platelet-like particle into the fibrin matrices, which are known to retract clots and dynamically and regionally stiffen the microenvironment, further influence cell viability.

Poster #: 39

Evolution of Stem Cell Production: Multi-sensor Array & Wireless Electronics for Real-time Cell Culture Monitoring

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Large-scale manufacturing of stem cells, crucial for personalized medicine, faces challenges in consistency, quality, and scalability due to limitations in current bioreactor technologies. Herein, we introduce a novel smart bioreactor system that integrates wireless, multivariate sensors into a flexible cell bag, enabling real-time, in situ monitoring of critical culture parameters such as pH, dissolved oxygen and glucose levels, and temperature. This innovation supports long-term monitoring over 30 days, facilitating dynamic assessment without interfering with the cell culture environment. Experimental results validate system accuracy, sensitivity, and reliability under continuous operation, showcasing its potential to transform stem cell manufacturing processes.

This presentation will highlight the system's impact on stem cell research and therapy, emphasizing its capacity for high-throughput manufacturing to produce high-quality stem cells. Future work aims to expand this technology to larger-scale operations, further advancing stem cell manufacturing capabilities.

Poster #: 40

Control of Enzyme Cascade Reactions within Synthetic Cell-like Multicompartment Protein Vesicles

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The intricate mechanisms of living cells, characterized by their multi-compartmentalization, regulate enzyme cascade reactions for the essential processes of life such as metabolism or signal transduction. Replicating these systems has been considered as one of the most critical tasks in synthetic cell exploration. Moreover, the development of artificial organelles holds significant importance in synthetic biology, as these structures mimic the compartmentalization seen in living cells, enabling precise control and optimization of biochemical processes. Globular protein vesicles (GPVs), vesicles self-assembled from globular fusion proteins, emerge as a promising platform in synthetic cell development, leveraging protein's biocompatibility and functions as the building blocks. Particularly, functional globular protein incorporated compartments represent promising artificial organelle platforms, offering the ability to localize enzymes within a defined space, thus enhancing efficiency and functionalities. In this study, we demonstrate the formation of multicompartment protein vesicles housing octopine dehydrogenase (ODH) incorporated vesicles and coacervates as artificial organelles. By pairing with pyruvate kinase, we perform enzyme cascade reactions to simulate pyruvate metabolism under anaerobic conditions in vitro. We foresee that the promise of these synthetic cell-like structures in mimicking vital cellular processes among specific confinements with recombinant fusion proteins will pave the way for progress in synthetic cell engineering and biotechnology.

Poster #: 41

Structurally decoupled hyaluronic acid-based 3D model to elucidate the impact of matrix metalloproteinase-mediated invasion of brain metastatic breast cancer cell spheroid

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Polymeric hydrogels have been extensively employed to study cancer cell-matrix interactions as they provide cancer cells with a relevant three dimensional (3D) context evidenced in vivo and enabling us to maintain the cellular phenotype in vitro. The ability to tune hydrogel properties has enabled recapitulation of several key aspects of the tumor microenvironment in vitro. In the context of breast cancer, it is known that cancer cells invade the tissue at the primary and metastatic site by degrading the native extracellular matrix (ECM) using matrix-metalloproteinases (MMPs) resulting in disease progression. Efforts have been made to model MMP-mediated invasion of cancer cells by incorporating MMP-cleavable crosslinks in the hydrogel structure, however, to our knowledge, model systems enabling effective decoupling between hydrogel mechanical properties and mesh size, while incorporating MMPs into the hydrogel matrix have not been reported. To address this need, we fabricated a structurally decoupled hyaluronic acid (HA) based 3D biomimetic model to specifically investigate the invasion of metastatic breast cancer cells mediated by MMPs. The hydrogels were fabricated using varying ratios of biologically sensitive (i.e., MMP cleavable peptide) and insensitive crosslinkers (i.e., Dithiothreitol (DTT) or polyethylene glycol dithiol (PEGDT)) to investigate the impact of incorporated MMP-cleavable peptides on the invasion of encapsulated MDA-MB-231Br metastatic breast cancer spheroids. We found that HA hydrogels crosslinked with various ratio of DTT/MMP or PEGDT/MMP exhibited comparable mechanical and physical properties as tested via rheological measurements, swelling ratio analysis, estimated mesh size, and permeability measurements. However, their degradation rate in the presence of collagenase enzyme was significantly altered and directly related to the concentration of MMP-cleavable peptide used to crosslink the hydrogel. Consistent with this, encapsulated MDA-MB-231Br spheroids in HA hydrogels with MMP sensitivity showed more invasiveness than those without MMP after 14 days of culture. Further, F-actin staining revealed invaded cells with a well-developed actin cytoskeleton and presence of invasive protrusions at the periphery of spheroids within HA hydrogels containing MMP cleavable peptides as opposed to those without MMP-cleavable peptide incorporation. Overall, these structurally decoupled HA hydrogels provide a platform to study MMP-mediated invasion of metastatic breast cancer spheroids.

Poster #: 42

Enzymatically-Degradable Hydrogel Microcarriers Modulate Secretome of Mesenchymal Stromal Cells

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Culture of mesenchymal stromal cells (MSCs) on enzymatically-degradable hydrogel microcarriers (μCs) would enable more efficient cell harvest following in vitro expansion and facilitate in vivo cell delivery. However, the effects of enzymatically-degradable μCs on modulating the secretory properties of MSCs, a main mechanism of action in vivo, remain underexplored . Therefore, we aimed to determine the effect of enzymatically-degradable hydrogel microcarrier formulation on MSC numbers and soluble factor secretion compared to standard non-degradable poly(styrene)-based microcarriers (Synthemax).

Degradable hydrogel μCs were fabricated with acrylated poly(ethylene glycol) conjugated to the MMP-cleavable peptide GGVPMSMRGGGK (PEG-VPM). PEG-VPM μCs degraded completely upon overnight incubation in collagenase (assessed via phase contrast microscopy). Additionally, PEG-VPM μCs (n=7) were subjected to microscale mechanical testing (20μN preload applied over 1 min followed by compression at 0.5% strain/s). PEG-VPM μCs exhibited a compressive modulus of 54.3 ± 15.8 kPa.

Changes in cell number and secretory activity were evaluated following culture of human MSCs (RoosterBio) for 4d on both PEG-VPM and Synthemax μCs (n=8). Via PicoGreen assay, fold changes in numbers of MSCs 24h and 4d after seeding on PEG-VPM μCs did not differ from that on Synthemax. Multiplex ELISA revealed significant differences in the abundance of secreted factors: MSCs cultured on PEG-VPM secreted 2.1-fold higher levels of the pro-regenerative factor hepatocyte growth factor compared to Synthemax, and the immunomodulatory cytokine interleukin-10 was secreted by MSCs on PEG-VPM but not Synthemax. Additionally, MSCs on PEG-VPM secreted significantly lower levels of several pro-inflammatory factors, including growth-regulated oncogene, interleukin-6, and interleukin-8.

In a follow-on study, pro-inflammatory cytokine licensing (used to augment cytokine secretion by MSCs) was investigated for its impact on MSC numbers on microcarriers. MSCs were cultured on PEG-VPM μCs, Synthemax μCs, and planar poly(styrene) with or without IFN-γ (0, 10, or 100ng/mL) for 4d (n=8). MSC numbers on each surface did not significantly vary with IFN-γ concentration.

Together, these results suggest that hydrogel carrier properties can influence soluble factor secretion from MSCs, and MSC adhesion is maintained in the presence of pro-inflammatory cytokine licensing. Further development of this system may advance both the manufacturing and therapeutic delivery of MSCs.

Poster #: 43

Independent tuning of hydrogel stiffness, viscoelasticity, and integrin engagement to study fibroblast-macrophage crosstalk

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Cells engage with their surrounding extracellular matrix (ECM) via integrin binding, which provides structural support and mechanical stimuli. These cues are converted to biochemical signals through the process of mechanotransduction, allowing cells to both sense and respond to their environment. Studies have shown that dysregulated changes in ECM mechanics can lead to diseases like cancer and fibrosis; however, traditional cell culture platforms for studying these diseases do not capture physiologic mechanics or components of ECM.

To better understand the contribution of changing ECM properties in pathogenesis, our group and others utilize hydrogels to recapitulate the microenvironment of native and diseased tissues. By modifying chemical motifs on a hyaluronic acid backbone, we leverage thiol-ene click chemistry to tune mechanical properties like stiffness and viscoelasticity. Additionally, we can incorporate various cell adhesion motifs that mimic those found in native ECM. While many material-based culture methods utilize the fibronectin-derived RGD peptide, studies show that this motif has lower binding affinity compared to longer peptide/protein fragment domains and can non-specifically bind multiple integrin subunits. Furthermore, these limitations in cell culture methods convolute the investigation of cell-cell interactions. In the context of lung fibrosis, where multiple mechanosensitive cells like macrophages and fibroblasts are driving disease and mechanics of ECM are changing throughout disease progression, there is a need to develop tunable platforms that decouple these factors.

To distinguish integrin-mediated cell activation from substrate mechanics, we have utilized fibronectin-derived fragments that demonstrate preferential integrin engagement (αvβ3 vs. α5β1). Incorporating these fragments into our previously developed, hyaluronic acid-based system allows us to independently tune stiffness, viscoelasticity, and integrin binding. Our group has shown that fibroblasts seeded on substrates that preferentially engage αvβ3 integrin demonstrate increased spreading, actin stress fiber formation, and focal adhesion size, indicative of myofibroblast activation. Here, we found that cadherin 11, a cell-cell adhesion molecule implicated in fibrotic macrophage-fibroblast crosstalk is upregulated in fibroblasts seeded on hydrogels with preferential αvβ3 engagement. We have previously found that fibroblasts co-cultured with M2 macrophages exhibit increased spreading, collagen-1, and cadherin-11 expression regardless of hydrogel mechanics, and anticipate that these trends will uphold on hydrogels with different integrin-specificities.

Poster #: 45

Neural-muscle cell co-cultures in aligned collagen-glycosaminoglycan (CG) scaffolds for skeletal muscle tissue engineering

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Volumetric muscle loss (VML) is the traumatic removal of skeletal muscle exceeding natural wound healing capabilities. Treatments are unable to overcome the detrimental effects of VML, including reduced force generation capacity and impaired locomotion. Biomaterials are emerging as promising therapeutic alternatives for skeletal muscle tissue engineering due to their ability to support cellular alignment, neural connection, and electrical excitability necessary for integrated myogenesis and neurogenesis.

We previously created biomaterials for skeletal muscle tissue engineering utilizing a directional freeze-drying process to fabricate aligned and highly porous collagen-glycosaminoglycan (CG) scaffolds made to mimic muscle architecture both in ECM composition and in microstructural organization. While previous work showed that these scaffolds could support in vitro myogenesis and limited functional recovery in vivo, we were interested in further exploring if these scaffolds could support simultaneous myogenesis and neurogenesis for improved functional regeneration. Inspired by recent studies on 2D substrates demonstrating expedited myogenesis when co-culturing muscle cells with neural cells, we hypothesized that seeding muscle-derived cell (MDC) and neural stem cell (NSC) co-cultures into a 3D aligned scaffold would be beneficial for skeletal muscle tissue engineering.

Primary rat MDC-NSC co-cultures were cultured to assess the promotion of robust myotube maturation and NMJ formation within 2D controls and 3D CG scaffolds. Myotube diameter, fusion index, and neuromuscular junction (NMJ) formation were quantified utilizing immunocytochemistry. Results show evidence of co-cultures positively impacting myogenesis on 2D surfaces with neural cells promoting faster maturation of MDCs. Ongoing work is assessing the influence of incorporating skeletal muscle-derived extracellular matrix components (e.g. laminin), with or without electrical stimulation, on further improving myogenesis.

Poster #: 46

Engineering Hydrogels to Investigate the Role of Extracellular Matrix Cues on Natural Killer Cell Functions

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Natural killer (NK) cells are of interest for immunotherapy due to their ability to detect and kill cancer cells without prior antigen priming. For NK cells to exert their cytotoxic functions, direct contact with cancer cells is needed; however, NK cells that extravasate to the tumor largely remain in the stroma of solid tumors and only a small fraction come into direct contact with tumor cells. The biochemical and mechanical properties of solid tumors are known to play a role in NK cell infiltration and activation; however, it remains unclear if these properties act as a barrier or a substrate. Traditionally, 2D in vitro culture systems are used to perform mechanistic studies of NK cell functions; however, these culture systems do not replicate the 3D tumor microenvironment and, incidentally, correlate poorly with in vivo and clinical studies. The Sharma Lab has established a 3D poly(ethylene) glycol (PEG)-based hydrogel system in which the biochemical and mechanical properties can be independently manipulated to study the effect of tumor extracellular matrix (ECM) on NK cell function. Through the inclusion of hyaluronic acid (HA), a glycosaminoglycan prevalent in lung tumors, we observed increased NK cell infiltration, suggesting that HA may act as a substrate in the tumor microenvironment. Alternatively, when we increased the stiffness of our hydrogel system, we observed a decrease in NK cell infiltration suggesting that solid tumors may utilize mechanotransduction pathways to avoid detection. Future studies will investigate the influence of tumor ECM components, like HA and stiffness, on NK cell activation and elucidate the mechanisms involved.

Poster #: 47

Microporous Particle Scaffolds for Mesenchymal Stromal Cell Expansion and Enhanced Immunomodulatory Function

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Background: Despite promising preclinical studies, challenges in reproducibly manufacturing therapeutic mesenchymal stromal cells (MSCs) at scale hinder their clinical translation. Hydrogel-based culture systems, like microporous particle (MP) scaffolds, have shown to offer a scalable solution while also enhancing MSC function(1,2). To establish MP scaffolds as an alternative to traditional MSC biomanufacturing, we expand MSCs within MPs and compare their immunomodulation to cells grown on traditional tissue culture polystyrene (TCPS). Our findings show MP scaffolds allow for scalable MSC expansion and enhance their immunomodulatory function.

Methods: VF was assessed by incubating MPs with fluorescent dextran (2000kDa) that diffuses into MP pores, and three regions of interest (ROI) were imaged using confocal microscopy (Zeiss LSM900). For cell culture, MSCs from three donors and two tissue sources (RB98-adipose, RB71-bone-marrow, RB62-adipose – RoosterBio) were cultured in 96-well plates within MPs using chemically defined media (CDM). Cell counts were acquired by quantifying dsDNA via PicoGreen. Immunomodulation was assessed via an indoleamine 2,3-dioxygenase (IDO) assay(3). Briefly, MSCs were grown in 2D or 3D for 24 hours, exposed to pro-inflammatory cytokines (50 ng/mL TNFα and IFNϒ) for 24 hours, and subsequently assayed for IDO activity.

Results: MPs exhibited consistent VF (~33%) across three replicates, demonstrating reliable scaffold preparation. After 4 days of MSC expansion in MPs, the three donors proliferated 2X, on average, although significant differences were observed between the three donors. Coefficients of variation (CV) were notably low for each donor in MPs (RB98: 2.17%, RB71: 1.65%, RB62: 1.81%), possibly due to assurance of consistent VF across scaffolds. IDO activity was significantly higher in MSCs within MPs for both control and primed conditions across all donors, excluding the primed RB71 group. These results highlight MP's potential to expand MSCs and enhance their immunomodulatory function in the presence and absence of priming with pro-inflammatory cytokines.

Conclusion: MP scaffolds demonstrated the ability to serve as a viable MSC expansion platform and enhance their immunomodulatory function across multiple donors.

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Poster #: 48

Hydrogel microcarriers reduce development of MSC senescence in small and large scale culture

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Mesenchymal stromal cells (MSCs) have great potential as a regenerative medicine therapy due to their immunomodulatory capacity. However, to reach therapeutic doses, large scale culture in bioreactors is required. One challenge hindering MSC scale-up is development of replicative senescence during expansion, which leads to growth arrest and decreases the quality of the cell product. To effectively scale up MSC culture in bioreactors, microcarriers are necessary prodive adequate surface area. Therefore, the hypothesis of this study is that PEGDA-based microcarriers can serve as substrates to reduce senescence of MSCs cultured in suspension bioreactors. PEGDA microcarriers incorporating the integrin engaging peptide RGD were fabricated using a microfluidic system. Commercially available plastic microcarriers (Synthemax) were used as a control. MSCs from two donors were seeded at 5000 cells/cm2 and cultured for 6 days in agitated 6-well plates or in duplicate PBSmini 0.1L bioreactors. Media was assayed for lactate (n=2-3), cell yields were quantified with picogreen (n=3) and cell counts (n=2), and cells were stained for -gal (n=4-6). Cells from bioreactors were co-cultured with macrophages and TNF- concentrations were assayed using an ELISA (n=5). In well plate cultures, similar trends in lactate accumulation and cell yields were seen between PEGDA and Synthemax microcarriers. When stained for senescence-associated -galactosidase, cells on Synthemax showed increased staining compared to cells on PEGDA with nearly 50% of cells staining positive for -gal compared to less than 10% on PEGDA. In bioreactors, lactate accumulation was similar between microcarrier types, but cell yields indicate that PEGDA may support improved growth at larger scale, while -gal staining trends were maintained. After bioreactor expansion, all cells significantly suppressed secretion of TNF- from macrophages, and PEGDA-cultured cells suppressed to a degree that was not significantly different than the negative control, indicating that PEGDA carriers support MSCs that retain immunomodulatory capacity. -galactosidase results indicate that PEGDA based microcarriers may be used to reduce development of senescence in MSC culture. From these studies, PEGDA microcarriers were shown to serve as an equivalent or superior surface vs commercially-available microcarriers for MSC culture at both small and larger scale.

Poster #: 49

T follicular helper-like cell differentiation, encapsulation, and culture in hydrolytically degradable microgels

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T follicular helper (Tfh) cells provide functionally crucial signals to B cells in secondary lymphoid tissues, like the lymph node, during an adaptive immune response. However, obtaining high quantities of functional Tfh cells to model these responses ex vivo can be difficult solely from primary tissue isolation. Here, we obtained naïve CD4+ T cells from a bulk human peripheral blood mononuclear cells (PBMC) sample and evaluated their encapsulation and maintained culture within hydrolytically degradable microgels. We then explored their differentiation into Tfh-like cell phenotypes using cytokine stimulation with and without biomaterial-cell interactions. Our microgels are generated using a microfluidic droplet generator composed of a PEG-4MAL biomaterial at <100 μm diameter. Optimized concentrations of ethylene glycol bis-mercaptoacetate (EGBMA), a hydrolytic crosslinker, within the microgel allows for highly tunable degradation kinetics. Isolated CD4+ T cells from human donors were resuspended in macromer densities of PEG-4MAL polymer solution and pre-functionalized with a fibronectin-mimic RGD peptide and FITC for in vitro tracking of microgels. Microgel degradation, swelling kinetics, and T cell viability in vitro were monitored by optical microscopy, and confocal imaging. T cell microgels were cultured with IL-12 and Activin-A to support differentiation into more Tfh-like phenotypes. Using flow cytometry, we evaluated the recovered Tfh cells and observed increases in CD40L expression after 5 days in culture, a key signal that Tfh cells provide to B cells. Media from cultured 2D and microgel conditions were also evaluated for secretion of IL-21 and other key T cell cytokines using ELISA and Legendplex. This research will establish foundational knowledge of T cell differentiation within hydrogels and will ultimately be applied to generate a functional T cell zone mimicking the human lymph node for exploration of adaptive immune responses in vitro.

Poster #: 50

SA-FasL Microgels Designed to Promote Immune Tolerance

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Type 1 Diabetes (T1D) affects 1.6 million Americans and accounts for over $15 billion in annual health care costs. T1D is characterized by the irreversible, autoimmune destruction of the insulin producing β-cells in the pancreas. Without the production of insulin, blood glucose levels increase causing life-threatening complications. Allogeneic transplantation of islets is the only treatment with curative intent. However, this is limited by lack of available donors and by graft failure in most patients within 5 years due to both acute and chronic rejection of the islets. Current immunosuppressive drugs contribute to graft failure by direct toxicity to transplanted islet β cells and by worsening peripheral insulin resistance. Therefore, there is a need to create methods to induce tolerance of transplanted islets to develop a cure for T1D. Previously demonstrated by our lab, we can co-deliver 200µm microgels that present Streptavidin-Fas Ligand (SA-FasL) to promote the immune acceptance of transplanted islets in non-human primates. Additionally, these 200µm microgels still induce mouse A20 cell apoptosis 2 months after being manufactured demonstrating their off-the-shelf potential. However, SA-FasL is only presented on the surface of the microgels so a downfall of the 200µm gels is that most of the space that they take up in the graft is not used to deliver SA-FasL. Therefore, we have developed smaller (50µm) microgels with SA-FasL that induce the apoptosis of mouse A20 cells like that of the larger microgels. Promoting immune acceptance of islets with these microgels pushes the field closer to developing a cure for T1D.

Poster #: 51

Exploring Effects of Surface Conjugated poly(ethylene) glycol on Pancreatic Islets: Assessing T cell Migration in a 3D Co-culture environment

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Background: Grafting long-chain poly(ethylene) glycol to pancreatic islets increases graft efficacy in both murine and non-human primate models. However, specific mechanisms for how PEG impairs graft rejection have yet to be fully elucidated. Herein, we utilized an in vitro immune screening platform to gain insight into how PEGylation modulates immune attack. Using an antigen specific system, we can interrogate impacts of PEG grafting on T cell mobility.

Methods: Pancreatic islets were derivatized with linear PEG (5000 Da, JenKem, Inc.) as previously described(2). OTI-GFP CD8 T cells were isolated, cultured, and activated for 48 hours as previously described before being cultured with free or PEGylated mOVA or B6 islets in decellularized pancreatic ECM hydrogel, prepared as described elsewhere and adapted for culture of immune cells and pancreatic islets3. Time-lapse confocal imaging of activated T cells and PEGylated islets in 3D culture was conducted over 16 hours. Murine antigen-specific models were compared to murine non-specific models to elucidate differences in migration patterns. T cell migration parameters quantified following time-lapse imaging using the ImageJ TrackMate plug-in. To evaluate PEGylated islet viability and function, Live/Dead imaging was performed using a LIVE/DEAD Viability Cytotoxicity Assay kit and static glucose stimulated insulin secretion (GSIS) assays were performed as described elsewhere.

Results: Interrogation of the impact of linear PEG grafting on effector CD8 T cell mobility found that effector CD8 T cells cultured with untreated islets show no differences in migration parameters when compared to T cells cultured with linear PEG formulations in either antigen-specific or non-specific models. This indicates that effector CD8 T cells are not repelled by the PEG grafted to the islet surface.

Conclusions: Both antigen specific and non-specific systems reveal that PEGylation has no impacts on effector CD8 T cell migration patterns following their interaction with the PEGylated islet. Thus, observed benefits of PEGylation in animal models is unlikely due to repelling of already activated immune cells. Future work will further investigate if PEGylation plays more a role in impairing the activation of T cells.

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Poster #: 52

PEG-Crosslinked Vinyl Azlactone Hydrogels with Tunable Architecture for Sequential Release of Multiple Immunomodulating Drugs

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Immunotherapy provides an exciting alternative to traditional chemotherapeutics for cancer treatment. However, current immunomodulating approaches to cancer treatment are hindered by their poor pharmacokinetics after intravenous administration and accumulation at off-target sites. Therefore, we proposed the use of an implantable hydrogel for the controlled release of multiple immunotherapeutic cargos in concert. Using poly(2-vinyl-4,4-dimethylazlactone) (pVDMA) and functionalized PEG crosslinkers, we were able to release multiple drug classes at different rates dependent on crosslinking composition and density. Further, we demonstrated burst release of a small molecule and sustained release of a monoclonal antibody from the same hydrogel.

Crosslinking density and composition were varied to construct a library of hydrogels with various properties. The gels were then incubated in buffer and measurements were taken to quantify the degradation and release of therapeutic agents over time.

We were able to release multiple drug classes from the hydrogels including small molecule, polymer-drug conjugate, protein, antibody, and nanoparticle. By tuning the crosslinking density and composition, differential release profiles were achieved for the drug classes dependent on their size and the composition of the hydrogel. Notably, while small molecules, polymer-drug conjugates, and proteins achieved near-complete release within an initial three-day window, antibodies manifested sustained release kinetics over a span of two weeks. Furthermore, nanoparticle release profiles exhibited pronounced sensitivity to crosslinking density, with densely crosslinked gels demonstrating reduced release efficiency compared to their lower crosslinked counterparts. Additionally, we have been able to demonstrate dual release of a small molecule and antibody from the same hydrogel, indicating the hydrogel could be used for immunotherapy that requires the release of multiple drug classes at different rates.

The ability to finely control therapeutic release across various drug classes presents an exciting avenue for the development of multifunctional therapies, capable of eliciting specific, localized immune responses. Overall, the programmable hydrogel platform holds promise for advanced materials with tunable degradability and controlled release, offering broad utility in biomedical applications. Further studies will measure the anti-tumor effects of immunotherapeutic agents released from these hydrogels in a mouse model.

Poster #: 53

Antigen-loaded extracellular vesicles to regulate adaptive immune responses and drive immunological tolerance

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Autoimmune diseases are driven by adaptive immune responses that are inappropriately directed towards an autoantigen. Current treatments primarily rely on broad immunosuppression, which leaves patients susceptible to viral infection and cancer progression without providing a permanent cure. Tolerogenic vaccines capable of tolerizing the immune system towards a particular autoantigen posit a potential treatment without the drawbacks of broad immunosuppression. Unfortunately, the clinical success of such therapies has been limited by their inabilities to: 1) adequately deliver sufficient antigen to the lymphatics, where T cell priming occurs and 2) spatiotemporally co-deliver antigen and tolerizing adjuvant to reprogram antigen presenting cells (APCs) towards a suppressive phenotype. To this end, we have developed an extracellular vesicle (EV)-based platform for the co-delivery of antigens and immunoregulatory cues. EVs are cell membrane enclosed nanoparticles (~50-200 nm) secreted by all cells as a means of intercellular communication via the transfer of various biomolecules. Additionally, many types of EVs are intrinsically immunosuppressive and involved in maintenance of immune tolerance. In this work, we aimed to exploit the size-dependent lymphatic trafficking and intrinsic immunosuppressive effects of exogenously administered EVs to induce tolerogenic T cell priming by APCs, leading to CD4+ regulatory T cell (Treg) proliferation and CD8+ effector T cell (Teff) deletion/anergy. EVs were isolated from HEK293SF-3F6, B16.F10, hTERT-MSC, and BeWo cells and functionalized with dibenzocyclooctyne (DBCO) moieties using a DBCO-PEG12-TFP Ester linker. Azide-functionalized moieties were ligated to DBCO-EVs via overnight incubation. EVs were characterized using NTA, western blot, and TEM. EVs were screened for immunosuppressive potential in vitro using bone marrow-derived dendritic cells (BMDCs). BMDCs were challenged with lipopolysaccharide (LPS) and then treated with EVs. Flow cytometry was used to quantify expression of CD80, CD86, CD40, and MHC II. The ex vivo biodistribution of EVs was evaluated by labeling DBCO-EVs with azide-Cy5 and interrogating the tissue- and cell-specific delivery using IVIS and flow cytometry, respectively. Peptide antigens were synthesized with N-terminal azido groups using solid phase peptide synthesis (SPPS). Antigen-loaded EVs were evaluated in vivo using OT-I and OT-II adoptive T cell transfer models as well as an experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS).

Poster #: 54

Utilizing protein nanosheets as a scaffold for therapeutic proteins in cancer immunotherapy.

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The efficacy of intratumorally administered cancer immunotherapies relies on the specificity and localization of therapeutic macromolecules within the tumor microenvironment. To this end, we propose protein nanosheets (pNS) for controlled and prolonged presentation of therapeutic proteins in the tumor. pNS are self-assembled two-dimensional protein materials with thickness less than 100 nm. Our synthesis methodology entails aqueous self-assembly through a straightforward process, wherein two fusion amphiphilic proteins are mixed, followed by end-to-end rotation for a predetermined duration. The distinct hydrophilic and hydrophobic moieties in the fusion proteins contribute to the alignment of protein assemblies at the air-water interface during pNS synthesis. The pNS are compatible with different sized globular proteins spanning 19.39-86.80 kDa and can be synthesized out of two different globular proteins. Modulating protein quantity during synthesis, along with rotation time and speed yields pNSs with approximate size ranges, definite pNS concentration and different concentrations of globular proteins on their surface. These pNS show lower diffusivity in mice-derived tumors imposed by their micro-scale size. They exhibit enzymatic activity comparable to soluble control and are structurally stable in the human pooled serum for a prolonged time. The versatility in the pNS synthesis process coupled with their limited diffusivity, high loading capacity and better stability make them a suitable platform for localized delivery of proteins and enzymes for tumor-targeted cancer immunotherapy.

Poster #: 55

Smart Prodrug Synthesis for Controlled Release of Active Sting Agonists

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Controlled drug release can be achieved through prodrug development, which enhances the therapeutic efficacy but reduces off-target effects. A new avenue for synthesis of smart prodrugs that are capable of releasing active sting agonists in a controlled manner is proposed herein. By following the principles of prodrug design and adopting stimuli-responsive linker molecules, we hope to achieve precise spatiotemporal control over drug release and consequently promote therapeutic outcome. Our strategy involves conjugation of sting agonists to biocompatible carriers by means of cleavable linkers that respond to certain environmental cues such as enzymes or external stimuli such as light, pH etc. These prodrugs become active in a spatiotemporal fashion. This leads into the selective liberation of an active sting agonist upon administration. Such a controlled release system maximizes not just drug efficiency but also minimizes systemic exposure thereby limiting toxicity. By pursuing this, we anticipate significant advancements in immunotherapy research.

Poster #: 56

Engineered hydrogel platform for the study of B cell-stromal interactions in Germinal Centers.

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Successful immune responses to vaccines or pathogens are characterized by the presence of high affinity antibodies and immunological memory. B cells, who are responsible in the production of humoral immune responses, can generate high affinity antibodies through Germinal Center (GC) structures found in Secondary Lymphoid Organs. However, the cells involved in the GC reaction go beyond B cells and T cells, it also involves non-hematopoietic (defined by the lack of CD45 expression) cells commonly named stromal cells. These cells are responsible for the maintenance of the lymphoid niche by secretion of survival factors, production of extracellular matrix (ECM), lymph node expansion and contraction, and antigen presentation. Although much of stromal immunology knowledge has been generated using animal models, not much success has been made when translating these findings in human ex vivo models. Therefore we propose the use of a synthetic or semi-synthetic hydrogel system to better understand B cell-stromal immunology. Stromal cells were obtained from tonsil tissue removed from pediatric patients and were processed to a single cell suspension with or without stromal cells prior to hydrogel encapsulation. First we conducted studies using a PEG-4MAL hydrogel system with ECM derived cues in the presence or absence of stromal immune cells. Our findings showed that in the presence of stromal cells the number of B cells, T cells and antibody secreting cells (defined by CD19+CD27++CD38++) was significantly increased. We also noted an increase in the concentration of B cell survival cytokine IL6 and chemokine CXCL12 after 12 days in culture in our hydrogel system in the presence of stromal cells. Due to the presence of collagen in native tissue, we hypothesized that the presence of collagen would further increase the survival of stromal cells and further increase the survival of other immune cells as well. Our findings demonstrated that when collagen is included in our synthetic hydrogel system, we saw a 2-fold expansion in B cells and stromal cell populations is increased. All together our results highlight the benefit of a combined semi-synthetic system for studying of B cells and stromal cell interactions ex-vivo.

Poster #: 57

Development of Self-Assembled Protein Vesicles Displaying Alpha C Antigen as a Novel Group B Streptococcus Maternal Vaccine for Enhanced Protection

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Protein vesicles self-assembled from recombinant fusion proteins are biocompatible, tunable, and stable, making them candidates for vaccine applications. Recombinant protein vesicles are self-assembled from Globule-Zipper-ELP protein complexes, where “Globule” is a globular protein, zipper is a coiled coil, and ELP is a thermoresponsive elastin-like-polypeptide that exhibits lower critical solution temperature phase behavior. Protein vesicles exhibit stability at physiological conditions with tunable size and allow protein antigens to be displayed on the surface with tunable antigen concentration and conserved antigen structure. We hypothesize that a protein vesicle-based subunit vaccine will enhance the strength of cellular and humoral immune responses compared to soluble antigen. In this work, a protein vesicle maternal vaccine was designed against Group B Streptococcus (GBS), as it is the leading cause of life-threatening infections in newborns. Stable vesicles were developed using Alpha C, a GBS antigen, and an ELP containing tyrosine at 5 guest residue positions. These vesicles exhibited long-term stability at physiological conditions, and by increasing the ZE/ZR ratio, the diameter was reduced to 50 nm to facilitate drainage into lymphoid organs and allow direct interaction of vesicles with follicular B cells for enhanced humoral responses. Alpha C vesicles elicited enhanced bone marrow dendritic cell activation of MCH II+ and CD86+ markers when compared to soluble Alpha C. Upon evaluation of the viability of functional protein vesicles displaying a GBS antigen, this vaccine design will be tested in mice and results of vaccination comparing Alpha C vesicles and soluble Alpha C will be presented, including humoral and cellular immune responses.

Poster #: 58

Adjuvanted lymph node-resident and lymph node-migratory antigen presenting cells differently influence the T-cell response to immune checkpoint blockade

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A key research problem in cancer immunotherapy is developing further understanding of factors that drive T-cell antitumor responses to immune checkpoint blockade (ICB). Increasingly, tumor-draining lymph nodes (TdLNs) have been understood to play a critical role in the T-cell response to ICB. This is because TdLNs facilitate the co-mingling of T-cells, tumor antigen, and antigen-presenting cells including conventional dendritic cells (cDCs) to enable T-cell activation and differentiation. cDCs can be delineated into resident cDCs, which access tumor antigen draining into the TdLN through lymphatic capillaries, and migratory cDCs, which take up tumor antigen in the periphery before trafficking to the TdLN. Previous work in our lab has used an engineered particle system to implicate resident and migratory cDCs in promoting distinct T-cell responses to antigen presentation in the TdLN, with resident cDCs associating with a more stem-like CD8+ T-cell phenotype and migratory cDCs associating with a more effector-like CD8+ T-cell phenotype. These phenotypes of CD8+ T-cell are known to respond differently to ICB. Inspired by this, we hypothesize that separately adjuvanting TdLN-resident and TdLN-migratory cDCs will enable delineation of their relative contributions to T-cell response to ICB. To investigate this, we react pyridyl disulfide-functionalized poly(propylene) sulfide nano- or microparticles with the TLR9 agonist CpG to create 30 nm CpG-NPs that can drain to TdLNs via lymphatics and 500 nm CpG-MPs that are transported to TdLNs by migratory cDCs. These CpG-NPs/MPs are injected through various routes of administration into B16F10 tumor-bearing mice and analyzed to determine their biodistribution (IVIS, flow cytometry) and their immunomodulatory and therapeutic effects when combined with ICB (immune phenotyping, tumor growth, survival). Preliminary results using 30 nm CpG-NPs in combination with ICB indicate that CpG-NP delivery to TdLN-resident cDCs is accompanied by an increase in cDC1 presence and maturation in the TdLN. This in turn couples with ICB to induce increased differentiation of stem-like CD8+ T-cells to effector-like CD8+ T-cells in the TdLN. Furthermore, this differentiation effect is accompanied by an increase in mobilization of antigen-experienced PD-1+ CD8+ T-cells into the blood when adjuvanting LN-resident cDCs in the TdLN but not the non-draining lymph node (NdLN).

Poster #: 59

Supramolecular peptide-protein granules for tunable intracellular protein delivery

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I prefer poster presentation only

The ability to deliver active proteins across the cell membrane and into the cytosol would provide access to druggable targets not available in the extracellular environment. However, proteins do not efficiently cross the cell membrane to enter the cytosol on their own. Here we will present the development of supramolecular peptide-protein granules for intracellular protein delivery. This approach utilizes charge-complementary molecules known as, “CATCH(+) peptides” and “CATCH(-) fusion proteins”. Alone, CATCH(-) and CATCH(+) remain in the soluble state, but combined form b-sheet fibrils. After introducing a crowder to the mixture, such as polyethylene glycol (PEG), Tween-80 micelles, or excess CATCH(+) peptide, 100-200 nm “CATCH(+/-) granules are formed at peptide concentrations ~10-fold lower than the critical fibrillization limit in dilute conditions (~200 mM). CATCH(+/-) granules are rapidly internalized by various adherent and suspended mammalian cell types including fibroblasts, HEK293, T cells, dendritic cells, monocytes, and neutrophils. Different CATCH-fusion proteins can be co-assembled into multicomponent granules with tunable composition. Multicomponent granules allow for co-delivery of both an “effector” (i.e., a protein that confers therapeutic function) along with a “selector”, where the latter can be used to enrich the sub-population of cells that have internalized the CATCH(+/-) granule. CATCH(+/-) granule internalization demonstrates greater delivery efficiency and lower cell death than other state-of-the-art methods, such as cell penetrating peptides or electroporation. Collectively, these data establish CATCH(+/-) granules as a simple and flexible nanomaterial platform cell engineering via intracellular protein delivery.

Poster #: 60

Multilayered Cryogel for Macrophage Modulation Toward Anti-Tumor Immunity

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The tumor microenvironment (TME) presents multiple dysregulated signals polarizing macrophages towards M2 functions, thus suppressing anti-tumor immunity. The spatiotemporal regulation of drug release in the TME is critical to tumor associated macrophage (TAM) activation toward M1 functions and the restoration of functional immunosurveillance. Immunomodulators, such as cytokines, offer promise in TME modulation and promote immune cell infiltration. To prevent toxicity from the systemic delivery of these immunomodulators, localized delivery is necessary. We have implemented an injectable cryogel (hydrogel fabricated at -20oC) to act as a delivery depot for inflammatory cytokines IFN-γ and IL-12, as well as CCL2, a chemokine that attracts pro-tumor, M2-like macrophages. The aim of this cytokine-loaded cryogel is to draw M2-like TAMs from the tumor and repolarize them toward M1-like inflammatory functions to induce anti-tumor immunity.

We first developed a single layer cryogel and evaluated its impact on tumor growth. Peritumoral injection of the cryogel system into FVB female mice with PyMT-MMTV mammary tumors resulted in significantly suppressed tumor growth, an increase in T cell infiltration, and an increase in the M1:M2 ratio of TAMs. This localized treatment primes the TME for subsequent T cell-based immunotherapies like immune checkpoint blockade.

To allow TAM attraction before their exposure to the inflammatory cytokines, we have developed a novel injectable multi-layered cryogel (MLC) composed of an inner layer and a peripheral layer. We loaded our inner layer with the inflammatory cytokines, while retaining the chemokine in the peripheral outer layer. This design ensures a burst release of CCL2 followed by sustained inflammatory cytokine release. Our MLC is also tunable, in that the number of layers, layer thickness, and drug dose can be altered to release the biologics with fine control.

The injectability of our MLC allows for localized, non-invasive delivery of our depot to target the therapeutic site with a strong modulatory dose in the TME that has limited systemic toxicity. We believe our cytokine and chemokine loaded MLC will better induce an inflammatory tumor microenvironment to suppress tumor growth and prime the tumor to be susceptible to other therapies, such as immune checkpoint blockade.

Poster #: 61

Optimization of siRNA Nanoparticles with Custom Polymeric Surfactants

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Small interfering RNA (siRNA) is a promising therapy for regulation of specific gene expression, but endolysosomal escape presents a barrier against intracellular bioavailability and silencing potency. Here, siRNA-loaded nanoparticles (si-NPs) are conceptualized, synthesized, and screened for intracellular siRNA delivery. The si-NPs are comprised of a poly(dimethylaminoethylmethacrylate-co-butyl methacrylate) (PDB) and poly(lactide-co-glycolide) (PLGA) core to complex with the siRNA and enable pH-dependent membrane disruption for endosome escape. New custom polymeric surfactants have been developed with increased anchoring to the si-NP core to promote stability while maintaining gene knockdown activity and cytocompatibility. The surfactants are diblock copolymers with an anchoring block that includes varied density and length of alkyl side groups in order to investigate and optimize the contributions of hydrophobic interactions in anchoring to the si-NP core.

A library of diblock polymeric surfactants was synthesized with controlled reversible addition fragmentation chain transfer (RAFT) polymerization and verified using 1H NMR and GPC. Polymeric si-NPs were formulated via flash nanoprecipitation using a confined impinging jets (CIJ) mixer using a range of concentrations and surfactants from the polymer library. PLGA, PDB, and siRNA were dissolved in acetonitrile and mixed with the surfactant dissolved in deionized water in the CIJ mixer. Dynamic light scattering (DLS) was performed to evaluate the size, polydispersity index (PDI), and zeta potential of the si-NPs, which were found to have a narrow size distribution and neutral charge. The viability and gene silencing activity of the si-NPs loaded with luciferase-targeting siRNA were then assessed in luciferase-expressing MDA-MB-231 cells 48 hours after treatment. Luciferase knockdown was achieved while maintaining cell viability. Further studies are ongoing to determine a lead si-NP formulation.

Poster #: 62

Development of ingestible molecular probes for non-invasive detection of sucrase-isomaltase deficiency

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I prefer poster presentation only

Sucrase-isomaltase (SI) is an important intestinal brush border glycosidase that breaks down dietary sucrose into the monosaccharides, glucose and fructose, for absorption in the small intestine. Individuals with congenital sucrase-isomaltase deficiency (CSID) have an intolerance to sucrose and experience chronic post-prandial symptoms such as gas, bloating, diarrhea, and pain due to fermentation of intact sucrose by the microbiome in the large intestine. CSID is currently diagnosed via endoscopy and collection of biopsy samples from the small intestine, which are used in ex vivo assays to measure SI activity. However, less invasive diagnostic tests are needed as CSID usually presents at an early age in pediatric patients. Breath tests are non-invasive and rapid alternatives and enable diagnosis via detection of disease-indicating volatile organic compounds in breath (i.e. breath biomarkers). In this work, we sought to develop a method to induce the exhalation of breath biomarkers reflecting intestinal SI activity for non-invasive detection of CSID. Glucose-based probes were synthesized containing covalently bound volatile reporter molecules. We hypothesized that ingested probes would release volatile reporters upon degradation by active SI in the small intestine and that liberated reporters would diffuse from the intestinal lumen into blood circulation and be exhaled after pulmonary gas exchange. Probe specificity for sucrase activity was confirmed by reacting probes with sucrase and other common intestinal glycosidases and analyzing the reaction headspace for signal from volatilized reporters using mass spectrometry. Volatile reporter signal was only observed when probes were reacted with sucrase. Non-specific probe degradation was also not observed in tissue homogenates from the mouse GI tract. When probes were administered via oral gavage into healthy mice, breath signal from exhaled reporters was observed during the time the probe transited the small intestine (0-1.5 h after oral gavage). No signal was observed from mice administered vehicle controls. Pre-treatment of mice with sucrase inhibitors reduce the induced breath signal, indicating that the breath signal is specifically driven by intestinal sucrase activity. Altogether, we have developed ingestible probes that can be used for breath-based assessment of intestinal sucrase activity to provide a non-invasive alternative to CSID diagnosis via endoscopy and biopsy.

Poster #: 63

An asparagine-rich polypeptide tag for the assembly of protein gels

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Certain amino acid sequences give rise to polypeptides that form supramolecular networks in response to specific external stimulus. Here, we have identified an asparagine rich polypeptide sequence, (GGGSGGGSGGNWTT)10 or "NGT," that when fused to superfolder green fluorescent protein (NGTsfGFP) or NanoLuc luciferase (NGTnL), assembled into supramolecular network at reduced temperatures. These fusion proteins were recombinantly expressed in Origami B (DE3) competent cells and purified in acceptable yields as soluble protein. Both NGTsfGFP and NGTnL retained the activity of the fused protein, demonstrated by NGTsfGFP emitting green fluorescence in blue light, and NGTnL emitting blue light in the presence of the chemical substrate furimazine. Oscillatory rheology characterized the resulting materials as a weak viscoelastic gel which exhibited self-healing after mechanical disruption and softening with increasing temperature. Gel formation was disrupted through mutation of the asparagine residues in the NGT sequence, installation of glucose onto the asparagine residues via Actinobacillus plueropneumoniae N-glycosyltransferase, or use of a chaotropic agent, suggesting a role of asparagine hydrogen-bonding in supramolecular network formation. Due to gel formation being mediated by NGT, NGTsfGFP and NGTnL could co-assemble into a two-component gel. This multifunctional gel emitted both green and blue light in the presence of furimazine, demonstrating bioluminescence resonance energy transfer between the nL and sfGFP domains. These data position NGT as a temperature-responsive polypeptide tag that can be used to create functional biomaterials from soluble fusion proteins synthesized by cell-based hosts.

Poster #: 64

Hemostatic and Wound-Targeting Antibacterial Silver-Composite Injectable Microgels Prevent Biofilm Growth in Synthetic Wound Media

Ethan Pozy, NC State University

I would like to be considered for a Rapid Fire Oral Presentation

The risk of bacterial infection is increased following traumatic injury due to both the disruption of physical barriers to the environment and dysregulation of the immune system. An estimated 65-80% of human infectious diseases are caused by biofilm-forming bacteria that incorporate extracellular material into networks that protect the pathogen from immune cells. Bacteria in biofilms undergo epigenetic and metabolic changes that greatly reduce the susceptibility of the microbes to antibiotic treatment. These biofilms are implicated in infected and non-healing chronic wounds. We have developed a platelet-mimicking nanosilver microgel composite comprised of a polyNIPAM microgel loaded with silver nanoparticles (AgULCs) that demonstrate antibiotic activity. These microgels are decorated with fibrin-specific antibodies to form platelet-like particles (AgPLPs) that bind to and increase the density of fibrin clots present at injury and augment hemostasis. Incubation with AgULCs and AgPLPs have been shown to halt the growth of S. aureus in broth microdilution assays, and incubation with AgULCs has demonstrated a dose-dependent reduction and prevention of S. aureus biofilm biomass and metabolism in a synthetic wound media that has been shown to drive similar genetic expression to bacterial wound infections in vivo. AgPLPs present a potential therapeutic platform to improve traumatic injury outcomes with hemostatic and antibacterial functionality.

Poster #: 65

The Art of War: S. aureus Biofilm Defensive Tactic Provides Offensive Opportunity

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Introduction:

S. aureus is a bacterial species that is highly infectious upon breach of the dermis. Bacteria persist in the wound site by generating a protective biofilm matrix. S. aureus secretes virulence factors that: recruit the host's machinery to produce fibrin via coagulase; manipulate fibrin into a protective biofilm matrix via Clumping Factor A; and commence biofilm degradation for dissemination via staphylokinase. Fibrin biofilms reduce antimicrobial therapeutic efficacy and stymie the immune system. Despite these defensive capabilities, the fibrin-binding tactic employed for biofilm formation is exploitable. We have

developed fibrin-based nanoparticles (FBNs) that can load antimicrobial therapeutics like vancomycin for dermal biofilm administration. FBNs can penetrate and be incorporated into the biofilm matrix by bacteria, providing a targeted delivery vehicle that increases therapeutic contact time.

Methods/Results:

FBNs were synthesized via enzymatic thrombin-fibrinogen reaction, followed by fibrin clot straining and sonication. FBNs were characterized by atomic force microscopy and NanoSight nanoparticle tracking analysis for dry height (~300nm) and diameter (~15nm), and hydrodynamic diameter (~215nm). Particles were loaded with vancomycin via a “breathing in” method and release was tracked over 144hrs, with a ~70% loading efficiency and sustained release profile. FBNs were incubated with S. aureus USA300 WT and ΔclfA strains, and demonstrated interaction with WT but not ΔclfA. A fibrin biofilm was formed over 24hrs by inoculating 30% human plasma and 546-labeled fibrinogen in RPMI media with GFP+ Newman strain, and resultant biofilms were treated with 647-labeled FBNs for 24hrs. FBNs were seen interacting with sequestered bacteria via confocal. A viability assay using vancomycin-free and -loaded FBNs (vFBNs) was conducted as in the prior assay. After 48hr treatment incubation, biofilms were stained with BacLight Green and imaged via plate reader and confocal. Green:red signal was compared, and 1024μg/ml vFBNs had a significantly greater efficacy than free drug. Similar results were seen in a murine dermal wound infection model.

Discussion:

S. aureus is an invasive pathogen that forms protective biofilms. vFBNs infiltrate fibrin biofilms, promoting enhanced bacterial killing compared with free drug in vitro and in vivo. vFBNs significantly reduce the required therapeutic dose to treat infection, limiting off-target effects of antimicrobials.

Poster #: 66

Sweet Corn Phytoglycogen Dendrimers as a Lyoprotectant for Dry-State Protein Storage

Junha Park, University of Florida

I would like to be considered for a Rapid Fire Oral Presentation

Protein biotherapeutics typically require expensive cold-chain storage to maintain their fold and function. Packaging proteins in the dry state via lyophilization can reduce these cold-chain requirements. However, formulating proteins for lyophilization often requires extensive optimization of excipients that both maintain the protein folded state during freezing and drying (i.e., “cryoprotection” and “lyoprotection”), and form a cake to carry the dehydrated protein. Here we show that sweet corn phytoglycogens, which are glucose dendrimers, can act as both a protein lyoprotectant and a cake-forming agent. Phytoglycogen (PG) dendrimers from 16 different maize sources (PG1-16) were extracted via ethanol precipitation. PG size was generally consistent at ~70-100 nm for all variants, whereas the colloidal stability in water, protein contaminant level, and maximum density of cytocompatibility varied for PG1-16. 10 mg/mL PG1, 2, 9, 13, 15, and 16 maintained the activity of various proteins, including green fluorescent protein, lysozyme, β-Galactosidase, and horseradish peroxidase, over a broad range of concentrations, through multiple rounds of lyophilization. PG13 was identified as the lead excipient candidate as it demonstrated narrow dispersity, colloidal stability in phosphate-buffered saline, low protein contaminants, and cytocompatibility up to 10 mg/mL in NIH3T3 cell cultures. All dry protein-PG13 mixtures had a cake-like appearance and all frozen protein-PG13 mixtures had a Tg’ of ~ -26 ℃. The lyoprotection and cake-forming properties of PG13 were density-dependent, requiring a minimum density of 5 mg/mL for maximum activity. Collectively these data establish PG dendrimers as a new class of excipient to formulate proteins in the dry state.

Poster #: 67

Materials characterization of a self-assembling elastin-like polypeptide fusion as a potential platform for immunotherapies

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Self-assembling polypeptides are a unique category of biomaterials that undergo spontaneous organization into nano- or microstructures due to the combination of precise interactions, such as hydrogen bonds, ionic and electrostatic interactions, π-stacking, and hydrophobic effects. Many of these self-assembling materials are stimuli-responsive and undergo morphological changes based on external cues such as temperature, pH, or enzymatic activity. Stimuli-responsive self-assembling materials have grown increasingly popular as platforms to improve vaccine delivery and immunogenicity by increasing plasma residence time, improving uptake, or targeting lymphoid tissues.

We have identified a novel self-assembling fusion protein, termed K2(E36)115, which forms a diverse array of nano and microstructures in a concentration and temperature dependent manner. The protein is

comprised of a short, zwitterionic peptide (K2) derived from an immunomodulatory class of drugs called Glatiramoids, as well as (E36)115, a long and hydrophobic elastin-like polypeptide (ELP). ELPs are a family of repetitive proteins that undergo lower critical solution temperature (LCST) phase behavior, wherein they transition from a soluble to coacervate state upon heating above a characteristic transition temperature (Tt). Below its Tt, K2(E36)115 forms a heterogeneous assembly of vesicles with variable size and configuration (unilamellar, multilamellar, multicompartmental), but same lamellar thickness. Above its Tt, it forms large coacervates that, remarkably, maintain a multilamellar structure.

In this work, we aimed to elucidate the self-assembling behavior of K2(E36)115 to identify lead formulations for further application as immunotherapies. We used dynamic light scattering, UV-Vis spectrophotometry, and cryo-electron microscopy to characterize the temperature and concentration dependence of nanoparticle assembly. To investigate the contributions of specific amino acids to ionic and π-stacking interactions, we designed a library of fusion proteins with single amino acid alterations in the parent peptide K2 and evaluated them for disruption in nanoparticle formation. In our ongoing work, we aim to characterize a second library of fusion proteins where the ELP component has varied length and hydrophobicity, to investigate how the features of the hydrophobic block influence morphology. Together, this information provides insight into the molecular characteristics governing the self-assembly of (K2)E36115 and guides further investigation into the development of a tunable, stimuli-responsive platform for immunotherapy development.

Poster #: 68

Engineering multiplexed breath biomarkers for rapid respiratory pathogen identification

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Respiratory infections are caused by a wide variety of pathogens, and pathogen identification is a crucial step for informed, targeted treatment. However, pathogen identification takes days due to time-intensive clinical assays that require culturing of microbes from patient samples. To eliminate this step, we are developing a diagnostic test that leverages existing pathogen growth inside the infected lungs. Together, the pathogen and host produce a repertoire of proteases in the infected tissue microenvironment that can be used for pathogen classification. To harness pulmonary protease activity for rapid pathogen

identification, we have developed nanosensors that can be administered into the lungs to induce exhalation of reporters for lung protease activities.

Nanosensors are comprised of volatile reporter molecules tethered to a nanocarrier via protease-cleavable peptide linkers. Upon peptide cleavage, liberated reporters vaporize and are subsequently exhaled for quantification in breath via mass spectrometry. Thus, nanosensors harness disease-associated protease activities to produce breath biomarkers. Biomarker signatures comprised of the activity of multiple proteases provide superior disease specificity to that of single proteases. Therefore, for pathogen identification, we have established multiplexed sensing capabilities in which nanosensors for orthogonal proteases are barcoded with volatile reporters of distinct mass.

To test our approach, we established three mouse models of lung infection with common respiratory pathogens: Staphylococcus aureus, Pseudomonas aeruginosa, or Klebsiella pneumoniae. Cleavage assays combining bronchoalveolar lavage fluid collected from infection models and a fluorogenic peptide substrate library revealed 10 orthogonally-cleaved peptides that can be used for pathogen identification. The 10-plex includes peptides that are cleaved by host and pathogen proteases. Using established linker chemistries, peptides were VOC-barcoded to develop a nanosensor array. Reaction of the nanosensor array with proteases in vitro confirmed that nanosensors are cleaved by targeted proteases with high specificity and that the magnitude of reporter signal reflects protease activity levels. Most importantly, multiplexed protease sensing in vitro was possible using the nanosensor array. We are currently assessing the ability of nanosensors to generate pathogen-specific breath biomarkers in mouse infection models. Success of this work will result in a breath test for rapid pathogen identification to facilitate informed, targeted treatment of lung infections.

Poster #: 69

Thrombin-sensitive platelet-like-particles for injury site-specific shape change

Krista Hilmas, North Carolina State University/UNC-Chapel Hill

Preventable mortality rates in the battlefield are primarily caused by combat-associated traumatic hemorrhage and trauma-induced coagulopathy. Consequently, hemorrhage control and resuscitation has been labeled a primary focus area of research towards material development for 'on-field' mitigation of combat trauma. First-generation platelet-like particles (PLPs) have been developed to induce clot contraction and stability by binding to fibrin fibers and exerting a strain on fibers as they collapse into an energetically favorable conformation, enhancing hemostatic benefits and wound healing outcomes. PLPs, however, are morphologically slower than native platelets due to their inability to respond to injury site-specific agonists. Therefore, second-generation thrombin-sensitive platelet-like particles (tsPLPs) have been developed to change shape in response to thrombin exposure at a wound site. Thrombin-sensitive nanogels (TSNs) are synthesized by crosslinking N-isopropylmethacrylamide (NIPMAM) with a thrombin-cleavable peptide sequence. These TSNs are then conjugated with a fibrin-binding motif through EDC/NHS chemistry to create tsPLPs that bind to fibrin and are amenable to shape change upon exposure to thrombin at a wound site. This study evaluated TSNs synthesized with a decreased initiator content, from 34.7% to 7.5% APS initiator, to increase the yield of TSNs and allow for greater scale up. TSNs were then evaluated before and after exposure to thrombin for changes in size and morphology. TSNs were incubated with thrombin (75U/mL) at room temperature, and the solution was spiked with Thrombin (100U/mL) daily. Aliquots of TSN/thrombin solution were taken at t=2hr, 4hr, 24hr, 48hr, and 96hrs for characterization by Atomic Force Microscopy (AFM) and NanoSight. Results showed an increase in size upon exposure to thrombin, with a size peak at the 2-hour timepoint, and a decrease in size at later timepoints, indicating degradation of TSNs by thrombin. Furthermore, tsPLPs were evaluated for their ability to induce clotting in an APTT assay and were found to have similar clotting times to first-generation PLPs. In conclusion, TSNs exhibit shape change deformation abilities, indicated by initial swelling of particles and further degradation, and tsPLPs retain clot contraction abilities similar to first-generation PLPs, without exposure to high levels of thrombin. Ongoing studies are evaluating particle deformation's effect on wound healing efficacy.

Poster #: 70

Optimizing a Nanoparticle Drug Delivery System to Treat Preterm Labor

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Biomaterials have a slew of uses across many medical disciplines. Among these applications, drug delivery methods have received ample attention for their obvious utility in treating complex diseases, mitigating off-target effects, and improving pharmacokinetics and pharmacodynamics. Poly(lactic-co-glycolic) acid (PLGA) has garnered great popularity – with FDA-approval this biopolymer is among the most used biomaterial in drug delivery applications. With robust heterogeneity across the field, our work aimed to characterize formulation parameters for PLGA nanoparticles (NPs) that tune particle size, loading efficiency, and surface modification while maintaining particle morphology and surface charge. We compare two formulations, using a simple oil-in-water emulsion technique. One with dicholoromethane (DCM) and polyvinyl alcohol (PVA), and the other with ethyl acetate (EtAc) and D-α-tocopheryl polyethylene glycol succinate (Vit E-TPGS). With serial centrifugation we show an ability to isolate multiple particle cohorts by size with tight polydispersity (PDI). However, the resulting EtAc/Vit E-TPGS NPs produced more variable NPs (PDI:0.30) in comparison to the DCM/PVA NPs (PDI:0.20). For drug loading, we successfully encapsulated two lipophilic molecules, indomethacin and nifedipine, in high efficiency (82±15% and 90±4%, respectively) without major effect on NP size. Using succinyl ester, we have shown preliminary studies coupling either antibody or small-peptide molecules to the particle surface. We used a 3x4 matrix approach to test both reactive to non-reactive polymer ratios and peptide to NP mass ratios. We show that greater peptide to NP mass ratio and lower reactive to non-reactive polymer ratios produce NPs with more peptide bound per particle, however this is accompanied by lower coupling efficiency. Moreover, we novelly report coupling efficiency on the NPs directly through either dot blots (for antibodies) or Ellman's assay (for small-peptide molecules). In future studies, we plan to explore another common chemistry, maleimide click-chemistry, with our peptide molecules and compare their efficiencies and total coupling to succinyl ester methods as reported here. This effort is to provide a resource to varying methods in the field to streamline development of these useful drug delivery systems.

Poster #: 71

Nanofibrous hydrogel preparation via electrospinning for drug delivery and wound healing

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With its high surface area and porous structure, a nanofibrous scaffold helps with protein adsorption, binding of ligands, diffusion of molecules, biomimicking of the extracellular matrix (ECM), and promoting stem cell differentiation. Electrospinning is a polymer processing method to obtain nonwoven fiber mats with fiber diameters ranging from tens of nanometers to hundreds of micrometers. This research presents a nanofibrous hydrogel incorporating hyaluronic acid (HA) prepared via electrospinning, a method that holds promise for drug delivery and wound healing applications. The process involves optimizing the components, including the effective polymer (HA), the carrier polymer (polyethylene oxide) that improves spinnability, the crosslinker (polyethylene glycol diacrylate), and the photo initiator (Irgacure 2959) to create an electrospun fibrous unwoven mat with continuous and uniform fibrous morphology. This mat is crosslinked using ultraviolet (UV) light and swelled under deionized water to form the nanofibrous hydrogel. The nanoscale morphology of the electrospun fiber mat, the mechanical properties of the nanofibrous hydrogel, and the release rate of drug molecules from the hydrogel under acid, base, or neutral solutions related to the formulation of polymer solutions for electrospinning are further studied. We find that a higher ratio of PEO improves fiber morphology but decreases the mechanical properties of the crosslinked hydrogel. Because cells can sense the stiffness of the surrounding matrix and respond accordingly, the tuning of mechanical properties of fibrous hydrogel facilitates versatile adaptation to the physiological environment. This hydrogel system has the potential for the application of wet healing dressing to reduce scar formation, degradable bioscaffold for long-term treatment of knee pain caused by osteoarthritis (OA), and cell therapy by promoting Mesenchymal Stem Cells (MSC) differentiation.

Poster #: 72

Addressing Immune Suppression in Acute Respiratory Distress Syndrome (ARDS) with Synthetic Nanoparticle Antibodies (SNAbs)

Shivani Vyas, Georgia Institute of Technology

Acute respiratory distress syndrome (ARDS) is a severe condition characterized by acute lung inflammation and respiratory failure, and results in a hospital mortality rate of approximately 40%.Immunosuppressive myeloid cells, termed myeloid-derived suppressor cells (MDSCs), play a vital role in shaping immune balance in acute respiratory diseases. MDSCs are involved in the pathogenesis of ARDS due to their contribution to the amplification of pulmonary inflammation and tissue damage. Current therapies utilized to deplete MDSCs, ranging from small molecule inhibitors to monoclonal antibodies (mAbs), encounter challenges in specifically targeting MDSCs, resulting in broad off-target effects and limited efficacy. The development of novel immunomodulatory therapies targeting MDSCs can potentially mitigate disease severity in these conditions. We have developed synthetic nanoparticle antibodies (SNAbs) to target and deplete MDSCs and restore beneficial host immune responses in models of ARDS. SNAbs are multivalent, bifunctional gold nanoparticles (AuNPs) featuring MDSC-targeting ligands and antibody-fragment crystallizable (Fc)-mimicking ligands. By bridging MDSCs with immune effector cells, SNAbs have demonstrated the capability to induce the depletion of MDSCs. We administered SNAbs to ARDS-challenged murine lung cells in vitro and through aerosolized delivery to a mouse model of ARDS. Following flow cytometry and cytokine analysis, we demonstrated that SNAbs can deplete MDSCs, reduce immunosuppressive mediator expression, and restore beneficial T cell responses.

Poster #: 73

Synthetic biomaterials as carrier for limbal stem cells derived from induced pluripotent stem cells for ocular surface regeneration.

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Limbal stem cell deficiency (LSCD) is a debilitating ocular surface disorder occurring due to loss or dysfunction of limbal stem cells (LSCs). Existing treatments for LSCD include transplantation of autologous limbus from the healthy eye in unilateral cases, or allogeneic limbal tissue from living or cadaveric donors when both eyes are affected using human amniotic membrane , the innermost layer of the placenta as the carrier. Scarcity, lack of reproducibility, risk of disease or infection transmission of amniotic membrane have made research necessary into alternative biomaterials compatible with LSCs. Also, treatment of LSCD is further exacerbated by limited supply of healthy LSCs especially in the case of bilateral LCSD. To address the existing limitations, we designed a graft with the essential properties and combining it with a viable source of LSCs to develop a treatment for LSCD. LSCs derived from induced pluripotent stem cells (iPSCs) have the advantage of being an unlimited and non-immunogenic source. We developed a xeno-free and chemically defined protocol for differentiating iPSCs to LSCs and observed the expression of relevant markers (PAX6, p63α,CK12). Electrospun synthetic membranes were laser perforated, plasma-treated, and biofunctionalized with Collagen-IV to mimic the corneal microenvironment with sufficient mechanical strength to withstand surgical manipulation and minimize the obstruction of vision. Laser perforations with different diameters and spacing allowed for regulation of the mechanical properties and light transmittance of the construct. The light transmittance of the construct after perforation was increased to 60%. Plasma treatment allowed more homogenous and profuse covering with Collagen-IV compared to non-plasma treated membranes which improved cell adhesion to the scaffold by providing binding sites that receptors of cells can interact with. We have made progress developing an iPSC-LSC seeded synthetic graft which has potential to address existing limitations of LSCD treatment.

Poster #: 74

One Step, Catalyst-Free Synthesis of a Mechanically Tailorable Organohydrogel and its Derived Underwater Superoleophobic Coatings

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Organohydrogels are an emerging class of soft materials that comprises the mechanical durability of organogels and the biocompatibility of hydrogels for prospective biomedical applications. In this work, we have introduced a facile, catalyst-free one-step chemical approach to develop an organohydrogel with impeccable anti-biofouling properties following the amine-epoxy ring opening reaction under ambient conditions. Hence fabricated mechanically tailorable organohydrogels have prospective applications in medical devices, drug delivery, and tissue culture. The affinity of the current organohydrogel to both organic solvents and water was exploited to incorporate the nitric oxide donor, S-nitroso-N-acetylpenicillamine (SNAP) from ethanol, and subsequently, the water-sensitive NO-releasing organohydrogels were observed to demonstrate excellent antibacterial activity against E. coli and S. aureus with 99.71% and 87.31% reduction in planktonic bacteria, respectively, without eliciting any cytotoxicity concerns. Moreover, the reported organohydrogel with remarkable water uptake capacity was extended as a coating on different medically relevant polymers to demonstrate transparent underwater superoleophobicity. Thus, the reported facile organohydrogel and its derived underwater anti-fouling coating can open avenues for utility in biomedical, energy, and environmental applications.

Poster #: 75

Combination Biomedical Therapeutics Utilizing Nitric Oxide and Zinc Molybdate

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Antimicrobial resistance (AMR) has been increasing at a steady pace for the last decade. It has been estimated that in the United States alone, AMR causes 2.9 million infections and 35,000 deaths annually. This threat has encouraged research into alternative therapies apart from conventional antibiotic use. Nitric oxide (NO) is an alternative therapy that has been extensively researched for its relevance as a broad-spectrum antimicrobial that pathogens cannot grow resistant to. In addition to synthetic NO donor molecules, researchers can utilize catalysts that take advantage of naturally occurring molecules to generate NO consistently over long periods. Materials containing metal ion coatings have shown dose-dependent NO generation as well as bactericidal killing. Herein, a common NO donor, S-nitroso-N-acetylpenicillamine (SNAP), was blended into a medical-grade polymer to create NO-releasing films. The films were then coated with a polymer solution containing zinc molybdate ($ZnMoO_4$) to create NO-releasing and -generating films with a bimetallic oxide outer layer confirmed with scanning electron microscopy (SEM) and elemental analysis. Bimetallic oxides represent multiple metal complexes with the ability to catalyze NO generation and kill bacteria similarly to mono-composite metals while maintaining high biocompatibility, something single metal ions do not display. The combination materials showed a release of relevant levels of NO (0.5-4.0 Flux) for at least 7 days while the $ZnMoO_4$-only films displayed dose-dependent generation of NO comparing the 1 and 3 wt% $ZnMoO_4$. Additionally, the inclusion of $ZnMoO_4$ on the surface of the material alone showed a 51.02% killing efficiency towards E. coli. Even more so, the combination of a NO-releasing and -generating surface showed an almost two-log reduction in adhered bacteria colonies, representing a 98.53% reduction efficiency. Further biological testing was completed to determine the biocompatibility of the combo material. When exposed to human fibroblast cells for 24 h, there was no visualized toxicity towards the cells from any of the material groups. This NO + bimetallic oxide material displayed comparable antibacterial and NO-generation properties as materials utilizing mono-composite metal ions while maintaining high biocompatibility, even showing proliferative effects. These characteristics make it an intriguing class of materials for use in the biomedical field.

Poster #: 76

Investigating in Vitro Angiogenesis in a Verapamil-Releasing 3D Printed Polymeric Scaffold

Amanda Bradley, University of Florida

BACKGROUND

Angiogenesis is key for successful tissue integration of cellular implants. Although not the intended use, verapamil has been shown to have an impact on in vivo vascularization. In order to improve the vascularization of porous 3D implants, this long-term study observes the direct impact of verapamil drug release from polymeric scaffolds on the formation of in vitro blood vessels.

METHODS

A reverse-cast method was used to generate 3D printed PDMS scaffolds loaded with verapamil (bulk concentration: 0%, 0.05%, and 0.25%). Negative space was filled with fibrin, NHLFs, and GFP-HUVECs, and media was replaced every 24h. Scaffolds were confocal imaged on d7, 14, and 21 and analyzed for percent vessel coverage in scaffold pores in ImageJ. Concurrently, verapamil elution data was collected daily and analyzed by multiple reaction monitoring (MRM).

RESULTS

Verapamil was successfully integrated into and released from a PDMS construct. Over 21 days, both 0.05% and 0.25% scaffolds display a first-order release profile with peak concentrations of 83.5 ng/mL and 1375.4 ng/mL respectively. After 8 days, both scaffolds reach steady-state averaging a daily release of 24ng/mL (0.05%v) and 356.6 ng/mL (0.25%v). Control and 0.05% scaffolds had significant increases in vessel coverage from d7-14, both having more coverage on d14 than 0.25% scaffolds. By d21, vessel coverage across all groups was the same. Visually, at d14 and 21, 0.05% scaffolds have thicker and more highly branched vessels.

CONCLUSIONS

Verapamil was successfully integrated into a PDMS scaffold, yielding first-order release with varied magnitude based on verapamil loading. Verapamil appears to have minimal impact on vessel coverage in vitro, however, there appears to be a shift in vessel thickness, length, and branching which future work aims to quantify.

Poster #: 77

PLG microparticles encapsulating retinoic acid accelerate muscle recovery in mice after hindlimb casting-induced muscle atrophy.

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Muscle atrophy is the loss of muscle mass from disuse, aging, or chronic illness. The recovery process starts with an activation of local and infiltrating immune cells, which creates a local inflammatory environment before attenuating towards an anti-inflammatory, wound-healing stage. The inflammatory stage utilizes cytokines secreted by pro-inflammatory macrophages such as interleukin-6 (IL-6) and is important for immune cell recruitment, removal of cellular debris, and to activate stem cells. However, IL-6 impedes repair systems if sustained and must be attenuated by anti-inflammatory cytokines and muscle-regenerative factors such as insulin-like growth factor-1 (IGF-1), produced by anti-inflammatory macrophages. We have shown that all-trans retinoic acid (ATRA) induces stem cell differentiation and induces macrophages to produce IGF-1 in culture. However, ATRA has poor oral bioavailability and a short half-life in in vivo systems. Thus, we developed particles for extended release. For this study, we hypothesized that ATRA delivery into the fascia of muscle would accelerate muscle growth after immobilization-induced atrophy in mice.

C57bl/6 male mice were given casts immobilizing the hindlimbs for 10 days. Upon cast removal, particles containing a high, medium, or low dose of ATRA or saline were injected into the intermuscular space between the gastrocnemius and tibialis anterior muscles. Mice were allowed to recover for 3-7 days, after which mice were euthanized and hindlimb muscles were collected for biochemical or histological analysis. H&E shows fiber cross-sectional area is larger in high- and low-loaded ATRA-PLG groups compared to saline at the 3- and 7-day timepoints. To understand the mechanism for increased fiber recovery, ELISAs of muscle lysate show IL-6 decreased at the 3-day timepoint in a dose-dependent manner, with a 50% decrease in the low-dose treatment group. Solei sections were immunofluorescently stained for macrophage markers CD206 (pan-macrophage), CD68 (anti-inflammatory), and CD163 (anti-inflammatory). Taken together, local delivery of ATRA-PLG may be a potential treatment for accelerating recovery after muscle atrophy. To further elucidate the mechanism for which ATRA is accelerating recovery, we intend to determine whether ATRA impacts signaling pathways associated with muscle growth and stem cell activation (proliferation, differentiation) using western blotting and immunofluorescent techniques.

Poster #: 80

Impacts of beta-sheet crystallinity on the mechanical properties of chemically and physically crosslinked silk fibroin hydrogels

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Silk fibroin (SF), obtained from Bombyx mori silkworm cocoons, is a natural biopolymer used in the formation of biomaterials due to its biocompatibility, controllable degradation rate, and tunable physical properties. In SF hydrogels, molecular weight, concentration, and crystallinity are all tunable parameters that affect the mechanical and physical properties of the resulting structures. SF is known to form physical crosslinks via secondary structure formation within the protein, resulting in ordered beta-sheet structures. Organization of beta sheet structures via hydrogen bonding yields crystalline regions amongst amorphous polymer chairs, decreasing optical transparency and increasing mechanical strength over time. Crystalline beta-sheet structures can be intentionally induced via application of shear forces or by increasing the temperature, but will form even without intentionality as the biopolymers interact within the hydrogel, aiming to minimize free energy. To interfere with the free energy landscape, we investigated the impact of solution parameters (concentration and molecular weight) on resulting hydrogel mechanical properties through shear rheology. Furthermore, we evaluated the extent to which chemical crosslinking minimizes physical crosslinking, exploring horseradish peroxidase enzymatic crosslinking. Physical crosslinking (beta-sheet content) was assessed through Fourier transform infrared (FTIR) spectroscopy and optical transparency. Gelation kinetics reveal the temporal profile of gelation for physical and chemical crosslinking strategies. Results confirm that SF molecular weight has the greatest impact on viscosity and the greatest impact on physical crosslinking dynamic moduli. The gelation kinetics of the chemical crosslinking chemistries are faster than the physical crosslinked hydrogels; however, the storage and loss moduli of the physically crosslinked hydrogels is significantly larger than the chemically crosslinked materials. Understanding the relationship between chemical and physical crosslinking in SF hydrogels enables temporal control over mechanical properties for future soft tissue engineering applications. Temporal control can also be modulated through additional chemical crosslinking methods in SF hydrogels, including methacrylation or norbornene functionalization.

Poster #: 81

Using 3D Engineered Tissue Model for Investigating the Role of CXCL7 in Colon Cancer Progression

Jannatul Ferdous Nipa, Auburn University

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Colorectal cancer (CRC) ranks among the top three prevalent cancers worldwide, with a high incidence in the US. 3D engineered tissue models are used for studying cancer behavior, enabling replication of tumor microenvironments which enhance our insight into tumorigenesis and therapeutic development. For our study, we used our 3D engineered tissue model to study the impact of chemokine ligand CXCL7 on CRC cells (HT-29 cell lines) by using transfected CRC cells with CXCL7 and an empty vector used as control. CXCL7 contributes to driving progression of various types of cancer, including CRC. Our preliminary findings suggest that CXCL7 chemokine promotes CRC cell proliferation, consistent with the clinical data of CRC patients. We examined the effect of CXCL7 on the proliferation and viability, and the change of colony and overall tissue size. The transfected cells were encapsulated in poly (ethylene glycol) (PEG)-fibrinogen hydrogels to provide a 3D environment for the cells. Cancer cell proliferation was assessed by measuring EdU incorporation in DNA after 8 days of encapsulation. Viability was observed on day 1, day 8 and day 15 post-encapsulation. 3D engineered tissues were cultured for 30 days to assess temporal differences between tissues formed with CXCL7 transfected and vector control HT29 cells. From the experimental outcomes we observed that engineered tissues formed with cells expressing CXCL7 displayed significantly higher growth and proliferation compared to the vector-transfected engineered tissues. Additionally, colony formation in CXCL7 transfected tissues showed noticeable increase in size when compared to the vector-transfected tissues on day 8 and day 15 (significantly on day 15) of culture. The viability of cells in the tissues formed with CXCL7 transfected cells was higher than that in the tissues formed with vector transfected cells (day 8 and day 15 of culture). Overall, this study demonstrated that CXCL7 expression impacts cellular behavior inside 3D engineered tissue, including HT29 cell colony development, viability and proliferation. This evaluation of the impact of CXCL7 on CRC growth within a 3D tissue engineered model opens opportunities for further investigation into it's potential as a therapeutic target and biomarker.

Poster #: 82

Hydrophilic Reactive Oxygen Species-Degradable Scaffolds for Wound Healing

Joshua McCune, Vanderbilt University

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Synthetic biomaterials represent a promising class of materials used for wound dressings. They are relatively inexpensive to produce and allow for fine control over physiochemical properties compared to biologic dressings. However, current synthetic wound dressings fail to fully optimize physiochemical properties as they are predominantly polyester-based materials subject to poorly controlled hydrolytic degradation and material-associated inflammation. We have previously developed a synthetic polythioketal urethane (PTK-UR) wound dressing which degrades in response to reactive oxygen species (ROS) in the wound environment. In porcine wound models, it was observed that critical wound healing factors such as tissue infiltration, vascularization, re-epithelialization, and reduced inflammation correlated with increasing scaffold hydrophilicity.

Our previous work was limited in the range of achievable hydrophilicity due to the synthetic approach and use of ethylene glycol monomers (EG). Here we have innovated a novel class of PTK materials capable of achieving significantly more hydrophilic wound dressings. These super hydrophilic PTK-URs have been validated to maintain morphological features (pore structure, pore size, and overall porosity) and degradation mechanisms previously optimized with the EG-PTK-URs. Despite comparable physical characteristics, these super hydrophilic scaffolds are more efficient at scavenging ROS, ultimately providing cytoprotection against oxidative stress in vitro. These scaffolds also exhibit larger swell ratios and improved moisture retention, a critical design feature for wound dressings. In pilot studies, these scaffolds have demonstrated exceptional healing responses in excisional wound models with more robust tissue infiltration, vascularization, and re-epithelialization. Currently, we are exploring the tunability of this new class of PTK-URs to allow for control over tissue responses and optimizing these next-generation scaffolds for evaluation in chronic wound models.

Poster #: 84

Large Engineered Cardiac Tissue Production using Tissue Engineered Pluripotent Stem Cell Microspheres as Building Blocks

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This study evaluated the use of differentiating 3D microspheroidal engineered heart tissues (µEHTs) as building blocks for macro-scale EHT production (>1 cm3). Currently, EHT production methods are complex and not easily scalable; advances are required for moving to manufacturing scale cell production and tissue assembly. We have previously shown that µEHTs can be formed by encapsulating human induced pluripotent stem cells (hiPSCs) in poly(ethylene glycol) fibrinogen (PF) hydrogel microspheres and differentiating these hiPSC-laden engineered tissue microspheres in scalable, suspension culture. Here, to investigate the ability to form larger EHTs from the µEHTs, µEHTs were assembled into millimeter scale tissues on differentiation days 3, 5, and 7. As a control, millimeter scale engineered tissues were also formed directly from hiPSCs as previously reported. Secondary encapsulated tissues were produced by adding differentiating µEHTs to PF precursor solution and photocrosslinking in millimeter scale molds. µEHTs were successfully encapsulated and remained viable to form millimeter scale tissues. For all tested secondary encapsulation timepoints, µECTs fused together to form larger composite tissues and underwent successful cardiac differentiation forming EHTs. Tissue contraction was observed in EHTs formed at all secondary encapsulation timepoints by day 20 and contraction/relaxation velocity temporally increased through day 40. All tissues appropriately responded to beta-adrenergic stimuli, one indicator of functional maturation. µEHTs encapsulated on day 5 had higher contraction and relaxation velocities compared to days 3 and 7, suggesting that day 5 secondary encapsulation yields better cardiac tissue function. To test macro-scale large tissue production using this method, µECTs from day 5 were secondarily encapsulated within a perfused large tissue bioreactor (1 cm3). Macro tissues were successfully formed from day 5 µECTs within the bioreactor and after five days of culture, cells remained viable when in proximity to the perfusion channels and showed positive staining for the cardiac marker alpha sarcomeric actinin. Overall, µECTs from differentiation day 3, 5, and 7 can successfully be employed as building blocks to produce larger tissues through secondary encapsulation in PF; the approach established here was demonstrated for use in macroscale CT production and has potential applications to 3D EHT printing.

Poster #: 85

3D Printable Biomaterials for Tissue Engineering Applications

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Pelvic organ prolapse (POP) is a prevalent condition among women, characterized by the descent of pelvic organs into the vaginal canal due to weakened pelvic floor muscles and connective tissues. Current treatments for POP often involve surgical intervention, including the use of tissue scaffolds to provide support and promote tissue regeneration. However, existing scaffolds face limitations in customization, biocompatibility, and mechanical strength. This research project proposes leveraging 3D printing technology to address these challenges and develop personalized, biocompatible, and mechanically robust tissue scaffolds for POP treatment. By utilizing advanced 3D printing techniques, such as material extrusion and digital light processing, we aim to fabricate scaffolds with tailored structures and properties, optimizing their effectiveness in supporting pelvic organs and promoting tissue regeneration. This innovative approach holds promise for revolutionizing POP treatment by providing patients with bespoke solutions that enhance treatment outcomes and improve quality of life.

Poster #: 86

Engineered 3D In Vitro Model of Colorectal Cancer in Insulin-Sensitive and Insulin-Resistant Microenvironments

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This work establishes an insulin-resistant (IR) 3D engineered colorectal cancer (3D-eCRC) tissue model to examine the role of IR in colorectal cancer (CRC) progression. CRC remains the third-most common cancer and the second-leading cause of cancer related deaths in the US. CRC has been epidemiologically linked with obesity; IR and inflammation could underlie this link. However, the mechanism is not well understood. To better understand this link, in vitro CRC models that more accurately mimic IR and associated inflammation are needed. Here, we developed a 3D-eCRC model that incorporates an IR microenvironment and examined the impact on CRC cell growth and migration.

To form 3D-eCRC tissues, HT29 CRC cells were encapsulated in poly(ethylene glycol) fibrinogen. To maintain cell viability in insulin-sensitive (IS) and IR culture conditions, 3D-eCRC tissues were cultured in media containing 50% CRC media and 50% of IS conditioned media (CM), IR CM, or control media.

To obtain IS CM, 3T3 L1 fibroblasts were differentiated into adipocytes. To obtain IR CM, we treated the differentiated matured adipocytes with tumor necrosis factor alpha and 1% hypoxic conditions, replaced the media, and then collected the CM after 24 hours.

On day 15 of culture, cell viability within the 3D-eCRC tissues in the control, IS and IR conditions was 51%, 54%, and 54%, respectively. IS and IR CM stimulated cell proliferation within the 3D-eCRC tissues; percentages of cells positive for the cell proliferation marker Ki67 were 6.3%, 16.5% and 12.0% in control, IS, and IR conditions, respectively. Examination of the morphology of resulting cell colonies revealed that 3D-eCRC tissues cultured in control media had smooth, round colonies, whereas IS and IR tissues showed rough, fragmented colonies.

Additionally, cells in IS and IR 3D-eCRC tissues were more migratory than control. From day 14 to day 15 of culture, 23,000, 29,000, and 44,000 cells migrated out of control, IS, and IR 3D-eCRC tissues, respectively.

In conclusion, our results suggest that IS and IR conditions impacted cell proliferation, migration, colony morphologies.

Future studies will examine gene expression and tissue staining to further understand the roles of IS and IR microenvironments on 3D-eCRC tissue properties.

Poster #: 87

Engineered Vascularizing Hydrogel to Deliver Stem Cell-Derived Beta Cells

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Type 1 Diabetes (T1D) is a chronic, autoimmune disease which results in the irreversible destruction of β-cells. The insulin produced by the β-cells is responsible for the regulation of blood glucose levels and without them, the blood glucose levels become dangerously high and multiple long-term health complications can arise. The direct injection of exogenous insulin is the current standard to regulate glucose levels but requires constant monitoring and still does not prevent the long-term complications of T1D which leads to an average 18-year reduction in life span. The transplantation of allogeneic islets shows promise as a cure, but donor availability and long-term survival of the cells have prevented its success. Induced pluripotent stem cell-derived β-cells (SCβ-cells) have emerged as an alternative cell source for implantations due to their scalability. Furthermore, the subcutaneous space is a clinically relevant transplant site that is minimally invasive compared to the intrahepatic site but has low vascularization. Hydrogels made from 4-armed maleimide-terminated poly(ethylene glycol) (PEG) are useful for controlled vasculogenic endothelial growth factor (VEGF) delivery. Subsequently, islet vascularization and engraftment in the epididymal fat pad and subcutaneous space. Changes in the polymer backbone and crosslinker affect the mode of degradation and delivery of the VEGF. Additionally, the dose of VEGF within the hydrogel needs to be optimized to ensure the formation of functional vessels. Therefore, we conducted experiments to assess the capability of different PEG/VEGF gel formulations in promoting vascularization in the subcutaneous space by transplanting SCβ-cells with different PEG/VEGF gels and evaluating the survival and engraftment of the cells. Information about vessel density and insulin secretion within the grafts will be used to select the most promising gel formulations to then be used in a diabetic mouse model.

Poster #: 88

Direct-Ink Write 3D printing of therapeutic poly (N- vinyl caprolactam) and hyaluronic acid-based scaffolds

Samina Yasmeen, Clark Atlanta University

I would like to be considered for a Rapid Fire Oral Presentation

Layer-by-layer printing is an ideal choice for fabricating 3D scaffolds using bioinks that support cellular and tissue growth such as photocrosslinkable methacrylated hyaluronic acid (meHA) however, it has limited printing properties. Therefore, there is a need to design hybrid bioinks to address this issue. In this work, we prepared bioinks containing trihybrid PVCL-meHA-allyl α-tocopherol (PMATP), PVCL-meHA-allyl carvacrol (PMCAR), PVCL-meHA-allyl curcumin (PMCURC), and PVCL-meHA-allyl catechin (PMCAT), consisting of polyvinyl caprolactam (PVCL) (P), meHA (M), and one of the functionalized antioxidants (ANTOX). Allevi 3D bioprinter was used to print bioink at 17°C, 45 psi, 6 mm/s, 30% crosslinking intensity, and a 37°C printing bed temperature. Fourier Transform Infrared (FTIR) spectroscopy and scanning electron microscopy (SEM) were employed to determine the crosslinking and morphological features, respectively. The 3D scaffolds were evaluated for swelling behavior printability antioxidant activity, thermoresponsive and rheological properties. High-resolution scaffolds (PMANTOX) were printed by covalent crosslinking of PVCL, meHA, and ANTOX, providing the bioink showed rapid sol-to-gel transition at 37 °C, structural fidelity, and long-term mechanical stability upon dual photocrosslinking. FTIR microscopic images confirmed the crosslinking of the trihybrid component. The rheological analysis of the scaffold revealed that PMCAT, which contains penta-allylated catechin, has the highest final storage modulus (G') of 2.1 × 105. SEM images reveal an open porous structure with a smooth surface of a successful printed multilayered hydrogel with a grid-like pattern. All scaffolds were fully rehydrated after 15 min, with an average swelling ratio of 113.4 %, indicating that the PMANTOX scaffolds can carry over twice their dry weight in water without disintegrating for 21 days. The 3D scaffolds displayed a narrow average size distribution of pores and strand diameters, with an average pore size of 0.993 + 0.04 mm and strand diameters average of 1.29 + 0.03 mm. In the 1-1-Diphenyl picrylhydrazyl (DPPH) assay, antioxidant activity was increased under 20 % O2, with PMATP exhibiting the highest antioxidant activity (24.48%). Further studies include long-term maintenance of 3D scaffold with proliferation of extracellular matrix proteins. This study introduces a novel direct ink write printing concept for 3D scaffolds, providing practical insights for developing 3D scaffolds for clinical applications.

Poster #: 90

Understanding tissue functionality and cell types formed during cardiac differentiation of hydrogel supported microspheres and scaffold-free aggregates

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One of the major roadblocks in disease modeling is the inability to produce chamber specific cardiomyocytes (CMs). To generate clinically relevant CMs, there is a need to better understand the different cell types produced when hiPSCs undergo cardiac differentiation. This work investigates the cell types produced when scaffold-free and hydrogel supported hiPSC tissues undergo the standard B27 cardiac differentiation protocol, as well as assess and compare the resulting functionality and CM yield of these tissues. Robust cardiac differentiation protocols have been around for a decade and 2D cardiac differentiation was found to yield almost exclusively left ventricular (LV) CMs; however, cardiomyocyte sub-type populations generated in 3D from this protocol are yet to be investigated. We have previously generated engineered cardiac microsphere tissues (MS) by encapsulating and differentiating hiPSCs within poly(ethylene glycol) fibrinogen (PF) 3D hydrogels. Additionally, self-aggregated hiPSC tissues have been successfully differentiated to yield CMs; however, detailed analysis of the cell types formed is needed. Following hiPSC encapsulation (MS) or shaker flask initiation of forced aggregation (aggregates), tissues were cultured for 3 days prior to initiation of differentiation. On differentiation day (DD) 10, a higher CM yield in MS compared to aggregates was observed by flow cytometry analysis for the cardiac troponin T (cTnT) marker. Contraction and relaxation velocities for MS were 40.1±9.7 μm/sec and 46.8±15.5 μm/sec respectively, compared to aggregates with 12.6±7.0 μm/sec and 23.1±6.2 μm/sec respectively. DD 15 tissue cryosections showed positive staining for cTnT and α-smooth muscle actinin. Tissues were optically mapped on DD 45; MS were able to be paced up to 3 Hz. Higher calcium transition velocities and lower calcium transition durations were observed in MS compared to aggregates. Preliminary single-cell RNA sequencing (scRNA-seq) data identified presence of cardiomyocytes, fibroblasts, and endodermal cells. To further investigate the different cell types formed during differentiation, DD 5 and DD 15 tissues were dissociated for scRNA-seq and samples are currently being sequenced. Overall, MS show higher functionality than aggregates and, therefore, are more advantageous for disease modeling and use in further studies to generate specific chamber-specific CMs.

Poster #: 91

Human stem cell-derived β-cells delivered with vasculogenic hydrogels reverse hyperglycemia in diabetic immunodeficient mice

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Type 1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing β-cells in the pancreas. The most promising therapy thus far is the transplantation of cadaveric islets, but this approach is severely limited by the lack of donors, poor engraftment, and the need for chronic immunosuppression. To address the lack of insulin-producing cells, human stem cell-derived β (SC-β) cells have become a promising scalable source of insulin-producing cells. This study focuses on hydrogel delivery vehicles for to promote engraftment and function of SC-β cells. SC-β cells were transplanted to the gonadal fat pad (gFP) of non-diabetic SCID-beige mice either with a polyethylene glycol (PEG) hydrogel delivering vascular endothelial growth factor (VEGF) (PEG-VEGF) or PEG gel without VEGF. Immunohistochemistry (IHC) images showed higher signal of C-peptide when SC-β cells were delivered with the PEG-VEGF carrier, indicating that the PEG-VEGF gel promoted engraftment and survival of the SC-β cells. We next transplanted 5.5•106 SC-β cells to the gFP of streptozotocin (STZ)-induced diabetic SCID-beige mice, both with and without PEG-VEGF hydrogels. At 21 days post-transplant, normoglycemia was restored in both treatment groups and persisted for 90 days. At 6 weeks post-transplant, an intraperitoneal glucose tolerance test (IPGTT) confirmed that the grafts responded to glucose stimuli in real-time, and blood serum samples showed the presence of circulating C-peptide. These results motivate current dosing studies to determine the marginal graft that can correct diabetes, and whether the PEG-VEGF gel carrier can promote vascularization of the marginal graft and reverse hyperglycemia. Success in these studies will be a step forward to future T1D correction studies incorporating immunomodulatory strategies in the omentum of immune competent and larger animal models.

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2024 Regional Symposia

Southwest Symposium: University of Texas at Austin

September 19 - 20, 2024

Program Agenda

Thursday, September 19, 2024

8:15 AM - 8:45 AM	Registration / Check-in
8:45 AM - 9:00 AM	Welcome by Site Chairs
9:00 AM - 10:15 AM	Session I - Cancer ***Daniel Harrington, UT Health School of Dentistry*** ***MK Sewell Loftin, University of Alabama Birmingham*** ***Elizabeth Lipke, Auburn University*** ***Stephanie Seidlits, University of Texas at Austin***
10:15 AM - 10:30 AM	Coffee Break
10:30 AM - 11:45 AM	Plenary Session I: ***Shana Kelley, Northwestern University*** ***Joel Collier, Duke University*** *(2024 Clemson Award for Basic Research Recipient)*
11:45 AM - 1:00 PM	Session II - Musculoskeletal ***Daniel Alge, Texas A&M University*** ***Elizabeth Cosgriff-Hernandez, University of Texas at Austin*** ***Akhilesh Gaharwar, Texas A&M University*** ***Teja Guda, University of Texas at San Antonio***

Program Agenda

Thursday, September 19, 2024 *(continued)*

1:00 PM - 2:00 PM	Lunch **"The American Heart Association's Role in Technology and Innovation,"** ***Dr. Patrick Dunn, Program Director of the Center for Health Technology & Innovation at the American Heart Association***
2:00 PM - 3:15 PM	Session III - Trainee Rapid Fire Talks
3:15 PM - 4:30 PM	Plenary Session II: ***Sarah Stabenfeldt, Arizona State University*** ***Danielle Benoit, University of Oregon***
10:15 AM - 10:30 AM	Coffee Break
4:30 PM - 6:30 PM	Poster Session and Reception

Program Agenda

Friday, September 20, 2024

8:30 AM - 9:00 AM	Registration / Check-in
9:00 AM - 10:15 AM	Session IV - Nervous System ***Zhengpeng Qin, University of Texas at Dallas*** ***Gabriela Romero Uribe, University of Texas at San Antonio*** ***Evan (Huiliang) Wang, University of Texas at Austin*** ***Michael Moore, Tulane University***
10:15 AM - 10:30 AM	Coffee Break
10:30 AM - 11:45 AM	Plenary Session III: ***Elazer Edelman, Massachusettes Institute of Technology*** *(2024 Founders Award Recipient)* ***Cynthia Reinhart-King, Rice University***
11:45 AM - 1:00 PM	Session V - Cardiovascular ***Jane Grande-Allen, Rice University*** ***Abhishek Jain, Texas A&M University*** ***Binata Joddar, University of Texas at El Paso*** ***Janet Zoldan, University of Texas at Austin***
1:00 PM - 2:00 PM	Lunch

Program Agenda

Friday, September 20, 2024 *(continued)*

2:00 PM - 3:15 PM	Session VI - Immunoengineering and Drug Delivery ***Isaac Adjei, Texas A&M University*** ***John Clegg, University of Oklahoma*** ***Jennifer Maynard, University of Texas at Austin*** ***Kevin McHugh, Rice University***

2024 Regional Symposia Chair:

Anita Shukla, PhD, Brown University

Southwest Regional Symposium Co-Chairs:

Stephanie Seidlits, PhD, University of Texas at Austin
Janet Zoldan, PhD, University of Texas at Austin

Program Committee:

John Clegg, PhD, University of Oklahoma
Teja Guda, PhD, University of Texas at San Antonio
Binata Joddar, PhD, University of Texas at El Paso
Kevin McHugh, PhD, Rice University
Shreya Raghavan, PhD, Texas A&M University

PLENARY SESSION I
Thursday, September 19

Dr. Shana Kelley
Northwestern University

PLENARY SESSION I
Thursday, September 19

Dr. Joel Collier
Duke University
(2024 Clemson Award for Basic Research)

PLENARY SESSION II
Thursday, September 19

Dr. Sarah Stabenfeldt
Arizona State University

PLENARY SESSION II
Thursday, September 19

Dr. Danielle Benoit
University of Oregon

PLENARY SESSION III
Friday, September 20

Dr. Elazer Edelman
Massachusetts Institute of Technology
(2024 Founders Award)

PLENARY SESSION III
Friday, September 20

Dr. Cynthia Reinhart-King
Rice University

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2024 Regional Symposia

September 19th - 20th

- **Northeast: Northeastern University**
- **Midwest: Case Western Reserve University**
- **Southeast: Georgia Institute of Technology**
- **Southwest: University of Texas at Austin**
- **Western: University of Colorado, Denver | Anschutz Medical Campus**
- **Northwest: University of Washington**

RAPID FIRES

Rapid Fire #: 1

Development of a Benchtop Pelvic Model for Testing Gynecological Devices

Ashley Hicks, The University of Texas at Austin

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Elizabeth Cosgriff-Hernandez, PhD - The University of Texas at Austin - cosgriff.hernandez@utexas.edu

The design and testing of gynecological devices are hampered by a lack of testing apparatuses that can be used to iteratively design devices. To address this critical need, we developed an anatomical and physiological benchtop testing apparatus that can simulate vaginal anatomy, temperatures, and pressures. We utilized this benchtop model to test the deployment and retention of a self-fitting vaginal stent. A 3D printed pelvis comprising of a cervix and vagina with labia was printed from biocompatible silicone (EcoFlex) at Lazarus3D™ based on a representative adolescent pelvic MRI. This model was encased in an acrylic chamber that enables testing of vaginal stent deployment (time, expansion) and retention under simulated pressure conditions. Heating tape was wrapped around the acrylic chamber to heat the vaginal canal to physiological temperatures. Temperature readings taken at three locations within the model (cervical, central, and introitus regions) were confirmed to be within physiological ranges (36°C 1°C). Pressures measured by the MizCure perineometer were within the range of pelvic floor muscle contraction (PFM) pressures reported clinically using this device (19 mmHg 1 mmHg). Utilizing this testing apparatus, we evaluated the design of a novel shape memory polymer (SMP) vaginal stent. The SMP stent can maintain a crimped shape for ease of insertion and expand upon changes in temperature and hydration to restore the vaginal lumen. Visualization of stent expansion and deformation was achieved through hysteroscopic imaging near the model introitus. Notably, the crimped SMP vaginal stent exhibited rapid expansion (~70% increase in cross-sectional area) within <5 minutes post-irrigation with warm water (~45°C), with minimal cross-sectional area reduction (8.3 7.0%, n = 3) under PFM contraction pressure. Stent diameter exhibited ~1% (1.1 7.5%, n = 3) decrease along the anterior-posterior dimension, with a corresponding 11% (11.0 3.6%, n = 3) increase distally. Results from this deployment study suggest that the SMP stent would successfully deploy and maintain the vaginal lumen under contractile forces in vivo. This study demonstrates the usefulness of this benchtop apparatus to model the temperatures and pressures of the vagina to facilitate robust design of future gynecological devices.

Rapid Fire #: 2

Development of UV-Crosslinkable Surface-Eroding Polymers for the Controlled Release of Immune Checkpoint Inhibitors

Heather Chia-Chien Hsu, Rice University

Heather Chia-Chien Hsu1 (ch108@rice.edu), Yosan Embafrash2 (yosansebahtu@gmail.com), Kevin J. McHugh1,2 (kevin.mchugh@rice.edu)

1Department of Chemistry, Rice University; 2Department of Bioengineering, Rice University

Immunotherapy has recently emerged as a prominent approach to cancer treatment, showcasing notable clinical successes. However, it still faces challenges such as its ineffectiveness against certain tumor and the risk of serious immune-related adverse events (irAEs) due to systemic administration. While local administration could be therapeutically beneficial, intratumoral injections are typically invasive and often require repeated dosing.

This project aims to develop a novel application of a UV-crosslinkable surface-eroding polymer to overcome two key issues in cancer immunotherapy: the lack of efficacy and the high off-target toxicity associated with systemic administration. This approach involves the development of a modified methacrylated poly (glycerol sebacate) (PGS-M) with improved hydrophobicity, evidenced by a contact angle of 82° (compared to the unmodified PGS-M with a contact angle of 52°), which is used to form the water-impermeable shell for a core-shell microparticle encapsulating biological immune checkpoint inhibitors, such as anti-PD-1. The particles made from this optimized material demonstrated the ability to resist infiltration by cholesterol esterase solution for 2 weeks and PBS for 4 weeks. This encapsulation ensures the protection of the payload from environmental degradation before its release for extended bioactivity.

In addition, we can leverage surface erosion to precisely modulate release kinetics by changing microparticle geometry, creating multiple microparticle population that provides a consistent series of customizable drug pulses with a single injection. Additionally, intratumoral administration of these microparticles acts as reservoirs, prolonging drug retention at the tumor site and establishing a locally high but systemically low drug concentration.

Ultimately, we aim to use this drug delivery platform to reduce dosing frequency and optimize drug release kinetics, thereby enhancing immunotherapy efficacy while mitigating side effects to provide a more effective treatment.

Rapid Fire #: 3

Liposome Coated Mesoporous Silica Nanoparticle Loaded with Forskolin Effectively Modulates Thermogenesis in Adipocytes in vitro and in vivo

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Maria A. Gonzalez Porras (maria.gonzalezporras@utsa.edu) - Department of Biomedical Engineering and Chemical Engineering, The University of Texas at San Antonio

Obesity is an ever-increasing health crisis in the US and can lead to various illnesses such as type 2 diabetes, hypertension, and stroke, yet many therapeutic treatments prove to be ineffective due to the lack of bioavailability at the physiological target site. We have successfully designed a liposome coated mesoporous silica nanoparticle (LCMSN) delivery system for forskolin (FSK), a water insoluble cAMP activator that has been used to increase thermogenic activity in adipose tissue. Briefly, dissolved FSK were mixed with liposome components and dried into a film. Dendritic mesoporous silica nanoparticles (MSN) were synthesized and loaded with FSK, which was then suspended in PBS, and fused with liposome film through electrostatic interactions to form LCMSN-FSK. The hydrodynamic size was measured to be ~140 nm, with a low PDI (<0.1), and a negatively charged surface (ζ=-28mV). The FSK loading percentages were 11% and 30% respectively, when liposomes were prepared with 10% or 25% FSK. Human adipose stem cells were differentiated to mature adipocytes and used to evaluate treatment efficacy. Cytotoxicity assays show no detectable toxicity (20-1000 ug/ml media). The uptake of fluorescently labeled LCMSN in adipocytes was evaluated using flow cytometry at different time points (3-48h) demonstrating maximum uptake at 24 h post treatment. Cell internalization was confirmed using 3D confocal microscopy images. Adipocytes treated with LCMSN-FSK resulted in an increase in UCP1 and Cox7A1 gene expression when compared to FSK or LCMSN alone. Furthermore, glucose uptake and lipolysis were also significantly elevated, demonstrating increased metabolic functional outcomes. For in vivo analysis, LCMSN-FSK was subcutaneously injected in inguinal white adipose tissue (iWAT). Biodistribution imaging using KINO Spectral Imaging system was performed in obese and non-obese mice. Retention of fluorescently labelled LCMSN-FSK was observed even until 48h post injection, while accumulation in heart, lung, liver, and kidney was not detected. Increase in lipolytic function and expression of UCP1 at the protein (WB) and gene (RT-PCR) levels were also detected. In conclusion, we have optimized a nanoparticle delivery system that is taken up by adipocytes, and effectively delivered forskolin to adipose tissue in mice which resulted in an increase in thermogenic activities.

Rapid Fire #: 4

Intracerebral Immunotherapy to Accelerate Neurological Recovery after Intracerebral Hemorrhage

Christopher Pierce, University of Oklahoma

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Abstract: Currently, there is an unmet treatment option for neurological disorders. Toward the goal of intracerebral delivery of disease-modifying therapeutics, we have developed a brain-tissue-inspired hydrogel depot to mimic native brain parenchyma, to locally deliver IL-4 and IL-10, modulating the immune system to improve neurological inflammation caused by diseases. The depot named “Parenchygel” is a novel combination of hyaluronic acid, heparin, poly(lactic-co-glycolic acid) nanoparticles, and chemically crosslinked with poly(ethylene glycol). We conducted a study to evaluate the feasibility of our therapeutic in rats induced with intracerebral hemorrhage, with a control group (no treatment, n = 5), surgical intervention (n = 5), carrier group (Parenchygel, n = 5) and a therapeutic group (Parenchygel, IL-4, and IL-10, n = 5). All rats received an intracerebral injection of 50 µL of blood obtained from the tail vein. The surgical intervention, carrier group, and therapeutic group had 50 µL hematoma evacuated the next day. The rats in the carrier group and therapeutic group were injected once with 20 µL of Parenchygel and monitored for 6 weeks. Rats were sacrificed after 35 days, the brains were removed, embedded in paraffin, and sectioned. Hematoxylin and eosin (H&E) staining was used to evaluate the health of tissues around the injection site and the hematoma volumes in rats. We evaluated neurologic deficits and improvements using the Modified Neurological Severity Score (mNSS) scoring on days 1, 3, 5, 7, 8, 13, 20, 27, and 34 post-surgery. A 2-way ANOVA revealed a statistically significant improvement in mNSS scoring for rats treated with the therapeutic compared to the control. The rats showed significant improvement on day 3 compared to the control where mNSS scores continued to increase until day 7. In conclusion, these results support that a single injection of Parenchygel with IL-4 and IL-10 therapeutic improves recovery from intracerebral hemorrhage.

Rapid Fire #: 5

Role of radiation-induced, GBM-secreted extracellular matrix in tumor treatment resistance and recurrence

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Background: Glioblastoma (GBM) is the most common and lethal type of brain tumors with median survival of 15 months. Patients' poor prognosis is mainly due to recurrence and development of treatment resistance. While the most common GBM treatment is radiation therapy, information about development of resistance to radiation therapy is scarce. Here, using hyaluronic-acid (HA) hydrogels as 3D scaffolds, we investigated the evolution of GBM-secreted extra cellular matrix and correlate that with development of radiation resistance in GBM. Method: HA hydrogels were formed via a UV-initiated, thiol-ene reaction. GBM cells are encapsulated as single cells, in different seeding densities in HA hydrogels to form tissue-engineered scaffolds (TE-pGBM). TE-pGBMs were irradiated with gamma radiation using a tabletop cell irradiator. Changes in ECM expression was investigated using RNA-sequencing (RNA-seq), matrisome proteomics, and confirmed using western blotting. GBM colony formation was investigated periodically, using bright field imaging and colony size was measured using an ImageJ script. Results: We first investigated the colony-formation ability of GBM cells in various seeding densities to establish the clonal expansion of GBM cells in 3D. Interestingly, at the lowest seeding density (50k/ml), GBM colonies' sizes in 10 Gy samples were similar to the no treatment control, suggesting an intrinsic resistance of GBM cells to radiation. To broadly investigate the post-radiation changes in GBM, we performed RNA-seq, 7-days post radiation. RNA-seq data clearly showed that radiation resulted in overexpression of ECM molecules such as Mucins, osteopontin, collagen IV, etc. On the other hand, collagen I was downregulated after radiation. Future: We will use computation oncology to correlate the ECM expression with GBM FLuc readings to determine what ECM molecules drive radiationresistance.

Rapid Fire #: 6

Macrophage Checkpoint Nanoimmunotherapy Has the Potential to Reduce Malignant Progression in Bioengineered In Vitro Models of Ovarian Cancer

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Most ovarian carcinoma (OvCa) patients present with advanced disease at the time of diagnosis. With its chemoresistant and invasive nature, the 5-year survival rate of metastasized OvCa is <30%, exposing a need for improved therapeutic targeting. Clinical biospecimen link poor prognosis to increased macrophage immune checkpoint signaling where CD47 (OvCa) binds macrophage checkpoint SIRPα to suppress antitumor immunity and enhance OvCa chemoresistance and metastasis, making this interaction a significant target for therapeutic discovery. Even so, previous attempts have fallen short, limited by CD47 antibody specificity due to its expression on several cell types in the body. Thus, we developed a new approach to disrupt CD47-SIRPα signaling by instead targeting the macrophages with short interfering RNA (siRNA) to reduce SIRPα expression. We specifically leverage the phagocytic nature of macrophages by encapsulating SIRPα siRNA (siSIRPα) in lipid-based nanoparticles (LNP) – which are preferentially taken up by macrophages – to aid in specificity and delivery.

We generated OvCa/macrophage co-culture heterospheroids on a hanging drop array as a representative model of cellular interactions in metastatic OvCa. In this model, we identified the presence of the CD47 and SIRPα proteins and observed increased carboplatin chemotherapy resistance and invasion (1.5-fold increases in IC50 and migration area) indicating a more malignant OvCa phenotype in the presence of

macrophages. Microfluidic techniques were used to prepare stable, reproducible LNP encapsulating siSIRPα which were characterized for optimal size, neutral charge, and efficient encapsulation. M0 macrophages quickly took up the particles and exhibited ~50% reduction in SIRPα gene and protein expression in heterospheroids, leading to reversal of pro-tumoral alternative activation in macrophages (reduction of IL-10/CD206, upregulation of NOS2/IL-1B/IL-12). Disrupting CD47-SIRPα signaling resulted in a 60% recovery of chemosensitivity and reduced OvCa invasion. Notably, siSIRPα LNP did not significantly alter chemoresistance or invasive potential in OvCa monospheroids, supporting that our therapy directly affects macrophages in the system, as desired. Additionally, LNP loaded with negative control siRNA did not reduce viability or activity of heterospheroids, suggesting there are no negative effects from the vehicle, itself. Ultimately, our results strongly indicate the potential of using CD47-SIRPα nanoimmunotherapy to reduce malignant progression of ovarian cancer.

Rapid Fire #: 7

Composite-Coated Endotracheal Tubes for Restoring Vocal Fold Function After Traumatic Intubation Injury

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Background and purpose: Endotracheal intubation is a common procedure, however, it often leads to laryngeal injury and complications including dysphonia, dysphagia, and difficulty breathing. If left untreated, these injuries can develop into glottic or subglottic stenosis which requires surgical intervention to improve airway patency. Therapeutic strategies in the acute phase of laryngeal wound healing could reduce fibrosis severity and allow the retention of vocal fold function. In this study, we investigated the efficacy of a dual drug eluting endotracheal tube for localized delivery of corticosteroids and small interfering RNA (siRNA) targeting smad3 for immediate reduction in pro-fibrotic transforming growth factor-beta 1 (TGFϐ1). Methods: Traumatic intubation was simulated in 9 Yorkshire crossbreed swine under direct laryngoscopy. Endotracheal tubes were first coated with dexamethasone-loaded polycaprolactone (PCL) fibers via electrospinning and then embedded in a four-arm polyethylene glycol acrylate matrix containing smad3 silencing siRNA. Composite-coated ETTs and uncoated regular ETTs were placed in the injured airway for 3, 7, or 14 days (n= 3 per timepoint). The local stiffness properties of the airway with injury and ETT placement were evaluated with normal indentation testing and assessed histologically by Masson's Trichrome. Results and Conclusions: Local stiffness outcomes (mean ± std. error) at 3 days were significantly greater with composite coated ETT placement (22.3 ± 0.692 N/m) than with the uncoated ETT placement (17.0 ± 0.757 N/m, $p< 0.0001$). At 7 and 14 days, tissue with uncoated ETT placement had significantly greater local stiffness than the composite coated ETTs ($p< 0.0001$ and $p= 0.001$, respectively). Histological assessment of collagen in vocal fold sections with injury and coated ETT placement indicated a decreased percentage area of collagen over time. Increased stiffness outcomes associated with fibrotic tissue formation impede vocal fold function, therefore, the goal of the study was to restore mechanical outcomes close to native properties by reducing inflammation and downregulating the TGF-β1 pathway. Our results indicated that dual delivery of dexamethasone and siRNA targeting smad3 reduced vocal fold stiffness over 14 days of intubation compared to uncoated ETTs.

Rapid Fire #: 8

Impact of Cardiac-Vascular Crosstalk on the Maturity of hiPSC-derived Cardiomyocytes in 3D Printed Cardiovascular Organoids

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Cardiovascular disease (CVD) has been the leading cause of death in the United States and much of the developed world for decades. This significant impact on public health necessitates the development of preclinical models that accurately recapitulate mature human heart function. Accordingly, there is a significant need for models of human cardiac tissue that elucidate the cell signaling pathways that drive normal CM development and reflect the physiological behavior of native, mature cardiac tissue. One approach for generating such models is using human induced pluripotent stem cells (hiPSCs) to generate hiPSC-derived cardiomyocytes (hiPSC-CMs). However, clinical relevance of hiPSC-CM models is greatly limited by several factors, with a principal limitation being the immaturity of hiPSC-CMs, as they are functionally closer to fetal CMs than adult CMs. While various approaches have used chemical and mechanical stimuli to increase hiPSC-CM maturity, such approaches largely focus on CM-only systems and fail to capture the importance of vasculogenesis, which coincides with maturation of cardiac tissue in embryonic development. As such, while hiPSCs have shown potential in cardiac tissue modeling applications, it is critical that heterogeneity resulting from cardiac differentiation and immaturity of hiPSC-derived CMs be addressed in order to advance clinical relevance of engineered cardiac tissue. To address the immaturity of hiPSC-CMs, I propose utilizing Freeform Reversible Embedding of Suspended Hydrogels (FRESH) bioprinting to engineer cardiovascular organoids. The bulk volume of these 3D tissue constructs will be comprised of hiPSC-CMs while a perfusable channel running through their center will be lined with hiPSC-ECs. This design will allow the hiPSC-CMs in the organoid to receive mechanical stimuli via perfusion through the channel as well as biochemical stimuli from the hiPSC-ECs, mimicking signaling from vasculature that drives CM maturation in the developing heart.

Rapid Fire #: 9

Evaluation of In Situ Assembled Granular Hydrogel Scaffolds for Neural Progenitor Cells Delivery in a Severe Spinal Cord Injury Model

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Spinal cord injury (SCI) is a neurological condition associated with a significant socioeconomic impact on patients and the healthcare system due to minimal treatment options. Neural progenitor cell (NPC) engraftment is a promising treatment option for the acute injury phase. We have previously identified in situ assembled poly (ethylene glycol) (PEG)-based granular hydrogels as a novel biomaterial scaffold that is suitable for the delivery of NPCs to SCI lesions. Importantly, testing in a mild acute injury model showed that these PEG-based granular hydrogels have good biocompatibility and support NPC differentiation toward astrocytic and neural lineages. The objective of this study was to evaluate their performance for NPC delivery in a severe injury model. In brief, PEG microgels were synthesized by thiol-norbornene click chemistry which reacts 4-armed PEG-amide-norbornene with enzymatically degradable (KCGPQGIAGQCK) and cell adhesive peptides (CGRGDS). These microgels were annealed using PEG-tetra-methyltetrazine (4PEG-mTz), which crosslinked the microgels by inverse electron demand Diels–Alder (iEDDA) click chemistry. These granular hydrogels were characterized by rheology, porosity and enzymatic degradation. Subsequently, to evaluate their utility for NPC delivery in a severe injury model of SCI, testing was performed in a cervical hemisection murine model. Immunostaining analysis performed at 2-weeks post-implantation revealed that the granular hydrogels resulted in enhanced NPC engraftment in injured sites and significantly larger graft volumes compared to NPC delivery in a fibrin matrix, which is the standard delivery material. This work demonstrates that in situ assembled poly (ethylene glycol) (PEG)-based granular hydrogels with 4PEG-mTz annealing linker is a promising scaffold for enhancing the NPC delivery to treat SCI.

Rapid Fire #: 10

Inorganic Nanomaterial-based Expandable Shape-Memory Hemostat for Treatment of Non-Compressible Hemorrhage

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Introduction. Blood loss due to uncontrollable hemorrhage causes around 80% of battlefield casualties and 40% of deaths in civilian settings. Uncontrolled bleeding due to truncal, junctional, and noncompressible hemorrhage can significantly increase the mortality rate from 0.1% to 20%. Due to the inability to perform pressure dressing, mortality related to noncompressible hemorrhage increases by a factor of four compared to other battlefield injuries. Failure to perform rapid patient care and transport to a medical facility within 30 minutes of injury increases morbidity by nearly 50% during the prehospital period (Hickman, 2018). In addition, the lack of biodegradability and inefficiency in administering commercially available hemostats raises the need to develop an alternative strategy for noncompressible hemorrhage.

Materials and Methods. Both shape-memory polyurethane (SMP) foam and shear-thinning nanocomposite hydrogel, combining gelatin (1% w/v) and nanosilicates (2% w/v), were synthesized using previous protocols (Singhal, 2012, Gaharwar, 2014). Following rheological analysis and sterilization, the biocompatibility of the nanocomposites was assessed using human endothelial cells via Alamar Blue assay, and clotting time and amount of blood loss were quantified using a rat-liver laceration model, followed by H&E and PTAH staining.

Results and Discussion. The hydrogels demonstrated shear thinning properties, promoting successful infiltration within SMP foam. No change in FTIR spectra and volume expansion profile indicated no alteration of the elemental properties of the samples after UV sterilization. Approximately 8-fold volume expansion was observed within the first 120 seconds. The SMP foams demonstrated high biocompatibility with an average cell viability greater than 85%. Further, in vivo, clotting time and amount of blood loss of composite were found to be 52.33 ± 4.93 s and 0.47±0.16 g, respectively, which are significantly lower than the negative control with 409±45.31 s and 1.14±0.56 g respectively. H&E and PTAH staining confirmed aggregation of erythrocytes at the injury sites, demonstrating stable blood clot formation. Our future study will include both in vitro and in vivo analysis, with different injury models.

Conclusion. We have developed a highly expandable novel hemostat with remarkable hemostatic ability, demonstrating promising potential to combat noncompressible battlefield injuries.

POSTERS

Poster #: 1

Lipid droplets as substrates for protein phase separation

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Bilayer membranes are critical interfaces for protein phase separation, which drives essential cellular phenomena from immune signaling to membrane traffic. Importantly, by reducing dimensionality from three to two dimensions, lipid bilayers can nucleate phase separation at substantially lower concentrations compared to those required for phase separation in solution. How might other intracellular lipid interfaces, such as lipid droplets, act as an interface for protein phase separation? Distinct from bilayer membranes, lipid droplets consist of a phospholipid monolayer surrounding a core of neutral lipids. Lipid droplets are energy storage organelles that protect cells from lipotoxicity and oxidative stress. As a biomaterial, lipid droplets display a greater density of packing defects compared to a phospholipid bilayer, and thus present a different molecular environment to which proteins can bind and assemble. Here we show that intrinsically disordered proteins can undergo phase separation on the surface of synthetic and cell-derived lipid droplets. Specifically, we find that model disordered domains, FUS LC and LAF1-RGG, separate into protein-rich and protein-depleted phases on the surfaces of lipid droplets. Owing to the hydrophobic nature of interactions between FUS LC proteins, increasing ionic strength drives an increase in its phase separation on droplet surfaces. The opposite is true for LAF1-RGG, owing to the electrostatic nature of its interprotein interactions. In both cases, protein-rich phases on the surfaces of synthetic and cell-derived lipid droplets demonstrate molecular mobility indicative of a liquid-like state. Our results show that lipid droplets can nucleate protein condensates, suggesting that lipid droplets provide an additional cellular interface upon which proteins could undergo phase separation, potentially helping to organize biological processes.

Poster #: 2

Solids and Liquids in Clathrin-Mediated Endocytosis

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Clathrin-mediated endocytosis, the major uptake pathway in eukaryotic cells, is essential for cell signaling and receptor recycling. The invagination of the plasma membrane is orchestrated by an interconnected network of initiator and adaptor proteins that ultimately recruit the clathrin coat. The initiator proteins, Eps15 and Fcho, arrive early at endocytic sites. These proteins catalyze endocytosis by recruiting critical adaptor proteins, yet are not engulfed by the developing coated vesicle. Instead, they remain at the plasma membrane, where they nucleate successive rounds of vesicle assembly. How do the initiator proteins avoid being incorporated into coated vesicles, despite their biochemical interactions with multiple adaptor proteins? Recently, our group and others have shown that endocytic initiator proteins form a liquid-like network, which serves as a flexible catalytic platform for vesicle assembly. Here we explore the role of this liquid network in orchestrating clathrin mediated endocytosis using in vitro assays. In particular we show that clathrin accumulates at the boundary of protein droplets, indicating solid-like clathrin is not miscible in the liquid-like network of adaptor proteins. Furthermore, we demonstrate that this behavior results in clathrin acting as a surfactant which limits the growth of the liquid-like network of endocytic adaptor proteins. This observation suggests a novel and simple explanation for the turnover of initiator proteins in endocytosis and for the uniform size of clathrin coated pits.

Poster #: 3

WITHDRAWN

Poster #: 4

WITHDRAWN

Poster #: 5

PEG Hydrogel/Shape Memory Polymer Foam Composite Biopsy Sealants with Multimodal Contrast

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Lung tissue biopsies can result in a leakage of blood and air from the biopsy tract which can threaten the patient with a collapsed lung. We have previously developed a lung biopsy tract sealant based on a thiol-ene crosslinked poly(ethylene glycol) (PEG) hydrogel and polyurethane-based shape memory polymer foam composite that will swell (hydrogel) and actuate (SMP foam) to seal the biopsy tract. Here, we describe the addition of a Gd chelate, covalently bound within the hydrogel network, and platinum marker bands to the composite for positive MR and CT contrast, respectively. This allows for tracking of the device and tumor post-biopsy. The peptide-based Gd chelate was synthesized via solid-phase peptide synthesis. Prior to characterization, the chelate solution was treated with chelex 100 resin to remove free Gd3+, as determined by a xylenol orange assay. Characterization of bulk PEG hydrogels after incorporation of the Gd chelate revealed that with an increase in concentration of Gd3+, there was a decrease in the swelling and increase in the equilibrium storage modulus due to the increase in crosslink density of the hydrogels. Composites were then fabricated via vacuum infiltration of the PEG hydrogel precursors along with the Gd chelate into the SMP foam followed by thermal curing. Gd3+ leaching testing with a xylenol orange assay showed that the composites did not leach Gd3+ after soaking in buffer for 1 week. In vitro MR imaging in a lung phantom and ex vivo MR imaging in bovine liver showed that lyophilized 0.5 mM Gd3+ composites soaked in PBS for 3 weeks maintained their intensity compared to 0.5 mM Gd3+ composites soaked for 24 hours and 0.5 mM Gd3+ composites soaked after fabrication (no drying) for 3 weeks. This suggests that lyophilization does not affect Gd3+ complexation and there is negligible Gd3+ leaching after 3 weeks. Furthermore, in vivo imaging of composites with platinum marker bands at each end provided intense CT contrast after implantation into a pig. With the addition of the Gd chelate and Pt bands, our biopsy sealant will be able to serve as a fiducial marker while also sealing the biopsy tract.

Poster #: 6

Understanding the Impact of PEG Immunogenicity on the Host Response to Hydrogel Implants

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Polyethylene glycol (PEG)-based hydrogels have attracted notable attention in the biomaterial engineering field. They are often considered as a "blank canvas" in designing hydrogel scaffolds for tissue engineering. They are also used in several FDA-approved medical devices. While this biomaterial has been widely regarded as biologically inert, concerns over PEG's immunogenicity have emerged in recent years, with an estimate of about 20-30% of the population possessing antibodies against PEG due to the exposure to drugs, cosmetics, and other PEG-containing commercial products. While an anti-PEG immune response has been found to reduce the efficacy of intravenously administered PEGylated drugs, the impact on the biocompatibility of PEG hydrogels has not been previously studied and is critically unexplored. In this study, the objective is to characterize the foreign body response (FBR) of PEG-based hydrogels via comprehensive in vivo testing to address the current knowledge gap. In the first phase, which included synthesizing and characterizing PEG-diacrylate (PEG-DA) conjugated to cell-adhesive motif "RGD", various concentrations of PEG-DA were assessed to determine three stiffness points as soft, intermediate, and stiff. Then, based on the obtained results, gel implants were produced to be placed subcutaneously in C57BL/6 mice, that had been sensitized against PEG through a series of PEG-KLH injections. Mice that had received saline injections were used as a control. Finally, these hydrogel implants were collected at three different time points: 1-week, 5-week, and 12-week. The collected samples were processed for histology (H&E, Masson's Trichrome) and evaluated by a pathologist. The findings thus far indicate that stiffness impacted the severity of the host response. In addition, while all groups exhibited a moderate inflammatory response at the 1-week time point, PEG-sensitized female mice had lower inflammation scores compared to their naive counterparts at 5 weeks, whereas PEG-sensitized males had higher inflammation scores at 5 weeks. These results suggest a sex-dependent difference in the impact of PEG sensitization on the resolution of inflammation. However, the immune response appeared to be resolving at the 5-week time point onward, and no significant differences between PEG-sensitized and control mice were apparent at the 12-week time point.

Poster #: 7

WITHDRAWN

Poster #: 8

Composite-Coated Endotracheal Tubes for Restoring Vocal Fold Function After Traumatic Intubation Injury

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Background and purpose: Endotracheal intubation is a common procedure, however, it often leads to laryngeal injury and complications including dysphonia, dysphagia, and difficulty breathing. If left untreated, these injuries can develop into glottic or subglottic stenosis which requires surgical intervention to improve airway patency. Therapeutic strategies in the acute phase of laryngeal wound healing could reduce fibrosis severity and allow the retention of vocal fold function. In this study, we investigated the efficacy of a dual drug eluting endotracheal tube for localized delivery of corticosteroids and small interfering RNA (siRNA) targeting smad3 for immediate reduction in pro-fibrotic transforming growth factor-beta 1 (TGFβ1).

Methods: Traumatic intubation was simulated in 9 Yorkshire crossbreed swine under direct laryngoscopy. Endotracheal tubes were first coated with dexamethasone-loaded polycaprolactone (PCL) fibers via electrospinning and then embedded in a four-arm polyethylene glycol acrylate matrix containing smad3 silencing siRNA. Composite-coated ETTs and uncoated regular ETTs were placed in the injured airway for 3, 7, or 14 days (n= 3 per timepoint). The local stiffness properties of the airway with injury and ETT placement were evaluated with normal indentation testing and assessed histologically by Masson's Trichrome.

Results and Conclusions: Local stiffness outcomes (mean ± std. error) at 3 days were significantly greater with composite coated ETT placement (22.3 ± 0.692 N/m) than with the uncoated ETT placement (17.0 ± 0.757 N/m, $p < 0.0001$). At 7 and 14 days, tissue with uncoated ETT placement had significantly greater local stiffness than the composite coated ETTs ($p < 0.0001$ and $p = 0.001$, respectively). Histological assessment of collagen in vocal fold sections with injury and coated ETT placement indicated a decreased percentage area of collagen over time. Increased stiffness outcomes associated with fibrotic tissue formation impede vocal fold function, therefore, the goal of the study was to restore mechanical outcomes close to native properties by reducing inflammation and downregulating the TGF-β1 pathway. Our results indicated that dual delivery of dexamethasone and siRNA targeting smad3 reduced vocal fold stiffness over 14 days of intubation compared to uncoated ETTs.

Poster #: 9

Integrin Targeting Multilayer Vascular Grafts for Sustained Thromboresistance

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Despite success in large diameter peripheral artery diseases, synthetic grafts are currently not used in small caliber applications due to high failure rates from thrombosis and intimal hyperplasia. To address both failure mechanisms, our lab has designed a multilayer graft consisting of a luminal hydrogel coating that promotes post-implantation endothelialization and an electrospun polyurethane mesh that matches arterial mechanical properties. Damage-resistant hydrogels were developed using a double network design with a long-chain polyether urethane diacrylamide (PEUDAm) as the first network and N-acryloyl glycinamide (NAGA) as the second network with enhanced hydrogen bonding to increase fracture resistance. In this study, we utilized redox-mediated initiation to apply conformal coatings of these new durable hydrogels to our electrospun grafts with high compliance. Bioactivity was conferred to these hydrogel coatings through incorporation of streptococcal collagen-like (Scl2-2) proteins modified with α1β1 and α2β1 binding sites to promote selective cell interactions for post-implantation endothelialization while maintaining acute thromboresistance. Surface stiffness assessments on hydrogel coatings were performed via nanoindentation to assess network formation and ensure the durable hydrogel coatings have appropriate stiffnesses to promote cell adhesion. Hydrogel coating stiffness was within similar moduli ranges as that of the basal lamina. Antifouling and acute thromboresistant properties of the hydrogels was confirmed as evidenced by decreased protein adsorption and minimal platelet adhesion, respectively. Sustained thromboresistance was evaluated by assessing the effect of Scl2 protein concentration on promoting endothelialization. It was shown that higher concentrations of protein led to increases in cell adhesion. Future work will explore the effect of sex and age on endothelial cell-material interactions to better inform the design of our integrin-targeting platform.

Poster #: 10

Model-directed Design of a High Compliance Vascular Graft with Improved Kink Resistance

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Nearly 400,000 coronary artery bypass grafting procedures are performed annually. Synthetic grafts are needed for 20% of patients that do not have a suitable autograft. To address the high failure rate of synthetic grafts, our lab developed an electrospun graft with improved compliance matching to address intimal hyperplasia-based failure. Although these grafts showed reduced markers for intimal hyperplasia in an ex vivo model, the grafts were prone to kink-based failures in vivo. To impart kink resistance, a polymeric coil was added. Although the coil improved the kink-radius from 11.3 ± 1.2 mm to 4.0 ± 0.9 mm, compliance was reduced from 8.1 ± 0.4x10-2 %/mmHg to 3.7 ± 0.6x10-2 %/mmHg demonstrating an inverse relationship. We hypothesize that tuning the coil thickness, spacing, and modulus can be utilized to balance the two properties. However, iteratively exploring the >4000 combinations would be prohibitively time-consuming. A model-directed approach allows for in silico testing to rapidly explore the design space for identification of optimized parameters. Two finite element models were developed in FEBio using an axisymmetric cylinder with helically wrapped coil reinforcement to analyze the impacts of the coil design on compliance and bend-based kinks. The models were validated using control grafts, grafts with a coil spacing of 4.6 mm and a thickness of 0.14 mm (CS1), and grafts with spacing of 2.5 mm and a thickness of 0.35 mm (CS2). Experimental compliance was within 10% compared to the model (control: experimental = 8.1 ± 0.4x10-2 %/mmHg, model = 8.0x10-2 %/mmHg; CS1: experimental = 6.2 ± 0.5x10-2 %/mmHg, model = 6.4x10-2 %/mmHg; CS2: experimental = 3.7 ± 0.6x10-2 %/mmHg, model = 3.4x10-2 %/mmHg). The bend-kink trends were similar between the model and experimental (control: experimental = 11.3 ± 1.2 mm, model = 9.9 mm; CS 1: experimental = 8.9 ± 1.7 mm, model = 7.8 mm; CS2: experimental = 4.0 ± 0.9 mm, model = 7.1 mm). Future work will utilize an optimization equation that compares model predictions to compliance and bend kink targets with the ability to weight each property based on the location of implantation for rapid identification of candidate graft parameters.

Poster #: 11

Interpenetrating Network Design to Increase Damage-Resistant Hydrogel Coatings for Cardiovascular Devices

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Poly(ethylene glycol) (PEG)--based hydrogel coatings have the potential to improve the thromboresistance of cardiovascular devices due to their innate antifouling properties. However, PEG diacrylate hydrogels are relatively brittle with low extensibility which makes them prone to damage during surgical implantation. When used as vascular grafts or heart valves, suturing damage can generate particles that can cause embolism and stroke. Therefore, our lab has developed a copolymer-interpenetrating network (IPN) by combining polyether urethane diacrylamide (PEUDAm) and N-acryloyl glycinamide (NAGA) to create a tough, damage-resistant hydrogel designed to mitigate multiple surgical damage mechanisms. The first network is comprised of a copolymer with PEUDAm that provides high extensibility for damage resistance and NAGA to incorporate sacrificial hydrogen bonds to increase fracture energy dissipation. The second network consists of a NAGA network that further enhances damage resistance. To assess the impact of introducing and increasing the concentration of sacrificial hydrogen bonds in the first network on suture damage resistance, we quantified particulate generation following suture passaging through hydrogels with different first-network NAGA concentrations. Suture damage assessments showed decreased generation of particulates with increasing concentrations of NAGA in the first network. Torque damage was also mitigated on all substrates regardless of hydrogel chemistry. Assessments of tension until fracture exhibited higher moduli with increasing concentrations of NAGA in the first network while maintaining high extensibilities for all networks. Tensile testing of the IPN also demonstrated that the moduli of each gel composition fall within the range of the requisite stiffness needed to promote endothelial cell attachment and migration. Collectively, these studies highlight the potential of this new tough hydrogel coating for cardiovascular devices.

Poster #: 12

Fatigue Testing of Hydrogel Electrodes for the Treatment of Ventricular Arrhythmia

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Ventricular arrhythmia (VA) is the leading cause of sudden death in the United States. VA results from abnormal conduction velocities in scarred and/or diseased myocardium and can lead to re-entrant currents. Our lab has designed an injectable hydrogel electrode that can be used in conjunction with a standard pacemaker to increase stimulation area across scarred tissue of the myocardium to permit painless defibrillation. Ionic hydrogels based on polyether urethane diacrylamide (PEUDAm) demonstrated active pacing in a porcine model. For long-term utility, the hydrogel electrode must demonstrate fatigue resistance and sustained conductivity. To characterize fatigue resistance, a custom tester apparatus was developed to test hydrogels under physiological conditions. As an initial indication of fatigue resistance, the hydrogel specimens maintained integrity for 500,000 cycles. Material properties were evaluated before and after fatigue testing, including swelling ratio and modulus, as a measure of crosslinking density. Changes in specimen dimensions were measured and tensile tests were also performed before and after fatigue testing. No change in sample length after cycling indicated that the hydrogel had minimal to no plastic deformation post-cycling. Similarly, no statistical change in swelling ratio, tensile strength, and modulus before and after fatigue testing indicates that this hydrogel is mechanically stable and fatigue resistant. Future studies will accelerate fatigue testing to predict longer term mechanical stability (~10 patient years).

Poster #: 13

Chemically Modified Rhamnan Sulfate Compounds as Therapeutics for Inflammation and Non-Alcoholic Fatty Liver Disease (NAFLD)

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Non-alcoholic fatty liver disease (NAFLD) is a progressive type of fatty liver disease characterized by fat accumulation in hepatic tissue that is not linked to excessive alcohol consumption. The disease encompasses early stages of non-alcoholic fatty liver (NAFL) to more severe stages of non-alcoholic steatohepatitis (NASH).8 It is currently the most common form of chronic liver disease worldwide, with a prevalence of approximately 25% in the general population (~1.8 billion people worldwide). While the fundamental mechanisms of NAFLD remain an area of active research, the current understanding of the disease suggest that increased de novo lipogenesis is the primary source of the lipid build up in NAFLD. The long term presence of increased lipids in the liver lead to lipotoxicity, chronic inflammation, fibrosis, cell death, and in severe cases, increased risk of hepatocellular carcinoma. There are currently no clinically approved therapies for NAFLD. Our group recently completed studies demonstrating that a marine polysaccharide, rhamnan sulfate (RS), can reduce atherosclerosis in mice when delivered as an oral therapeutic and had potent anti-inflammatory activity. An unexpected and intriguing result from these studies was that RS had a strong effect in reducing NAFLD in the livers of female mice, including a reduction in lipid accumulation, inflammation and NAS liver morphology.

To enhance the activity of RS for treating inflammation and NAFLD, we used chemical synthesis to create a library of chemically modified RS compounds. To identify modifications that improve efficacy for treating NAFLD, we screened the derivatives for in vitro activity in reducing steatosis in Hep2G cells and inflammation in endothelial cells and Hep2G cells. From these screens, we identified compounds that had increased anti-inflammatory and anti-steatosis activity compared to native RS. We performed further studies on the lead compounds and demonstrated that they reduced endothelial cell expression of inflammatory markers following stimulation with TNFα. In addition, the compounds blocked functional inflammatory activity and reduced monocyte adhesion to inflamed endothelial cells and prevented shedding of cell surface proteoglycans. Overall, our work supports that chemically modified forms of RS may be promising therapeutics for the inhibition of inflammation and lipogenesis in NAFLD.

Poster #: 14

Introduction of Corrugation to Electrospun Vascular Grafts Improves Kink Resistance

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Coronary artery bypass grafting is performed on nearly 400,000 patients annually in the United States. Autografts are the gold standard for treatment but are unavailable in 20% of patients due to multiple bypass surgeries or patients with peripheral artery disease. Synthetic grafts made of ePTFE and Dacron demonstrate unsatisfactory outcomes in small diameter applications, with 40% of grafts failing within five years, a significant portion of which is attributed to intimal hyperplasia. Intimal hyperplasia is attributed to a mismatch in compliance. We previously developed an electrospun mesh with compliance matching to the native vascular that showed promising results in in vivo and ex vivo models for mitigating the progression of the adverse tissue response. Unfortunately, the grafts failed when implanted in vivo due to graft kinking. Corrugation has been utilized in clinically used grafts to impart kink-resistance. We thus developed methods to fabricate corrugated vascular grafts. Corrugation was imparted on the grafts utilizing post-fabrication molding. After removal of the spun neat graft from the mandrel, grafts were slipped over SLA printed corrugated molds, onto which a monofilament was wrapped over the graft and mold to anneal and set shape. Kink resistance testing was conducted by bending grafts around templates of varying radii till a 50% reduction in graft diameter appeared. Results of kink resistance testing indicated that corrugated grafts had a kinking radius of 5 ± 1 mm, nearly half of the control graft kinking radius of 10 ± 1 mm. Compliance testing of the corrugated vascular grafts indicated a reduction in compliance to 5.5 ± 0.7 %/mmHg × 10-2 compared to the control grafts at 8.5 ± 0.4 %/mmHg × 10-2 demonstrating an inverse relationship between kink-resistance and compliance. Although the corrugated graft exceeds compliance of veinous autografts such as the great saphenous vein, it falls below target compliance matching. We hypothesize that changes to the corrugation geometry, such as amplitude and spacing, can be utilized to balance kink resistance and compliance to achieve desired targets. Future work will utilize changes in geometry and model-directed fabrication to accelerate and optimize corrugation design.

Poster #: 15

WITHDRAWN

Poster #: 16

HYACINTH: A HYdrogel-based, Adhesive, Contraction-INductive, Therapeutic Hemostatic Material for Postpartum Hemorrhage

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Introduction: Postpartum hemorrhage (PPH) is a life-threatening childbirth complication affecting up to 6% of deliveries annually and is the leading cause of maternal morbidity and mortality (Evensen 2017). Further, a 2016 Government Accountability Office report noted that the risk of PPH is higher in military versus civilian populations (GAO-16-596). The most common cause is uterine atony, wherein the uterus cannot contract against bleeding vessels (Wormer 2022). Oxytocin is the gold standard treatment (Escobar 2022); however, prolonged exposure desensitizes the uterus (Vrachnis 2011). Our previous nanocomposite hydrogel promotes blood clotting (Gaharwar 2014), but postpartum physiology poses challenges of high blood flow. We aim to augment this hydrogel with wet-tissue adhesion and uterotonic drug release to develop a HYdrogel-based, Adhesive, Contraction-INductive, Therapeutic Hemostatic material ("HYACINTH").

Materials and Methods: HYACINTH is fabricated by modifying our previous gelatin-nanosilicate hydrogel (Gaharwar 2014). Conjugation of catechol-containing dopamine to gelatin lends wet tissue adhesivity. Adhesion is assessed in burst strength, lap shear, and submerged adhesion testing. Nanosilicates are loaded with uterotonic misoprostol (Gibbins 2013) and the release profile is quantified. Injectability is probed via rheology and mechanical testing. Hemostatic assays confirm procoagulant activity. Uterotonic ability will be studied in vitro with myometrial cells and tissue strips and further evaluated in a rat model.

Results: Preliminary results show successful dopamine addition and dry adhesion. Injectability is maintained with power law indices less than 1 to indicate a shear-thinning fluid and forces less than 30 N for rates up to 20 mL/min. HYACINTH can also fill irregular cavities. A model drug (alizarin red) demonstrates sustained release over 72 hours. Clotting time is consistent between HYACINTH and the unmodified hydrogel. Future results will validate HYACINTH under wet conditions, with a burst strength of at least 200 mmHg to withstand physiological blood pressure.

Conclusions: HYACINTH poses strong potential as a multimodal material by addressing both the symptoms (i.e., hemorrhage) and underlying cause (i.e., uterine atony) of PPH. Further work will optimize adhesion with hemostasis and drug release. Ultimately, we aim to develop a material to reduce both immediate mortality and long-term morbidities for civilian and military mothers.

Poster #: 17

In Vitro and Ex Vivo Assessment of a Hemostatic Hydrogel for Postpartum Hemorrhage

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The US has one of the highest maternal mortality rates among developed countries with approximately 11% of those deaths being attributed to postpartum hemorrhage (PPH). Currently, PPH is treated according to its severity, with extreme cases involving invasive procedures that can cause long-term damage or removal of the uterus entirely. As such, there is a need to evaluate novel biomaterials for minimally-invasive treatment, especially using in vitro and ex vivo testing. In this project, we aim to evaluate a hemostatic hydrogel through both a series of in vitro hemostatic assessments and a novel ex vivo model of a postpartum uterus. The hemostatic hydrogel under evaluation is composed of nanosilicate and dopamine-conjugated gelatin.

In our in vitro assessments, we are exploring clotting times across varying concentrations of each component through an inversion test. The clot strength will be determined through rheology and thromboelastography. We will examine the samples with SEM imaging to qualitatively describe the platelet activation and red blood cell adhesion.
These in vitro characteristics will be further tested in an ex vivo model which considers the anatomy, physiology, and physicochemical makeup of the uterus. Our design criteria specify that the model must be transparent and must have a surface that does not contain any components of the hemostatic hydrogel. Further, we desire the ability to incorporate a network to simulate an appropriate blood pressure and flow rate.

So far, we have observed that the hydrogels composed of 15% gelatin and a range of 4% to 15% nanosilicate were statistically equivalent to a clinical control and demonstrated a significant improvement over the negative control. These preliminary results help inform the material choices for our ex vivo model. With this model, we have selected materials capable of producing a complex geometry. Further development of this model will include adding external pressure and the ability to produce a hemorrhagic environment. As we develop the model, we will rely on the in vitro hemostatic assessments to gauge the efficacy of the model. These studies will contribute to the overall evaluation of and optimization of the hemostatic hydrogel into an effective treatment for PPH.

Poster #: 18

Multicenter Trial of Chlorinated Polyethylene Elastomer (CPE) for Maxillofacial Prosthetics

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A randomized, controlled, phase III, double-blind, single crossover clinical trial at the University of Louisville School of Dentistry, M.D. Anderson Cancer Center, University of Texas School of Dentistry Houston, and Toronto Sunnybrook Regional Cancer Centre was conducted of thermoplastic chlorinated polyethylene (CPE) extraoral maxillofacial prosthetic material, originally developed at Gulf South Research Institute in New Orleans, compared to medical-grade silicone rubber. Of 28 patients completed, the two materials were chosen equally by the 9 first-time users, but silicone was preferred by previous users of the silicone material. Future studies must use patients with no previous experience in test materials. Each prosthesis of ear, nose, or orbit took a week or more to fabricate by anaplastologists in 2- or 3-part gypsum molds. Eighteen inclusion and exclusion criteria were required. Detailed questionnaires and blinding conditions were effective. A quality-of-life study of the Toronto Outcomes Measure of Craniofacial Prosthetics was also included in the trial design. Supported by 5-U01 DE014543 from the NIDCR, NIH.

Poster #: 19

Multicenter Trial of Chlorinated Polyethylene Elastomer (CPE) for Maxillofacial Prosthetics

Lawrence Gettleman, University of Louisville, School of Dentistry

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A randomized, controlled, phase III, double-blind, single crossover clinical trial at the University of Louisville School of Dentistry, M.D. Anderson Cancer Center, University of Texas School of Dentistry Houston, and Toronto Sunnybrook Regional Cancer Centre was conducted of thermoplastic chlorinated polyethylene (CPE) extraoral maxillofacial prosthetic material, originally developed at Gulf South Research Institute in New Orleans, compared to medical-grade silicone rubber. Of 28 patients completed, the two materials were chosen equally by the 9 first-time users, but silicone was preferred by previous users of the silicone material. Future studies must use patients with no previous experience in test materials. Each prosthesis of ear, nose, or orbit took a week or more to fabricate by anaplastologists in 2- or 3-part gypsum molds. Eighteen inclusion and exclusion criteria were required. Detailed questionnaires and blinding conditions were effective. A quality-of-life study of the Toronto Outcomes Measure of Craniofacial Prosthetics was also included in the trial design. Supported by 5-U01 DE014543 from the NIDCR, NIH.

Poster #: 20

Biodegradable Microparticles Exhibiting Pulsatile Release for Single-Injection Vaccination

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Despite the proven utility of vaccines for combating infectious diseases, there are still 1.5 million vaccine-preventable deaths each year, predominantly in low- and middle-income countries (LMICs). Two major contributors to these deaths are low patient accessibility and the cost of vaccine distribution, which are exacerbated by the need for multiple injections administered over time. Therefore, a single-injection vaccine that confers immunity after one administration could improve vaccine access in LMICs and greatly reduce the number of annual vaccine-preventable deaths. Studies have shown that leveraging antigen exposure kinetics to exponentially increase doses over the priming period can result in a more robust immune response by better mimicking the body's response to native infection. In this study, we developed a vaccine delivery system capable of simulating the antigen kinetics of a native infection to enhance the immune response, potentially leading to the creation of a single-administration vaccine.

Many vaccine delivery approaches utilize biodegradable microparticles, composed of polymers such as poly(lactic-co-glycolic acid) (PLGA), to encapsulate antigens. However, these particles typically exhibit release kinetics that decrease over time, the direct opposite of native infections. We developed a process called Particles Uniformly Liquified and Sealed to Encapsulate Drug (PULSED), a microfabrication method that yields PLGA particles that release encapsulated antigen in a pulse after a period of time that is controlled by the polymer properties. Altering the composition and properties of the PLGA such as blending PLGAs with different molecular weights, adding porogens, or adding excipients, allows the release time to be fine-tuned to a desired timeline. Using these methods, we have established microparticle formulations that release their payload around hour 8, day 3, day 6, day 8, and day 14. By combining multiple microparticle populations with different delays in release time, we can generate release profiles that mimic the antigen kinetics of a native infection in a single injection. The ultimate goal with this technology is to decrease the number of vaccine preventable deaths each year by lowering the barriers to immunization in LMICs.

Poster #: 21

Near-Infrared Light-Responsive Hydrogel for on-demand Dual Delivery of Proangiogenic Growth Factors

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Achieving precise spatiotemporal control over proangiogenic factors release is crucial for vasculogenesis, the process of de novo blood vessel formation. Although various strategies have been explored, there is still a need to develop cell-laden biomaterials with finely controlled release of proangiogenic factors at specific locations and time points. Here, we have developed a near-infrared (NIR) light-responsive collagen hydrogel comprised of gold nanorods (GNRs)-conjugated liposomes containing proangiogenic growth factors (GFs). We demonstrated that this system enables on-demand dual delivery of GFs at specific sites and over selected time intervals. Liposomes were strategically formulated to encapsulate either platelet-derived growth factor (PDGF) or vascular endothelial growth factor (VEGF), each conjugated to gold nanorods (GNRs) with distinct geometries and surface plasmon resonances at 710 nm (GNR710) and 1064 nm (GNR1064), respectively. Using near infrared (NIR) irradiation and two-photon (2P) luminescence imaging, we successfully demonstrated the independent release of PDGF (107 pg/ml/% of scanned area) from GNR710 conjugated liposomes and VEGF (219 pg/ml/% of scanned area) from GNR1064-conjugated liposomes. Our imaging data revealed rapid release kinetics, with localized PDGF released in approximately 4 minutes and VEGF in just 1 and a half minutes following NIR laser irradiation. Importantly, we demonstrated that the release of each GF could be independently triggered using NIR irradiation with the other GF formulation remaining retained within the liposomes. This light-responsive collagen hydrogel holds promise for various applications in regenerative medicine where the establishment of a guided vascular network is essential for the survival and integration of engineered tissues.

Poster #: 22

Controlled Release of Small Molecules and Biologics from a Supramolecular Peptide Hydrogel Enabled with Boronic Acid Dynamic Covalent Chemistry

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Many drugs require frequent administration to reach and maintain therapeutic levels, which can contribute to poor patient adherence and reduced treatment efficacy. Administering larger doses can prolong efficacy, but this is not an effective strategy for drugs with narrow therapeutic windows. Controlled release systems, such as biodegradable microparticles, osmotic pumps, and transdermal patches, have been developed to help reduce dosing frequency and improve patient outcomes. These systems must overcome several challenges, including a high initial burst release, a low drug loading capacity, favorable device biocompatibility, and payload stability. They would also be more clinically appealing if the manufacturing were low cost, scalable, and administration is minimally invasive. Supramolecular peptide hydrogels are a promising class of biomaterials that have been evaluated in preclinical studies and clinical trials. Multidomain peptides (MDPs) are a class of hydrogel-forming peptides composed of natural amino acids that offer favorable biocompatibility and self-assemble into β-sheet-rich nanofibers. MDP hydrogels are shear-thinning and self-healing allowing for their minimally invasive administration through small-bore needles. Minimal changes to the sequence pattern can result in hydrogels with diverse chemical and biological properties, allowing the material to be fine-tuned for each application. However, the duration of drug release from MDP hydrogels is often limited by rapid diffusion. So far, we have overcome this challenge by developing MDPs with N-terminal modifications to enable dynamic covalent bonding with boronic acids (BAs) to enhance drug retention. We demonstrated that this self-assembling boronate ester release (SABER) hydrogel platform is compatible with four clinically relevant BA-containing drugs in vitro and used two of these drugs, bortezomib and GSK656, to demonstrate enhanced in vivo release kinetics. We further demonstrated the ability of SABER peptides to deliver phenylboronic acid (PBA)-modified insulin, which resulted in normoglycemia for 6 days in one injection. Inspired by the macromolecular delivery, we aimed to further extend the release with biologics by improving the boronate ester equilibrium. We extensively screened BA derivatives and identified several compounds with substantially improved equilibria, up 4.5-fold increase, to further prolong release, which could extend glycemic correction in diabetic mice or be used in other applications requiring long-term treatment.

Poster #: 23

Delivery of Misoprostol via Lipid Coated Mesoporous Silica Nanoparticles for the regulation of BNIP3/L Mitophagy in Fat Cells during Cancer Cachexia

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Cancer-associated cachexia (CAC) is a multifactorial metabolic syndrome characterized by systemic inflammation and ongoing loss of fat and muscle mass that cannot be reversed by nutritional supplementation. It is common in advanced cancers, associated with poor treatment response and reduced quality of life, and ultimately, is implicated in approximately 30% of cancer deaths. White adipose tissue (WAT) is one of the first organs to be affected by cancer cachexia and preserving WAT protect muscle in murine cachexia models. Despite the clinical relevance, the cellular mechanisms behind tissue loss in cancer cachexia remain unclear. Mitophagy, a vital cellular process, regulates mitochondrial quantity by eliminating dysfunctional or surplus mitochondria through autophagy machinery. Excessive mitophagy in WAT can lead to increased lipolysis, inflammation, and subsequent fat waste. Mitophagy receptors, BNIP3 and BNIP3L/Nix, play crucial roles in this process by recognizing autophagosomes. We hypothesize that hypoxia/pseudohypoxia-driven BNIP3/L-mediated mitophagy is increased in fat cells exposed to cachexia inducing cancer conditioned media, and that this can be reversed by treatment with mitophagy inhibitors encapsulated in lipid coated mesoporous silica nanoparticles (LCMSN). We used human adipose stem cells (hASC) and exposed them to B16F10 cancer conditioned media (CCM) for 48 h. For treatment, we used misoprostol (Known BNIP3 inhibitor, primarily used in the prevention of stomach ulcers from NSAIDs) alone or encapsulated within LCMSN for 24 h. hASC exposed to CCM exhibited a significantly higher expression of BNIP3/L measured by RT-qPCR, in comparison to untreated hASC. Misoprostol treatment alone and delivered with LCMSN, reversed the increase of both BNIP3/L levels observed in cells exposed to CCM. BNIP3 protein levels in treated and untreated groups were confirmed by immunohistochemistry. Finally, confocal images of mitochondria stained with MitoTracker Red showed more fragmented mitochondria in ASC exposed to CM compared to control and Misoprostol treated cells. This data suggests that the delivery of Misoprostol via LCMSN for targeted delivery to adipose tissue cells, modulate mitophagy and could be effective in the prevention or treatment of cancer-associated cachexia.

Poster #: 24

Design of a Self-assembled Polymeric Oral Delivery Platform for Therapeutic Proteins

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Autoimmune diseases are characterized by the failure of an organism to tolerate its own cells and tissues which can lead to inflammation and tissue dysfunction. One of the most relevant therapies to treat these diseases are monoclonal antibodies. However, because of their high molecular weight, low bioavailability, and susceptibility to degradation, their administration is limited to parenteral routes. Out of the numerous alternative administration routes that have been proposed, oral delivery offers the most advantages regarding costs, accessibility, and reduced discomfort, which can increase patient compliance. Nevertheless, the oral route has significant biological and physicochemical challenges such as the presence of proteolytic enzymes, drastic changes in pH, as well as the mucus and the epithelial cell layer. All these challenges have to be overcome to successfully deliver monoclonal antibodies across the intestinal epithelium into the bloodstream. One approach to protect them from these barriers is to use polymeric nanocarriers based on block copolymers. Thus, in the present work we developed pH-responsive self-assembled polymersomes based on block copolymers to deliver monoclonal antibodies orally.

Diblock copolymers were synthesized using reversible addition–fragmentation chain-transfer polymerization and carbodiimide-mediated coupling reactions. Poly(methacrylic acid) (PMAA) and poly(ethylene glycol) (PEG) were selected due to their pH-responsive properties and stealth abilities, respectively. Different degrees of polymerization were examined to study the effect of the hydrophilic weight fraction on the micellar polymorphism. Analysis by Fourier-transform infrared spectroscopy and 1H nuclear magnetic resonance spectroscopy confirmed the polymerization of PMAA and the successful addition of the PEG chain. Since PMAA has a low pKa, strategies to improve the stability of the system at higher pH values have been implemented. The pKa of the system was increased by copolymerizing PMAA with hydrophobic comonomers, thus enhancing its suitability for oral delivery applications. In future studies, the loading and release capacity of the self-assembled systems will be studied as well as their cytocompatibility with Caco-2 cells. By combining the material design of self-assembled nanocarriers with monoclonal antibodies and their successful delivery through the oral route, the proposed work has the potential to impact the scientific community and the lives of many patients.

Poster #: 25

Synthesis and Characterization of PEG-Chitosan Hybrid Hydrogels with Polymerized Gentamicin

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Bacterial infections are one of the most common post-operative complications of surgical procedures due to the creation of a semi-closed wound that requires intensive care to limit infection. Despite these efforts, many patients will still develop infections that will delay wound healing and recovery. To address this problem, the objective of this project is to develop hydrogel systems functionalized with antibacterial moieties. Chitosan, a chitin-derived amino polysaccharide was chosen for hydrogel synthesis due to its inherent antibacterial properties and was functionalized with norbornene groups (Chit-NB) to permit crosslinking with 4-arm PEG-thiol (PEG-SH) via thiol-ene click chemistry. Chitosan was reacted with carbic anhydride to modify the primary amine in chitosan and create amide linkages for the norbornene groups. Further modification of chitosan can also be achieved in this reaction through the esterification of hydroxyl groups, creating ester-linked norbornene groups. Additionally, a polymerized form of gentamicin (Gent-MBA) was synthesized by reacting gentamicin with N, N'-Methylenebisacrylamide (MBA) through an Aza-Michael addition reaction and is being investigated to incorporate into the hydrogel network. This reaction takes place with a 2:1 molar ratio of MBA to gentamicin, resulting in a hyperbranched polymer morphology. Our Chit-NB and Gent-MBA functionalization were characterized and confirmed by nuclear magnetic resonance. The antibacterial activity of Gent-MBA was characterized by incubation in bacterial cultures of E. coli to determine the zone of inhibition. Future work will integrate these two components into a hybrid hydrogel network. Hydrogels will be characterized through rheology and swelling ratio experiments, and drug release experiments will evaluate the delivery of gentamicin. The sustained release of our polymerized gentamicin, coupled with our inclusion of chitosan, is expected to provide sustained antibiotic properties that can be leveraged to prevent bacterial infection in a variety of applications.

Poster #: 26

Electrospraying Methods to Modulate Antimicrobial Release from Polymeric Microspheres

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Chronic wounds impact over 50 million patients worldwide with an increasing prevalence as a result of an aging population and rising rates of diabetes. Two significant challenges in treating chronic wounds are maintaining proper moisture balance and controlling infection. We previously developed a polyethylene glycol-based hydrogel foam through a manual foaming procedure that enables moisture balance to address both dry and exudative wounds. However, chronic wounds are frequently impacted by infection that induces persistent inflammatory responses, slow wound healing, and poses a threat of limb amputation. Current treatments for chronic wound include antibiotics and antimicrobial dressings, but face issues like antibiotic resistance and patient sensitivities to antimicrobial agents. Recent studies have highlighted Gallium maltolate (GaM) as a new effective antimicrobial agent. However, the high burst release of GaM via direct incorporation into the hydrogel network would necessitate frequent dressing changes, leading to higher treatment costs and patient impact. To address this need, we developed GaM-loaded poly(lactic-co-glycolic acid) microspheres for sustained infection control. Microspheres were fabricated using electrospraying with varying GaM loading ratios (15%, 25%, 37.5%). Microsphere diameter was altered by modulating electrospraying parameters including solution viscosity, needle gauge, voltage, and flow rate to further tune GaM release. An automated MATLAB algorithm was developed to characterize microsphere sizes distributions. Microspheres were fabricated to 2.30 ± 0.68 µm (~2µm), 4.24 ± 1.33 µm (~4µm), and 5.84 ± 1.67 µm (~6µm). GaM release was characterized using a transmembrane release system following incorporation of the microspheres into the hydrogel foam. For a 25% GaM loading, foams with the 2µm, 4µm, and 6µm microspheres had first-day GaM burst releases of 55%, 40%, and 25%, respectively. For different GaM-loading, the 37.5% GaM loading had a 60% burst release within 24 hours and a 90% release over 5 days, whereas lower loadings had smaller burst releases and more prolonged releases. Thus, larger microsphere size and lower GaM loading resulted in more sustained GaM release profiles for sustained infection control. Future work will evaluate the efficacy of the hydrogel foam system with loaded GaM microspheres in an infected equine lower extremity wound model.

Poster #: 27

Efficacy of Gallium Maltolate in Treatment of Chronic Wound Infections

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Chronic wounds, an increasingly prevalent problem, are hindered by conditions like diabetes and peripheral vascular disease, with bacterial infections posing a significant challenge to healing. Current treatments that rely on topical antibiotics or antimicrobial agents like silver or iodine face limitations due to antibiotic resistance and toxicity concerns. Gallium maltolate (GaM) has emerged as a promising antimicrobial agent, but its applications in wound dressings remain largely unexplored. Our lab has developed a hydrogel foam-based wound dressing with GaM-loaded microspheres for sustained wound infection management. In this study, the antibacterial activity of GaM was evaluated. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) was determined using a microdilution assay of both methicillin-susceptible Staphylococcus aureus (MSSA) and methicillin-resistant Staphylococcus aureus (MRSA). The effect of GaM on native wound cells were studied on human dermal fibroblasts (hDFs) with a cell viability assay. The identified half-maximal inhibitory concentration (IC50) was then used to calculate the selectivity index (IC50/MIC). The antimicrobial activity of GaM was compared to two commonly used antibiotics, gentamicin sulfate and vancomycin hydrochloride. GaM exhibited higher MIC and MBC than the antibiotics for MSSA; whereas, gentamicin displayed a higher MIC against MRSA compared to GaM. Assessment of hDF viability following treatment with GaM, vancomycin, and gentamicin was used to identify the selectivity index. No significant adverse effects of GaM on dermal fibroblasts were shown at MIC, showing an acceptable selectivity index. Overall, GaM demonstrates potent antimicrobial activity against bacterial infections with a clinically-relevant selectivity index. Further investigation will include a comprehensive assessment of bacterial resistance against GaM to evaluate its long-term in vivo effectiveness.

Poster #: 28

WITHDRAWN

Poster #: 29

Development of UV-Crosslinkable Surface-Eroding Polymers for the Controlled Release of Immune Checkpoint Inhibitors

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Immunotherapy has recently emerged as a prominent approach to cancer treatment, showcasing notable clinical successes. However, it still faces challenges such as its ineffectiveness against certain tumor and the risk of serious immune-related adverse events (irAEs) due to systemic administration. While local administration could be therapeutically beneficial, intratumoral injections are typically invasive and often require repeated dosing.

This project aims to develop a novel application of a UV-crosslinkable surface-eroding polymer to overcome two key issues in cancer immunotherapy: the lack of efficacy and the high off-target toxicity associated with systemic administration. This approach involves the development of a modified methacrylated poly (glycerol sebacate) (PGS-M) with improved hydrophobicity, evidenced by a contact angle of 82° (compared to the unmodified PGS-M with a contact angle of 52°), which is used to form the water-impermeable shell for a core-shell microparticle encapsulating biological immune checkpoint inhibitors, such as anti-PD-1. The particles made from this optimized material demonstrated the ability to resist infiltration by cholesterol esterase solution for 2 weeks and PBS for 4 weeks. This encapsulation ensures the protection of the payload from environmental degradation before its release for extended bioactivity.

In addition, we can leverage surface erosion to precisely modulate release kinetics by changing microparticle geometry, creating multiple microparticle population that provides a consistent series of customizable drug pulses with a single injection. Additionally, intratumoral administration of these microparticles acts as reservoirs, prolonging drug retention at the tumor site and establishing a locally high but systemically low drug concentration.

Ultimately, we aim to use this drug delivery platform to reduce dosing frequency and optimize drug release kinetics, thereby enhancing immunotherapy efficacy while mitigating side effects to provide a more effective treatment.

Poster #: 30

Liposome Coated Mesoporous Silica Nanoparticle Loaded with Forskolin Effectively Modulates Thermogenesis in Adipocytes in vitro and in vivo

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Obesity is an ever-increasing health crisis in the US and can lead to various illnesses such as type 2 diabetes, hypertension, and stroke, yet many therapeutic treatments prove to be ineffective due to the lack of bioavailability at the physiological target site. We have successfully designed a liposome coated mesoporous silica nanoparticle (LCMSN) delivery system for forskolin (FSK), a water insoluble cAMP activator that has been used to increase thermogenic activity in adipose tissue.

Briefly, dissolved FSK were mixed with liposome components and dried into a film. Dendritic mesoporous silica nanoparticles (MSN) were synthesized and loaded with FSK, which was then suspended in PBS, and fused with liposome film through electrostatic interactions to form LCMSN-FSK. The hydrodynamic size was measured to be ~140 nm, with a low PDI (<0.1), and a negatively charged surface (ζ=-28mV). The FSK loading percentages were 11% and 30% respectively, when liposomes were prepared with 10% or 25% FSK.

Human adipose stem cells were differentiated to mature adipocytes and used to evaluate treatment efficacy. Cytotoxicity assays show no detectable toxicity (20-1000 ug/ml media). The uptake of fluorescently labeled LCMSN in adipocytes was evaluated using flow cytometry at different time points (3-48h) demonstrating maximum uptake at 24 h post treatment. Cell internalization was confirmed using 3D confocal microscopy images. Adipocytes treated with LCMSN-FSK resulted in an increase in UCP1 and Cox7A1 gene expression when compared to FSK or LCMSN alone. Furthermore, glucose uptake and lipolysis were also significantly elevated, demonstrating increased metabolic functional outcomes. For in vivo analysis, LCMSN-FSK was subcutaneously injected in inguinal white adipose tissue (iWAT). Biodistribution imaging using KINO Spectral Imaging system was performed in obese and non-obese mice. Retention of fluorescently labelled LCMSN-FSK was observed even until 48h post injection, while accumulation in heart, lung, liver, and kidney was not detected. Increase in lipolytic function and expression of UCP1 at the protein (WB) and gene (RT-PCR) levels were also detected. In conclusion, we have optimized a nanoparticle delivery system that is taken up by adipocytes, and effectively delivered forskolin to adipose tissue in mice which resulted in an increase in thermogenic activities.

Poster #: 31

Peristalsis drives a malignant mechanotransduction response in KRAS mutant colorectal cancer cells

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Colorectal cancer (CRC) begins as a polyp on the inner lining of the colon, which can progress malignantly over time. Within the tumor microenvironment, mechanical forces can influence the polyp-carcinoma transition and in CRC, cells are continuously exposed to peristalsis forces. Genetic mutations in the KRAS gene can also drive CRC initiation and progression. Therefore, we hypothesized that activating KRAS mutations lead to increased progression metrics and tumorigenicity in the context of peristalsis-related mechanobiology.

We custom-built a peristalsis bioreactor capable of exposing cancer cells to peristalsis in vitro. The peristalsis bioreactor was tuned to replicate the mechanics of the colon (0.4 Pa shear and 15% strain). Four cell lines were tested: KRAS mutant (1) HCT116 and (2) LS174T; KRAS Wild Type (3) RKO and (4) PDX1. Cells were exposed to peristalsis or static conditions for 24 hours.

Peristalsis enriched LGR5+ cancer stem cells in HCT116 (1.8-fold) and LS174T cells (2.8-fold), compared to static controls. However, wild type RKO and PDX1 cells saw no significant increases in LGR5 downstream of peristalsis. In vivo studies with KRAS mutant HCT116 cells resulted in increased growth rates in peristalsis exposed cells compared to static controls. Conversely, in KRAS wild type RKO cells resulted in no significant changes in growth rates. Further, peristalsis significantly increased gene expression of several Wnt ligands involved in the enrichment of LGR5+ cancer stem cells in KRAS mutant HCT116 cells (1.6-2.5-fold). Interestingly, KRAS wild type RKO cells also demonstrated increases (1.0-1.3-fold) in all Wnt ligands. However, when evaluating β-catenin activation (nuclear translocation) specifically using immunofluorescence, HCT116 cells demonstrated significant increases (1.25-fold) in peristalsis compared to static controls while RKO cells showed no significant changes (1.01-fold). CRISPR gain of function strategies introduced into KRAS wild type RKO cells also demonstrated LGR5+ cancer stem cell enrichment and increased β-catenin activation in response to peristalsis.

This work demonstrates that active KRAS mutations play an important role in the mechanotransduction of peristalsis in both cancer stem cell emergence and β-catenin activation in CRC. Our continued work aims to define the specific players connected to KRAS mutation status and these increased progression metrics in CRC.

Poster #: 32

Tunable hydrogel networks by varying secondary structure of hydrophilic peptoids provide viable 3D cell culture platforms for hMSCs

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Hydrogels are attractive for use as 3D extracellular matrix (ECM) mimics due to their high-water content and similar physical properties to natural tissue. Using hydrogels, cells have been shown to respond to different matrix stiffnesses, but the way stiffness is changed is by altering the crosslinking density of the matrix, which may also affect other network properties that are crucial to cells such as permeability. Therefore, to understand cellular behavior in a 3D environment, a system where stiffness is decoupled from other properties is highly needed. Based on this necessity, we developed a hydrogel with tunable stiffness by altering the molecular rigidity of the crosslinkers that connect the network. Peptoids, oligomers of N-substituted glycines, were used as crosslinkers. Due to their N-substitution, peptoids can form helical secondary structures that resemble those found in polyproline type I helices, which are more rigid than disordered peptoids. However, a major limitation of helical peptoids is that they are hydrophobic. Therefore, a series of hydrophilic helical peptoids that allow simultaneous hydrogel formation and 3D cell encapsulation have been engineered. We characterized the helicity of the peptoid crosslinkers using circular dichroism and compared them to disordered peptoids or peptoids with disrupted helices. Incorporation of these crosslinkers into hyaluronic acid hydrogels enabled successful decoupling of stiffness from network connectivity. The developed platform enabled 3D encapsulation of human Mesenchymal Stem Cells (hMSCs) as they are very attractive due to their potential in therapeutic applications. High cell viability was found for all hydrogel conditions. Future work will measure cytokine secretion from the encapsulated hMSCs to explore the potential of our hydrogels as scaffolds for therapeutic production.

Poster #: 33

Characterizing Sex-Based Differences in hiPSC-Derived Cardiac Cell Metabolism

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During cardiac development, cellular metabolism switches from glycolysis to oxidative phosphorylation (OXPHOS) to support increased energy demands and promote contractility. Current human induced pluripotent stem cell (hiPSC) cardiac differentiation methods result in metabolically immature cell populations which derive most of their energy through glycolysis. Importantly, this metabolic immaturity leaves hiPSC-derived cardiomyocytes (hiPSC-CMs) unable to meet the significant energy needs of contracting adult cardiac tissue, thus limiting their therapeutic application. Moreover, limited understanding of the role of hiPSC donor sex on engineered cell and tissue functionality, despite known cardiac sexual dimorphisms, hinders this technology. Previous work has demonstrated the addition of fatty acids (FAs), critical CM energy substrates, drives the metabolic switch towards OXPHOS in hiPSC-CMs. This work theorizes that male and female hiPSC-CMs will respond differently to this intervention, based on known sex-based differences in native cardiac metabolism. Two hiPSC lines (one male and one female) of similar donor demographic (~30yo, Japanese, Reprogrammed Fibroblast) were used to gather metabolic maturation data, characterized as the ratio between OXPHOS and glycolysis. We observed changes in metabolic pathway preference in response to palmitic and oleic acid supplementation. Initial results show sex discrepancies in FA preference and baseline metabolic maturation, as measured by oxygen consumption rate, optical redox imaging, and gene expression.

Poster #: 34

Uncovering Heterogeneity in hiPSC-Derived Endothelial Progenitors from Different Differentiation Protocols

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Human induced pluripotent stem cell-derived endothelial progenitors (hiPSC-EPs) have shown considerable promise as an easily expandable and patient-specific cell source for vascular tissue engineering. Considerable work has focused on differentiating these cells and characterizing their vasculogenic potential both in vitro and in vivo. However, most research in the field does not consider the heterogeneity that exists within endothelial cells. To that end, we investigated differences in hiPSC-EPs produced from two differentiation protocols that drive differentiation using either small molecules (SM Protocol) or growth factors (GF Protocol). Following encapsulation of SM- and GF-derived CD34+-hiPSC-EPs in Collagen/Norbornene-modified hyaluronic acid interpenetrating polymer network hydrogels, we found that although cells from both protocols were able to form vasculature within 7 days of culture, GF-derived cells formed denser and more interconnected vasculature. In addition, we observed that GF-derived cells secreted lower levels of matrix metalloproteinases and Collagen IV, indicating decreased extracellular matrix remodeling relative to SM-derived cells. This was corroborated by RNAseq, which also showed that GF-derived cells are significantly more migratory. Taken together, this suggests that GF-derived cells have a higher propensity for undergoing an endothelial-to-mesenchymal transition. This demonstrates that although both SM- and GF-derived cells were selected for the same surface marker and successfully undergo angiogenesis in 3D hydrogels, there exists significant differences on both the gene and protein level. If this cell type is to be used for tissue-engineered vasculature, researchers need to perform similar characterization to better understand the cell types that they have generated.

Poster #: 35

Impact of Donor Age on Microvasculature Self-Assembly of induced Pluripotent Stem Cell Derived Endothelial Progenitors

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Current vascular tissue-engineering using induced pluripotent stem cells (iPSCs) predominantly relies on somatic cells obtained from newborn or fetal donors. However, iPSCs sourced from aged individuals exhibit epigenetic differences in angiogenic genes that may hinder their utility in iPSC derived vasculature. We hypothesized that, due to these differences, iPSCs from aged donors will exhibit decreased vasculogenic potential.

Methods: We differentiated iPSCs into endothelial progenitors (iEPs) and encapsulated them in an interpenetrating polymer network hydrogel containing norbornene-functionalized hyaluronic acid and Collagen I at cell densities and hydrogel stiffness optimal for vasculogenesis. Seven days after encapsulation, the hydrogels were fixed, and the resulting microvasculature imaged. We then utilized a previously developed computational pipeline to quantify several vasculogenic properties.

Results: We derived iEPs from four separate iPSC lines: 2 sourced from neonatal donors (ND) and 2 from mature donors (MD). Irrespective of donor age, differentiation yielded ~15% (n=4) iEPs of the total cell population. The two ND lines developed a similar plexus, whereas this structure was diminished in one adult line and absent in the second. Between iEPs matched for sex and somatic cell origin, there is a 50% decrease in vessel volume fraction and number of links between vessels, a 66% reduction in the percentage of vessels connected to the largest vessel, and a 43% reduction in branch points between vessels.

Conclusion: Overall, these data suggest MD iEPs are less likely to form dense interconnected microvasculature as compared to ND iEPs. Interestingly, the average diameter did not change across all cell lines. This suggests that while MD iEPs exhibited cell clustering, they were less likely to branch out and interconnect with other clusters of iPSC-EPs to form interconnected networks.

Poster #: 36

Matrix-Mechanical Memory: EMT Dynamics in PDAC

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Our bodies have the remarkable capacity to generate and maintain a wide range of highly specialized organs from a single type of cell: the stem cell. And yet, this singular ability also endows cancer stem cells (CSC) with an equivalent potential for unfettered growth and differentiation. In Pancreatic ductal adenocarcinoma (PDAC), CSCs are thought to drive tumor initiation, progression, and recurrence, likely contributing to one of the highest mortality rates of any malignancy with a 5-year survival rate under 5%. Despite the breadth of resources devoted to developing effective therapeutics, there remains a significant gap in our understanding and ability to target the heterogeneous nature of this deadly malignancy. One key feature of the PDAC tumor microenvironment (TME) is the desmoplastic reaction, comprising a variety of cell types and extracellular matrix (ECM) components that greatly increase tissue stiffness. Researchers have begun to resolve the extracellular cues that shift stem cells (SCs) into a metastatic state. For example, it has been repeatedly demonstrated that mechanical stiffness of the local environment not only guides natural stem cell differentiation down the appropriate tissue lineage but is also a critical factor driving the epithelial to mesenchymal transition (EMT). However, the site of tumor initiation is often mechanically distinct from that of the metastatic tumor microenvironment, which may contribute to the failure of current treatments. Our initial results demonstrate cellular “memory” after exposure to certain synthetic matrices. Therefore, we hypothesize that mechanical properties at the primary site drive epigenetic changes dictating pancreatic CSC behavior at the metastatic site. With our alginate-based model, we have successfully achieved normal- and PDAC-mimetic stiffnesses of 0.2kPa and 1.0kPa. Mia Paca 2 on stiff gels exhibit fibroblast-like morphology, while those on soft gels maintain rounded morphology in cellular aggregates. Preliminary results demonstrate how the initial mechanical environment affects stemness and metastasis at the gene level, including differential expression of EMT-associated LAMA4, LTBP4, FSCN1, PHB1, GFRA3, PXB1, SMAD5-AS1, and CHST13. This research supports the narrative that TME stiffness can independently drive pancreatic CSC behavior and lays the foundation for further investigation of therapeutic strategies targeting the heterogeneous nature of PDAC.

Poster #: 37

Matrix mechanics regulates temporal changes in mesenchymal stroma

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Introduction:

Cellular infiltration into matrices, successive proliferation, and differentiation of stem cells is a well-coordinated sequence involving mechanical and biochemical cues. While musculoskeletal extracellular matrix is primarily Collagen I (Col), tissue damage results in replacement by a transient fibrin (Fib) matrix within a blood clot. Our objective is to observe temporal changes of cellular behavior that occur and determine if differences in mesenchymal stroma is driven by matrix mechanics, cellular response, or a combination. The results of this study will inform the temporal support available for rudimentary vascular networks to form within regenerating stroma.

Materials/Methods:

Mesenchymal stem cells (MSCs) were seeded within fibrin, collagen:fibrin (col:fib) and collagen hydrogels (fibrin at fibrinogen density of 10 mg/mL (F10), collagen at a concentration of 2, 4, and 6, mg/mL (C2, C4, C6), col:fib gels at a ratio of 25 Col:75 Fib at each of the previously listed concentrations (C2F10, C4F10, and C6F10)). The study was carried out over 14 days. Following fixation, samples were stained with Actin and DAPI and imaged, cryo-sectioned and sample stiffness characterized. Statistical analysis was performed using one-way ANOVA across material composition for n=3/group/time-point and Kruskal-Wallis' post hoc test for significance at $p<0.05$.

Results/Conclusion:

Matrix density clearly impacted cell morphology and proliferation early and throughout the study. Collagen at increased concentration prevented infiltration of MSCs and had a rounded morphology, unlike with the addition of fibrin, where cells formed tracks and spindle like morphology prior to highly spread-out morphologies at later times. Changes in matrix stiffness are a combination of initial hydrogel proteins, degraded hydrogel matrix, cellular contributions, and secreted matrix, all of which could potentially be within the 10 kPa to 1 MPa range measured. By day 14, there were no significant differences within matrix stiffness, indicating that either enough matrix was produced or enzymatic activity resulted in matrix degradation to an appropriate level of stiffness.

We found that fibrin is crucial from a mechanical perspective in allowing cellular infiltration/proliferation and resultant matrix remodeling over time. It's anticipated that temporal changes in matrix stiffness and stromal proliferation will synergistically support which hydrogels are most conducive for concurrent vascularization.

Poster #: 38

Self-assembly Nucleopeptide Hydrogels for Stem Cell Culture

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Human induced pluripotent stem cells are adult somatic cells that have been reprogrammed to a pluripotent, embryonic like state. They have vast clinic potential, from drug discovery to regenerative medicine. Due to this potential, there is a current trend of hiPSC based therapies moving to clinical trials. However, these cells are notoriously hard to culture, due to the complexity of maintaining pluripotency without effecting chromosol integrity, and this remains a major barrier to translation.

Matrigel is currently the gold standard for all aspects of hIPSC culture. Matrigel, derived from the ECM of mouse sarcoma, has many properties ideal for both 2D and 3D culture. It's bioactive, biodegradable, perfusable, and easy to use. However, due to its source, Matrigel is subject to high batch-to- batch variability and xenophobic concerns. In addition, the numerous unknown and variable minor components compound results of cellular studies.

In addition to the positive attributes of Matrigel, the ideal hiPSC scaffold system is also easily tunable, enabling the same system to be used for expansion and differentiation. Small molecular weight hydrogelators consisting of a nucleotide attached to a short amino acid sequence innately meet many of these requirements. Like a hydrogel spice rack, we aim to develop a base nucleopetide gelator that can be matched with different modulators to create a user-tunable scaffold.

While much work has been done on the development and characterization of our base nucleopeptide, thymine tri-phenylalanine, the effect of the addition of modulator nucleopeptides is still largely unknown. My current work centers around the synthesis and gelation characterization of the RGD containing nucleopeptides Thy-FFFGRGDS and Ade-FFFGRGDS. We hypothesize Ade-FFFGRGDS:ThyFFF gels will be more stable due to Watson-Crick interactions

LCMS analysis confirmed successful production via solid phase peptide synthesis. Results of buffer-mediated gelation indicate Thy-FFFGRGDS more readily associates with ThyFFF in the cell-compatible pH range. In addition, pH-switch trigged gelation of Ade-FFFGRGDS:ThyFFF gels onset at a higher pH compared to Thy-FFFGRGDS: ThyFFF gels. Further study of gelation bounds, long term stability, and cellular studies are needed for more concrete conclusions.

Poster #: 39

Optimizing Vascular Tissue Regeneration: Engineering Stiffness-Controlled Norbornene-Modified Hyaluronic Acid Hydrogels for Enhanced Maturation of hiPSC-Derived Endothelial Progenitors

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Engineering biomaterials to mimic the extracellular matrix (ECM) is crucial for vascular tissue engineering, particularly for facilitating the maturation of endothelial progenitors (EPs) into endothelial cells (ECs). We've shown that the microenvironment's stiffness can control the microvascular plexus architecture formed by encapsulating human-induced pluripotent stem cell-derived endothelial progenitors (hiPSC-EPs) in various stiffened hydrogels. To further understand this, we explored the biomechanics of hiPSC-ECs by encapsulating them in norbornene-modified hyaluronic acid (norHA) hydrogels, adjusting stiffness by varying degradable peptides (DGD) crosslinker amounts. We used these hydrogels to study differentiation, spreading, proteoglycan deposition, and Hippo pathway activation in hiPSC-EPs.

We formulated norHA hydrogels with DGD concentrations from 25% to 100% relative to available norbornene groups, establishing a gradient of mechanical stiffness. Studies showed the equilibrium swelling ratio decreases linearly as peptide crosslinker concentration increases, indicating a tighter network with higher crosslinking densities. Rheometry showed an increase in hydrogel stiffness, from about 200 Pa at 25% DGD to 800 Pa at 100% DGD, showing a near-linear relationship between crosslinker concentration and stiffness. FRAP assays suggested diffusivity reduction with more crosslinking, though the significance of these differences is under investigation. CD31/CD34 imaging confirmed endothelial cells' activation in all groups, with low or no CD34 expression, indicating successful maturation from EPs to ECs. The investigation of YAP/TAZ activation through imaging and analysis is ongoing.

We are developing an assay to study proteoglycan deposition across the four stiffness-varying groups, highlighting its significance in endothelial maturation. This aspect of the research will reveal a stiffness-dependent modulation of proteoglycan profiles, which are critical for ECM remodeling, cell adhesion, and signaling. This study's expected outcomes and impact are significant, as they will provide novel insights into the microenvironmental factors that promote vascular tissue development and regeneration. Understanding the relationship between hydrogel stiffness, proteoglycan deposition, and endothelial cell behavior opens new avenues for designing biomaterial scaffolds that more effectively support vascularization, potentially improving the success of tissue-engineered constructs in clinical applications.

Poster #: 40

Engineering Immunomodulatory Materials: Indole-functionalized Gelatin Hydrogels

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The development of immunomodulatory biomaterials is currently of high interest for mitigating the foreign body reaction, stimulating tissue regeneration, and enhancing vaccine efficacy. An exciting avenue of exploration falls in leveraging the properties of indole, a tryptophan metabolite, which has been shown to reduce inflammation in the gut and could be exploited to engineer immunomodulatory biomaterials. To this end, the objective of this project was to synthesize indole-functionalized hydrogels. To achieve this goal, gelatin was chosen as a versatile platform to conjugate indole to its backbone. Gelatin was first functionalized with norbornene to facilitate crosslinking via click chemistry between thiol and norbornene groups. Norbornene functionalization was achieved by reacting gelatin with carbic anhydride, thereby attaching norbornene to the gelatin backbone through amide bonds. Subsequently, hydrazide groups were added to facilitate indole conjugation via Schiff base chemistry. Similar to the norbornene functionalization, hydrazide was attached to the backbone of gelatin through an amide bond formation when reacted with carbohydrazide. The aldehyde group on the indole then reacts with the amino group of the hydrazide to form the Schiff base. The presence of these functional groups was confirmed through nuclear magnetic resonance (NMR) spectroscopy and further validation was performed using a fluoraldehyde assay. The hydrogels were prepared using a gelatin weight percent of 10% and then characterized using rheology to determine the storage modulus. Future tests to be conducted will evaluate the efficacy of indole-functionalized gelatin hydrogels in mitigating inflammation in epithelial cells and macrophage polarization. This research holds significant implications for the development of immunomodulatory biomaterials with broad biomedical applications, particularly in tissue engineering.

Poster #: 41

Overlapping contributions of matrix mechanics and hypoxia on glioblastoma phenotype

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Glioblastoma (GBM) is a uniformly lethal brain cancer due to poor treatment response and high rates of recurrence. GBM cells diffusely invade into the surrounding brain tissue, which is softer than the tumor bulk, and inevitably remain after surgical tumor resection. These remaining cells then seed recurrent tumors. Previously, we identified a metabolic shift in GBM cells cultured in 3D brain-mimetic matrices, where cells cultured in soft matrices mimicking the stiffness of noncancerous brain tissue (G' = 100 Pa) displayed a more glycolytic metabolism and were more invasive compared to cells cultured in stiff matrices mimicking the stiffness of the tumor bulk (G' = 1 kPa), which were more proliferative (Sohrabi, et al. Cell Reports 2023). While these observations were made at 21% (atmospheric) O2, hypoxia is known to affect cell metabolism and GBM progression. Thus, we cultured GBM spheroids in 3D matrices with defined elastic moduli in 21% or 1% O2 to identify how these effects interact to determine GBM behavior. Although the influence of matrix stiffness and hypoxia on GBM cell metabolism and phenotypic behavior likely have overlapping effects, they have typically been studied without appropriate decoupling to understand the contribution of each factor. Our data shows that GBM cells from multiple patient-derived cell lines are more apoptotic in 1% O2 conditions regardless of matrix stiffness. In soft matrices, 1% O2 had varying effects on invasive behavior based on cell line but generally increased invasion. Multiplex bead-based ELISAs (Luminex) were used to investigate signaling changes in relevant pathways such as Src, Ras/Raf/MAPK, and Akt. GBM cells cultured in soft and stiff matrices in 1% or 21% O2 displayed distinct intracellular signaling patterns over 7 days in culture, suggesting important interplay between these two microenvironmental factors in controlling GBM cell behavior. Future work will incorporate these data into a partial least squares regression model to identify key signaling targets regulating the microenvironmental effects on GBM cell phenotype.

Poster #: 42

Understanding Adipose Stem Cells Characteristics and Motility in the Endometrial Cancer Environment

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The incidence of endometrial cancer is increasing year after year and is mainly attributed to advanced age, estrogen exposure, and recently, the obesity epidemic, whose profile has modified the advanced age range, affecting women at an earlier age. A meta-analysis has revealed that each 5 units increase in body mass index (BMI) is associated with a 50% increased risk of developing endometrial cancer. In US, endometrial cancer accounts for 40% of all cancer diagnoses, so it is expected to surpass lung cancer and colorectal cancer by 2030 and become the third most common cancer in women. Recent data has shown that adipose stem cells (ASCs), infiltrate the endometrial microenvironment of obese, but not non-obese, patients, with elevated numbers of ASCs correlating with increased obesity. The purpose of this study is to analyze the altered ASC behavior in an endometrial tumor microenvironment that can be targeted for therapeutic purposes. Using RT-PCR, atomic force microscopy, cyTOF, and a 2D migration or scratch assay, we investigated the impact of the oncogenic endometrial environment on ASCs. Specifically, we assessed the following: 1) ITGs gene expression, 2) Nanomechanical properties, and 3) Motility. ASCs were exposed to conditioned media (CM) derived from an endometrial cancer cell line (KLE) and non-oncogenic endometrial epithelial cells (EECs, EME6/7). In 2D studies, exposure of ASCs to CM revealed significant increases in the expression of ITGA5 and ITGA7 in those exposed to KLE CM compared to non-oncogenic EME6/7 CM ($p<0.05$) or control media ($p<0.0001$). Moreover, ASCs exposed to KLE CM exhibited lower stiffness and higher adhesion and deformation compared to the other groups. Finally, when exposed to CM, ASCs demonstrated a 45% increase in motility rate compared to the control. These findings suggest that altered ASC behavior could potentially contribute to a microenvironment conducive to oncogenicity, particularly in obese patients with endometrial cancer.

Poster #: 43

Breast cancer tumor and cardiovascular microphysiological system to determine the role of HER2 in cardiac dysfunction

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In 2022 The American Cancer Society estimated there were more than 300,000 new cases of breast cancer with a projection of more than 43,000 deaths in the US. Despite its high prevalence, breast cancer mortality rates have decreased in recent years. This is due to improved cancer chemotherapy treatments and diagnostics. However, women that are undergoing breast cancer treatment can experience life-threatening adverse effects such as myocardial dysfunction, cardiovascular disease (CVD), and heart failure. Specifically, HER2-targeted therapies have improved the outcome of patients with HER2-positive breast cancer, however, these therapies are associated with cardiotoxicity. Consequently, CVD has become the leading cause of death in breast cancer survivors, and is in part due to the use of chemotherapeutic agents. In vitro microphysiological systems (MPS) provide a platform to find improved, personalized breast cancer treatments while reducing the detrimental impact to the heart. The central hypothesis of this project is that a physiologically relevant breast cancer tumor and cardiovascular microphysiological system (MPS) will provide a more effective in vitro model to determine the mechanism of cardiomyocyte dysfunction with chemotherapeutic inhibition of HER2. Within this study we demonstrate the detrimental effects of HER2-targeted chemotherapeutics on 3D engineered cardiac and breast cancer tumor MPS models.

Poster #: 44

Electrically Conductive Hydrogels for Central Nervous System Repair

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As of 2018, approximately 300,000 people are currently living with a spinal cord injury (SCI) in the United States and there are 17,700 new cases each year. Along with the physical and psychological consequences causing an overall lower quality of life, a SCI patient faces a lifetime of medical care ranging from $1.2-5.1 million. Unfortunately, no cure for SCI currently exists creating an urgent, yet unmet, need for new therapies that can restore function. To repair the spinal cord, there are several considerations including, but not limited to, modulation of the scar that forms, cell-based therapies and initiating endogenous repair processes. Two approaches have shown promise in limited clinical trials: 1) neural stem cell (NSC) grafts and 2) biomaterials. However, significant barriers to the clinical translation of both therapies remain. For grafts, poor cell survival after transplantation and inadequate differentiation into functional spinal circuitry have limited success. Biomaterials typically suffer from either a lack of biocompatibility with native tissue or electrical conductivity that can mediate the endogenous signaling in the area. To address these limitations, this project proposes to develop electrically conductive hydrogels based on hyaluronic acid (HA) as scaffolds for transplanted cell grafts after SCI to create a platform for evaluating applied stimulation protocols in a controlled in vitro setting to identify procedures that maximize formation of functional neural networks. Several studies have demonstrated that HA-based biomaterials can both attenuate formation of an inflammatory scar after SCI and promote survival and differentiation of human NSCs. While various approaches to impart electrical conductivity in HA-based hydrogels have been explored, these materials have significant limitations including cytotoxicity, poor conductivity, mechanical mismatch with spinal cord tissue and complex fabrication methods. This project addresses these issues by incorporating a highly conductive graphene-based material within the HA-based hydrogel to form conductive scaffolds for NSCs. Ultimately, a multi-pronged approach that incorporates populations of either NSCs or mature neural phenotypes within an electrically conductive biomaterial vehicle can be combined with rehabilitative training and paired electrical stimulation to kickstart plasticity of neuronal circuitry that is spared after injury.

Poster #: 45

Extracellular Matrix Influence on Dormancy: Insights from a 3D Bioengineered Model of Microscopic Colorectal Cancer Liver Metastasis

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Colorectal cancer (CRC) liver metastasis (CRLM) is responsible for two-thirds of CRC deaths. Survival depends on successful hepatic resection and adjuvant chemotherapy; however, most patients relapse within 18 months after delayed outgrowth of microscopic residual disease. Clinically undetectable, dormant microscopic metastases have not been well-studied to sufficiently inform in vitro modeling, driving a need for new models to understand the mechanisms by which the liver harbors these nests and subsequently permits their delayed invasive outgrowth. We previously established a metastasis model to mimic metastatic clustered seeding and behavior with 3D tumor spheroids and decellularized porcine ECM scaffolds. Here, we apply these techniques to establish and characterize a model of microscopic CRLM dormancy. Decellularized scaffolds uphold the biochemical composition of fresh liver tissue as evidenced by maintenance of major amide bands (A, B, I, II, III) in the FTIR spectra. Notably, these spectra resemble that of collagen, which can also be identified in Masson's Trichrome-stained liver scaffold sections. Ultimately this suggests the scaffold's ability to mimic liver tissue, specifically imposing the influence of a collagen matrix on CRC micrometastases in vitro. To create micrometastases, we first evaluated a dormant phenotype in HCT116 CRC cells. Serum starvation (2%) resulted in smaller spheroids, which was confirmed to be the result of dormancy rather than increased cell death by significant reductions in Ki67 protein expression (75% decrease) and several genes related to cell cycle progression. CRC spheroids seeded onto the liver scaffold, exhibited similar changes as those driven by serum starvation. Specifically, CDKN2B was increased 1.6-fold while CCND1, MKI67 and AURKA experienced 0.73, 0.43 and 0.65-fold changes, respectively, indicating the presence of the collagen matrix arrests cell cycle. Interestingly, we saw similar trends in CDKN2B, MKI67 and AURKA expression following oxaliplatin chemotherapy, demonstrating the potential for our model to further mimic dormancy specifically driven by chemotherapy treatment. Further study will assess functional consequences of matrix- and chemotherapy-driven cell cycle arrest with the goal of aiding in mechanistic discovery to inform diagnostic research of recurrent CRLM. Once established, this model can also be applied for high throughput drug screening for personalized therapeutic development.

Poster #: 46

Mimicking the viscoelastic nature of brain tissue to study neural cell behavior using a hyaluronic acid-based interpenetrating polymer network.

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Brain is the softest, most viscoelastic tissue in mammals and these mechanical properties are known to influence the phenotypes of brain cells. However, conventional hydrogels for 3D cultures are elastic and not viscoelastic. Furthermore, hydrogels that do exhibit viscoelastic properties, rarely provide the ability to tune the elasticity (G') independently of the viscosity (G''), making it impossible to decouple the effects of each mechanical component on cell behavior. To address this deficiency, we have developed a hyaluronic acid (HA)-based interpenetrating polymer network (IPN) hydrogel platform, in which G' and G" can be tuned independently, including to approximate the mechanical properties of native brain tissue. The IPN includes a covalent network consisting of photocrosslinked HA (thiol-norbonene) to control the elasticity of the hydrogel and a non-covalent network of dynamically crosslinked HA (hydrazone bonds) to regulate the viscosity. Addition of the dynamic network increases viscoelasticity (tan(δ)) of the biomaterial threefold over that of the covalent network alone, while maintaining the same storage modulus (G') of the static, covalent network. In IPN conditions, we observed an increase in the cell proliferation of patient-derived human glioblastoma tumor cells, which may more closely represent in vivo tumor growth. Additionally, we found that increased viscoelasticity can drive the differentiation of mouse neural progenitor cells to a mature neuron state. With the addition of a viscous network, our IPN model is a better representation of the mechanics of native brain tissue and can be used to better understand how tissue mechanics affect neural cell behavior.

Poster #: 47

Role of radiation-induced, GBM-secreted extracellular matrix in tumor treatment resistance and recurrence

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Background: Glioblastoma (GBM) is the most common and lethal type of brain tumors with median survival of 15 months. Patients' poor prognosis is mainly due to recurrence and development of treatment resistance. While the most common GBM treatment is radiation therapy, information about development of resistance to radiation therapy is scarce. Here, using hyaluronic-acid (HA) hydrogels as 3D scaffolds, we investigated the evolution of GBM-secreted extra cellular matrix and correlate that with development of radiation resistance in GBM.

Method: HA hydrogels were formed via a UV-initiated, thiol-ene reaction. GBM cells are encapsulated as single cells, in different seeding densities in HA hydrogels to form tissue-engineered scaffolds (TE-pGBM). TE-pGBMs were irradiated with gamma radiation using a tabletop cell irradiator. Changes in ECM expression was investigated using RNA-sequencing (RNA-seq), matrisome proteomics, and confirmed using western blotting. GBM colony formation was investigated periodically, using bright field imaging and colony size was measured using an ImageJ script.

Results: We first investigated the colony-formation ability of GBM cells in various seeding densities to establish the clonal expansion of GBM cells in 3D. Interestingly, at the lowest seeding density (50k/ml), GBM colonies' sizes in 10 Gy samples were similar to the no treatment control, suggesting an intrinsic resistance of GBM cells to radiation.

To broadly investigate the post-radiation changes in GBM, we performed RNA-seq, 7-days post radiation. RNA-seq data clearly showed that radiation resulted in overexpression of ECM molecules such as Mucins, osteopontin, collagen IV, etc. On the other hand, collagen I was downregulated after radiation.

Future: We will use computation oncology to correlate the ECM expression with GBM FLuc readings to determine what ECM molecules drive radiationresistance.

Poster #: 48

Macrophage Checkpoint Nanoimmunotherapy Has the Potential to Reduce Malignant Progression in Bioengineered In Vitro Models of Ovarian Cancer

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Most ovarian carcinoma (OvCa) patients present with advanced disease at the time of diagnosis. With its chemoresistant and invasive nature, the 5-year survival rate of metastasized OvCa is <30%, exposing a need for improved therapeutic targeting. Clinical biospecimen link poor prognosis to increased macrophage immune checkpoint signaling where CD47 (OvCa) binds macrophage checkpoint SIRPα to suppress antitumor immunity and enhance OvCa chemoresistance and metastasis, making this interaction a significant target for therapeutic discovery. Even so, previous attempts have fallen short, limited by CD47 antibody specificity due to its expression on several cell types in the body. Thus, we developed a new approach to disrupt CD47-SIRPα signaling by instead targeting the macrophages with short interfering RNA (siRNA) to reduce SIRPα expression. We specifically leverage the phagocytic nature of macrophages by encapsulating SIRPα siRNA (siSIRPα) in lipid-based nanoparticles (LNP) – which are preferentially taken up by macrophages – to aid in specificity and delivery.

We generated OvCa/macrophage co-culture heterospheroids on a hanging drop array as a representative model of cellular interactions in metastatic OvCa. In this model, we identified the presence of the CD47 and SIRPα proteins and observed increased carboplatin chemotherapy resistance and invasion (1.5-fold increases in IC50 and migration area) indicating a more malignant OvCa phenotype in the presence of macrophages. Microfluidic techniques were used to prepare stable, reproducible LNP encapsulating siSIRPα which were characterized for optimal size, neutral charge, and efficient encapsulation. M0 macrophages quickly took up the particles and exhibited ~50% reduction in SIRPα gene and protein expression in heterospheroids, leading to reversal of pro-tumoral alternative activation in macrophages (reduction of IL-10/CD206, upregulation of NOS2/IL-1B/IL-12). Disrupting CD47-SIRPα signaling resulted in a 60% recovery of chemosensitivity and reduced OvCa invasion. Notably, siSIRPα LNP did not significantly alter chemoresistance or invasive potential in OvCa monospheroids, supporting that our therapy directly affects macrophages in the system, as desired. Additionally, LNP loaded with negative control siRNA did not reduce viability or activity of heterospheroids, suggesting there are no negative effects from the vehicle, itself. Ultimately, our results strongly indicate the potential of using CD47-SIRPα nanoimmunotherapy to reduce malignant progression of ovarian cancer.

Poster #: 49

Cell-internalized synthetic nanogels for the induction of therapeutic phenotypes in murine macrophages

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Nanogels are three-dimensional nanostructures formed through crosslinking of hydrophilic polymers in water. Recent studies have shown that, once internalized by macrophages, nanogels can intrinsically effects on macrophages' metabolism, transcriptome, and function. This study presents the development, characterization, and immunomodulatory evaluation of stimulus-responsive nanogels synthesized using activators regenerated by electron transfer atom transfer radical polymerization (ARGET ATRP).

We synthesized a small library of 20 nanogel formulations incorporating one of two cationic (DMAEMA and DEAEMA) and one of five hydrophobic (MMA, BzMA, CHMA, tBMA, and BMA) methacrylic monomers, at ratios of 75:25 and 25:75 cationic:hydrophobic co-monomer. Nanogels were synthesized by a miniemulsion technique, using bis(2-methacryloyl)oxyethyl disulifde as a crosslinking agent and a poly(ethylene glycol MW=2,000) methacrylate macromer for surface modification.

Fourier Transform Infrared Spectroscopy (FT-IR) was utilized to validate incorporation of feed monomers. Dynamic Light Scattering (DLS) was used to evaluate particle diameter and zeta potential as a function of environmental pH. Cytotoxicity and cell viability were evaluated using LDH and MTS assays, respectively. Flow Cytometry analysis of nanogel-treated on Raw264.7 macrophages (200 g/mL, 24h) for markers of a classical/inflamed (CD86, MHCII, iNOS) or alternative (CD163, CD206, Arg-1) was used to determine nanogel-induced changes to murine macrophage phenotype.

FT-IR analysis confirmed the successful incorporation of feed monomers and complete extraction of unreacted monomer, as evidenced by the disappearance of double bond peaks associated with acrylic monomers. Most nanogels had a particle size below 200 nm, exhibiting pH-dependent behavior. They also displayed a positive surface charge, which was reduced, approaching neutrality, at pH 9. Remarkably, cytotoxicity assays demonstrated no toxicity at both 50 and 200 µg/ml concentrations after 24h treatment, with approximately 80% viability showing no significant difference compared to the untreated control sample. This highlights the biocompatibility of the synthesized nanogels. Preliminary analysis of the flow cytometry data revealed a significant difference in the expression of pro-inflammatory and anti-inflammatory markers for certain nanogels compared to untreated macrophages.

In conclusion, we have laid the groundwork for a tailored drug delivery platform for macrophage modulation. Future studies will utilize ELISA and RNA-Seq analysis to further interrogate the mechanism of intrinsic nanogel immunomodulation of macrophages.

Poster #: 50

Nanogel-Cytokine Conjugates: A Platform Technology Approach for Macrophage Immunomodulation

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Cytokines show promise in modulating macrophage activity to treat life-threatening immune diseases. However, their effectiveness is hindered by severe side effects and frequent dosing. Research exploring biomaterials for systemic cytokine delivery, like PLGA or lipid nanoparticles, faces challenges such as immunogenicity and reliance on organic solvents for synthesis conditions, making them less than ideal for delivering protein therapeutics. Inspired by hydrogel properties that mimic physiological environment, and to overcome limitations associated with drug encapsulation, we developed a novel delivery platform: poly (acrylamide-co-methacrylic acid) P(Aam-co-MAA) nanogels conjugated to therapeutic cytokines.

Our study demonstrated that Interferon-gamma (IFNγ), Interleukin 10 (IL-10), and Interleukin 4 (IL-4) maintain their ability to modulate Raw264.7 macrophage activity even after conjugation, as compared to free cytokines. We performed multiple stimulation regimens, such as co-stimulation, treatment, and prophylaxis, to mimic different micro-physiological environments that macrophages are exposed to. We observed an increased expression of MHC II, CD86, and iNOS pro-inflammatory markers in the presence of IFNγ ($p<0.05$) that were differentially lowered in the presence of IL-4 and IL-10. The expression of these markers, and anti-inflammatory CD206, CD163, and Arg1, varied significantly between treatment regimens and timepoints.

To characterize the unconjugated nanomaterial, we compared the co-localization of nanogels with Raw264.7 macrophages and mouse whole blood. While the nanogels did not co-localize with any white blood cell (WBCs) ex-vivo after a 3-hour incubation at 0.3 mg/ml, they significantly co-localize with adherent macrophages. Interestingly, this differed from PLGA nanoparticles, which showed significant co-localization with WBCs. Furthermore, we demonstrated that the nanogels exhibit no toxicity to Raw264.7 macrophages at concentrations up to 3 mg/ml. This was supported by a significant increase in WBC death in blood treated with PLGA nanoparticles ($p<0.01$), but not with P(Aam-co-MAA) nanogels ($p>0.05$), compared to the untreated control.

Our results indicate that we have successfully developed a safe cytokine delivery platform with remarkable ex vivo whole blood distribution, retaining cytokine activity after conjugation. Both elements are essential for developing safe and effective cytokine therapeutics. Future studies will focus on assessing in-vivo biodistribution of the nanomaterial and evaluating the therapeutic efficacy of the conjugates in inflammatory mouse models.

Poster #: 51

Impact of Molybdenum Disulfide (MoS2) Nanoparticles on Macrophages

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Molybdenum disulfide (MoS2) nanoparticles have gained attention in recent years due to their physicochemical properties, adaptability and potential biomedical applications. Previous research on human mesenchymal stem cells has revealed that MoS2 nanoparticle treatment decreases oxidative stress, as evidenced by decreased reactive oxygen species (ROS) production, associated with increased mitochondrial health.

While reduction of ROS production can have protective benefits against oxidative damage, ROS formation is an essential component of phagocytotic antimicrobial activity. However, mitochondrial health and energy production is an essential aspect of functional macrophage phagocytosis. Therefore, balancing ROS production with mitochondrial health in MoS2-treated macrophages is crucial.

This study investigates the impact of MoS2 nanoparticles on macrophages, a critical component of the innate immune system and primary immune response. Macrophages were exposed to varying concentrations of MoS2 nanoparticles to assess cytotoxicity and viability assays indicated a dose-dependent cytotoxic effect, with higher concentrations (≥100 μg/mL) significantly reducing cell viability. Thus, treatment of a concentration at 25 μg/mL was selected for this study, as it does not significantly affect cellular viability. Additionally, the expression of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, are markedly changed in MoS2-treated primary macrophages. Primary macrophages treated with 100nm MoS2 nanoparticles demonstrate a shift in macrophage polarization, the effect of which is still being studied.

These findings suggest that while MoS2 nanoparticles hold promise for therapeutic applications, understanding the mechanisms underlying these responses, specifically regarding the immune system, is crucial for optimizing the safe use of MoS2 nanoparticles in biomedical contexts. Future research will focus on surface modifications to mitigate adverse effects while harnessing the beneficial properties of MoS2 nanoparticles.

Poster #: 52

Intracerebral Immunotherapy to Accelerate Neurological Recovery after Intracerebral Hemorrhage

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Abstract: Currently, there is an unmet treatment option for neurological disorders. Toward the goal of intracerebral delivery of disease-modifying therapeutics, we have developed a brain-tissue-inspired hydrogel depot to mimic native brain parenchyma, to locally deliver IL-4 and IL-10, modulating the immune system to improve neurological inflammation caused by diseases. The depot named “Parenchygel” is a novel combination of hyaluronic acid, heparin, poly(lactic-co-glycolic acid) nanoparticles, and chemically crosslinked with poly(ethylene glycol). We conducted a study to evaluate the feasibility of our therapeutic in rats induced with intracerebral hemorrhage, with a control group (no treatment, n = 5), surgical intervention (n = 5), carrier group (Parenchygel, n = 5) and a therapeutic group (Parenchygel, IL-4, and IL-10, n = 5). All rats received an intracerebral injection of 50 µL of blood obtained from the tail vein. The surgical intervention, carrier group, and therapeutic group had 50 µL hematoma evacuated the next day. The rats in the carrier group and therapeutic group were injected once with 20 µL of Parenchygel and monitored for 6 weeks. Rats were sacrificed after 35 days, the brains were removed, embedded in paraffin, and sectioned. Hematoxylin and eosin (H&E) staining was used to evaluate the health of tissues around the injection site and the hematoma volumes in rats. We evaluated neurologic deficits and improvements using the Modified Neurological Severity Score (mNSS) scoring on days 1, 3, 5, 7, 8, 13, 20, 27, and 34 post-surgery. A 2-way ANOVA revealed a statistically significant improvement in mNSS scoring for rats treated with the therapeutic compared to the control. The rats showed significant improvement on day 3 compared to the control where mNSS scores continued to increase until day 7. In conclusion, these results support that a single injection of Parenchygel with IL-4 and IL-10 therapeutic improves recovery from intracerebral hemorrhage.

Poster #: 53

Investigating the Capacity of Polyelectrolyte Complex Micelles to Sequester Double-stranded RNA

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Polyelectrolyte complex micelles (PCMs) are a class of self-assembled nanoparticles that are assembled by mixing a polyanion and a neutral-cationic block copolymer in an aqueous solution. PCMs show significant promise for nucleic acid delivery due to their hydrated nature and programmable features. Nucleic acids, due to their inherently anionic phosphate backbone, are not only sequestered but play an active role in assembly and therefore contribute to micellization and morphological control. However, nucleic acids do not simply behave like an anionic polymer due to their ability to form secondary structures through base pairing and base stacking. Furthermore, despite their like charges, DNA and RNA have shown changes in PCM formation. Specifically, double-stranded (ds) RNA fails to assemble PCMs under the same conditions of dsDNA, posing an enormous limitation of PCMs for delivering short-interference RNA (siRNA), which is predominately double-stranded and holds unique advantages in gene targeting and silencing. Understanding the influence of nucleic acid chemical and physical properties on PCM assembly and behavior is crucial for their advancement. Through systematic studies of polyelectrolyte chemistry, block length, and ionic-pairing interactions, we are uncovering the driving forces behind RNA polyelectrolyte assembly. Using a combination of small angle x-ray scattering, optical microscopy, electron microscopy, and all-atom molecular dynamic simulations, we are building structure-property relationships and a molecular understanding of the driving forces behind RNA polyelectrolyte complexation and PCM formation. These studies expand knowledge in the science of self-assembly and will help direct the design of future nucleic acid delivery systems.

Poster #: 54

Effect of Poly(NIPAM) Nanoparticles on the Viability of Osteoblast-Like Cells

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Osteoporosis affects approximately 10 million Americans, and is characterized by low bone mineral density and deterioration of the bone tissue architecture resulting in bone fragility. The weakened bone tissue structure can thus lead to the increase in fracture incidence, however, due to the compromised tissue, healing of the fracture is often difficult to achieve. In severe osteoporotic cases, diseased bone fractures may require surgical repair. For this reason, tissue engineering strategies have been investigated using cells, biomaterials, and biomolecules to intervene when a patient's bone quality is compromised to promote bone tissue remodeling during injury. Thus, poly(N-isopropylacrylamide)-based nanoparticles were investigated for bone tissue engineering applications, and their viability was determined using osteoblast-like cells.

Briefly, N-isopropylacrylamide (NIPAM), 2-aminoethyl methacrylate hydrochloride (AEMA), 2-hydroxyethyl methacrylate (HEMA), and N,N'-methylenebisacrylamide (crosslinking agent) were combined in a round bottom flask with water, purged with nitrogen, and heated to 70°C. Then, the solution was initiated with ammonium persulfate and left to react for 4 hours, after which the nanoparticles were purified by dialysis, lyophilized, and stored at room temperature for future use. The poly(NIPAM-co-AEMA-co-HEMA) nanoparticles were characterized using dynamic light scattering to determine their zeta potential, polydispersity, and hydrodynamic diameter. After successful synthesis and characterization, the synthesized nanoparticles (0.25-1.0 mg/mL) were used to test the viability of MG-63 and U2-OS cells under standard cell culture conditions for up to 24 hours. Controls were cells cultured in parallel under similar conditions, but not exposed to the nanoparticles. At the prescribed time point, cell viability was determined using the CellTiter 96 Aqueous One Solution Cell Proliferation assay. Dynamic light scattering results revealed that in 0.1X PBS nanoparticles are positive in surface charge, monodisperse, swollen at room temperature, and collapsed at body temperature. Cell exposure to nanoparticles resulted in grater than 80% viability at all concentrations of nanoparticles tested. Moving forward, the osteoblast-like cells can be further studied in conjunction with various biomaterials for applications in tissue engineering and drug delivery.

Poster #: 55

Development of Chemical Tags for Universal Lipid Nanoparticle Visualization and Tracking in 2D and 3D Imaging

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To achieve effective disease diagnosis and treatment, precise delivery of therapeutic agents to specific cells or sub-cellular compartments is crucial. Lipid nanoparticles have emerged as promising nano-carriers for drugs and genes. However, understanding their transport and distribution within cells and tissues post-administration remains limited due to the lack of methods for visualizing and tracking them at high resolution or over large tissue volumes. In this project, we developed a fluorescent peptide tag for lipid nanoparticles tailored for compatibility with visualization and tracking using advanced optical fluorescence imaging techniques that involve lipid disruption steps, such as cell permeabilization or tissue clearing. We named these tags SPARKLE (Strategic Peptide Anchored, Retained, and Kept after Lipid Elimination). Lipid nanoparticles labelled with SPARKLE tags can be crosslinked and fixed within cells and tissues. In contrast to conventional lipophilic dye tags that wash out during lipid disruption, the SPARKLE tag will be retained and fluorescently mark the position of lipid nanoparticles. Initially, we optimized the design and conjugation scheme of the SPARKLE tags. Subsequently, as proof of concept, we demonstrated the utility of SPARKLE tags by tracking lipid nanoparticle uptake in vitro within cells using the cell painting assay, and within tissues using CLARITY tissue clearing and 3D lightsheet microscopy. We anticipate that SPARKLE tags will provide insights into how lipid nanoparticles interact with biological environments in the body, aiding in understanding their journey to the desired target and the release of cargo, ultimately benefiting patients.

Poster #: 56

WITHDRAWN

Poster #: 57

Near-Infrared Afterglow Luminescence Amplification via Albumin Complexation of Semiconducting Polymer Nanoparticles for Surgical Navigation in Ex Vivo Porcine Models

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Afterglow imaging, leveraging persistent luminescence following light cessation, has emerged as a promising modality for surgical interventions. However, the scarcity of efficient near-infrared (NIR) responsive afterglow materials, along with their inherently low brightness and lack of cyclic modulation in afterglow emission, has impeded their widespread adoption. Addressing these challenges requires a strategic repurposing of afterglow materials that improve on such limitations. Here, we have developed an afterglow probe, composed of bovine serum albumin (BSA) coated with an afterglow material, a semiconducting polymer dye (PFODBT/SP1), called BSA@SP1 demonstrating a substantial amplification of the afterglow luminescence (∼3-fold) compared to polymer-lipid coated PFODBT (DSPE-PEG@SP1). This enhancement is believed to be attributed to the electron-rich matrix provided by BSA that immobilizes SP1 and enhances the generation of $^1O_2$ radicals, which improves the afterglow luminescence brightness. Through molecule docking, physicochemical characterization, and optical assessments, we highlight BSA@SP1's superior afterglow properties, cyclic afterglow behavior, long-term colloidal stability, and biocompatibility. Furthermore, we demonstrate superior tissue permeation profiling of afterglow signals of BSA@SP1's compared to fluorescence signals using ex vivo tumor-mimicking phantoms and various porcine tissue types (skin, muscle, and fat). Expanding on this, to showcase BSA@SP1's potential in image-guided surgeries, we implanted tumor-mimicking phantoms within porcine lungs and conducted direct comparisons between fluorescence and afterglow-guided interventions to illustrate the latter's superiority. Overall, our study introduces a promising strategy for enhancing current afterglow materials through protein complexation, resulting in both ultrahigh signal-to-background ratios and cyclic afterglow signals.

Poster #: 58

Molybdenum disulfide Nanoflowers Promote Intercellular Mitochondrial Transfer

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Common pathologies of human disease can be traced back to the dysfunction of each and any of the organelles that make up our cells. In recent years, the phenomenon of intercellular organelle transfer has been explored in a number of cell types and variations such as a mitochondrion originating in one cell being transported to another. Cell-to-cell mitochondrial transfer plays an essential role in regulating system development and maintaining normal tissue homeostasis under physiological and even rescue in pathological conditions. The phenomenon of intercellular organelle transfer has been explored in a number of cell types and variations such as a mitochondrion originating in one cell being transported to another. Mitochondria play a central role in cellular metabolism; thus, not surprisingly, its dysfunction is associated with the pathology of several common pathologies including neurodegenerative diseases, pulmonary fibrosis, and cardiomyopathies. The objective of this proposal is to explore the possibility of transforming cells into bio-factories of organelles, eliminating the need for treatments that are limited to temporary relief and replace dysfunctional organelles entirely. We have recently designed and characterized a novel molybdenum (Mo) disulfide (MoS2) nanoflower with predefined atomic vacancies for biological applications. We found that these particles have the unique ability to increase mitochondrial respiration and copy number in treated cells through atomic vacancies in the structure. This allows cells to act as factories for mitochondrial biogenesis, a low cost and low barrier-to-entry method to increase mitochondrial numbers. Preliminary data suggests that we can effectively engineer cells to produce healthy, energetic mitochondria in excess and deliver them to other cells through isolation and co-culture.

Poster #: 59

Functionalized Hybrid Nanoparticles for Reactive Oxygen Species Scavenging in the Central Nervous System

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At the cellular level, reduction-oxidation reactions are vital to energy generation pathways such as oxidative phosphorylation. During these processes, nicotinamide adenine dinucleotide (NAD+) works as an upstream regulator for maintaining homeostasis with the concentration of Reactive Oxygen Species (ROS) that are formed. However, in some cases the concentration of ROS exceeds the physiological norm, resulting in oxidative stress which can damage surrounding cell functions. This leads to an upregulation of the inflammatory response that can impact a variety of autoimmune diseases, including Multiple Sclerosis (MS). It has been shown that patients with MS have significantly lower concentrations of NAD+ combined with high ROS levels. Current treatment methods for autoimmune and neurodegenerative diseases focus on the management of symptoms, but there is a distinct lack of solutions that aim at developing methods to reverse the disease state. By creating a hybrid nanoparticle capable of targeting ROS within the central nervous system (CNS), promise exists for potential treatments to return functionality in the CNS.

For this reason, gold nanoparticles, known for their nano-catalytic properties, were formed by combining gold chloride and trisodium citrate. The molar ratio of trisodium citrate to gold chloride were varied from 1:1 and 4:1 to affect diameter. Then, a polymer shell coating was added to the gold nanoparticles to act as further ROS scavengers with the polymeric formulation varying to test polydopamine, polythioesters, and polypropylene sulfide. Lastly, the nanoparticles were functionalized with L-DOPA (a neurotransmitter precursor) to allow for interactions with the large amino acid transporter 1 (LAT1) that is abundant on brain endothelial cells to promote passage through the blood brain barrier. Monodisperse particles were synthesized and confirmed using dynamic light scattering, transmission electron microscopy, and scanning electron microscopy. Future studies will investigate ROS scavenging, compatibility with oligodendrocytes, and nano-catalyst functionality of the nanoparticles.

Poster #: 60

Cellular and Noncellular Influences on Lipid Nanoparticle Tropism in Liver

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Hepatocellular carcinoma (HCC) is currently ranked as the second leading cause of cancer-related mortality worldwide, causing more than 700,000 deaths a year. The liver is important in blood filtration and metabolism and for this reason is a prime target for drug delivery, with lipid nanoparticles (LNPs) showing promise as carriers due to their natural hepatic tropism. However, LNP efficacy is decreased in HCC due to the limited understanding of liver physiology in cancerous environments. This project proposes to address this gap by using a tissue-clearing technique known as CLARITY along with a chemically tagged LNP for comprehensive visualization of LNPs in the liver using 3D optical microscopy. CLARITY allows whole organ 3D fluorescent imaging, offering a better perspective into LNPs' interaction with cellular and non-cellular components. First, we aim to optimize the chemical tags on the LNPs to make them compatible with the CLARITY tissue-clearing technique. Next, we apply our chemically tagged LNPs to quantify LNP accumulation in altered physiological states, including fibrosis, cirrhosis, and HCC. The project's success will be measured by achieving high-quality images covering at least 50% of the entire liver while preserving cellular and fluorescence components, blood networks, immune cells, and the intact LNP chemical tag. Beyond the scientific contributions to nanoparticle research, this project holds broader implications. It provides insights into liver physiology in cancer and can be applied to study LNP localization and efficacy in other organs and diseases.

KEYWORDS: lipid nanoparticles, hepatocellular carcinoma, drug delivery, tissue clearing, biodistribution, therapeutic delivery, light-sheet imaging, chemical tag, liver tropism.

Poster #: 61

WITHDRAWN

Poster #: 62

Cytocompatibility and Intrinsic Immunomodulation of Poly (Methacrylic Acid) Co-Polymer Nanogels to Macrophages

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Nanoscale hydrogels (nanogels) are of significant research interest in the field of drug delivery due to their biocompatibility, hydrophilic nature, and modular properties. In context of inflammatory diseases, the physical interactions between nanoparticles and immune cells, and their ability to provoke an immune response can have major impact on the safety and efficacy of a therapeutic. These effects are specifically relevant for macrophages due to their inherent ability to phagocytize nanogels and modulate the immune response. In this project, we sought to develop a biodegradable nanoscale hydrogel library and assess how different polymer formulations influence macrophage viability and phenotype.

We synthesized a biodegradable hydrogel library, where nanogels were co-polymerized from different monomer feed ratios of methacrylic acid (MAA), hydroxyethyl methacrylate (HEMA), and acrylamide (Aam) by inverse emulsion free radical polymerization. We characterized the hydrodynamic diameter and zeta potential of the nanogels using dynamic light scattering (DLS), and their chemical composition using Fourier-Transform Infrared Spectroscopy (FTIR). Toxicity to and metabolic activity of Raw264.7 macrophages was assessed using LDH and MTS assays after a 48-hour incubation with up to 1.5mg/mL of nanogels.

DLS data indicated that all nanogel formulations were within the range of 70-100nm in diameter. LDH assay showed that no formulation exhibited significant dose-dependent cytotoxicity when compared to untreated groups ($p>0.05$). MTS assay showed a 25% metabolic activity increase with increasing treatment concentrations of p(HEMA-co-MAA) and p(HEMA-co-Aam-co-MAA) formulations. Preliminary data did not show a significant increase in the expression of CD86, MHCII, and CD206 markers on Raw 264.7 macrophages when compared to untreated group after a 24-hour incubation with 0.5 mg/mL of p(Aam-co-MAA) formulations.

These findings indicate that nanogels' composition directly affects their physicochemical characteristics and leads to altered metabolic activity of nanogel-loaded macrophages. These data provide an initial framework to predict macrophage viability and phenotype from the known physicochemical characteristics of new nanogel formulations.

Poster #: 63

Geometry and Surface Modification Effects on the Sensitivity of Anisotropic Gold-Hydrogel Biosensors

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Biosensors are analytical devices that use a transducing component to emit a signal based on a molecular recognition event between a bound receptor and identifying biomarker/analyte. Of the various transducers that have been used for developing biosensors, gold nanoparticles (AuNPS) have been increasingly popularized in the last two decades. This popularity can be attributed to their optical properties including localized surface plasmon resonance (LSPR). The LSPR is dependent on many factors including geometry, composition, and the refractive index of the surrounding medium. Due to the high refractive index sensitivity of the AuNPs, analyte binding results in LSPR shifts, acting as a "label-free" biomarker detector.

While there have been several studies investigating sensitivity of gold-based biosensors, there still lacks a comprehensive study on how the plasmonic resonances are affected by the sensing environment and the binding dynamic of biomolecules in anisotropic geometries. In this work, we investigated the influence of various factors on hybrid gold nanoparticle-hydrogel based biosensors in the pursuit of increasing their sensitivity towards detecting the proteins bovine serum albumin (BSA) and immunoglobulin G (IgG). The gold biosensors consisting of anisotropic AuNPs (e.g., bipyramids, nanorods, nanostars) were coupled to/embedded in charged poly(N-isopropylacrylamide) (PNIPAM)-based hydrogels through functionalization methods such as, self-assembled monolayers (SAM) and polyelectrolyte coating. These surface modifications were explored to compare the effect each geometry will have on the LSPR and their influence on the hybrid biosensor's ability to detect surrounding proteins. At each stage of the development of these biosensors, the sensitivity of the sensing capabilities of the LSPR were compared and analyzed. It was found that the AuNPs alone were limited in their detection of the proteins and prone to aggregation. Once functionalized with the surface modifications and hydrogels we observed less issues with aggregation and greater shifts in the LSPR wavelength at lower concentrations of the proteins. These results obtained facilitate an evolving study optimizing plasmonic-based sensing through AuNP geometry and functionalization.

Poster #: 64

WITHDRAWN

Poster #: 65

Drug Delivery Platforms for the Treatment of Ocular Diseases

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Posterior eye segment diseases affect millions of people worldwide and, unfortunately, the gold-standard treatment that is used for most is in the form of intravitreal injections. In addition to being highly inconvenient and uncomfortable, requiring highly skilled ophthalmologists to administer them creates an important source of inequity in eyecare access. As a result, the ocular field is increasingly invested in developing topical treatments for such diseases as a less invasive and more accessible alternative. However, a key challenge to topical formulations is that the bioavailability of drugs administered to the eye via topical formulations is very low, with only 5-10% of the active therapeutic entering the eye. To address this challenge, our goal is to develop a topical drug delivery platform capable of increasing drug bioavailability. Specifically, in this study we have optimized mucoadhesive and thermosensitive biomaterial formulations for their use in topical ocular drug delivery.

In situ gel formulations were fabricated using Pluronic F-127 as a temperature sensitive material. Chitosan, alginate, and poly(acrylic acid) were selected as mucoadhesive agents and were incorporated into the Pluronic F-127 material at different concentrations to obtain mucoadhesive and temperature sensitive formulations. The formulations were evaluated for optical transparency using ultraviolet-visible spectroscopy. Additionally, their viscoelastic behavior was analyzed using rheology. Lastly, release of a model therapeutic from the formulations was analyzed using side-by-side diffusion cells and dialysis.

As expected, gelling temperature of the biomaterial formulations decreased as the concentration of Pluronic F-127 increased. All the formulations had excellent optical properties with light transmittance values above 80% and the presence of the 3 mucoadhesive agents showed minimal impact on both the gelling temperature of the formulations and their optical properties. The diffusional release profiles obtained show that these in situ gel formulations have the potential to retain drugs for a longer time than normal saline based formulations.

Developing these formulations is a critical milestone in the development of topical formulations for the treatment of posterior eye segment diseases. Future work will seek to incorporate nanoparticles in the formulations to further optimize drug delivery and to develop a more targeted and retinal specific approach.

Poster #: 66

Matrix mechanics and external mechanical stimuli: two foundational elements for bone regeneration

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Osteogenesis and the simultaneous development of a blood vessel network are foundational interconnected events necessary for bone regeneration. The mechanical environment of bone, both internal matrix mechanics and external mechanical stimuli, play a crucial role in bone regeneration. Under normal physiological conditions, bones experience load and strain. Both mechanical stimuli are necessary for bone regeneration. However, little is known about their uncoupled effects. Thus, the objective of this study is to isolate and investigate the effect of load, with the use of hydrostatic pressure, and internal matrix mechanics, by varying scaffold porosity and casted gel composition, in bone regeneration. A well characterized trabecular bone model developed by our laboratory consisting of an 8x8 mm porous cylindrical hydroxyapatite scaffold was prepared by template coating and sintering. Scaffolds were made with varying porosities, 45, 60 and 80 pores per inch (ppi), and seeded with 400,000 Lewis rat derived Mesenchymal Stem Cells (MSCs)/scaffold. Microvascular Fragments (MVFs) were isolated from adipose pads of Lewis rats and seeded within the porous scaffolds in either 25:75, 50:50 or 75:25 fibrin:collagen gels at 20,000 MVFs/mL to provide a vessel source to support vascular network formation. Scaffolds were exposed to either static culture conditions or one of two levels of cyclic hydrostatic pressure, 20 or 40 kPa at 0.5 Hz, using the MechanoCulture TR (CellScale, Ontario, Canada) for up to 14 days. Supernatant media was collected after days 1,3,7,10 and 14 and Alkaline Phosphatase (ALP) activity assessed. At days 1, 7 and 14, scaffolds were fixed and stained with Lectin I to evaluate scaffold pore vascularization. A three-way ANOVA was conducted across pore size, gel composition and pressure at each time point, with Tukey's test for post-hoc analysis. Matrix mechanics (collagen:fibrin gel blend) seem to overwhelm the effect of pore size on osteogenic differentiation and vessel network formation. Results indicate a time dependent coordination between bone regeneration and vessel network formation, as demonstrated by how ALP concentration trends match those of vessel network formation associated parameters, independent of pore size and accelerated by higher applied biophysical hydrostatic stimulation.

Poster #: 67

Durable and Antimicrobial Electrospun Wrap for Bone Regeneration

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Grafting procedures used to treat large bone fractures are plagued with rapid graft resorption and persistent infection that inhibit graft consolidation. An engineered bone wrap loaded with antibiotics has the potential to reduce graft resorption by acting as a pseudo-periosteum and providing sustained infection control. To this end, an electrospun bone wrap was designed to support tissue integration and prolong the release of antibiotics. A wrap was electrospun from polycaprolactone (PCL) with a range of fiber diameters (0.3, 2, 5, and 10 µm) to obtain a balance of mechanical properties and tissue integration. To increase the fiber diameter, the flow rate, distance, and concentration of PCL were increased while the voltage was decreased. To fabricate 0.3 and 10 µm fibers, the solvent was changed from hexafluoroisopropanol to include 5% pyridine and 80:20 of chloroform and dimethylformamide, respectively. The 0.3 µm and 2 µm fibers exhibited limited cell infiltration due to reduced pore size. The 10 µm fibers exhibited a suture retention strength of less than 2.0 N (ISO 7198). However, the 5 µm fiber wrap supported cell infiltration throughout a 500 µm wrap depth after 4 weeks and met suture retention strength standards, thus balancing tissue integration and mechanical properties. Histological analysis of the 5 µm wrap inserted in the subcutaneous space of a Sprague Dawley rat revealed tissue integration within the top 50 µm after only 1 week. The chosen microarchitecture was loaded with 5wt% gentamicin and tested using a Kirby Bauer disk diffusion assay for antibacterial efficacy. The loaded wrap created a zone of inhibition greater than 17 mm in osteomyelitis-specific methicillin-susceptible Staphlyococcus aureus (MSSA), showing retained antimicrobial activity after electrospinning. The loaded wrap released gentamicin above the minimum bacterial concentration (1.5 µM) for up to 8 weeks. In the future, a loaded wrap inserted in an osseous defect model for 6 weeks will assess tissue integration and infection control. Altogether, this electrospun wrap works to improve the durability of the induced membrane and infection control to avoid revision surgery and amputation.

Poster #: 68

Spatial Characterization over Time in a Rat Femoral Critical-Sized Defect Model

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Purpose/Objective: Bone critical-sized defects (CSD) are characterized by their inability to heal on their own without the aid of a bone graft or an adequate substitute. Defect size and wound environment are factors contributing to CSDs, yet little is known about the mechanisms that are inhibited and normally drive bone regeneration in most bone defects. Without this knowledge, CSDs may not be addressed adequately with tissue engineering methods. Here, we aim to address this gap in knowledge by investigating and tracking the temporal and spatial changes in morphology, cell presence, and bone growth in a 6 mm critical-sized femoral defect rat model.

Methodology: In 8 skeletally mature Lewis rats, 4 males and 4 females, a 6 mm CSD was created on the left femur and mechanically stabilized with a plastic PEEK fixator and k-wires. 4 rats were euthanized at 1 and 3 weeks and the affected femora excised. Collected femora were sectioned and stained with Movat Pentachome stain to observe changes in bone development and morphology. Slides were imaged at 40x and % area of cartilage, fibrin, hypertrophic chondrocytes, and bone was determined using the Trainable Weka Segmentation plug-in on ImageJ software. Each tissue section was vertically segmented into 10 1mm segments and analyzed individually for area of tissues of interest.

Results: At 1 week post-surgery, the defect consisted of cartilage, fibrin, and hypertrophic chondrocytes. Additionally, there was an increase in formation of these tissues on the proximal end of the defect compared to the distal side. At 3 weeks, there was an absence of a fibrin clot and hypertrophic chondrocytes within the defect and an increase in cartilage formation evenly distributed between the distal and proximal sides. The data shows a change in tissue presence and distribution over 1 and 3 weeks in the CSD model, and a lack of bone formation in the defect at 3 weeks which was expected. Conversely, hypertrophic chondrocytes, which are a pre-curser for bone development, are present at 1 week. Future and current work includes investigating sub-critical defect sizes to determine differences in bone remodeling between sub-CSD and CSD defects.

Poster #: 69

Optimizing a Brain Inspired Bioink for 3D-Printed Neural Models

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Neurological disorders such as neurodegenerative diseases and stroke are a leading cause of death and disability worldwide. Therapeutic development for neurological diseases is limited by inadequate models, which ideally should mimic the semipermeable nature of the human blood brain barrier (BBB) and interplay of different neural cells in the human brain parenchyma. New 3D in-vitro models comprised of human cells and engineered biomaterials are an alternative model, which can better mimic human brain anatomy, gene expression and cellular behavior than 2D cell cultures or rodent models. With the advent of three-dimensional (3D) bioprinting, it is now feasible to make bio-mimetic, personalized, functional tissue constructs; with precise spatial control over depositing a printable hydrogel or bioink. Herein, we engineered a brain-inspired bioink using a mixture of methacrylated hyaluronic acid (HAMA), methacrylated gelatin (GelMA), polyethylene glycol diacrylate (PEGDA) and poly-(lactic-co-glycolic acid) nanoparticles (PLGA NP) which mimics the brain extracellular matrix composition and stiffness while enabling local delivery of encapsulated drugs.

This brain-inspired hydrogel was optimized for application as a 3D-extrusion based printable bioink. Successful methacrylation of the HA precursor was confirmed by the presence of characteristic methacrylation peaks using NMR and FT-IR. Successful incorporation of all bioink components in cured gels was confirmed by FT-IR. Quantitative rheological assessments were conducted under compression and shear stress (i.e., separate frequency and amplitude sweeps) (n = 5 technical replicates). The compressive modulus of the hydrogels increased linearly with the GelMA concentrations in the hydrogel (0-10 wt%). Amplitude sweeps revealed a linear viscoelastic region (LVER) of up to 10% oscillation strain, after which the loss modulus (G'') superseded the storage modulus (G'), indicating fluid-like viscoelastic behavior necessary for printing. Cytocompatibility characterization of the hydrogel toward surface-seeded brain endothelial cells (hCMEC/D3, Milipore) was evaluated by LIVE/DEAD staining which revealed 98.9% ± 1.15%, 98.93% ± 0.93%, and 98.04% ± 0.26% viability at 24, 48, and 72 hours respectively, post-seeding and revealed no statistically significant differences (One-way ANOVA) amongst the hydrogel compositions. Thus, the brain-inspired bioink whilst maintaining high cell viability, can be used to fabricate viable functionalized 3D bio-printed constructs which combines aspects of neuroimmunology and additive manufacturing.

Poster #: 70

Co-Electrospinning a Multifunctional Wrap to Treat Infection and Improve Bone Regeneration

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The Masquelet Technique relies on an induced membrane to act as both a physical barrier to reduce graft resorption and guide bone regeneration. However, the membrane is vulnerable to tearing, persistent infection that inhibits graft consolidation, and receding vasculature following graft implantation. To address these complications, we have developed a multifunctional electrospun wrap consisting of two different fiber populations. Polycaprolactone (PCL) fibers loaded with gentamicin structurally reinforces the friable membrane and provides sustained antibiotic release over 8 weeks for infection control. Decellularized extracellular matrix (dECM) fibers from porcine small intestinal submucosa is used to enhance vascularization. To combine the benefits of each fiber population in a single multifunctional wrap, a method to co-electrospin PCL and dECM was developed. The dECM suspension and PCL solution were spun on opposite sides of a rotating collection mandrel in order to collect both fiber populations simultaneously. As the dECM fibers have a 5-times slower collection rate than the PCL, the dECM was spun from a custom 3D printed multi-nozzle adapter with five outputs to match the higher rate of the PCL. When positioned in parallel, the multiple positively charged dECM jets repelled and prevented collection. A positively charged ring is being designed to constrict the jet repulsion and facilitate collection. Composite wraps with targeted mass ratios of 1:5, 1:3, and 1:1 SIS to PCL will be spun with the composition of the mesh analyzed with Fourier-transformed infrared spectroscopy and selective mass loss studies. We will identify the optimal mass ratio that balances durability, infection control, and vascularity in vitro and a contaminated osseous defect model. The handling properties will be evaluated via tensile and suture retention testing and the angiogenic properties via a chorioallantoic membrane assay. The new co-spinning methodology developed here enables the fabrication of a multifunctional wrap that combines the necessary properties of PCL and dECM to address the medical shortcomings of the Masquelet Technique.

Poster #: 71

Pyridostigmine Bromide Exposure Results In Neuroinflammation Impairing Enteric Neural Stem Cell Regeneration In Gulf War Illness

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The enteric nervous system (ENS) is a central controller of gut motility. Nerves of the ENS are maintained and regenerated upon injury by p75+ enteric neural stem cells (ENSC). Gulf War Veterans suffer from significant disruptions in colonic motility, due to toxic exposure during combat that manifests as a multisymptomatic disorder called Gulf War Illness (GWI). In our previous work, we demonstrated acute neuro-inflammation within the ENS due to exposure to Pyridostigmine Bromide (PB), taken prophylactically against nerve gas attacks. However, Gulf War veterans have impaired colonic motility persisting decades after their transient toxic exposure during combat. Thus, we hypothesized that the regenerative ENSC compartment is altered upon toxic exposure to PB. The objective of our work is to demonstrate that neuro-inflammation extends to the p75+ ENSC compartment, persistently impairing the regeneration of the ENS in GWI. C57BL/6 mice were administered PB orally for 7 days and allowed 30 days to recover to mimic delayed symptom onset following exposure in combat leading to GWI. Colon explants from GWI mice were assessed for real time force generation (motility) and demonstrated a 36.87 ± 1.18% increase of force (hypercontractility). Histological and immunofluorescent preparations of whole colon mounts demonstrated a loss of βIII-Tubulin ENS networks (mature neurons), with an associated loss (50.38 ± 0.67%) in p75+ expression within the myenteric plexus. Also associated with p75+ ENSCs were a 4.72-fold increase of inflammatory CD40 expressing macrophages in GWI, compared to control. A multiplex cytokine array revealed a 2.46-fold increase of IL-6 within GWI mice and a 2.43-fold increase of inflammatory gene, Nos2, compared to control mice. Our results show that a significant loss of ENSCs is observed along with hallmarks of inflammatory insult in the colon. This loss of ENSCs translated to a lack of diverse subsets of enteric nerves required to maintain colonic motility. We observed hyper-contractility in colons, typically resulting in chronic diarrhea and other functional gastrointestinal disorders often observed in Gulf War veterans. Our future work will focus on isolating macrophage-enteric nerve-enteric glia crosstalk, to identify therapeutic targets to improve veteran quality of life.

Poster #: 72

The Enderstruder: An accessible open-source syringe extruder compatible with Ender series 3D printers

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Bioprinting enabled the precise spatiotemporal deposition of cell-containing bioinks, opening new avenues of research in tissue engineering and regenerative medicine. However, the widespread adoption of bioprinting technology is hindered by the high cost of commercial bioprinters and an expanding closed ecosystem, which has led to custom bioprinting modifications to 3D printers. Although several open-source extruder designs currently exist, only one extruder has been specifically adapted for the affordable open-source Ender series, a line of machines that are widely available. Here, we introduce the Enderstruder, a cost-effective extruder attachment that uses a standard 10 mL BD syringe, positions the stepper motor at gantry level, enhances x-axis stability with a linear rail, and uses the originally included stepper motor, resulting in simplified assembly. The Enderstruder consists of a 3D printed core, syringe carriage, and herringbone gears. Alongside the Enderstruder, we present a rigorous iterative process to fine-tune printing profiles for high-viscosity biomaterial inks. To help implement our work, we also provided fully editable Cura profiles for five commonly used biomaterials. We then employed the Enderstruder to print established calibration patterns as well as complex shapes using these bioinks. Printability assessments were conducted using ImageJ, focusing on analyzing the circularity value of pores in logpile lattices present in the calibration prints. We observed results that aligned with those in existing literature, validating the applicability of our design. In future iterations we will add a UV light and temperature control to handle photosensitive polymers that are viscous at room temperature.

Poster #: 73

The application of iPSC-derived hMSC secreted products in a tissue-engineered tendon scaffold for ACL reconstruction

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Anterior cruciate ligament (ACL) tears account for 50% of knee injuries. While reconstruction can restore stability, 25% of patients undergo surgical revision due to poor graft healing. Two processes of graft healing include tendon graft-to-bone tunnel healing and graft mid-substance remodeling. However, excessive inflammation and insufficient bone tunnel engraftment inhibit healing. While autografts are common for reconstruction, donor site morbidity negatively impacts patient quality of life. Therefore, incorporating anti-inflammatory and osteoinductive therapies into a tissue-engineered scaffold would eliminate the need for an autograft and drive graft healing. In this study, we designed a decellularized tendon scaffold, incorporating human mesenchymal stem cell (hMSC)-derived osteogenic extracellular matrix (OCM) or anti-inflammatory extracellular vesicles (EVs) into a coating of nanoengineered ionic-covalent entanglement (NICE) hydrogel. Previous studies reported the superior osteogenic potency of OCM to bone morphogenic protein 2 (McNeill, 2020), and the immunosuppressive effects of hMSC-derived EVs (Hai, 2018). The incorporation of OCM into NICE was also established (Sears, 2020). To create the scaffold, rabbit tendons were decellularized using trypsin/EDTA and a lysis buffer of peracetic acid and Triton X-100. OCM or EVs were incorporated into NICE, coated on the scaffolds, and crosslinked using UV light. Decellularization was confirmed through the absence of cell nuclei in H&E-stained sections and the negligible presence of genetic material determined by DNA quantification. High neutral red and MTS uptake in primary rat tendon cells cultured with the scaffold confirmed cytocompatibility. H&E staining was used to visualize and determine coating thickness. Immunofluorescence was used to visualize the incorporation of OCM or EVs into NICE and confirm even distribution. Pulling a NICE-coated scaffold through a mock bone tunnel confirmed that NICE coatings can withstand the reconstruction procedure. This study presents the incorporation of osteogenic and anti-inflammatory hMSC-derived cell products into a decellularized tendon scaffold for ACL reconstruction using NICE coatings. Future work will characterize the therapeutic efficacy of incorporated therapies in vitro and in vivo, using a rabbit ACL reconstruction model.

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Poster #: 74

Designing Cost-effective Open-source High Resolution Multi-Material DLP Bioprinters

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Bioprinting is an emerging and promising method to print structures using biomaterials. A significant problem that stagnates exploration in bioprinting is the printing limitations associated with the commercially available bioprinters and their high costs. Digital Light Processing (DLP) printing has revolutionized various industries due to its ability to produce complex geometries with high precision. However, the commercially available DLP printers have limited features that can't be modified and are expensive. To address these issues, this project details how to construct an affordable multi-material high-resolution custom DLP printer. The printer was made by purchasing different parts online and generating necessary custom parts using an FDM printer. A custom software was created using LABVIEW to enable a range of controls on the custom DLP printer. The customized printer is capable of printing different inks at different points along the same layer and at different points at other layers. Furthermore, it allows the printing of a structure of varying stiffness. This finding will help overcome some major limitations of DLP printing and will allow the printing of many complex geometries.

Poster #: 75

Emulsion-Based Synthesis of PEG Microparticles using Michael Addition Chemistry

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Hydrogel microparticles have emerged as versatile tools in biomedical research and clinical practice, facilitating therapeutics such as targeted drug delivery, cell encapsulation, and scaffold formation for tissue repair. Current methodologies for hydrogel microparticle fabrication, such as microfluidics, micropatterning, and electrospraying, demand costly materials and significant energy expenditure, limiting scalability. The objective of this project was to develop a scalable one-pot synthesis method for PEG microparticles using light mineral oil as the continuous phase and study the effects of different variables on various particle properties. Using emulsion as our fabrication method, coupled with mineral oil as the continuous phase, enables the production of a substantial quantity of particles, alleviating cost concerns. To fabricate PEG microparticles using Michael addition chemistry, a PEG precursor solution was prepared using 4-arm 20kDa PEG-VS, 4-arm 10kDa PEG-SH, and 50mM TEOA adjusted to a pH of 5. This solution was then vigorously vortexed for 30 seconds in an organic phase consisting of mineral oil with 1% SPAN 80 and 0.2 M TEA. Subsequently, the mixture was promptly placed on a nutator for 30 minutes. The particles were then washed twice with 70% ethanol and twice with PBS. From the results, we found that the minimum thresholds for particle formation were 0.2M TEA and a vortex speed of 7.5. Significant differences in particle size were observed with variations in oil:water ratios, PEG concentrations, and thiol:ene ratios. However, no significant differences were noted in particle size with changes in vortex speed or SPAN 80 concentrations. Trends indicated that particle size tended to increase with higher PEG concentrations, oil to water ratios, and thiol:ene ratios. The microparticles synthesized through this one-pot method offer versatility for various applications, such as encapsulating enzymes and drugs, 3D printing, and can also be used as building blocks for granular hydrogels. They can also be tailored based on the crosslinking chemistry, size, and stiffness requirements.

Poster #: 76

WITHDRAWN

Poster #: 77

3D Microphysiological System to Model Breast Cancer Tumor Migration through the Blood Brain Barrier and Evaluate the role of ICAM-1 and VCAM-1 in Metastasis

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Breast cancer is the most common cancer in women and the 2nd leading cause of cancer-related death. Breast cancer treatment outcomes have improved, but metastasis to the brain remains an issue, reducing quality of life and overall survival. According to the American Society of Clinical Oncology, breast cancer is the second most common cancer that metastasizes to the brain. Of patients who developed breast cancer metastasis to the brain, approximately 11.5% were related to the human epidermal growth factor receptor 2–positive (HER2-positive) breast cancer subtype. To reach the brain, metastatic breast cancer cells need to bypass the specialized and regulatory microvascular blood brain barrier (BBB). ICAM-1 and VCAM-1 are intercellular adhesion proteins utilized by migratory breast cancer cells to strongly adhere to the brain endothelial cells that comprise the BBB. Once bound, these breast cancer cells can then bypass the BBB by disrupting the tight junctions in between brain endothelial cells. In vivo animal models have limited ability to represent human physiology and are not easily scalable. Microphysiological systems allow for the creation of reproducible, high throughput 3D in vitro human tissue models. We hypothesize that the expression of CAM adhesion proteins will be altered in migratory HER2-positive SKBR3 breast cancer cells and HBEC-5i brain endothelial cells when cocultured inside a custom microfluidic device. Our preliminary data demonstrates that HER2-positive SKBR3 cells retain HER2 expression and aggregate when cultured in a 3D hydrogel microphysiological system (MPS). The goal of our project is to utilize fluorescent microscopy, flow cytometry, and real time reverse transcription polymerase chain reaction (rRT-PCR) to characterize the expression of ICAM-1 and VCAM-1 intercellular adhesion proteins following the interaction between HER2-positive SKBR3 breast cancer cells and HBEC-5i brain endothelial cells within a 3D microfluidic platform to study cell migration in vitro. Future applications of this breast cancer

MPS include incorporation of patient-specific tissue models and evaluation of anti-metastatic chemotherapeutics for personalized medicine approaches.

KEYWORDS: breast cancer, brain metastasis, blood brain barrier (BBB), microfluidic, 3D in vitro model, microphysiological system (MPS)

Poster #: 78

Shape-dependent granular hydrogel-based bio-ink for extrusion bioprinting

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Jammed microgel-based inks have overcome the limitation of bio-ink choices in extrusion printing by allowing the printing of many soft biomaterials with self-supporting characteristics and improved biological functions. However, the reported jamming properties that control the flow within the printing nozzles are limited to spherical microgels. Some critical limitations due to the sphericity of the microgels are poor scalability, poor versatility, and the need for oil and additives. The arrangement of spherical microgels in a close-packed lattice restricts the interaction between individual spheres and prevents any directional variation in the printed material. Here, we present an in-depth analysis of the jamming details of microgel-based inks developed using different complex structures of microgels. The digital light processing (DLP) printer was used to prepare different shapes of GelMA microgels. Jamming was performed using an extrusion printer with different shapes and ratios of hydrogel microparticles. The results of this study could be utilized to enhance the ability to print hydrogel microparticles. They will provide an essential toolbox for the 3D bioprinting of architected hydrogels on a larger scale.

Poster #: 79

Dynamically Cross-Linked Granular Hydrogels for 3D Printing and Therapeutic Delivery

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Granular hydrogels, or densely packed microgel fragments, have recently emerged as promising biomaterials for tissue engineering and 3D-printing applications. These materials address the limitations of bulk hydrogels while exhibiting desirable properties such as injectability and high porosity. However, their structural stability can be improved with post-injection interparticle cross-linking. In this study, we developed granular hydrogels with interparticle cross-linking through reversible and dynamic covalent bonds. The reversible crosslinking between microgel fragments allows for efficient shear-thinning and self-healing properties as microgels will separate in response to high shear during injection and the crosslinks will reform once at rest. To achieve this, chondroitin sulfate was first functionalized with methacrylate groups (CSMA), followed by the separate addition of aldehyde groups and hydrazide to form CSMA-ALD and CSMA-ADH. Mixing the two microgels together facilitates crosslinking through reversible hydrazone bonds. Confocal and fluorescence imaging were performed to determine particle size and

porosity. Strain and shear rate sweeps as well as strain recovery tests were performed using rheology. Injection force was quantified for different needle sizes using a mechanical tester. Extrusion printing was used to print 2D shapes with high fidelity in addition to multilayer geometries. The ability of the granular hydrogels to load and release biomolecules was assessed using FITC-BSA as a model. Finally, the granular hydrogel ability to attract cell infiltration and maintain viability was assessed in vitro. Based on our findings, the interparticle crosslinking through dynamic covalent hydrazone bonds led to significant improvements in granular hydrogel mechanical strength, injectability, and self-healing properties. Additionally, the use of a natural polymer in chondroitin sulfate allowed for positive cell interactions and its inherent charge facilitated high therapeutic loading and sustained release. Overall, this granular hydrogel is overall a promising tool for tissue engineering applications as it can be injected with low force to preserve encapsulated cells, in addition to its ability to load and release therapeutics.

Poster #: 80

Modelling PD using Fibrinogen coated Scaffolds for the Differentiation of Human Neural Progenitor Cells into Dopaminergic Neurons

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Parkinson's disease (PD), a progressive central nervous system disorder characterized by involuntary movements, stands as a challenge in neurodegenerative research, whose main cause is the relentless degeneration of dopaminergic (DA) neurons within the central nervous system. A viable option for studying the early onset and pathogenesis of Parkinson's disease is to take advantage of the potential of in-vitro modeling approaches developed using DA neurons derived from human induced pluripotent stem cell (hiPSC)-derived neural progenitor cells (NPCs). Therefore, our team utilized electrospun (ES) nanofibrous polycaprolactone (PCL) scaffolds in facilitating the adhesion and targeted differentiation of hiPSC-NPCs, into dopaminergic (DA) neurons. This study aimed to explore the efficiency of ES PCL scaffolds coated with extracellular matrix (ECM)-based biomaterials, including Cell Basement Membrane (CBM), Matrigel, and Fibrin (in a diluted and more concentrated form), towards promoting the differentiation and maturation of NPCs into a mature DA neuronal phenotype. We hypothesized that the ES PCL scaffolds coated with diluted fibrin would enhance the differentiation and maturation of NPCs into DA neurons, in comparison with other biomaterial coatings. The ES PCL scaffolds only, were characterized using Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Fourier Transform Infrared Spectroscopy (FTIR-ATR) to study the scaffold’s physicochemical properties. For cell adhesion and differentiation, NPCs were seeded on the ES PCL scaffolds after which they were differentiated and allowed to mature into DA neurons. To confirm their differentiation into a DA neuronal phenotype, immunocytochemistry targeting Tyrosine Hydroxylase (TH), an enzyme that catalyzes the conversion of L-tyrosine to L-Dopa, was performed to analyze the level of this DA marker expression as well as SEM which was performed to validate the differentiation of cells and elucidate any morphological alterations. Additionally, microelectrode array (MEA) wells were utilized to record neuronal firing and confirm

communication signals from differentiated and matured DA neurons. Preliminary results show increased cellular processes as well as increased TH expression in cells differentiated atop fibrin-coated scaffolds. This ongoing study will yield an advanced tissue-on-a-chip model for studying neurodegeneration in DA neurons using both healthy and diseased phenotypes.

Poster #: 81

Suspension Electrospinning of Decellularized Extracellular Matrix: A New Approach to Retain Bioactivity

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Decellularized extracellular matrices (dECM) have strong regenerative potential due to their complex composition. However, current clinical options for dECM are limited to freeze-drying its native form into sheets. Electrospinning is a versatile scaffold fabrication technique that allows control of macro- and microarchitecture. It remains challenging to electrospin dECM while maintaining bioactivity. This has led researchers to either blend it with synthetic materials or use enzymatic digestion to fully solubilize the dECM. Both strategies reduce the innate bioactivity of dECM and limit its regenerative potential. Our lab has developed a new method of suspension spinning that does not require additives and preserves bioactivity. Systematic study of electrospinning parameters was used to identify key processing steps and rheological properties necessary to fabricate dECM fiber scaffolds with retained regenerative capacity. Small intestinal submucosa (SIS) was decellularized using standardized protocols and electrospinning suspensions prepared using hexafluoroisopropanol. Homogenization was utilized to enhance particle interaction and increase elastic behavior of the suspension. Rheological analysis shows that the increase in particle interaction creates a viscoelastic solid that persists to larger strain amplitudes, facilitating electrospinning. A crossover strain amplitude of the loss and storage modulus greater than 100% was a strong predictor of dECM suspension spinnability. The versatility of the dECM suspension electrospinning method was demonstrated by electrospinning SIS with three common decellularization techniques (Abraham, Badylak, and Luo) and dECM derived from three tissue origins (intestinal submucosa, heart, and skin). Bioactivity retention was indicated by a similar cell response between the SIS sheet and electrospun SIS for all assays. For angiogenic properties, an endothelial cell tube formation assay and a chorioallantoic membrane assay both showed the retention of angiogenic capacity. To examine immunomodulation, a macrophage-SIS contact and subsequent gene expression study was performed. Macrophage activation was similar in native and electrospun SIS, with a preference towards tolerogenic

polarization (IL10, CHI3L3, VEGF). Interestingly, macrophages contacting electrospun SIS expressed 10-fold more TGFβ. Collectively, this work provides a user's guide to electrospin dECM with bioactivity retention without the use of additives. This has broad utility in tissue engineering and regenerative medicine applications.

Poster #: 82

Microgel-Embedded DLP Bioink for Biomedical Applications

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Digital Light Processing (DLP) utilizes photopolymerization for 3D printing by projecting 2D images onto a photosensitive polymer using UV light, enabling the rapid fabrication of complex constructs. It offers the advantage of restoring, repairing, or regenerating tissues and organs. In DLP printing, adjusting the mechanical properties of bioinks can improve the stability of printed constructs and better replicate the characteristics of specific tissues. However, most DLP bioinks are based on single polymers, which have limitations in modulating properties, thereby limiting their versatility. To overcome these challenges, previous studies have focused on tuning mechanical properties by using emulsion-based bioinks, but the polydispersity of microgels generated through emulsion-based methods limited the reproducibility of reliable results. In this study, we employ a novel microfluidic technology to prepare microgels and subsequently integrate them into a bioink matrix to reinforce better mechanical properties, leading to higher stability of the printed construct. Owing to its remarkable printability and biocompatibility, Gelatin methacryloyl (GelMA) has been selected for preparing the microgels. We varied the volume fraction of microgels added to the ink to assess the effects on printability, gelation time, and compressive modulus. The results indicated that with higher volume fractions of microgel, the printability and the compressive modulus decrease, while gelation time increases. Further, we employed computational simulations to analyze how printed structures perform under compressive force with varying volume fractions of microgels in the bioinks. Additionally, cells will be encapsulated within the microgel structures to engineer bioinks with enhanced biological functionality. We hypothesize that microgels offer a protective microenvironment for encapsulated cells, shielding them from external stresses, which will eventually lead to enhance their viability during and after the bioprinting process. Our study aims to address the challenge of rapidly preparing bioink with different properties that are required for the 3D printing of multilayer materials by introducing microgels into the bioink matrix. Through computational simulations and cell encapsulation within microgels, our approach provides a more intuitive and holistic demonstration of the influence of microgels on the mechanical properties of printed structures, facilitating the fabrication of cell-laden, multilayer structures with higher stability for various biomedical applications.

Poster #: 83

Impact of Cardiac-Vascular Crosstalk on the Maturity of hiPSC-derived Cardiomyocytes in 3D Printed Cardiovascular Organoids

Sogu Sohn, The University of Texas at Austin

Janet Zoldan, The University of Texas at Austin, zjanet@utexas.edu

Cardiovascular disease (CVD) has been the leading cause of death in the United States and much of the developed world for decades. This significant impact on public health necessitates the development of preclinical models that accurately recapitulate mature human heart function. Accordingly, there is a significant need for models of human cardiac tissue that elucidate the cell signaling pathways that drive normal CM development and reflect the physiological behavior of native, mature cardiac tissue. One approach for generating such models is using human induced pluripotent stem cells (hiPSCs) to generate hiPSC-derived cardiomyocytes (hiPSC-CMs). However, clinical relevance of hiPSC-CM models is greatly limited by several factors, with a principal limitation being the immaturity of hiPSC-CMs, as they are functionally closer to fetal CMs than adult CMs. While various approaches have used chemical and mechanical stimuli to increase hiPSC-CM maturity, such approaches largely focus on CM-only systems and fail to capture the importance of vasculogenesis, which coincides with maturation of cardiac tissue in embryonic development. As such, while hiPSCs have shown potential in cardiac tissue modeling applications, it is critical that heterogeneity resulting from cardiac differentiation and immaturity of hiPSC-derived CMs be addressed in order to advance clinical relevance of engineered cardiac tissue. To address the immaturity of hiPSC-CMs, I propose utilizing Freeform Reversible Embedding of Suspended Hydrogels (FRESH) bioprinting to engineer cardiovascular organoids. The bulk volume of these 3D tissue constructs will be comprised of hiPSC-CMs while a perfusable channel running through their center will be lined with hiPSC-ECs. This design will allow the hiPSC-CMs in the organoid to receive mechanical stimuli via perfusion through the channel as well as biochemical stimuli from the hiPSC-ECs, mimicking signaling from vasculature that drives CM maturation in the developing heart.

Poster #: 84

Evaluation of In Situ Assembled Granular Hydrogel Scaffolds for Neural Progenitor Cells Delivery in a Severe Spinal Cord Injury Model

Yu-Chi Huang, Texas A&M University

Spinal cord injury (SCI) is a neurological condition associated with a significant socioeconomic impact on patients and the healthcare system due to minimal treatment options. Neural progenitor cell (NPC) engraftment is a promising treatment option for the acute injury phase. We have previously identified in situ assembled poly (ethylene glycol) (PEG)-based granular hydrogels as a novel biomaterial scaffold that is suitable for the delivery of NPCs to SCI lesions. Importantly, testing in a mild acute injury model showed that these PEG-based granular hydrogels have good biocompatibility and support NPC differentiation toward astrocytic and neural lineages. The objective of this study was to evaluate their performance for NPC delivery in a severe injury model. In brief, PEG microgels were synthesized by thiol-norbornene click chemistry which reacts 4-armed PEG-amide-norbornene with enzymatically degradable (KCGPQGIAGQCK) and cell adhesive peptides (CGRGDS). These microgels were annealed using PEG-tetra-methyltetrazine (4PEG-mTz), which crosslinked the microgels by inverse electron demand Diels–Alder (iEDDA) click chemistry. These granular hydrogels were characterized by rheology, porosity and enzymatic degradation. Subsequently, to evaluate their utility for NPC delivery in a severe injury model of SCI, testing was performed in a cervical hemisection murine model. Immunostaining analysis performed at 2-weeks post-implantation revealed that the granular hydrogels resulted in enhanced NPC engraftment in injured sites and significantly larger graft volumes compared to NPC delivery in a fibrin matrix, which is the standard delivery material. This work demonstrates that in situ assembled poly (ethylene glycol) (PEG)-based granular hydrogels with 4PEG-mTz annealing linker is a promising scaffold for enhancing the NPC delivery to treat SCI.

Poster #: 85

Inorganic Nanomaterial-based Expandable Shape-Memory Hemostat for Treatment of Non-Compressible Hemorrhage

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Introduction. Blood loss due to uncontrollable hemorrhage causes around 80% of battlefield casualties and 40% of deaths in civilian settings. Uncontrolled bleeding due to truncal, junctional, and noncompressible hemorrhage can significantly increase the mortality rate from 0.1% to 20%. Due to the inability to perform pressure dressing, mortality related to noncompressible hemorrhage increases by a factor of four compared to other battlefield injuries. Failure to perform rapid patient care and transport to a medical facility within 30 minutes of injury increases morbidity by nearly 50% during the prehospital period (Hickman, 2018). In addition, the lack of biodegradability and inefficiency in administering commercially available hemostats raises the need to develop an alternative strategy for noncompressible hemorrhage.

Materials and Methods. Both shape-memory polyurethane (SMP) foam and shear-thinning nanocomposite hydrogel, combining gelatin (1% w/v) and nanosilicates (2% w/v), were synthesized using previous protocols (Singhal, 2012, Gaharwar, 2014). Following rheological analysis and sterilization, the biocompatibility of the nanocomposites was assessed using human endothelial cells via Alamar Blue assay, and clotting time and amount of blood loss were quantified using a rat-liver laceration model, followed by H&E and PTAH staining.

Results and Discussion. The hydrogels demonstrated shear thinning properties, promoting successful infiltration within SMP foam. No change in FTIR spectra and volume expansion profile indicated no alteration of the elemental properties of the samples after UV sterilization. Approximately 8-fold volume expansion was observed within the first 120 seconds. The SMP foams demonstrated high biocompatibility with an average cell viability greater than 85%. Further, in vivo, clotting time and amount of blood loss of composite were found to be 52.33 ± 4.93 s and 0.47±0.16 g, respectively, which are significantly lower than the negative control with 409±45.31 s and 1.14±0.56 g respectively. H&E and PTAH staining confirmed aggregation of erythrocytes at the injury sites, demonstrating stable blood clot formation. Our future study will include both in vitro and in vivo analysis, with different injury models.

Conclusion. We have developed a highly expandable novel hemostat with remarkable hemostatic ability, demonstrating promising potential to combat noncompressible battlefield injuries.

Poster #: 86

Development of a Benchtop Pelvic Model for Testing Gynecological Devices

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Elizabeth Cosgriff-Hernandez, PhD - The University of Texas at Austin - cosgriff.hernandez@utexas.edu

The design and testing of gynecological devices are hampered by a lack of testing apparatuses that can be used to iteratively design devices. To address this critical need, we developed an anatomical and physiological benchtop testing apparatus that can simulate vaginal anatomy, temperatures, and pressures. We utilized this benchtop model to test the deployment and retention of a self-fitting vaginal stent. A 3D printed pelvis comprising of a cervix and vagina with labia was printed from biocompatible silicone (EcoFlex) at Lazarus3D™ based on a representative adolescent pelvic MRI. This model was encased in an acrylic chamber that enables testing of vaginal stent deployment (time, expansion) and retention under simulated pressure conditions. Heating tape was wrapped around the acrylic chamber to heat the vaginal canal to physiological temperatures. Temperature readings taken at three locations within the model (cervical, central, and introitus regions) were confirmed to be within physiological ranges (36°C 1°C). Pressures measured by the MizCure perineometer were within the range of pelvic floor muscle contraction (PFM) pressures reported clinically using this device (19 mmHg 1 mmHg). Utilizing this testing apparatus, we evaluated the design of a novel shape memory polymer (SMP) vaginal stent. The SMP stent can maintain a crimped shape for ease of insertion and expand upon changes in temperature and hydration to restore the vaginal lumen. Visualization of stent expansion and deformation was achieved through hysteroscopic imaging near the model introitus. Notably, the crimped SMP vaginal stent exhibited rapid expansion (~70% increase in cross-sectional area) within <5 minutes post-irrigation with warm water (~45°C), with minimal cross-sectional area reduction (8.3 7.0%, n = 3) under PFM contraction pressure. Stent diameter exhibited ~1% (1.1 7.5%, n = 3) decrease along the anterior-posterior dimension, with a corresponding 11% (11.0 3.6%, n = 3) increase distally. Results from this deployment study suggest that the SMP stent would successfully deploy and maintain the vaginal lumen under contractile forces in vivo. This study demonstrates the usefulness of this benchtop apparatus to model the temperatures and pressures of the vagina to facilitate robust design of future gynecological devices.

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September 19–20, 2024

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2024 Regional Symposia

Western Symposium: University of Colorado Denver | Anschutz

September 19 - 20, 2024

Program Agenda

Thursday, September 19, 2024

8:00 AM - 8:30 AM	Set-up
8:30 AM - 8:45 AM	Registration / Check-in
8:45 AM - 9:00 AM	Welcome by Site Chairs
9:00 AM - 9:30 AM	*Invited Talk I:* ***Katie Sikes, Colorado State University***
9:30 AM - 10:45 AM	Plenary Session I: ***Shana Kelley, Northwestern University*** ***Joel Collier, Duke University***
10:45 AM - 11:00 AM	Coffee Break
11:00 AM - 11:30 AM	*Invited Talk II:* ***Christopher Snow, Colorado State University***
11:30 AM - 12:00 PM	*Session I: Engineering Cells and Their Microenvironments*
12:00 PM - 1:00 PM	Lunch
1:00 PM - 1:30 PM	*Invited Talk III:* ***Roberta Sabino, University of Wyoming***

Program Agenda

Thursday, September 19, 2024 *(continued)*

1:30 PM - 2:15 PM	Session II: Drug Delivery & Immune Engineering
2:15 PM - 3:30 PM	Plenary Session II: ***Sarah Stabenfeldt, Arizona State Univeristy*** ***Danielle Benoit, University of Oregon***
3:30 PM - 3:50 PM	Break
3:50 PM - 5:00 PM	Session III: Tissue Engineering and Rapid-Fire Poster Presentations I
5:00 PM - 7:00 PM	Poster Session I

Program Agenda

Friday, September 20, 2024

8:00 AM - 8:25 AM	Registration / Check-in
8:25 AM - 8:30 AM	Welcome by Site Chairs
8:30 AM - 9:30 AM	*Invited Talks IV and V:* ***Karin Payne, CU Anschutz*** ***Sayantani Basu, Tolmar***
9:30 AM - 10:45 AM	Plenary Session III: ***Elazer Edelman, Massachusetts Institute of Technology*** ***Cynthia Reinhart-King, Rice University***
10:45 AM - 11:00 AM	Coffee Break
11:00 AM - 12:00 PM	Session IV: Immune Engineering
12:00 PM - 1:00 PM	Lunch
1:00 PM - 2:30 PM	Panel Discussion: Navigating Starting a Career in Academia or Industry - ***Panelists: Wyatt Shields University of Colorado, Boulder; Justin Schaffer, Colorado School of Mines; Vitaly Kheyfets, University of Colorado, Anschutz Medical Campus; Michael Mestek, Medtronic; Micheal Pink, Biodesix***

Program Agenda

Friday, September 20, 2024 *(continued)*

2:30 PM - 2:50 PM	Break
2:50 PM - 3:50 PM	*Invited Talks VI and VII:* ***Wyatt Shields, University of Colorado, Boulder*** ***Jessica Weaver, Arizona State University***
3:50 PM - 5:00 PM	Session V: Engineering Models of Type 2 Diabetes and Rapid-Fire Poster Presentations II
5:00 PM - 5:15 PM	Break / Walk to Poster Session
5:15 PM - 7:00 PM	Poster Session II
7:00 PM - 7:30 PM	Awards Ceremony

2024 Regional Symposia Chair:

Anita Shukla, PhD, Brown University

Western Regional Symposium Co-Chairs:

Nikki Farnsworth, PhD, Colorado School of Mines
Chelsea Magin, PhD, University of Colorado Denver | Anschutz

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Jason Burdick, PhD, University of Colorado Boulder
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Karin Payne, PhD, University of Colorado Anschutz
Melissa Reynolds, PhD, Colorado State University
Jessica Weaver, PhD, Arizona State University
Katie Boncella (SFB Student Chapter President), University of Colorado Denver | Anschutz

PLENARY SESSION I
Thursday, September 19

Dr. Shana Kelley
Northwestern University

PLENARY SESSION I
Thursday, September 19

Dr. Joel Collier
Duke University
(2024 Clemson Award for Basic Research)

PLENARY SESSION II
Thursday, September 19

Dr. Sarah Stabenfeldt
Arizona State University

PLENARY SESSION II
Thursday, September 19

Dr. Danielle Benoit
University of Oregon

PLENARY SESSION III
Friday, September 20

Dr. Elazer Edelman
Massachusetts Institute of Technology
(2024 Founders Award)

PLENARY SESSION III
Friday, September 20

Dr. Cynthia Reinhart-King
Rice University

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2024 Regional Symposia

September 19th - 20th

- **Northeast: Northeastern University**
- **Midwest: Case Western Reserve University**
- **Southeast: Georgia Institute of Technology**
- **Southwest: University of Texas at Austin**
- **Western: University of Colorado, Denver | Anschutz Medical Campus**
- **Northwest: University of Washington**

SESSION I: ENGINEERING CELLS AND THEIR MICROENVIRONMENT

11:30 AM - 11:45 AM

ENGINEERING PHOTO-ADDRESSABLE HYDROGELS TO INVESTIGATE FIBROBLAST PLASTICITY IN FIBROBLASTIC FOCI IN 3D

Mikala Mueller, University of Colorado Denver Anschutz

Mikala C. Mueller; Department of Bioengineering, University of Colorado Denver, Anschutz Medical Campus; mikala.c.mueller@cuanschutz.edu, Alicia Vaquero Maria; Department of Bioengineering, University of Colorado Denver, Anschutz Medical Campus, Michael Nott; Department of Bioengineering, University of Colorado Denver, Anschutz Medical Campus; michael.2.nott@ucdenver.edu, Rachel Blomberg; Department of Bioengineering, University of Colorado Denver, Anschutz Medical Campus; rachel.blomberg@cuanschutz.edu

Chelsea Magin; Department of Bioengineering, University of Colorado Denver, Anschutz Medical Campus; Department of Pediatrics, University of Colorado, Anschutz Medical Campus; Division of Pulmonary Sciences & Critical Care Medicine, Department of Medicine, University of Colorado, Anschutz Medical Campus; chelsea.magin@cuanschutz.edu

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive interstitial lung disease that causes scar tissue formation within the interstitial region of the lung causing a rapid decline in respiratory function. The regions of active in the lung are called fibroblastic foci. Foci are characterized by aberrant, proliferative myofibroblasts that continuously deposit abnormal amounts of extracellular matrix proteins increasing the stiffness of the tissue causing a mechanotransduction feedback loop keeping the myofibroblasts present. Fibroblastic foci are distinct regions with volumes ranging from 1.6 x 104 µm3 to 9.9 x 107 µm3 and focus frequency ranging from 0.9 to 11.1 foci per mm3 with no evidence of interconnectedness between foci. To model the fibroblastic foci in vitro, we have engineered a biomaterial platform that enables dynamic stiffening and softening with precious control to replicate the 3D geometry of a fibroblastic focus. A photodegradable crosslinker was incorporated to study fibroblast plasticity in response to changes in matrix modulus and determine if decreasing the stiffness of the altered region also decreases the fibrotic activation. A 4-arm 5 kg/mol poly(ethylene glycol)-alpha-methacrylate macromer was crosslinked with an MMP9-degradable peptide and an o-nitrobenzyl ether-based photodegradable dithiol crosslinker. The hydrogels were initially measured to be within the range of healthy lung stiffness (1-5 kPa), were dynamically stiffened over 4-fold to mimic fibrosis with spatiotemporal control, and then can be softened back into the healthy range. Due to the arrangement of the hydrophobic groups on the photodegradable crosslinker, the hydrogels exhibit viscoelastic properties with tan(δ) of 0.040 ± 0.005 compared to the non-photodegradable hydrogel with tan(δ) of 0.005 ± 0.003. We have analyzed serial sections of human fibrotic lung tissue to recreate 3D renderings of fibroblastic foci which can be stiffened using two-photon laser scanning lithography into these novel biomaterials. Currently, work is being done to optimize the stiffening and softening of these materials with two-photon laser scanning lithography. Transitioning from a fibrotic state back to the stiffness of healthy lung tissue enables a novel investigation of the fibroblastic response to a mechanical target, potentially returning pathologically activated fibroblasts to homeostasis.

11:45 AM - 12:00 PM

Influence of Cell Seeding Density and ECM Type on Engineered Heart Tissue Behavior

Mackenzie Obenreder, University of Colorado Boulder

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Dr. Jason A. Burdick, University of Colorado Boulder, Jason.Burdick@colorado.edu

Heart disease is the leading cause of death worldwide. In recent years, in vitro cardiac models known as Engineered Heart Tissues (EHTs) have been developed to better understand the etiology and pathology of cardiac diseases using human induced pluripotent stem cell (iPSC) derived cardiomyocytes (CMs). Multiple groups have developed EHT models; however, there is no standard method to generate EHTs and features such as the type of extracellular matrix (ECM) are variable across groups. To fill this gap in knowledge, this work elucidates the influence of cell seeding density and ECM composition on iPSC-CM EHT properties. Our EHT model is generated using iPSC-derived cardiomyocytes in a Digital Light Processing (DLP) printed mold with flexible posts generated using a poly(ethylene glycol) diacrylate resin. The iPSC-CMs are mixed (10 and 60 million cells/mL) within an ECM solution of either type 1 collagen (1 mg/mL) or fibrinogen (4 mg/mL) and then added to the mold for culture. After 10 days of compaction, the EHTs are matured for two weeks using maturation media. The effect of cell seeding density and ECM was compared before (day 22) and after (day 36) maturation. iPSC-CM EHTs with low and high seeding densities (10 and 60 million cells/mL) had comparable functional contractility outcomes of action potential duration (APD), force, and relaxation time before maturation was induced. After maturation, both seeding densities achieved the expected results of increased force and relaxation time; however, only the high seeding density had an increased APD and a decreased time to peak associated with mature iPSC-CMs. To explore the role of ECM, the iPSC-CM EHTs were generated with 1 mg/mL collagen or 4mg/mL fibrinogen, which underwent similar compaction during the 10 days in culture and had comparable results in nearly all functional contractility outcomes before and after maturation. EHTs generated with fibrinogen exhibited a 65.2% greater force of contraction compared to collagen at the day 22 timepoint. By elucidating the effects of cell density and ECM composition over time, we will be able to advance current EHT models and provide the field a platform to study a variety of cardiac diseases and therapies.

SESSION II: DRUG DELIVERY AND IMMUNE ENGINEERING

1:30 PM - 1:45 PM

Particle Shape Modulates Macrophage Phenotype: Insights for Cell-mediated Drug Delivery Systems

Matthew Kwan, University of Colorado Boulder

Matthew M. C. Kwan (University of Colorado Boulder, matthew.kwan@colorado.edu), Nicole B. Day (University of Colorado Boulder, nila1495@colorado.edu), Iain R. Konigsberg (University of Colorado Anschutz Medical Campus, IAIN.KONIGSBERG@cuanschutz.edu), Evan Thoresen (University of Colorado Boulder, evan.thoresen@colorado.edu), Celeste Busch (University of Colorado Boulder, celeste.busch@colorado.edu), Elizabeth J. Davidson (University of Colorado Anschutz Medical Campus, ELIZABETH.DAVIDSON@cuanschutz.edu), Abigail G. Harrell (University of Colorado Boulder, abha3025@colorado.edu), Ivana V. Yang (University of Colorado Anschutz Medical Campus, IVANA.YANG@cuanschutz.edu), C. Wyatt Shields IV (University of Colorado Boulder, Charles.Shields@colorado.edu)

Cell-mediated drug delivery systems are emerging as a new approach to deliver bioactive drugs packaged in micro/nanoparticles to specific, pathologic sites. Circulating immune cells like macrophages have the capacity to enhance particle delivery in vivo through chemotaxis, and once delivered, the cells can participate in the intended therapeutic effect as well. However, particles attached to the surface or internalized are known to exert stresses on macrophages, causing macrophages to polarize between M1-like (proinflammatory) to M2-like (anti-inflammatory) phenotypes. Despite this understanding, little is known about the underlying relationship between particle shape and macrophage phenotype. It has been demonstrated that spherical particle geometries are rapidly phagocytosed in the phagosome within minutes to hours; however, certain non-spherical particle geometries such as discoids are capable of frustrating phagocytosis by macrophages for days, resulting in cell spreading along the particle surface due to inhibited formation of actin structures. Herein, we study the interaction of poly(D,L lactic-co-glycolic acid) discoidal (major axis 7.7 ± 0.5 μm, thickness 276.44 ± 31.8 nm) and spherical (diameter 2.3 ± 0.3 μm) particles on C57BL6-derived primary macrophages to respectively promote or suppress phagocytosis. Discoidal and spherical particles of a fixed volume were fabricated using microcontact printing and homogenization techniques, respectively. Macrophage-disc (MΦ-D) and -sphere (MΦ-S) complexes were manufactured by associating particles in vitro and profiled with RNA-seq, assay for transposase-accessible chromatin with sequencing (ATAC-seq), flow cytometry, and multiplexed ELISA. MΦ-D displayed greater phenotypic shifts compared to MΦ-S. Notably, MΦ-D showed significant differential gene expression [Irf1 (7.93-fold), Il1b (0.80-fold), Vegfa (1.24-fold)], protein biomarkers [Arg1 (31.42-fold), iNOS (3.25-fold), CD40 (0.39-fold)], and secreted cytokines [IL-6 (12.54-fold), TNFα (14.22-fold), CXCL10 (1.08-fold)] compared to unstimulated controls. MΦ-S, on the other hand, displayed significant but less pronounced differential gene expression [Irf1 (1.28-fold), Il1b (0.87-fold), Vegfa (0.76-fold)], protein biomarkers [Arg1 (1.56-fold), iNOS (1.37-fold), CD40 (1.07-fold)], and secreted cytokines [IL-6 (15.91-fold), TNFα (1.52-fold), CXCL10 (0.07-fold)] compared to unstimulated controls. Our work will be useful for designing macrophage-mediated drug delivery systems by enabling the rational selection of cell-particle pairings for the promotion of specific phenotypes that are desirable for certain disease manifestations.

1:45 PM - 2:00 PM

Magnetic biohybrid microrobots for macrophage transport, activation, and imaging

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Background: Adoptive cell transfers (ACTs) are rapidly growing in popularity due to the ability of cells to interact with diseased cells more specifically than small molecule drugs or injected particles. However, ACTs often lack methods to control the localization and function of cells within the body after injection. We have developed a class of non-spherical magnetic microrobots that bind to macrophages to remotely guide cellular phenotypes and transport in physiological environments. For applications involving intravenous delivery, we developed nanodisc "backpacks" that stick to macrophages for spatial control by a gradient magnetic field. For applications involving viscous barriers, we developed helical particles that facilitate cellular transport by swimming in rotating magnetic fields.

Methods: Biodegradable polymer particles were fabricated using standard lithographic techniques: soft lithography (PLGA backpacks) or two-photon lithography (poly(β-amino ester) helices) to encapsulate the small molecule drug resiquimod, which drives the phenotype of macrophages towards an antitumor phenotype. Magnetic field-responsiveness was imparted through entrapping superparamagnetic iron oxide nanoparticles (SPIONs) within the polymer matrix or surface coating by metal evaporation.

Results: Particle aspect ratio and size enables surface binding to macrophages and evasion of phagocytosis. The slow release of resiquimod drives potent M1-like cellular activation by enhanced expression of iNOS and 10-fold increase in IL-12 release on the fifth day compared to free drug. The magnetic properties of the backpacks enabled transport through fluid and tissue environments in vitro in response to a gradient magnetic field. Further, we show that SPIONs within the backpacks serve as tracers for magnetic particle imaging (MPI), a non-invasive imaging modality, with a limit of detection of 5e4 backpacks and accuracy of quantification within 2% for complexes injected into a murine tumor. For macrophage delivery through viscous biological barriers, such as mucus or aqueous humor, we found that the location of macrophage binding to the helical particles gave rise to distinct modes of locomotion, enabling transport as fast as 50 μm/sec through viscous media.

Conclusions: The combination of sustained macrophage polarization, field-responsive transport, and high-resolution monitoring highlights the potential of magnetic cell-based biohybrid robots to improve the performance of ACTs.

2:00 PM - 2:15 PM

Engineering microparticles by coacervation for pulmonary drug delivery

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Non-invasive delivery of therapeutics to the lungs can enhance overall safety and efficacy by ensuring the local accumulation of administered drugs, thus minimizing off-target effects. Presenting a unique opportunity, greater than 200 billion capillaries in the human lungs are ≥ 10 microns in diameter. Thus, intravenously administered microparticles passively accumulate in the lung microvasculature, owing to the anatomy of the pulmonary system. To leverage this anatomic feature for drug delivery, we engineered microparticles by charge-driven interaction, also called coacervation, between solutions of chitosan containing small molecules and hyaluronic acid. Dropwise addition of chitosan to hyaluronic acid solutions resulted in the self-assembly of complexes that formed microparticles, which were 32.6 ± 2.7 μm (n=3) in diameter and possessed a Zeta potential of -16.0 ± 0.6 mV (n=3). Using doxycycline as an example of a small molecule drug, we demonstrate an encapsulation efficiency of 31.0 ± 0.5 % (n=3). The loading content present in 10 mg of microparticles was 5.4 ± 0.1 mg (n=3). Further, we demonstrate that doxycycline is steadily released over a 7-day period. To determine the biodistribution of intravenously administered microparticles, we incorporated Cyanine 7 dye (Cy7) in our biomaterial system for in-vivo imaging. Within an hour of intravenous administration, administered microparticles accumulated in the lungs of healthy rats, with minimal Cy7 signals present in the liver, spleen, heart and kidneys. Our strategy is advantageous over inhalational therapeutics that are limited by the epithelial and mucus barriers present in the respiratory tract; as well as therapeutics administered directly into the bloodstream or via nanoparticles, which accumulate in the liver and spleen. Moreover, the biocompatibility of chitosan and hyaluronic acid makes our translatable approach clinically relevant, enabling the local delivery of therapeutics to the lungs of patients.

SESSION III: TISSUE ENGINEERING

3:50 PM - 4:05 PM

Synthetic hydrogel and degradable extracellular matrix supported 3D model for endometriosis

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Endometriosis is a chronic condition affecting one in nine women and occurs when endometrial lesions form outside the uterus. Endometriosis is frequently linked to infertility in women, is accompanied by excruciating chronic pain, and can invade and compromise abdominal organs. Laparoscopic surgery is currently the only method to obtain a confirmatory diagnosis of endometriosis, and average time to diagnosis is 7 years after symptom onset. Additionally, treatment methods are limited to hormonal therapies, which have limited efficacy. As such, it is critical to develop methods to improve the diagnosis and treatment of endometriosis.

In this study, we aim to engineer targeted endometriosis imaging and therapeutic approaches using high affinity nanobody-decorated magnetic resonance imaging agents and lipid nanoparticles (LNP). To develop our nanobody candidates, which will be generated using a custom phage display technique, we evaluated human endometriosis single-cell RNA-seq datasets and identified PAEP and IL20RA as proteins with high differential expression in endometriotic lesions. We used immunofluorescence and flow cytometry to identify and quantify these targets in normal endometrial and endometriotic cell lines and are validating our targets in primary patient samples.

We next developed an endometriosis organoid model using an extracellular matrix-mimicking synthetic poly(ethylene glycol) (PEG) hydrogel culture system and normal endometrial and endometriotic epithelial and stromal cell lines. Multicellular organoids were formed by day 3 with an architecture comparable to native endometrium and endometriosis, with a stromal core and an epithelial crust. Endometriotic cells and organoids exhibited higher metabolic activity than normal primary endometrium, and all cells exhibited high viability out to at least 7 days. Preliminary experiments with two untargeted LNP compositions incorporating GFP in the LNP membrane and luciferase mRNA cargo demonstrated enhanced uptake of LNP in endometriotic organoids relative to normal endometrial organoids via quantitative luciferase expression; we confirmed internalization within our organoids via GFP imaging. Future and ongoing studies will establish a humanized endometriosis mouse model in NSG mice to enable in vivo testing of nanobody targeting to implanted organoid lesions

4:05 PM - 4:20 PM

Effects of Hyaluronic Acid and A7R Peptide on Angiogenesis and Osteogenesis in Growth Plate Injury Models in vitro

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The growth plate is a cartilaginous tissue at the end of long bones that allows bones to elongate over time. Injury to the growth plate is common when children sustain fractures in their long bones, and the damage can create lasting growth complications that require many successive surgeries. Cartilage does not naturally regenerate, so bone tends to grow in the injury site, forming a “bony bar” that prevents the bone from growing normally. A big difference between bone and cartilage is that bone is vascular, which means that angiogenesis, the formation of blood vessels, must occur before osteogenesis. Anti-angiogenesis drugs have been shown to reduce blood vessel formation when encapsulated in a hydrogel matrix, but the effects of insoluble factors attached to the matrix have not been tested. This study aims to evaluate the impact of hyaluronic acid and anti-angiogenic peptides on angiogenesis and “bony bar” formation. Hyaluronic acid (HA) with varying molecular weights (10 kDa, 100 kDa, and 1 MDa) and an anti-angiogenic peptide (A7R with sequence ATWLPPR) with varying concentrations were attached to alginate-chitosan polyelectrolyte complexes (PECs) to assess their impacts on cell behavior and gel properties. These materials were used to separately culture human mesenchymal stems cells (hMSCs), for impact on cell differentiation, and human umbilical vein endothelial cells (HUVECs), for impact on angiogenesis, in vitro. Cells cultured in PECs containing the lowest and highest molecular weight of HA generally showed lowered expression of angiogenic genes compared to the 100 kDa HA PEC after two weeks of culture. However, after three weeks of culture in all PECs containing HA, HUVEC expression of angiogenic genes was significantly lowered. For hMSC cultures, the 10 kDa HA had the greatest effects on chondrogenic gene expression and differentiation. Similarly, the A7R PECs caused downregulation in osteogenic gene expression and dose-dependent upregulation of some chondrogenic genes. The results suggest that A7R peptide can prevent osteogenic gene expression and that fragmented HA (MW 10 kDa) can prevent angiogenic gene expression. In future work, the materials will be tested on growth plate injury rat models in vivo for limb length and bone volume.

SESSION IV: IMMUNE ENGINEERING

11:00 AM - 11:15 AM

Tolerogenic Human Placental Trophoblast Survival and Engraftment in a Xenograft Model

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Cell and tissue transplantation is hindered by graft rejection, which necessitates lifelong chronic systemic immunosuppression. Placental pregnancy is the only natural state of de novo tolerance against allogeneic tissue in adult mammals, and is mediated by placental trophoblast cells, which use diverse mechanisms to induce antigen-specific tolerance. Understanding trophoblast tolerogenic mechanisms holds potential implications for eliminating immunosuppression in allogeneic transplantation.

In this study we aimed to evaluate the potential of human trophoblast model cell line, JAR, to evade graft rejection via tolerogenic soluble factors in an immune competent xenograft model (C57BL/6 recipient). To conduct this, we validated the survival of JAR cells macroencapsulated in spiral devices via live/dead imaging and metabolic activity in vitro over 7 days of culture. To evaluate successful engraftment in vivo in the absence of immune rejection, encapsulated and unencapsulated JAR cells transfected with Nanoluciferase were transplanted into immune deficient NSG mice in the subcutaneous (SUBQ) or epididymal fat pad (EFP) sites with a vasculogenic VEGF-delivering degradable hydrogel to enhance local vascularization. Unencapsulated groups maintained higher luminescent signal than encapsulated groups, increasing gradually over time, and formed palpable tumors SUBQ over 3 weeks. Overall, encapsulated JAR cells exhibited highest signal relative to day 0 in the EFP (~213%) compared to the SUBQ (~74%). Next, we evaluated unencapsulated and encapsulated nanoluciferase JAR survival in an immune competent C57BL/6 xenograft model. JAR were transplanted in SUBQ and EFP sites, with and without vasculogenic degradable hydrogel. The EFP groups experienced rapid rejection, with a reduction to near-baseline signal in all groups by day 10. In the SUBQ groups, rejection was observed between day 14-28, except for the encapsulated with vasculogenic hydrogel group, which demonstrated significantly elevated signal comparable to the NSG group out to day 63. Encapsulated non-tolerogenic control human HEK cell controls were rapidly rejected within 10 days, suggesting that trophoblast-secreted soluble factors contributed to JAR survival.

Overall, trophoblast in vivo imaging survival demonstrated high cell survival in immune compromised mice and comparable survival in immune competent mice in the SUBQ site. Ongoing studies are evaluating mechanisms of trophoblast survival and immune modulation via histology and flow cytometry.

11:15 AM - 11:30 AM

Macroporous Gelatin Scaffolds for Lymph Node Stromal Cell-Mediated Immunomodulation in Type 1 Diabetes

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Autoimmune diseases (AD) like Type 1 Diabetes (T1D) are caused by the disruption of T-cell tolerance to self-antigens (Ags), thereby unleashing autoreactive T-cells that attack self-tissues. AD affect 8% of the US population, and their incidence is increasing. Current clinical treatments are limited to systemic T-cell approaches with adverse side effects and restricted efficacy. Therefore, there is a need for localized, Ag-specific therapies that selectively target autoreactive T-cells. A promising strategy involves Fibroblastic Reticular Cells (FRCs). FRCs are lymph node (LN) stromal cells that build the LN reticula and act as non-professional Ag-presenting cells. Upon inflammation, FRCs expand the LNs to facilitate T-cell proliferation and upregulate Ag-presenting machinery and immune checkpoint molecules with limited expression of co-stimulatory molecules. FRCs are thus more likely to regulate rather than activate Ag-specific T-cells. However, their therapeutic potential for Ag-specific immunomodulation for AD remains unexplored. We aim to use biomaterials as templates for the therapeutic application of FRCs in AD. We engineered 3D FRC-based reticula using macroporous gelatin scaffolds with pore diameters that mimic the FRC reticula in normal (>50 μm) and inflamed (>150 μm) LNs to study the effects of FRC reticular pore size on FRC and T-cell interactions and immunomodulation. Our scaffolds are biocompatible and promote FRC viability, reticular formation, extracellular matrix secretion, and local retention for at least 21 days after subcutaneous transplantation in T1D mouse models. In vitro, co-culture of CD8+ and CD4+ T-cells in scaffolds with FRCs and T1D peptide Ags promoted engagement of Ag-specific T cells (proliferation and CD25 and CD44 upregulation) with reduced cytotoxic and increased anergic and regulatory phenotypes. These results suggest an Ag-specific immunomodulation of T cells by our 3D FRC platform. Current studies are unveiling the optimal scaffold pore size for FRC engraftment for in vivo recruitment and immunomodulation. Future work will also evaluate whether incorporating T1D Ags onto scaffolds enables localized and prolonged delivery of Ags for FRC uptake and presentation to T-cells compared to ex vivo Ag pulsing. Our platform could provide a local, retrievable, Ag-specific immunomodulatory therapy to treat AD like T1D by selectively suppressing and/or regulating Ag-specific autoreactive T-cells.

11:30 AM - 11:45 AM

Characterization of Sex-specific Hormone and Gene Expression Alterations in Experimental Model of Traumatic Brain Injury

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Purpose/Objective: Traumatic Brain Injury (TBI) results from a blow or jolt to the head and leads to complex pathologies that can extend years after the initial injury. Unfortunately, current treatments for TBI focus on managing symptoms such as edema and intracranial hemorrhage rather than targeting the pathologies that lead to neurodegeneration. Additionally, little is known about how TBI pathology differs between sexes and how alterations in circulating sex steroid hormones contribute to chronic TBI pathologies. To address these challenges, our group employs both male and female models to assess sex-dependent pathologies of traumatic brain injury. In this project, our goal is to evaluate sex differences in circulating sex steroid hormones alterations, BBB disruption and neuroinflammation after TBI.

Methods: We used a well-established mouse controlled cortical impact (CCI) model to induce a moderate TBI over the primary somatosensory cortex in C57BL/6J mice. Male (n=10) and female (n=12) cohorts were injured at 9-10 weeks of age followed by tissue and blood collection at 24 and 72 hrs post-injury. Plasma samples were analyzed with an LC-MS/MS hormone panel for testosterone, androstenedione, estradiol, estrone, progesterone, DHEA and corticosterone. For the RNA-sequencing, a 4 mm cortical punch was taken directly over the injury penumbra and contralateral hemisphere at sacrifice.

Results: Notably, we observe sex-specific alterations in circulating sex steroid hormones after TBI at both 24 and 72 hours. Females exhibit decreases in progesterone, androstenedione, and testosterone at 24 and 72 hrs compared to naïve females, while males exhibit increases in DHEA and decreases in estradiol at 72 hrs compared to naïve males. Additionally, RNA-sequencing reveals sex- and timepoint-specific gene sets when compared to sex-matched controls. Furthermore, functional enrichment analysis of these gene sets shows the female 24-hour group had significant upregulation in genes associated with innate immune trafficking and activation compared to other groups.

Conclusion/Future Work: Our results indicate there are sex-specific responses to traumatic brain injury, including sex specific alterations in steroid hormone profile and gene expression. We aim to further elucidate hormone-related pathologies by conducting a correlation analysis using gene expression and hormone level.

11:45 AM - 12:00 PM

Extracellular Matrix Regulation of the Neutrophil Infectious Response

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As the body's first line of defense against disease and infection, neutrophils must efficiently navigate to sites of inflammation; however, neutrophil dysregulation contributes to the pathogenesis of numerous diseases, including fibrosis and cancers. While it is known that neutrophils are mechanosensitive cells, our understanding of how the extracellular matrix (ECM) affects their responses during infection is incomplete. This is due, partly, to a lack of experimental systems with the ability to control ECM composition while maintaining a physiologically relevant infectious microenvironment. Specifically, the endothelial cells that line blood vessels are both mechanosensitive and play a critical role in neutrophil activation. Therefore, in addition to directly impacting neutrophil function, changes to the ECM could indirectly influence neutrophils through the blood vessel. To capture these interactions, we used our infection-on-a-chip microfluidic device, which contains a model blood vessel lumen surrounded by collagen, to study how collagen density affects neutrophil function.

Collagen concentration in the ECM varies in healthy and diseased tissues. Additionally, tissues susceptible to infections, such as the lungs, skin, and liver, are more prone to infections when diseased. Therefore, we sought to study how collagen concentration affects neutrophil functionality in response to Pseudomonas aeruginosa. By varying collagen concentration from 2 to 6mg/mL we found neutrophils extravasate more into 4mg/mL collagen than 2 or 6mg/mL collagen in the presence of an endothelium. In the absence of an endothelium no difference in neutrophil extravasation was observed, suggesting an endothelial cell-dependent mechanism by which the neutrophils differentially extravasated. Furthermore, neutrophils migrate further and faster as the collagen concentration in ECM decreased. This could be due to decreased pore size with increasing collagen concentration, restricting neutrophil movement. However, this difference was not observed for neutrophils that had not extravasated through an endothelium, suggesting an endothelial cell-dependent mechanism by which neutrophil migration is regulated. Lastly, no differences in reactive oxygen species production across the collagen concentrations were observed. We are now seeking to investigate how enzymatic crosslinking of collagen, as seen in vivo, affects the ECM and, consequently, the neutrophil response. Together these results demonstrate the importance of studying neutrophil-ECM interactions in microphysiological systems.

SESSION V: ENGINEERING MODELS OF TYPE 2 DIABETES

3:50 PM - 4:05 PM

Extracellular matrix stiffness mediates insulin secretion in pancreatic islets via mechanosensitive Piezo1 channel regulated Ca2+ dynamics

Chelsea Johansen, Colorado School of Mines

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The pancreatic islet is surrounded by ECM that provides both biochemical and mechanical cues to the islet β-cell to regulate cell survival and insulin secretion. Changes in ECM composition and mechanical properties drive β-cell dysfunction in many pancreatic diseases. While several studies have characterized changes in islet insulin secretion with changes in substrate stiffness, little is known about the mechanotransduction signaling driving altered islet function in response to mechanical cues. We hypothesized that increasing matrix stiffness will lead to insulin secretion dysfunction by opening the mechanosensitive ion channel Piezo1 and disrupting intracellular Ca2+ dynamics in mouse and human islets. To test our hypothesis, mouse and human cadaveric islets were encapsulated in a biomimetic reverse thermal gel (RTG) scaffold with tailorable stiffness that allows formation of islet focal adhesions with the scaffold and activation of Piezo1 in 3D. Our results indicate that increased scaffold stiffness causes insulin secretion dysfunction mediated by increases in Ca2+ influx and altered Ca2+ dynamics via opening of the mechanosensitive Piezo1 channel. Additionally, inhibition of Piezo1 rescued GSIS in islets in stiff scaffolds. Overall, our results emphasize the role mechanical properties of the islet microenvironment plays in regulating function. It also supports further investigation into the modulation of Piezo1 channel activity to restore islet function in diseases like type 2 diabetes (T2D) and pancreatic cancer where fibrosis of the peri-islet ECM leads to increased tissue stiffness and islet dysfunction.

4:05 PM - 4:20 PM

Changes in extracellular matrix stiffness mediate pancreatic islet function: insights into glucose metabolism and mitochondrial dynamics

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In the pancreas, the islet is surrounded by a specialized extracellular matrix (ECM) which regulates cell survival and insulin secretion from islet β-cells. Little is known about how mechanical properties, like stiffness, of the ECM regulate islet function in health and disease. Previous research conducted on islets from a rat model of type 2 diabetes (T2D), characterized by increased ECM stiffness and reduced insulin secretion, revealed changes in mitochondrial morphology marked by increased mitochondrial fragmentation. Mitochondrial morphology is regulated by membrane potential (ΔΨ), with alterations in ΔΨ influencing fusion-fission dynamics crucial for maintaining mitochondrial function. Insulin secretion in the β-cell is tightly controlled by metabolism and mitochondrial function; however, the mechanisms underlying mechanotransduction regulation of glucose metabolism and mitochondrial dynamics have not been well studied in intact islets. We hypothesize that increasing matrix stiffness will yield a depolarized ΔΨ, an increase in mitochondrial fragmentation, and impaired insulin secretion. To test our hypothesis, mouse and human cadaveric islets were encapsulated in PEG-maleimide with an RGD-cysteine binding ligand crosslinked via Michael-type addition to PEG-dithiol. This 3D PEG hydrogel mimics the islet microenvironment, where 3wt% PEG gels (0.204±0.007 kPa) represent healthy pancreas stiffness and 7.5wt% (2.25±0.153 kPa) represents stiffness seen in T2D ECM. Our results indicate that increased scaffold stiffness causes a 57% reduction in insulin secretion stimulation index ($p=0.029$), a 220-240% decrease in TMRE fluorescence intensity ($p=0.0043$) indicating depolarization of the ΔΨ at 2mM and 20mM glucose respectively, and mitochondrial fragmentation. Overall, our results emphasize the role mechanical properties of the islet microenvironment plays in regulating insulin secretion and mitochondrial function. It also supports further investigation into the modulation of glucose metabolism and mitochondrial dynamics to restore islet function in diseases like T2D where fibrosis of the peri-islet ECM leads to increased tissue stiffness and islet dysfunction.

RAPID FIRES

Drug Delivery

4:20 PM - 4:26 PM

Designing polycaprolactone nanocapsules to target and promote pancreatic β-cell survival during the onset of type 1 diabetes

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The current gold standard for the treatment of T1D does not provide a cure but relies on continuous administration of exogenous insulin to regulate hyperglycemia and manage associated symptoms. Clinical interventions to prevent pancreatic β-cell stress or death during T1D onset are promising curative strategies that could halt or reverse the disease progression. Systemic administration of peptide therapeutics such as δV1-1 with excellent potential to promote β-cell survival often leads to severe off-target effects and reduced efficacy due to non-specific targeting and poor cellular uptake. We hypothesize that encapsulating δV1-1 in polycaprolactone nanocapsules coated with targeting peptides can lead to the preferential targeting of pancreatic β-cells, thereby enhancing the therapeutic efficacy of δV1-1. To test our hypothesis, Cy5, δV1-1, or δV1-1 conjugated to cell-penetrating TAT peptide were encapsulated in polycaprolactone nanocapsules using emulsification solvent evaporation method. The nanocapsules were coated with either glucagon-like peptide-1 receptor agonist Exendin-4 conjugated to hyaluronic acid (HA-Ex4) or an antibody against the human-specific β-cell marker, ectonucleoside triphosphate diphosphohydrolase 3 (ENTPD3 AB). The coated nanocapsules were characterized and evaluated in vitro using isolated mouse and human cadaveric islets treated with or without cytokines and in vivo with NOD mice. Our results showed that coatings of nanocapsules with targeting peptides facilitate selective uptake of the Cy5 by the pancreatic β-cells in both in vitro and in vivo models. A significant proportion of Cy5 nanocapsules coated with ENTPD3 AB were trafficked into the pancreatic islets with more than 70% delivered to the insulin-positive β-cells. Targeted delivery of δV1-1 by the nanocapsules improves the effectiveness of δV1-1 to protect pancreatic cells against cytokine-induced death when compared to the action of free δV1-1. Also, the viability percent of cytokine-treated islets in the presence of HA-Ex4 coated δV1-1 nanocapsules was not statistically different from the untreated islets. Conclusively, these data showed that targeted delivery of δV1-1 to the β-cells by the polycaprolactone nanocapsules enhanced its therapeutic efficacy. It also supports further preclinical investigation of these nanocapsules in promoting pancreatic β-cell survival in mouse models of T1D onset.

4:26 PM - 4:32 PM

Co-assembly of block and statistical copolymers into mixed micelles augment pDNA delivery performance and polyplex serum stability

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Gene therapy has demonstrated tremendous therapeutic promise for inherited diseases as well as highly prevalent diseases such as cancer, diabetes, or age-related macular degeneration. To mediate efficient gene delivery into target cells, numerous synthetic nanocarriers have been engineered. Cationic polymers have been extensively studied due to their low cost, availability, and the ability to readily condense bulky anionic payloads such as plasmids (pDNA) but their therapeutic applicability is limited by polymer toxicity and interactions with serum proteins. Earlier, we synthesized block (B), gradient (G), and statistical (S) copolymers to elucidate the role of polymer microstructure on polyplex stability and gene delivery efficiency. We hypothesized that mixed micelles, co-assembled of block copolymers with statistical/gradient copolymers, will outperform conventional micelles as well as linear polymers in maintaining colloidal stability, promoting efficient pDNA delivery, and minimizing toxicity. Block copolymers were co-assembled with either gradient or statistical copolymers using different mixing ratios (B/G and B/S) to form mixed micelles, which were then complexed with pDNA via electrostatic interactions, forming mixed micelleplexes. We compared the toxicity, serum stability, and pDNA delivery efficiency as a function of B/G and B/S mixing ratios. Morphological analysis using transmission electron microscope indicated that the size distribution and aspect ratio of mixed micelleplexes were highly sensitive to the mixing ratios used (B/G and B/S). Dynamic light scattering verified that all mixed micelleplexes were sized between 150 to 200 nm, which favors high cellular uptake. Mixed micelleplexes were less toxic than polyplexes and conventional micelleplexes. Mixed micelleplexes formed from block and statistical copolymers performed best among all the combinations tested. Interestingly, while the B/G mixing ratio governed the pDNA delivery efficiency of block: gradient mixed micelles, the delivery efficiency of block statistical mixed micelles was insensitive to B/S mixing ratio. To explain this trend, we performed static light scattering measurements and calculated pDNA loading per micelleplexes as a function of mixing ratio. Our study reveals design rules for rationally co-assembling copolymers with varied monomer spatial distribution (block/gradient/statistical) into mixed micelles that exhibit gene delivery performance superior to that of conventional micelles (formed by block copolymers only).

4:32 PM - 4:38 PM

Using Triazole Crosslinks and a Monofunctional Hydrazide to Engineer an Injectable Hydrazone Crosslinked Hydrogel for Cell Delivery

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Covalent Adaptable Networks (CANs) can enable self-healing and injectable properties in hydrogels for noninvasive drug and cell delivery. The alkyl-hydrazone crosslink is one such chemistry used in hydrogel CAN systems as it is biocompatible and biorthogonal compared to other clinically used amine-involved reactions such as imine ligation. Due to its adaptable nature and the reactivity of aldehydes with primary amines, the alkyl-hydrazone crosslinked hydrogels lack stability in protein rich environments. Additionally, hydrogels utilizing hydrazone crosslinks and biopolymers such as Hyaluronic Acid (HA) typically require low polymer content and low molecular weight polymers to enable injectability due to polymer entanglements. To achieve enhanced stability, a high molecular weight (>60 kDa) hyaluronic acid alkyl-hydrazone crosslinked hydrogel, comprised of HA functionalized with hydrazides (HA-Hyd) and HA functionalized with aldehydes (HA-Ald), was modified by introducing slow reacting triazole crosslinks. By reacting HA-Hyd with a small amount of benzaldehyde-poly(ethylene glycol)3-azide, HA-Hyd effectively becomes dual functionalized with hydrazides and azides which enables the formation of the triazole bond via a biorthogonal and slow reacting strain-promoted azide-alkyne cycloaddition (SPAAC) reaction with an 8 arm poly(ethylene glycol) (PEG) functionalized with bicyclononyne. With increasing percent of bonds formed through SPAAC, the stability of the dual crosslinked hydrogel in media was increased, as observed through reduced visual changes in shape and by measuring the relative HA erosion over a 14 day period. In situ shear rheology and force extrusion studies demonstrated the slow crosslink formation of SPAAC bonds enabling a temporary state of injectability that was similar to an alkyl-hydrazone crosslinked hydrogel. To improve the injectability of the hydrazone and triazole crosslinked hydrogel, a monofunctionalized hydrazide, methyl-PEG4-hydrazide (m-PEG4-Hyd), was reacted with HA-Ald to selectively slow down the alkyl-hydrazone crosslinks. By selecting concentrations of m-PEG4-Hyd that significantly slowed network evolution, tunable extrusion force was achieved. Decreasing extrusion forces with m-PEG4-Hyd enabled improved viability of post-extruded rat mesenchymal stem cells (rMSCs) inside the dual crosslinked hydrogel. Additionally, rMSCs encapsulated inside the hydrogel with high post-extrusion viability secreted relatively more VEGF and relatively less MCP-1 and TIMP-1 than rMSCs encapsulated inside a hydrogel with low post-extrusion viability.

4:38 PM - 4:44 PM

Ionic liquid-embedded adhesive hydrogel system for tunable transdermal drug delivery

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Background: Hydrogels are an effective platform for administering drugs to cells in the skin due to their biocompatibility, flexible chemistry, and mechanical similarity to living tissues. However, mechanisms governing drug release are often inextricably linked to bulk properties of the hydrogel, and drug transport across the stratum corneum is limited. There remains a need for a simple transdermal delivery system that enables multimodal release of immunomodulatory drugs for a variety of skin diseases.

Methods: We describe a tissue-adhesive hydrogel embedded with drug-loaded microparticles and ionic liquid to enable tunable release of drugs in a manner independent from the properties of the hydrogel. This hydrogel was designed to sustainably deliver localized treatments to skin while adherent, after which it can be removed by peeling. The hydrogel was synthesized using a one-pot free radical polymerization to create a biocompatible and nondegradable polyacrylamide gel containing adhesive galloyl groups, capable of encasing multiple populations of particles to release drugs in a multimodal manner. Embedded silicone particles were synthesized using a nucleation and growth technique. Ionic liquids, comprising an anion and a bulky, asymmetric cation, were incorporated to mediate drug transport across the stratum corneum for applications where the skin barrier is not compromised, such as by surgical resection or a wound site.

Results: We demonstrate multimodal drug release by designing particles that can release resiquimod (a macrophage-stimulating drug) over several hours and palbociclib (a T-cell-stimulating drug) over several days. We show cell activation by promoting polarization of macrophages in vitro and ex vivo toward anti-tumor phenotypes, with significantly higher expression of macrophage inflammatory markers (iNOS, CD86, and MHCII) compared to free drug over five days. Furthermore, we show that that ionic liquids embedded in the hydrogel can facilitate the transport of the small molecule drug ruxolitinib (a JAK1/2 inhibitor) across the stratum corneum, resulting in higher drug efficacy than clinical creams and enabling the treatment of skin disease with intact or hyperkeratinized stratum corneum.

Conclusions: The multimodal nature of this hydrogel system has implications in treating a variety of skin disorders and delivering vaccines at well-defined rates.

Immune Engineering

4:44 PM - 4:50 PM

Dissolved Gases from Elevated Pressure in the Lungs Elicit Innate Immune Cell Responses

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Conventional dogma suggests that decompression sickness (DCS) is caused by nitrogen bubble nucleation in the blood vessels; however, recent studies indicate that the abundance of bubbles is not indicative of DCS severity. Since immune cells are known to respond to chemical and environmental cues, we hypothesized that dissolved gasses from elevated hydrostatic pressures drive aberrant immune cell phenotypes in the vasculature while diving. We describe a system to determine this response using a custom hyperbaric chamber and human lung-on-a-chip devices. The hyperbaric chamber was designed with a compressor connected to an ASME-code pressure tank equipped to generate pressures up to 7.5 atm, corresponding to dive depths of 67.2 m (220 ft). Two-channel lung-on-a-chip devices were seeded with primary human alveolar and microvascular cells in the top and bottom channels, respectively. Human blood samples extracted from healthy donors were infused in the lung-on-a-chip devices, exposed to alveolar gas mixtures, and pressurized to either 1.0 atm or 3.5 atm for one hour followed by decompression at a fixed rate. Phenotypes of neutrophils, dendritic cells, and monocytes were determined by flow cytometry and multiplexed ELISA. Results indicate an immune response occurs at 3.5 atm compared to 1.0 atm controls. From the flow cytometry data, dissolved gases activated several phenotypic markers of innate immune cells (e.g., as indicated by an elevated expression of CD41a and MPO in neutrophils and CD80 in monocytes and dendritic cells). The cytokine secretion data showed distinct differences between effects from increased oxygen vs. nitrogen partial pressures, corresponding to compressed alveolar air vs. compressed oxygen-reduced air commonly used in diving (e.g., significant differences were observed in the secretion of IL-1α, IL-1β, GM-CSF, IFNα, and IFNγ). This work suggests innate immune reactions may play a role in DCS, which has implications in identifying high-risk individuals and may allow for new means of mitigating DCS. In future work, we will perform epigenetics and transcriptomics on cells in devices to determine the pathways of gene expression that mediate the cellular responses observed.

4:50 PM - 4:56 PM

Engineering trophoblast extracellular vesicle-delivering hydrogels for localized tolerance induction

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Cell therapies are a promising method to treat diseases such as type 1 diabetes; however, this approach necessitates systemic immunosuppression to prevent graft rejection, which imparts severe risks such as cancer and infection. Strategies such as cell encapsulation and localized delivery of tolerogenic factors within the cell graft site targeting the direct and indirect antigen presentation pathway, respectively, have been explored to prevent graft rejection. In pregnancy, the placental trophoblast uses a combined approach of inert surface to block direct antigen recognition and secreted soluble factors, such as extracellular vesicles (EVs), to mitigate indirect antigen recognition of the allogeneic fetus and induce tolerance. Trophoblast EVs contain a multitude of tolerogenic factors which influence the behavior of immune cells. In this work, we engineered a synthetic tunable hydrogel-based trophoblast EV delivery system for sustained delivery of EVs within an encapsulated cell transplant site to induce localized graft tolerance in a similar manner to placental pregnancy.

We first characterized EV delivery kinetics from degradable and nondegradable synthetic poly(ethylene glycol) (PEG)-based hydrogels, where EVs are chemically linked directly to the hydrogel matrix with or without a hydrolytic linker; tethered EV release was compared against passive hydrogel entrapment. EV delivery was evaluated in vitro and in vivo using longitudinal IVIS imaging, where matrix-tethered EV delivery was extended relative to untethered and passively entrapped EVs. In vitro immune cell studies demonstrate that hydrogel-delivered trophoblast EVs reduced natural killer cell (NK-92) activation in vitro, and upregulated TNFalpha secretion in THP-1 M0 macrophages. Imaging studies demonstrated significant EV uptake by M0 macrophages. Ongoing studies are evaluating hydrogel-delivered EV influence on THP-1 M1 macrophages, and primary human PBMCs via luminex cytokine panels. Histological analysis of in vivo EV delivery and localization to the graft site and draining lymph nodes are ongoing. Future studies will investigate the localized immune response at graft site through flow cytometry and histology.

Engineering Cells and Their Microenvironments

4:20 PM - 4:26 PM

Photo-responsive hydrogel to study 4D mechano-transduction during intestinal tissue homeostasis

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Background: Mammalian gut epithelial lining has a crypt-villi architecture and serves many crucial functions. The epithelium experiences rapid cellular turnover and regenerates continuously from multipotent intestinal stem cells (ISCs) at the crypt base. During homeostasis, the ISC niche exists together with differentiated cells. Anomalies in this homeostatic regulation can cause diseases like colorectal cancer (CRC). While molecules secreted by the surrounding mesenchymal cells constitute vital biochemical signals in guiding cell fate and organization, the role of mechanical cues during ISC differentiation has only been recently identified. However, the spatiotemporal role of mechano-transduction in the crypt formation process is not fully deciphered due to lack of appropriate in vitro models. More importantly, the role of cell nuclei in integrating the extracellular mechanical cues with genetic transformation during differentiation is unknown.

Methods: We employed an interdisciplinary approach integrating murine ISC derived primary organoids, novel photo-responsive hydrogels, sophisticated microscopic techniques like light-sheet imaging and in vivo tissue sections. The hydrogel system consists of poly(ethylene glycol) (PEG) chains functionalized with nitrobenzyl-azide and dibenzylcyclooctyne (DBCO). Upon mixing of the two PEG macromers, the azide reacts with DBCO via a strain promoted alkyne-azide cycloaddition (SPAAC) bio-click reaction that can be used to encapsulate ISCs and grow organoids. Uniquely, the ortho-nitrobenzyl (oNB) crosslinks can be cleaved with a 405nm confocal laser, softening the hydrogel at predefined regions, thus directing crypt formation of controlled dimensions at specified points in space and time allowing high resolution time-lapse microscopy.

Conclusion: Our spatio-temporally controllable crypt-growth approach revealed noticeable changes in the nuclear envelope architecture and composition as ISCs differentiate. We identified changes in the chromatin accessibility of the cells due to altered histone methylation levels. This is accompanied by changes in localization of cell nuclei with respect to the epithelium which is also observed in vivo. These observations indicate that the nuclei might play an active role in the process of crypt formation and differentiation. Cumulatively, we showcase the design and utilization of a novel photo-responsive biomaterial platform for studying intestinal crypt homeostasis and pathology.

4:26 PM - 4:32 PM

Inducing synthetic cryptobiosis in mammalian cells via reversible intracellular hydrogelation

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Some organisms can withstand extreme environmental conditions by undergoing transient vitrification of their cellular contents, providing protection against irreversible aggregation of intracellular proteins and other biomolecules. Typically, this “cryptobiotic” process relies on molecular crowding by polysaccharides or disordered proteins, but human and other mammalian cells lack this capability. In this work, we transfect mammalian cells with reactive poly(ethylene glycol) macromers to form degradable intracellular hydrogels as a synthetic mimic of cryptobiosis, which we term “biostasis.” Using fluorescence correlation spectroscopy, cells containing hydrogels are shown to have restricted intracellular mobility on the nanoscale, resulting in decreased cell cycle activity and cytoskeletal remodeling while retaining high viability in both conventional 2D cell culture and 3D culture of multicellular epithelial spheroids. By forming intracellular hydrogels with photodegradable crosslinks, we show that on-demand degelation via light exposure reverses biostasis. The reversal process is monitored by label-free multiphoton fluorescence lifetime imaging to probe NADH mobility and binding as a proxy for bioenergetics. Finally, we show that biostasis treatment results in increased cellular viability in the presence of harsh stimuli, including peroxides, hypo-osmolar media, and an apoptosis-inducing peptide ligand. Taken together, these results demonstrate that our approach for inducing synthetic cryptobiosis offers a novel and potentially transformative method for controlling cell cycle activity and protecting mammalian cells from a variety of stressors. This technology holds significant promise for applications in multiple fields, including cellular preservation, biophysics, and regenerative medicine.

4:32 PM - 4:38 PM

FUNCTIONALIZING HUMAN DECM FOR INCORPORATION INTO 3D PULMONARY FIBROSIS MODELS

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Idiopathic pulmonary fibrosis (IPF) is an incurable lung disease characterized by dysregulated fibroblast activation and excessive extracellular matrix (ECM) deposition, culminating in critical lung dysfunction. Existing preclinical models inadequately simulate the nuanced pathogenesis of IPF, primarily due to their reductive representation of pulmonary architecture, thereby hindering advancements in disease comprehension and therapeutic intervention. To overcome this limitation, we have engineered an advanced 3D lung model incorporating induced pluripotent stem cell (iPSC)-derived human epithelial cells and fibroblasts within hybrid-hydrogels containing decellularized ECM (dECM) from control and fibrotic lung tissues. These models aim to emulate the intricate cellular interactions and ECM dynamics underlying IPF pathogenesis, providing a biomaterial-centric and physiologically relevant framework for elucidating disease mechanisms and evaluating potential treatments.

Protocols for dECM preparation from control and fibrotic lung tissues were first optimized for incorporation into dynamically tunable poly(ethylene glycol) alpha-methacrylate (PEGαMA) hydrogels. This process entailed systematic decellularization, pepsin-mediated digestion (10 mg dECM/mL Pepsin-HCl for up to 96 hours), and chemical functionalization to enable a thiol-ene Michael addition reaction with PEGαMA. Analytical assessments were performed at 12-hour intervals throughout the digestion phase. A bicinchoninic acid (BCA) assay measured total protein quantification, ninhydrin assays evaluated primary amine concentrations, and SDS-PAGE quantified protein fragmentation. Results showed a 48-hour digestion period for both healthy and fibrotic lung tissues led to the highest levels of soluble protein and free primary amines. Subsequently, naturally occurring primary amines on the 48-hour-digested dECM were converted to thiols via 2-iminothiolane (Traut's reagent) at 25-, 50-, 75-, or 100-molar excess. Conversion was quantified through ninhydrin and Ellman's assays and functionalization at a 75-molar excess resulted in high levels of thiolation that did not significantly increase with increasing molar excess. Current work is focused on characterizing dynamic stiffening of human control and fibrotic hybrid-hydrogels with the goal of recapitulating healthy (1-5 kPa) and fibrotic (>10 kPa) lung tissue mechanics. Future directives will concentrate on characterizing cellular responses to biochemical and biophysical cues within these hybrid-hydrogels, further elucidating IPF pathophysiology and facilitating the development of innovative treatment modalities.

Tissue Engineering

4:38 PM - 4:44 PM

Aberrant Tissue Mechanics and Mechanotransduction During Heart Development in Down Syndrome

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Individuals with Down syndrome (DS, trisomy 21) account for 70% of all cases of patients diagnosed with an atrioventricular septal defect (AVSD), the most serious of structural heart defects that arise from endocardial cushion defects1. To determine the underlying phenomena driving septation in Down syndrome, we proposed two goals: (1) to determine extracellular matrix (ECM) composition and tissue stiffness of the endocardial cushions using the Dp16 mouse model of Down syndrome; and (2) to use these findings to interrogate cell mechanotransduction of trisomy 21 induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) in response to substrate stiffness and cyclic mechanical stretch. We hypothesized that in DS, upregulation of type VI collagen and hyaluronic acid in the endocardial cushion increases cushion stiffness, altering cellular mechanotransduction and ultimately leading to differences in cell proliferation and gene expression that perturb heart development. Results found that endocardial cushions of the Dp16 mouse model of DS have an increased stiffness compared to WT. Furthermore, iPSC-CM with trisomy 21 exhibited decreased proliferation following culture on substrates of increasing stiffness, and following cyclic mechanical stretch, DS

iPSC-CM developed stress fibers, disorganized sarcomeres, and a decreased expression of mature cardiac markers. Yet cyclic mechanical stretch of control iPSC-CM induced sarcomere alignment and increased mature cardiac gene expression compared to static conditions These data argue that tissue mechanics, driven by upregulation of ECM proteins, lead to increased endocardial cushion stiffness in the Dp16 mouse, and that iPSC-CM with trisomy 21 aberrantly respond to changes to stiffness and stretch, ultimately proposing a novel avenue to investigate congenital heart defects in the Down syndrome population.

4:44 PM - 4:50 PM

4D Granular Composites to Program the Shaping of Cartilage Tissue

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Background and purpose: Cell-cell interactions and extracellular matrix (ECM) remodeling drives tissue shape transformations and is essential for the functional evolution of tissues. 4D biomaterials (providing shape transformations) have been coupled with cells to mimic tissue morphogenesis processes; however, hydrogels often restrict cell-cell interactions and lack synchronous degradation with tissue evolution that result in improper tissue formation. Granular composites (spheroids and microgels) have recently been demonstrated to intrinsically possess cell-cell interactions, allow contraction via fusion, and easily deposit and remodel ECM. Here, we demonstrate a programmable, 4D approach that layers granular composites of various remodeling rates to elicit tissue shape transformations for engineering complex topological tissues.

Methods: Norbornene-modified hyaluronic acid (degradable or non-degradable) microgels were fabricated via batch emulsion, crosslinked with UV, and washed. MSCs were seeded into microarrays and allowed to form spheroids over 2 days. Spheroid and microgels were mixed at a 35:65 volume ratio where remodeling rate and ECM deposition was monitored. Composite remodeling rate was varied by modulating individual microgel degradation or microgel population degradation via microgel mixtures. Granular composites of different remodeling rates were cast layered and allowed to culture. Bend angle, tissue mechanics, ECM deposition have been evaluated at the terminal timepoint.

Results and conclusions: Degradable and non-degradable NorHA microgels were fabricated and demonstrated to fully degrade by day 3, 14 or never. Swelling 60% by end coincided with degradable microgel formulations. Granular composites (pure microgel populations or mixed microgel populations) demonstrated varying rates of remodeling. Generally, the higher concentration of NorHA in the system leads to reduced remodeling and heterogeneous ECM deposition. Layered granular composites of varying remodeling resulted in rapid tissue shape transformations (i.e. bending >300°) over a 28-day period compared to controls (<100°). Together, a programmable 4D granular composite approach has been developed to elicit tissue shape transformations for engineering complex topological tissues.

POSTERS

Poster Number: 1

Exploring Human Mesenchymal Stromal Cell-Matrix Interactions through Nascent Protein Deposition in Bottlebrush Polymer Hydrogels

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In native extracellular microenvironments, cells synthesize and deposit various proteins to create and remodel the surrounding extracellular matrix (ECM) leading to eventual tissue creation. Hydrogels provide a platform to study cell behavior in three dimensions (3D). Prior studies in the field have investigated how hydrogel stiffness, composition, and stress-relaxation influences cell protein deposition. A key mechanical property of native cell microenvironments is the balance between strain-stiffening and stress-relaxing mechanical properties which are inherent to fibrous extracellular matrix components. Although stress-relaxation has been extensively studied, the impact of strain-stiffening biomechanical cues on cellular ECM deposition has yet to be fully understood. This work utilizes a bottlebrush polymer hydrogel system that the lab has previously developed to vary strain-stiffening behavior across a range of critical stresses (2-75 Pa). Human mesenchymal stromal cells (hMSCs) were encapsulated within the bottlebrush polymer hydrogels to determine the influence of strain-stiffening properties on protein secretion. Fluorescent noncanonical amino acid tagging (FUNCAT) was used to visualize the amount, morphology, and location of the nascent proteins secreted by cells within the bottlebrush polymer hydrogels, as a function of critical stress. The encapsulated hMSCs were cultured in methionine-free media supplemented with a methionine analog, L-Azidohomoalanine (AHA), for three and six days. After fixing, copper-free azide-alkyne cyclo-addition was used to click a fluorophore onto the proteins containing AHA for visualization. Nascent protein organization varied depending on the critical stress. The deposited protein was pericellular to the spherical cell body outside the biologically relevant regime (critical stress >25 Pa). Interestingly, inside the biologically relevant regime (critical stress <25 Pa), a more fibrous/punctate protein morphology was observed that was deposited further from the cell body and protrusions. Therefore, cells cultured in high-critical stress hydrogels are hypothesized to interact with their own deposited matrix rather than the surrounding hydrogel network. In contrast, the cells cultured in low critical stress hydrogels are hypothesized to interact equally with the deposited proteins and surrounding hydrogel matrix. This work highlights how the organization of deposited proteins can be altered by modulating the biomechanical feedback of the surrounding microenvironment.

Poster Number: 2

Development and Evaluation of Targeted Polycaprolactone Nanocapsules for Selective Delivery to Pancreatic β-Cells in Type 1 Diabetes

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Type 1 diabetes (T1D) is a chronic autoimmune condition marked by immune-mediated destruction of pancreatic β-cells. While research into the protection and proliferation of -cells has advanced, the lack of therapies that selectively target -cells has hindered therapeutic efficacy and progress in developing new treatments for T1D. To circumvent this problem, we have developed a novel polycaprolactone (PCL) nanocapsule (NC) that can be coated with interchangeable targeting ligands for β-cell targeted drug delivery. We hypothesize that NCs coated with a targeting ligand will selectively target human islet -cells. Initially focusing on Exendin-4 (Ex4), a GLP1 agonist, conjugated to hyaluronic acid (HA) for β-cell targeting, we subsequently explored ectonucleoside triphosphate diphosphohydrolase 3 (ENTPD3) antibody targeting due to its specificity for human β-cells. Our NCs averaged 258.3 nm +/- 5.46 nm in size. Coating NCs with 0.2 mg/mL fluorescently labeled HA and washing via centrifugation revealed 0.0022 mg/mL HA remained on the NCs, demonstrating stability of NC coating with peptides. NCs were loaded with Cy5 fluorophore and coated with ENTPD3 or HA-Ex4 and HA alone or guinea pig IgG antibody as negative controls. These NCs were used to treat cadaveric human islets for 24- 48-hours and islets were subsequently stained with NucBlue for cell nucleus identification and FluoZin-3 for -cell identification. NC+ cells were manually counted in ImageJ by locating FluoZin-3 positive cells with co-localized Cy5 signal, indicating NC internalization. We found HA-Ex4 and guinea pig IgG coated NC uptake in 5% and 7% of insulin+ -cells respectively, while ENTPD3-coated NCs showed uptake in 17% of imaged -cells . Additionally, NCs injected via tail vein into NOD-SCID mice were shown to target the pancreas in vivo, where NCs coated in HA-Ex4 were enriched in -cells by ~400% compared to HA only coated NCs 24h post injection (p=0.014). Pancreas specific uptake of NCs was confirmed by IVIS imaging. Our results indicate that NCs can be coated with targeting peptide for specific delivery to -cells in vitro and in vivo. The results from this study present a novel strategy for selective delivery of therapeutic cargo to β-cells, that may lead to novel treatments for T1D.

Poster Number: 3

Reversible Intracellular Gelation of MCF10A Cells Enables Programmable Control Over 3D Spheroid Growth

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In nature, some organisms can survive in extreme environments by inducing a biostatic state within the organism's molecular structure. Synthetic biostatic states in adherent mammalian cells have been previously achieved via intracellular network formation. This is attained by using bio-orthogonal strain-promoted azide-alkyne cycloaddition (SPAAC) reactions between functionalized poly(ethylene glycol) (PEG) macromers introduced to the cell by a lipofectamine aided transfection. These macromers are able to spontaneously crosslink within the cytosol. In this work, the effects of intracellular network formation in a complex 3D epithelial MCF10A spheroid model are explored. Transfected cells are encapsulated in a proteinous 3D matrix, Matrigel, and overall spheroid area is reduced by ~50% compared to controls. Interestingly, intracellular network formation also induced a change in cell cycle state. Network formation results in a higher quiescent cell population indicated by the loss of phospho-Rb and a gain in p21 expression within single cell analysis of spheroids. After lipofectamine-aided transfection with SPAAC macromers, the formed network reduces overall bioenergetic (ATP/ADP) levels and functional metabolic rates, while also inducing quiescence -like effect in the mitochondrial electron transport chain. These effects are confirmed by Fluorescence Lifetime Imaging Microscopy (FLIM) and Seahorse Cell Metabolic Analysis. To enable reversibility of the observed biostasis effect within the model, a photodegradable nitrobenzyl moiety is incorporated into an azide containing macromer. This allows the PEG network to experience photoinduced degradation. The degraded network allows for continued proliferation and a return to normal spheroid growth. Following light exposure at day 12, growth and metabolic rates return to control levels, while SPAAC treated spheroids that did not receive light exposure (i.e., spheroids containing intact intracellular networks) remain smaller and less metabolically active through this same period. These results demonstrate that photodegradable intracellular polymer networks in 3D spheroid culture is a novel method that can control metabolic states and induce a reversible quiescent state.

Poster Number: 4

Characterization of low-cost nanoprecipitation system for nanoparticle fabrication

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Statement of Purpose:

Nanoparticles (NPs) have a variety of tunable characteristics that make them efficient drug delivery tools. Diameter is an important characteristic that directly impacts blood clearance, biodistribution, and biological barrier penetration. NPs within the range of 10-200 nm are preferred as this enhances extravasation and bioavailability. Nanoprecipitation is a popular fabrication technique effective for small drug encapsulation. It involves dissolving a biomaterial and drug in an organic solvent that is mixed in a stirring aqueous solution. Under adequate parameters, NPs will spontaneously nucleate and form a colloidal suspension.

In this study we developed and characterized a low-cost, open-source syringe pump that can fabricate sub-100nm lipid nanoparticles (LNPs) while encapsulating a small drug (diclofenac). The LNPs are made of a 10:48:40:2 blend of 1,2-distearoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-3-trimethylammonium-propane, cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[azido(polyethylene glycol)-2000], respectively.

Methods:

An open-source syringe pump was adapted for this application; the pump was assembled with 3D-printed components. The stepper motor was controlled by an Arduino board and a compatible driver. For the biomaterial injection, a gastight glass syringe was coupled with a 20cm section of fused silica tubing (100µm inner diameter). The organic solution was prepared by dissolving 4mg of lipids with 1.2-2mg of diclofenac in ethanol. The syringe injected the organic solution at 1.5-3 ml/min into deionized water stirring at 400-1200rpms. The aqueous to organic ratio ranged between 45:1 to 5:1. A quarter factorial design of experiments (DOE) was conducted to assess the variables and their effects on the LNP physical properties. The LNP size, polydispersity, and zeta potential was assessed via dynamic light scattering. The combination of parameters generating the smallest LNP were subsequently employed for drug encapsulation (diclofenac). The encapsulation efficiency (EE) was evaluated via ultraviolet-visible spectroscopy.

Results:

The DOE demonstrated that all variable combinations yielded sub-100nm LNPs. The formulation of A:O of 5:1, injection rate of 3ml/min and stirring rate of 400rpms yielded the smallest LNPs (55.58nm). We observed an increase in LNP size between 98.5 to 110.5nm when encapsulating diclofenac at an EE of 33.5% to 26.85%, respectively. This study demonstrated the feasibility of a cost-effective method for LNPs synthesis and drug encapsulation.

Poster Number: 5

Engineering 3D Lung Models with Magnetically Labeled Fibroblasts

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Idiopathic pulmonary fibrosis (IPF) is a chronic disease that progressively affects the lung, leading to respiratory failure. The extracellular matrix (ECM), the proteins and other molecules supporting the cells in our tissues, plays an important role in lung development, repair, and disease. Biophysical and biochemical signals produced by the ECM regulate the function of various cells including fibroblasts in the lungs. Fibroblasts are important lung structural cells providing production and repair of the ECM. There is an increased ECM deposition during IPF that results in increased tissue stiffness and a positive feedback loop of fibroblast activation. The Magin lab has advanced technologies for engineering models of lung tissue and created hybrid-hydrogels to mimic, decouple, and study biochemical and biophysical changes in the cellular microenvironments. In my project, I decellularized human lung from healthy and fibrotic donors and incorporated the resulting proteins and other molecules into 3D hybrid hydrogel models. In these models, we recreated lung geometry by magentically aggregating a layer of fibroblasts around stem cell-derived epithelial cell spheroids. We confirmed the decellularizaion process by analyzing the residual DNA that was below 50 ng/mg in both healthy and fibrotic samples. We selected fibroblast densities and aggregation time to create the structure that best replicates distal lung architecture. By embedding these structures in hybrid-hydrogels containing either healthy or fibrotic dECM, we will investigate the role of both cellular and extracellular components in the progression of IPF. This model will allow for patient-centered research by using human cells and dECM to study fibrotic phenotypes in a system that mimics the geometry, composition, and biomechanical properties of lung tissue.

Poster Number: 6

Tailoring Cationic Polymer Microstructures for Improved Nucleic Acid Delivery

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Viral vectors have proven safe and efficacious in clinical gene therapy, but their exorbitant costs motivate the development of synthetic nanocarriers, such as polymers. Thanks to their manufacturability, cationic polymers are promising for nucleic acid delivery, but their efficiency and safety profiles are highly sensitive to their physiochemical properties and interactions with nucleic acids, plasma proteins, and cellular targets. In this study, we explore how polymer microstructure engineering – synthesizing statistical, block, and gradient copolymers to vary the spatial distribution of cationic and neutral monomers –tunes plasmid (pDNA) delivery efficiency, hemocompatibility, and cytotoxicity. Careful physiochemical characterization revealed distinct behaviors among polymer microstructures. For instance, we compared polyplex sizes under different environmental conditions (water and 10% serum) and established that block copolymers granted the most serum-stable polyplexes. Similarly, block polyplexes are cytocompatibility but fall short in cellular internalization and transfection efficiency. In contrast, statistical polyplexes excel in cellular internalization and transfection at the cost of cell viability, whereas gradient polyplexes strike an optimal balance between transfection efficiency and cytotoxicity. Overall, our work highlights the importance of tailoring polymer microstructure to optimize multiple competing objectives such as serum stability, transfection efficiency, and cytocompatibility.

Poster Number: 7

Photopolymerizable dual-crosslinked boronate ester hydrogels to study cellular responses to rapid stress relaxation

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Viscoelasticity is an important characteristic of biomaterials, influencing many cellular responses such as morphology, proliferation, migration, and extracellular matrix (ECM) deposition. As a result, interest has grown in designing viscoelastic biomaterials as synthetic matrices for more physiologically relevant in vitro tissue models. While current synthetic ECMs cover a wide range of stiffnesses and rates of stress relaxation, fast-relaxing materials (half times on the order of seconds, resembling the native properties of

brain or adipose tissue) are relatively under-explored, partly due to a limited number of chemical and physical crosslinking methods that can achieve such fast relaxation. In some applications, covalent adaptable networks utilizing boronate ester crosslinks have been developed to access these material properties. While these systems enable fast-relaxing synthetic ECMs, most modern viscoelastic biomaterials have two fundamental limitations: lack of homogeneity due to fast crosslink formation and difficulty tuning the degree of stress relaxation. In this work, we design and characterize a fast-relaxing dual-crosslinked PEG hydrogel that addresses both limitations and apply this system to cell and organoid culture. Uniquely, our system utilizes a combined competitor/crosslinker molecule which produces highly homogeneous photopolymerizable hydrogels, affording spatiotemporal control over network formation and facile manipulation of material (visco)elasticity by altering the ratio of elastic to viscoelastic crosslinks. Cells encapsulated in this material display remarkable cellular remodeling and contraction of the matrix when cell-adhesive peptides are incorporated into the hydrogel network. By developing improved fast-relaxing biomaterials, we can investigate how mechanobiological cues such as the degree of stress relaxation affect cell behavior in hydrogels with varied stiffness. Finally, the photopolymerizable nature of this system is compatible with light-based additive manufacturing techniques, potentially improving not only the physiological relevance of fast-relaxing tissue models, but also allowing for biofabrication-based approaches to manufacturing tissue-scale cellularized architectures.

Poster Number: 8

Synthesis and in vitro applications of polymeric CO-releasing molecules

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Although known for its toxicity, carbon monoxide (CO) also exhibits therapeutic potential against a variety of diseases including cancer, autoimmune disorders, and sepsis. Nevertheless, the precise mechanism underlying CO's therapeutic action remains elusive due to the lack of non-toxic CO delivery methods with controlled and triggerable CO release. A novel platform of CO-releasing polymers (CORPs) has been designed and synthesized leveraging the CO releasing moiety diphenyl cyclopropenone (DPCP). DPCP and DPCP-derived polymers release CO gas via photodecarbonylation upon exposure to light. The DPCP analogue has been polymerized with hydrophilic moieties to improve water solubility and allow for biological applications. This presentation will discuss the synthesis and application of these new polymers in-vitro.

Poster Number: 9

Engineering Hydrogel Biomaterials to Study the Role of Integrin Binding and Environmental Stiffness in AT2-to-AT1 Lung Cell Differentiation

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Chronic respiratory diseases such as emphysema and pulmonary fibrosis are characterized by changes in extracellular matrix (ECM) composition and stiffness. These biochemical and biophysical alterations may influence differentiation of alveolar type 2 (AT2) lung progenitor cells into alveolar type 1 (AT1) cells. AT1 cells are responsible for gas exchange in the lung. AT2 cells have been shown to express mainly laminin-binding integrins, such as α6β1 and α3β1 in vivo. Integrins such as αvβ6, αvβ3, and α2β1 have been implicated in the differentiation of AT2 to AT1 cells and αvβ3 has been described in the fully differentiated AT1 cells. Our understanding of how these cues influence differentiation is limited by current alveolar models that rely on Matrigel, limiting physiological relevance and our ability to assess the impact of both ECM signaling and stiffness on AT2 cell stemness and differentiation. We have designed new peptide-functionalized hydrogel biomaterials to evaluate the influence of microenvironmental stiffness and ECM composition on AT2-to-AT1 differentiation. Induced pluripotent stem cell (iPSC)-derived AT2 cells (iAT2s) were seeded onto poly(ethylene glycol) alpha methacrylate (PEGαMA) hydrogels of three distinct stiffnesses, ranging from healthy (E = 3.96 kPa ± 0.196) to fibrotic (E = 20.27 kPa ± 0.546) as well as tissue culture polystyrene (TCPS). The effect of these cues on differentiation was analyzed through qPCR and immunofluorescent staining for AT2 (LAMP3, ABCA3, STFPA2, SFTPC), AT2-to-AT1 transitional (KRT8, KRT17, AREG, CLDN4), and AT1 markers (AGER, PDPN, SPOCK2, GPCR5A). Preliminary experiments show that iAT2s seeded onto TCPS with exposure to serum expressed greater KRT17. Furthermore, in a preliminary run of iAT2s on hydrogels with stiffnesses of 4 kPa, 12 kPa, and 20 kPa, data suggests that higher stiffness generally leads to greater AT1 gene expression (AGER, PDPN, SPOCK2, GPRC5A) compared to lower stiffnesses through day 7 and SFTPC expression, denoting AT2 identity, is greater in lower stiffnesses through day 7. Using this hydrogel model, we can separately study the effects of stiffness and integrin binding on both AT2 differentiation and stemness. Future studies could use this model to construct environments capable of supporting alveolar cell populations for drug testing and more accurate lung tissue engineering.

Poster Number: 10

Photocatalytic Targeted Release of CO as a Therapeutic Agent

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In recent years, there has been a growing interest surrounding the use of carbon monoxide (CO) in therapeutic settings. Methods of delivery include direct CO inhalation, which inherently lacks dosing control. The newer delivery approach is to use chemically bound CO which can be isolated in solids improving control, however current systems have toxicity issues. Hence, a need has arisen to create a system which allows for targeted and quick release of CO in the body at controlled locations with little to no adverse side effects. This poster will look at the synthetic approach and optimization of new polymeric CO releasing molecules with photocatalytic capabilities and near IR light triggered CO release.

Poster Number: 11

Nonlinear Elastic Microenvironments During Osteogenic Differentiation Promote Osteocytogenesis and Dendritic Network Formation

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The unmineralized collagen I-rich osteoid supports the transition of osteoblasts into osteocytes during bone formation as osteoprogenitor cells become encased and buried within this nonlinear elastic, fibrous matrix. Inspired by how strain-stiffening extracellular microenvironments play a critical role in cellular transformations in vivo, poly(ethylene glycol)-based bottlebrush polymer hydrogels were engineered to mimic the strain-stiffening mechanical properties found in the unmineralized osteoid. Previous work has shown that these covalently crosslinked bottlebrush polymer hydrogels can recapitulate strain-stiffening biomechanical microenvironments at biologically relevant stresses. By tailoring the bottlebrush polymer length in the hydrogel network, the critical stress associated with the onset of stiffening can be systematically varied to occur outside and within a biologically relevant stress regime (BRSR). When the critical stress of the material is within the BRSR, encapsulated human mesenchymal stromal cells (hMSCs) adopted a unique protrusion-rich morphology. This morphology was elucidated to be driven by cell-matrix interactions and regulated through actomyosin dynamics. In this work, the bottlebrush polymer hydrogels are used as a model culture platform probe how nonlinear elastic mechanical properties regulate cell fate. To date, there have been few successful attempts to monitor and support the hMSC-to-osteoblast-to-osteocyte transition using a three-dimensional culture platform in vitro. Using the bottlebrush polymer hydrogels as an osteoid-mimetic synthetic culture platform, a 6-week osteogenic differentiation protocol was used to pre-differentiate hMSCs into osteoblast-like cells for 2 weeks on 2D followed by 4 weeks of continued differentiation encapsulated within 3D bottlebrush hydrogel networks or collagen I controls. In the bottlebrush hydrogels, the pre-differentiated osteoblasts are observed to form dendritic protrusions which fuse into a functional dendritic network over the course of 4 weeks. These protrusion-rich morphologies coincide with an upregulation of E11 and DMP-1 (pre-osteocyte markers) and the formation of connexin 43 positive gap junctions. Fluorescence recovery after photobleaching experiments reveal the real-time recovery of calcein in photobleached cells from surrounding cells through functional gap junctions. The nonlinear elastic mechanical properties of the bottlebrush polymer hydrogels drive the up-regulation of a protrusion-rich morphotype promoting osteocytogenesis, and these engineered synthetic extracellular microenvironments hold great potential as a in vitro 3D culture model for osteocytes.

Poster Number: 12

Biocompatible, photocurable poly(β-amino ester) nanoparticles with programmable shape, degradation and mechanical stiffness for drug delivery

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Over the past several decades, numerous synthetic polymer systems have been considered to enhance the delivery of drugs for treating disease. Such polymer systems have been established to control cross linking, encapsulate drugs of various hydrophilicities, and degrade at prescribed rates. However, few of these polymer systems also enable the manufacturing of nanoparticles with well-defined shapes. This critical gap in capability is a major impediment to the needs of next-generation drug-delivery vehicles, as particle shape has been shown to play an important role in nanoparticle delivery and fate. To address these needs, we developed methods to fabricate micro/nanoparticles from UV-curable polymers with tunable shape and mechanical properties. Our research has explored poly(beta-amino ester) (PBAE) synthesis from 1,4-butanediol with either isobutylamine or benzhydrazide at various molar ratios of diacrylate:amine, ranging from 1.2:1 to 10:1. The addition of diphenyl (2,4,6-trimethylbenzoyl)phosphine oxide (TPO) allows the pendant diacrylate groups to crosslink when exposed to UV light, enabling a myriad of unique nanoparticle shapes through photolithography (e.g., discoids, hexagonal prisms) using a mask aligner. Particle height is tunable by using different spin speeds when spin coating the polymer onto a silicon wafer. The various molar ratios achieve different molecular weights, which results in varying crosslink densities and a range of elastic moduli (i.e., ~3 to 750 MPa). We manipulated PBAE chemistry to temporally control polymer degradation in an accelerated degradation study. In 1M NaOH at 37°C, some compositions showed full degradation in just a few days, suggesting the material is degradable at physiological timescales, while other compositions showed <10% degradation after seven weeks. To find the degradation time of microparticles in physiological conditions, we investigated the degradation of 8 unique PBAE compositions in PBS at 37°C. We observed similar programmable degradation. Further, we used an MTT assay to assess the effect of different diacrylate:amine molar ratios on biocompatibility. We observed that none of the studied PBAEs were cytotoxic. Altogether, our work establishes photocurable PBAEs as a biocompatible material that enables the formation of nanoparticles with tunable shape and mechanical properties, readily available for drug delivery and related applications.

Poster Number: 13

Anisotropic shape remolding of dithiolane crosslinked microgels

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Widespread deployment of granular biomaterials in tissue engineering has resulted from their unique properties, including porosity, tunability, injectability, and 3D printability. However, granular hydrogels have typically been restricted to spherical particles, with limited deployment of more intricate particle morphologies, typically requiring the individual templating of microgels through microfluidics or molds. Here, we use a dithiolane crosslinked hydrogel system for the fabrication of spherical microgels through a batch emulsion photopolymerization. Owing to the dithiolane crosslinking, these materials have inherent dynamic disulfides present at the crosslinks, which we harness to reconfigure the particle shape. Utilizing unconfined compression between parallel plates, we transform these particles into anisotropic disks through photoinitiated radical-mediated disulfide exchange. Analysis of this procedure indicates regions of effectively zero curvature of the regions of the microgel surface in contact with the parallel plates, while the curvature at the boundary regions is increased. When cultured in the presence of C2C12 myoblasts, the cells were found to localize to areas of higher curvature on the disk-shaped microgel surface. This preferential localization impacts the cell-driven assembly of large supraparticle scaffolds, with spherical particles assembling without specific junction structures, while disk microgels assemble interfaces preferentially on their curved faces. These findings present a distinctive spatiotemporal process for the rapid transformation of microgels into anisotropic shapes, offering new avenues to explore shape-driven mechanobiological cues during and after granular hydrogel assembly.

Poster Number: 14

Fabrication of a printable PLGA nanoparticle-loaded hydrogel for in vitro glioblastoma drug delivery models

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Bioprinting has enabled tissue engineers to control the spatiotemporal deposition of cells in an extracellular matrix-like material with increasingly sophisticated precision. The Crosby Lab at Southwestern University and the Clegg Lab at the University of Oklahoma seek to use these developments in polymer modification, nanoparticle synthesis, and open-source hardware to fabricate a novel hydrogel for treating neurological diseases. Specifically, we aim to partly recapitulate the brain parenchyma's physical and chemical characteristics for deployment in in vitro drug delivery models with a focus on models of glioblastoma. We incorporated gelatin methacryloyl (GelMA), methacrylated hyaluronic acid (HAMA), PEGDA, and blank PLGA nanoparticles to achieve this goal and mimic local therapeutic delivery. To ensure that our in vitro tissue scaffold captures the viscoelastic properties of brain tissue, we conducted compression and swelling tests on hydrogel samples. We performed these tests on five 8 mm diameter cylindrical samples consisting of GelMA, GelMA-HAMA (GH), GelMA-HAMA-PEGDA (GHP), and GelMA-HAMA-PLGA (GHPP). GelMA was synthesized by adding methacrylic anhydride (MAAnh) in a buffered solution according to the one-pot method. HAMA was synthesized by dissolving sodium hyaluronate in ultrapure water, adding MAAnh, and maintaining a basic pH with NaOH over two days. PEGDA was purchased from a commercial supplier. The PLGA nanoparticles were synthesized by dissolving PLGA in acetone and adding the mixture dropwise to a poly(vinyl alcohol) solution. The compression testing was performed on a Univert CellScale. The swelling tests were conducted in separate DI water baths over several days. Weight measurements were taken for the first few hours, and diameter measurements were recorded over the total duration. Results from compression testing indicated that GHP exhibited the highest compressive modulus, and that the addition of nanoparticles did not influence the final modulus. However, adding the nanoparticles enhanced the extrudability of the hydrogel while significantly improving printability. Adding HAMA reduced the swelling of the pristine GelMA hydrogel, and the additions of PEGDA and PLGA had little effect on the swelling ratio. This data and future rheological testing will further inform the encapsulation of primary glioblastoma cells alongside doxorubicin-loaded nanoparticles in a drug delivery microenvironment.

Poster Number: 15

Islet survival under cytokine stress is mediated by laminin interactions in a 3D reverse thermal gel scaffold

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Pancreatic islets are directly surrounded by a dense network of proteins and polysaccharides known as the extracellular matrix (ECM). Type 1 diabetes (T1D) is characterized as an autoimmune-mediated destruction of insulin-producing β cells within the islet. Infiltrating immune cells in the pancreas secrete high levels of pro-inflammatory cytokines that disrupt islet function and lead to β-cell death. The peri-islet ECM provides a barrier against infiltrating immune cells and is crucial for islet survival. Specifically, laminin is an ECM component that has been shown to protect β cells from cytokine-induced apoptosis via specific transmembrane integrins. To investigate this, we have employed a 3D biomimetic hydrogel functionalized with full-length laminin. This reverse thermal gel (RTG) spontaneously forms a 3D scaffold at body temperature and can easily be reverse gelled to extract the cells for biochemical analysis. We have encapsulated islets in an RTG polymer alone or functionalized with laminin (RTG-LAM) and treated with a pro-inflammatory cytokine cocktail of TNF-α, IL-1β and IFN-γ to show that islet interactions with laminin promote islet survival.

Poster Number: 16

Islet survival under cytokine stress is mediated by laminin interactions in a 3D reverse thermal gel scaffold

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Pancreatic islets are directly surrounded by a dense network of proteins and polysaccharides known as the extracellular matrix (ECM). Type 1 diabetes (T1D) is characterized as an autoimmune-mediated destruction of insulin-producing β cells within the islet. Infiltrating immune cells in the pancreas secrete high levels of pro-inflammatory cytokines that disrupt islet function and lead to β-cell death. The peri-islet ECM provides a barrier against infiltrating immune cells and is crucial for islet survival. Specifically, laminin is an ECM component that has been shown to protect β cells from cytokine-induced apoptosis via specific transmembrane integrins. To investigate this, we have employed a 3D biomimetic hydrogel functionalized with full-length laminin. This reverse thermal gel (RTG) spontaneously forms a 3D scaffold at body temperature and can easily be reverse gelled to extract the cells for biochemical analysis. We have encapsulated islets in an RTG polymer alone or functionalized with laminin (RTG-LAM) and treated with a pro-inflammatory cytokine cocktail of TNF-α, IL-1β and IFN-γ to show that islet interactions with laminin promote islet survival.

Poster Number: 17

Synovial fibroblasts support vascular function in both health and inflammatory disease

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The synovium is a vascularized, joint-lining tissue present in the knee, as well as in other articulating joints. After an acute trauma, the synovium undergoes marked changes in cellular composition. While advances in single cell sequencing have elucidated these cellular changes, the distinct roles of such cells have yet to be identified in both health and disease (inflammation). In this study, we aimed to develop a microphysiologic model of the human synovium to determine the specific role of synovial fibroblasts in maintaining vascular health. And so, endothelialized vessels were engineered within collagen gels (one of the primary matrix components of the synovium), exposed to IL-1B (inflammation/injury), and functional outcomes (permeability, collagen remodeling, synoviocyte-vessel colocalization) were assessed over 8 days in culture. We first discovered that the vessels matured rapidly with the addition of synoviocytes. Next, we introduced the pro-inflammatory cytokine IL1B daily to the cell culture media, and reassessed permeability at Day 8. Interestingly, the lumens without synoviocytes demonstrated a strong increase in permeability in response to IL1B treatment. However, the lumens surrounded by synoviocytes maintained their permeability. To probe the mechanisms behind this finding, we fabricated vessels using FITC-labeled collagen, enabling tracking of matrix remodeling. On Day 8, we imaged the bottom half of the vessels, and binarized the images to quantify collagen accumulation around the vessel periphery. These images highlight how endothelial cells alone remodel their environment; however, the synoviocytes elevate this endothelial cell-mediated response. In comparison to the -IL1B baseline, +IL1B appears to diminish the layer of collagen around the vessels. As mentioned, we believe that synoviocytes instruct the endothelial cells to remodel their surrounding environment, creating a ‘basement membrane,’ as we see no remodeling in the absence of endothelial cells. Lastly, we observed significantly more synovial fibroblasts surrounding our healthy vessels, in comparison to the +IL1B group. Future work will identify cell-secreted factors from both synoviocytes and endothelial cells that contribute to matrix remodeling. Overall, this model is the first-of-its-kind to investigate the role of synovial fibroblasts in health and inflammatory disease, focusing on synovial-vascular interactions.

Poster Number: 18

Immunomodulatory vaccine for traumatic brain injury

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Traumatic brain injury (TBI) affects over 2.5 million people each year in the US. Moderate-severe TBI is linked to increased risk of cognitive decline after injury, yet therapeutic interventions are limited. Therefore, we examined the efficacy of immunomodulatory polyesters of alpha-ketoglutarate (paKG) particles dosed prophylactically prior to TBI in a mouse to diminish chronic inflammation. This formulation aimed to generate antigen-specific memory T cells to myelin proteolipid protein (PLP) and modulate the immune metabolism resulting in altered phenotype.

paKG microparticles (MPs) encapsulated the glycolytic inhibitor PFK15 alone or in combination with the peptide antigen PLP 139-151. C57BL/6J male mice received subcutaneous injections of particles or saline at 17 and 3 days prior to controlled cortical impact (CCI). Day 7 and 28 post-injury, the brain, spleen, and lymph nodes were processed to assess immune cell profiles by flow cytometry. Brain tissue was analyzed with Nanostring.

Day 7 post-injury, flow cytometry revealed alternatively activated antigen presenting cells at the injury site and in secondary lymphoid organs. paKG(PFK15+PLP) increased cells or frequency of immunosuppressive PLP-specific central memory Th2 and Treg in the brain versus no treatment. Moreover, at day 28 post-injury paKG(PFK15+PLP) and paKG(PFK15) decreased expression of PLP specific T-cell receptors on Th1 and Th17 cells in cervical lymph nodes versus no treatment. Nanostring proteomic analysis at day 7 post-injury revealed that paKG(PFK15+PLP) exhibited upregulation in proteins associated with autophagy, potentially indicating neuroprotective effects. Rotarod motor behavior assay indicated functional impact of paKG(PF15+PLP) by increase in motor learning acutely post-TBI observed when compared to paKG(PFK15) and saline control. The open field test on day 27 showed significantly greater distance traveled with paKG(PFK15+PLP) in mice treated with paKG(PFK15+PLP) compared to paKG(PFK15) and naïve control, which may indicate lower anxiety.

This study was the first to our knowledge that assessed a vaccine-based approach to prime the immune system prior to TBI. This revealed that the circulating immune profile, infiltrating immune cells after TBI, and local neuroinflammatory process can be modulated with the vaccine, shifting away from a pro-inflammatory state. This resulted in modest behavioral outcomes, underscoring the critical importance of the neural-immune crosstalk in neurotrauma.

Poster Number: 19

Combinatorial Macrophage Modulation Using Cavitating Mesoporous Silica Nanoparticles

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Solid tumors avoid immune recognition by recruiting macrophages and driving their polarization toward anti-inflammatory phenotypes, which protect the tumor from killer T cells and promote tumor growth. Therefore, reprogramming tumor-associated macrophages toward a pro-inflammatory phenotype proposes a promising method of treating so-called “cold” tumors. To realize this strategy, we developed a mesoporous silica nanoparticle system that can (1) deliver the immunomodulatory drug resiquimod (R848) to macrophages and (2) act as seeds for cavitating microbubbles, which results in damage to cancer cells and release of damage-associated molecular patterns (DAMPs). Mesoporous silica nanoparticles were synthesized using a modified Stöber process and were coated with an alkyl moiety, creating a hydrophobic surface. Under high intensity focused ultrasound (HIFU), the surface facilitates the formation of cavitation bubbles. The violent collapse of these bubbles has been shown to lyse cancer cells and release DAMPs. Using this system, we studied the combined effects of resiquimod and DAMPs on the phenotypes of macrophages cultured in tumor-like conditions (tumor-conditioned media and hypoxia). We tested our drug delivery system on 4T1 murine mammary carcinoma cells in vitro due to its reputation for being highly nonimmunogenic. Our results show that CD86, MHC II, and iNOS were all upregulated in macrophages treated with R848-loaded particles actuated under HIFU. However, IL-12 secretion was severely dampened compared to cells treated with R848 or DAMPs separately. We also found our material control of particles without R848 or HIFU stimulation induced a slight inflammatory phenotypic shift in macrophages, mainly due to upregulation of CD86 and iNOS and downregulation of CD206. Future work will entail understanding how this material can prime the immune system for relapsing tumors, which could give rise to an innovative method for cancer vaccination. Overall, this work shows that hydrophobically modified mesoporous silica nanoparticles, under the influence of HIFU, can enhance macrophage effector functions like T cell activation through the upregulation of CD86 and MHC II and macrophage-mediated cytotoxicity from secretion of nitric oxide through iNOS. Together, these effector functions offer a new route of malignant cell clearance in cold tumors.

Poster Number: 20

Modulating Immune Responses in Vaccine Immunotherapy: The Impact of Adjuvant Crystallinity

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Biomaterials can act as pro- or anti-inflammatory agents; however, the effect of the degree of crystallinity of biomaterials on immune responses is poorly understood. Herein, we demonstrate that the adjuvant-like behavior of covalent organic framework (COF) biomaterials depends on its crystallinity, which controls anti-tumor immunity in mouse melanoma models. An inverse relationship was observed between COF crystallinity and activation of mice and human dendritic cells (DCs) and antigen-presentation by mice DCs. Also, amorphous COFs upregulated NFkB, TNFa, and RIG-I signaling pathways compared to crystalline COFs, and chemotaxis-associated gene unc5c was found to be inversely correlated with crystallinity. Furthermore, it was determined that COFs with the lowest crystallinity admixed with OVA antigen were able to prevent B16F10 expressing chicken ovalbumin (OVA) tumor growth in 60% of mice, and the lowest crystalline COFs admixed with TRP2 antigen were able to prevent YUMM1.1 tumor growth in 50% of mice. The lowest crystalline COFs also induced antigen-specific pro-inflammatory T-cell responses in B16F10-OVA tumors. These results demonstrate that the crystallinity of biomaterials is an essential factor to consider when designing immunotherapy for pro- or anti-inflammatory applications.

Poster Number: 21

Biodistribution Assessment of Macrophage-Nanoparticle Complexes in B16-F10 Melanoma Cancers

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Systemic administration of nano- and microparticles offers limited control of their site-specific accumulation. As a result, adoptive cell therapy (ACT) is an attractive alternative. Among the types of cells used in ACT, macrophages are a potential therapeutic platform because they can navigate biological barriers and respond to inflammatory signals throughout the body unlike synthetic particles. Macrophages can adopt multiple phenotypes that are beneficial in homeostatic contexts, but this plasticity can pose challenges for engineering consistent therapeutic behaviors when reprogramming macrophages ex vivo and readministering for ACT applications. In particular, we have shown that macrophages associated with microparticles of discrete geometries shape impact their phenotype through differential changes in chromatin accessibility, gene expression, biomarkers, and cytokine secretion. Therefore, characterizing their biodistribution profiles is essential to evaluate their potential as an ACT modality. Here, we assessed the in vivo biodistribution of macrophages associated with particles of two shapes, discoidal and spherical, in B16-F10-tumor-bearing C57BL/6 mice. This is accomplished by associating discoidal or spherical particles to macrophages in vitro, staining cells with VivoTrack 680 (a lipophilic near-infrared dye), and administering complexes via a single tail vein injection. We used a homogenization method to track the biodistribution of the stained cells into native tissue types including the lungs, liver, spleen, kidneys, brain, heart, and B16-F10 tumor. We developed a quantitative fluorescence-based biodistribution method by determining VivoTrack 680 fluorescence saturation in bone marrow-derived macrophages, generating fluorescent standard curves of VivoTrack 680 in each organ homogenate extract, and calculating extraction efficiencies for each whole organ. Fluorescence of the homogenate extract was then measured and the percentage of cell infiltration was reported based on respective standard curves. This quantitative method was complemented with spatiotemporal analysis using an in vivo imaging system (IVIS). Our work reveals that the shape of nanoparticles, when bound to macrophages, affects their accumulation in B16-F10 tumors, which aligns with our multi-omics analyses of macrophage activation states induced by particle association. Our work observes that particle shape can modulate macrophage phenotype and downstream trafficking of macrophages to tumors, thus having implications for controlling biodistribution profiles in macrophage-mediated ACT.

Poster Number: 22

Scalable, high throughput biomanufacturing of hydrogel encapsulated cell products

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Clinical islet transplantation, a cell therapy-based treatment for insulin-dependent diabetic patients, would eliminate the pain and cost burden of daily insulin injections, as well as the risk of long-term secondary complications, by restoring native insulin signaling. While clinical successes have demonstrated the feasibility of achieving insulin independence via this therapy, the necessity of long-term immunosuppressive drugs limits the widespread applicability of this procedure. As a result, researchers have investigated devices to isolate transplanted cells from the recipient immune system, including hydrogel macroencapsulation devices which prioritize whole graft containment, retrievability, and safety. To generate macroencapsulation devices with complex geometries in a high-throughput and scalable manner, we have developed a hydrogel injection molding technique. This technique can be automated to minimize labor costs and enhance sterility of the procedure, and be fully integrated into a biomanufacturing workflow. A critical component of this system is the requirement of hydrogel component mixing prior to injection within the molding system. To achieve this, we have engineered two prototype microfluidic mixing systems, a ‘paddle’ and ‘channel’ mixer. Here, we (1) use fluid dynamic modeling to predict the efficacy of hydrogel mixing and turbulent flow within the devices and minimize laminar flow, and (2) evaluate the mixers in vitro for mixing efficiency.

Solidworks Computational Fluid Dynamics software was used to simulate Newtonian and Non-Newtonian fluid flow for PEG and alginate hydrogels, respectively. A range of flow rates at varying ratios within the inlets were evaluated to achieve homogenous mixing at the outlet within the simulation. To validate in silico observations, we 3D printed our “paddle” and “channel” microfluidic mixer designs and used food coloring to label first water, then hydrogel components in the inlets. Images of fluid flow at the outlet are used to quantify the degree of mixing in each of the tested solutions. We are currently evaluating whether our mixer designs generate laminar or turbulent flow in vitro and under what conditions. Finally, future studies will incorporate a cellular channel within our microfluidic mixer flow and evaluate cell viability and function post-encapsulation.

Poster Number: 23

FAP-Responsive Hydrogel for Cardiac Repair Post-Myocardial Infarction

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Despite recent advances in pharmacological and reperfusion treatments, many patients will experience heart failure after myocardial infarction (MI), making cardiovascular disease the leading cause of death worldwide. Hydrogels are being explored for post-MI treatment due to their mechanical support of the tissue, as well as their ability to deliver a variety of therapies (e.g., cells, drugs) for local and sustained presentation. Hydrogels can also be customized for disease-specific applications, such as the introduction of stimulus-responsive features that trigger the release of an encapsulated therapeutic. With this in mind, fibroblast activation protein α (FAP), a membrane-bound protease, is specifically upregulated in the heart post-MI, and provides an opportunity to deliver therapeutics on demand based on local FAP activity. To create an FAP-responsive hydrogel, hyaluronic acid (HA) was modified with maleimide (MA) groups, which selectively react with thiols at the appropriate pH. MAHA was reacted with an FAP-responsive peptide (GCNSGPSNCG) containing thiols on both ends for crosslinking or dithiothreitol as a control. To evaluate hydrogel degradation, recombinant FAP was added in two concentrations (0.1 ug/ml, 0.001 ug/ml) and compared to phosphate buffer saline, and the solutions were collected over time (1, 3, 5, 24, 72, 120, 168 hrs) and measured for HA release via uronic acid assay. The results indicated that FAP-responsive gels degraded in an FAP concentration dependent manner, with less degradation observed in buffer over the time period measured. Further, the introduction of a non-degradable crosslinker also limited hydrogel degradation. These findings indicate the tunability of hydrogel degradation based on local enzyme concentration, which could be used to release encapsulated therapeutics on demand, which is important due to the heterogeneity of expression of FAP within MI patients. Future studies will adapt the hydrogel design to introduce injectability for easy tissue delivery and explore FAP-dependent release of therapeutics.

Poster Number: 24

Additive Manufacturing of Highly Entangled Polymer Network Tissue Adhesives

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Tough hydrogels are a broadly useful class of materials for biomedical applications due to their robust mechanical properties and ability to form strong adhesions with wet tissues. Toughness is important for adhesives to dissipate stress under loading and to maintain contact with tissues. Load dissipation is typically introduced into soft materials through double network hydrogel design; however, double network hydrogels suffer from high hysteresis and require multiple steps to fabricate, limiting their utility in biomedical applications with repeated loading and 3D printing techniques. Further, most hydrogels designed as tissue adhesives are presented as uniform materials, which can limit adhesion on non-planar interfaces. To address these concerns, we looked towards the additive manufacturing of single-network hydrogels that are highly entangled to improve toughness. Others have introduced polymer chain entanglements within single network hydrogels to synergistically improve stiffness and toughness, yet attaining such dense entanglements through lithography-based additive manufacturing (e.g., digital light processing, DLP) remains elusive. Here, we introduce a facile strategy that combines light and dark polymerization through controlled initiator and light presentation to allow constituent polymer chains to densely entangle as they form within 3D printed structures. This generalizable approach occurs at room temperature and avoids the need for additional post-processing steps with light or heat to increase network conversion, and allows the additive manufacturing of highly entangled hydrogels and elastomers that exhibit 4 to 7-fold higher extension energies in comparison to traditional DLP alone. Towards the engineering of advanced tissue adhesives with controlled structures due to additive manufacturing, we printed adhesives with ridges, microfluidic networks, and programmed meta-material designs to develop adhesives with controlled levels of adhesion, depots for drug delivery, and directional adhesion, respectively.

Poster Number: 25

Engineering Large Anisotropic Meniscal Microtissues via Digital Light Processing Printed Molds

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Introduction

The menisci are highly organized fibrocartilaginous tissues that provide load distribution, joint lubrication, and shock absorption in the knee. Meniscal injuries are among the most common musculoskeletal injuries and may lead to pain and early onset of osteoarthritis if left untreated. Although meniscectomies are often used for treatment, meniscal tissue removal has long term biomechanical consequences, motivating the development of new therapies. Our approach leverages advances in 3D printing and the fabrication of microtissues to produce implantable meniscal constructs that recapitulate the anisotropic structure of healthy tissues.

Methods

To accomplish this, digital light processing was used to fabricate molds with posts spaced at a 4:1 aspect ratio and varied dimensions (e.g., rectangular versus curved) using a poly(ethylene glycol) resin. Suspensions of meniscal fibrochondrocytes (MFCs) in collagen were added to the molds and cultured for up to two weeks. Images were acquired during culture to quantify construct width via ImageJ and select constructs were fixed and stained for nuclei (DAPI) and actin (phalloidin) and analyzed for nuclei/actin alignment (Directionality plugin in ImageJ). Cytochalasin D was investigated to alter MFC actin polymerization and limit construct contraction and necking.

Results

All constructs cultured in rectangular molds of various lengths (6, 12, 24 mm) underwent visible contraction and collagen reorganization, with rapid increases in nuclei and actin alignment. However, over time the constructs underwent undesired necking, where contraction continued and altered construct shape. To address this, curvature was incorporated into molds to increase initial construct volume while maintaining post spacing. This approach decreased necking during 7 days of culture relative to constructs cultured in rectangular molds. Further, cytochalasin D was potent in also limiting construct contraction and necking. With these tools now available, ongoing work is to culture larger constructs for longer periods (e.g., 8 weeks) in chondrogenic media and assess extracellular matrix deposition, construct mechanical properties, and integration with meniscal tissue.

Poster Number: 26

Quantifying Thrombogenicity: A Bioanalytical Protocol for the Absorbance-Based Assessment of Vascular Implants with Human Plasma

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Statement of purpose: Assessing thrombogenicity is critical for the evaluation of various biomaterials, especially those that interface with whole, flowing blood. Cardiovascular diseases are the leading cause of death globally. Coronary artery disease, cerebrovascular disease, and peripheral vascular diseases are the most common and are often treated with a vascular stent or graft. This protocol aims to standardize the assessment of such biomaterials using human plasma by quantifying the light absorbance of the biomaterial in the plasma to determine its thrombogenicity.

Methods: To evaluate the thrombogenicity of a vascular implant biomaterial, we longitudinally section the tubular vascular implant (stent or graft) into small pieces and place them in a low-adhesion 96 well plate. Using human plasma rich in or depleted of platelets, we measure the absorbance of light passing through the plate over an hour and plot the resulting curve. This procedure is used to quantify the thrombogenicity of a biomaterial using platelet-rich plasma (PRP) or platelet-poor plasma (PPP). Included in our tests are vascular implants from top brands, such as vascular stents, including bare metal stents and drug-eluting stents, vascular grafts, and stent-grafts. We investigate the impact of a multitude of parameters, including relative efficacy of various anticoagulants, antagonists, and surface coating molecules, on the thrombogenicity of these vascular implants.

Results: This procedure provides a facile, fast, high-throughput means to quantify the thrombogenicity of a vascular implant using the absorbance readings of separated plasma. Higher absorbance values correspond with more thrombogenesis.

Conclusions: This protocol establishes a standardized way to compare the thrombogenicity of different biomaterials using human plasma and get quantitative results.

Poster Number: 27

A 3D in vitro fibrin model to evaluate how strain heterogeneity alters ECM deposition

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Dermal fibroblasts responses to mechanical stimuli in the context of dermal wound healing has been the focus of both in vitro and in vivo studies. Still, how ECM deposition and dermal fibroblast-generated mechanical forces are influenced by the heterogenous mechanical environment in the wound have not been adequately described. To create more accurate computational models of cell behavior and dermal wound healing outcomes and ultimately inform better treatments, we developed an in vitro 3D dermal wound model using heterogeneous fibrin gels and primary normal human dermal fibroblasts (nHDFs). By creating fibrin gels with regions of differing fibrin density, we are emulating the heterogenous mechanical environment in the wound and surrounding tissue during dermal wound healing.

We previously published the mechanical and microstructural properties of 2 and 4 mg/mL fibrin gels and incorporated them into a multiscale fibrin mechanics model. Using these results, we created a 3D in vitro model to evaluate nHDF behaviors in 2 and 4 mg/mL fibrin gels and at their interface. Using a biopsy punch to remove an inner region of a larger fibrin gel and filling it with a different concentration fibrin, we created a repeatable heterogenous model. Using fluorescently labeled fibrinogen, we evaluated the microarchitecture of the fibrin interface. We incorporated nHDFs in the fibrinogen solution to create cellular models. Over 2 weeks, nHDFs fully infiltrated an initially acellular inner region. Additionally, we tested the feasibility of quantifying fibroblast-synthesized ECM in fibrin gels using liquid chromatography – tandem mass spectrometry. We resolved the deposition of major ECM components such as type I collagen and fibronectin by NIH 3T3 cells despite abundant fibrin.

By confocal imaging fluorescent bead-embedded gels and using digital image correlation, future studies will quantify local strains in bulk and interface regions to quantify changes in local stiffness in these regions and correlate this data with proteomics to evaluate the distribution and identities of nHDF deposited ECM proteins. Additionally, we will expand this approach to heterogenous collagen-fibrin gels to further evaluate how ECM composition and strain heterogeneities alter cell behavior to inform computational models and improve understanding of dermal wound healing processes.

Poster Number: 28

Optimizing Regenerative Cell Infiltration in Vascular Grafts: Enhanced strategies to engineer pore microstructures during fabrication

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Introduction: In tissue engineering, the goal is to create scaffolds that seamlessly integrate into the human body, guiding native tissue regeneration while slowly degrading. A crucial aspect of this process is achieving scaffold infiltration of functional cells. The initial phase centered on the material aspect, particularly different polymer behaviors, including degradation rates. Production methods represent another modifiable parameter to fine-tune the device structure, especially its porosity. Additionally, post-fabrication techniques can refine the microstructure and enhance interaction with the human body. The investigation originated with a control group, PCL+PEG-NB coaxially electrospun and air-dried. Ultimately, four groups were created by systematically altering one or more parameters and examined to assess their impact.

Methods: Coaxial electrospinning was used to achieve a strong core (PCL/PLCL) surrounded by a functionalized sheath (PEG-NB). The mixed condition was fabricated by directly blending sheath and core solutions before electrospinning. Samples were soaked in PBS for 24 hours prior air-drying or freeze-drying. Surface topography, including fiber structure and porosity, was investigated by SEM. Uniaxial tensile testing was adopted to determine the impacts of different parameters on the mechanical properties of the graft. The groups were implanted subcutaneously and explanted at various time points (1, 4, and 16 weeks). Histological and fluorescent analyses were adopted to visualize tissue morphology and cellular penetration.

Results: Freeze-dried samples demonstrate higher Young's Modulus, and, more importantly, higher porosity, subsequently, increased cell infiltration than their air-dried counterparts. PLCL shows significantly higher degradation than PCL, PLCL was overall weaker and had a less rigid structure than the PCL as shown by mechanical tests and SEM images. Mixed fibers also displayed increased degradation compared to the control and were shown to be slightly weaker in tensile tests.

Conclusions: Through systematic experimentation, we have uncovered the benefits of freeze-drying in enhancing scaffold porosity and cell infiltration, while also highlighting the importance of selecting polymers with suitable degradation rates. Work is still ongoing to determine optimal fabrication parameters. Moving forward, these insights can guide the development of advanced vascular grafts with improved regenerative capabilities, paving the way for more effective clinical applications in tissue engineering.

Poster Number: 29

Engineering entanglement of microfiber hydrogels

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Gelatin fibers have shown promise in the fabrication of tissue engineering scaffolds due to their general biocompatibility and cell adhesion properties. However, such scaffolds often fail to mimic the toughness of natural tissues, and rather exhibit high extensibility and low stiffness due to insufficient crosslinking, or brittleness due to over crosslinking. Motivated by molecularly entangled hydrogels where entangled chains act as crosslinks that can slide past each other under strain to dissipate stress, we investigated the entanglement of gelatin microfibers to improve scaffold mechanical properties, namely stiffness and extensibility. Wet spinning was used to fabricate gelatin fibers, which allows for micrometer control of fiber size and alignment, and has the potential for industrial scale capacity. Hyaluronic acid (HA) was used as a carrier polymer during wet spinning, to template reactive gelatin precursors and improve microfiber production. Gelatin was modified with lipoic acid, which allows crosslinking with light without a photoinitiator and enables the single step fabrication of stable wet spun microfiber hydrogels. Stretching the fiber and photocrosslinking while still in the coagulation bath aligned and stabilized the structures during collection. Taking inspiration from living highly entangled structures, we are currently developing methods to entangle gelatin fibers, where the tuning of features of gelatin fibers such as length, diameter, and level of entanglement can alter mechanical properties. This engineered physical entanglement of microfiber gelatin fibers is an innovative approach to (i) improve our understanding of entanglement, where gaps in knowledge of how entanglement affects fracture, fatigue, and friction exists and (ii) develop translatable scaffolds to improve tissue repair. Further, the incorporation of multi-fibers (more than one type of fiber) and the introduction of inter-fiber chemical crosslinking could expand on the available properties possible to better mimic features of natural tissues.

Poster Number: 30

Spatiotemporal hydrogel swelling induces basal curvature within intestinal monolayers to drive villi formation

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Intestinal stem cell derived tissues have been utilized to investigate morphogenesis and tissue mechanosensing in both 2D and 3D context, however in vitro tissue morphologies that mimic the native intestine (containing both crypt and villi structures) have to date only been formed utilizing scaffold guided monolayer growth. Here we utilize spatiotemporally defined hydrogel surface swelling to initiate villi morphogenesis within initially planar intestinal stem cell derived monolayers. First, poly(ethylene glycol) (PEG) based synthetic hydrogels of 1.6 kPa shear storage modulus, containing peptides to facilitate cell adhesion (GFOGER) and cell mediated protein remodeling (BM), were formed to contain allyl sulfide crosslinks to enable exchange based photoresponsive behavior (1.6 kPa-G-BM). Hydrogels were formed through a strain promoted azide alkyne cycloaddition reaction between an 8 arm 40 kDa PEG functionalized with dibenzocyclooctyne (Peg8DBCO) and an allyl sulfide bis (Peg11 Azide) crosslinker. Hydrogel crosslink degradation was afforded by equilibrating hydrogels in solution containing a monofunctional thiol (glutathione) and a photoinitiator, lithium phenyl-2,4,6trimethylbenzoylphosphinate (LAP), and subsequently irradiating with light (365 nm, 5 mW cm-2). Spatiotemporal hydrogel irradiation using lithographic based techniques with circular mask features of 50, 75, 100, and 200 m diameter and irradiation times of 40, 60, 80, and 100 sec resulted in spatially controlled hydrogel swelling that increased with pattern size and irradiation time with values ranging from 0.025 – 0.1 um-1. Intestinal monolayers grown on 1.6 kPa-G-BM hydrogels were irradiated at confluence to induce spatiotemporal defined hydrogel swelling. Cells within the monolayer overlaying irradiated regions were found to have increased basal curvature leading to the formation of protrusions of defined size. Lastly, the differentiation of these monolayers was investigated under static and bulk flow conditions, and the height of protrusions was found to increase when cultured flow conditions. Importantly, monolayers were observed to compartmentalize in response to differentiation following spatiotemporal hydrogel swelling, with enterocytes expressing Aldolase B/C accounting for a majority of the cells within the protrusions, while LGR5eGFP expressing stem cells were found to localize within inter-pattern regions.

Poster Number: 31

Hyaluronic Acid Attenuates Collagen Fiber Strain Recovery in Tendon

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Tendons perform a critical function by transmitting forces from muscle to bone. The extracellular matrix (ECM) provides tendons with the mechanical structure necessary to bear loads. While fibrillar type I collagen serves as the primary load-bearing element, proteoglycans and glycosaminoglycans (GAGs) surround collagen fibers, aiding in cellular communication and biochemical regulation, and are hypothesized to affect fiber sliding and the tendon mechanical response. Hyaluronic acid (HA) is widely used as a tendon therapeutic and biocompatible scaffold despite little being known on its role in tendon mechanics. The objective of this study was to measure the effect of HA on multiscale tendon mechanics to improve understanding of HA interactions with collagen fibers and surrounding ECM.

ECRL tendons from adult mice were harvested and incubated in either buffer (control) or hyaluronidase (Hyal) to digest endogenous HA. Tendons were mechanically tested using new methodology I developed that leverages laser ablation of collagen fibers in situ to perform fiber-scale mechanical characterization within intact tissue. It includes our custom soft-tissue testing system integrated with a multiphoton microscope to enable tendon loading to a prescribed force, holding for macroscale stress relaxation, then ablating two cuts across an individual collagen fiber within the tendon and imaging, via second harmonic generation, the contraction of the center fiber segment as the load is removed. Macroscopically, Hyal and control tendons showed no differences in cross-sectional area or stress relaxation (N=5/group). At the fiber scale, Hyal tendons demonstrated significantly less elastic strain recovery, the normalized change in fiber length from loaded to immediately after ablation ($p<0.01$), as well as total strain recovery after 5 minutes ($p<0.05$). Viscoelastic strain recovery, the normalized change in fiber length from after ablation to after 5 minutes, was lower in Hyal tendons, however the results were not significant ($p=0.06$). These results indicate that Hyal treatment decreased collagen fiber sliding but did not alter bulk tissue viscoelasticity.

Ongoing work in this study aims to confirm the effect of HA on collagen fiber mechanics by reintroducing exogenous HA to control and Hyal treated tendons, which can inform how current HA-based treatments can affect tissue mechanics and biocompatible scaffolds.

Poster Number: 32

Highly entangled photodegradable poly(acrylamide) networks enable spatiotemporal control over tough and low-hysteresis hydrogels

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Hydrogels consist of a hydrophilic network of crosslinked polymer chains. Typically, covalent crosslinks are used to mechanically stabilize the topology of network-forming polymers, but this results in embrittlement of the resulting material. Tanglemers are an emerging class of polymer networks that are characterized by dense entanglements between long polymer chains with sparse covalent crosslinks, leading to relatively high stiffness while maintaining toughness, extensibility, and elasticity. Although tanglemers have been previously prepared with degradable crosslinks, it is unclear what effect spatial variation of covalent crosslinks may have on these materials. Here we show the synthesis and characterization of a highly entangled polyacrylamide hydrogel system containing photodegradable covalent crosslinker, enabling tunability and spatiotemporal patterning of swelling, entanglement mobility, and modulus. Originally, both degradable and nondegradable gels have a comparable modulus and work of fracture, low hysteresis, and strain rate independent properties. We also observe birefringence through the strained hydrogel which is attenuated as covalent crosslinks are photodegraded, suggesting that covalent crosslinks facilitate alignment between long polymer chains under strain. Further, using photomasks or laser patterning to precisely vary the density of covalent crosslinks allows for the fabrication of materials with spatially graded properties. Overall, our results expand our understanding of highly entangled polymer systems and outline a method by which they can be made to accommodate spatial manipulation of covalent crosslink density. By interfacing known techniques to modulate standard hydrogels with properties unique to tanglemers, we allow for expanded use of the material properties space and foresee the development of new tissue mimetics or biomedical devices.

Poster Number: 33

WITHDRAWN

Poster Number: 34

Hyaluronic Acid Hydrogels: Mechanics at the Macro and Micro Scale

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Introduction: Bulk hydrogels are commonly employed in tissue engineering and drug delivery applications due to the tunability of their biophysical and biochemical properties; however, their uniform structure and nano-scale porosity often limit cell invasion, soluble factor diffusion, and ECM deposition. As an alternative, hydrogel microparticles, known as "microgels," have emerged as a promising approach to overcome these limitations when packed into granular hydrogels. Granular hydrogels exhibit inherent porosity due to microgel packing and are injectable due to microgel flow under loading. Despite this interest and the importance of local mechanics in mechanobiology studies, there has been little work to compare the mechanical properties of hydrogels when processed as either bulk hydrogels or microgels. To address this and using norbornene-modified hyaluronic acid (NorHA) as an exemplary hydrogel, we have engineered an active-feedback micropipette aspiration device to quantify the mechanics of individual microgels, which is then compared to traditional methods to quantify bulk hydrogel mechanical properties (e.g., uniaxial compression testing).

Methods and Results: Hyaluronic acid (HA) was chemically modified with reactive norbornenes via an aqueous esterification route with an organic triazine coupling agent (4-(4,6-dimethoxy-1,3,5-traizin-2-yl)-4-methyl-morpholinium chloride (DMTMM)). NorHA modification extents were controlled via time of reaction, and ~30% modification was obtainable, as characterized by 1H NMR. NorHA underwent thiol-ene crosslinking either as (1) bulk hydrogels or (2) microgels fabricated via oil-in-water batch emulsion (350RPM) in the presence of a di-thiol crosslinker (dithiothreitol (DTT)), photoinitiatior, and UV light. Polymer concentration was varied (1, 3, 5 wt%), and bulk mechanics demonstrated typical increases in compressive moduli (~3, 33, and 82 kPa) and decreased swelling ratios (39, 26, 19%), respectively. To test microgel mechanics, individual microgels were aspirated at varying pressures, and deformation was recorded to calculate the elastic modulus. Preliminary data demonstrates that average bulk mechanical properties were conserved in the microgel form; however, more testing is required to make this conclusion. Additionally, microgel size was found to be proportional to microgel elastic modulus. Together, this project enables the investigation of biomaterial mechanical properties in both the bulk and micro scale, empowering future microgel design.

Poster Number: 35

Degradation of Chlorothalonil by Catalytic Biomaterials

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Chlorothalonil (2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile) is a halogenated fungicide originally introduced in the United States in 1966, and currently applied to a variety of crops, most notably potatoes, turfgrass, and cherries. In recent years, it was banned in the European Union due to its carcinogenicity, embryo lethality, and high chronic oral toxicity in mammals, among other effects. However, chlorothalonil is still widely used in other parts of the world, including the United States, and has been found in countless drinking water sources around the globe. While previous studies have investigated its degradation in drinking water, this has proved a difficult problem to solve. Chlorothalonil dehalogenase (Chd), originally isolated from the bacterium Pseudomonas sp. CTN-3 in 2010, offers a potential solution. Chd performs the first step in the degradation of chlorothalonil, forming the product 4-OH-chlorothalonil. This study shows that Chd is active when encapsulated in tetramethylorthosilicate (TMOS) gels using a sol-gel method. Encapsulation has been confirmed after incubation with the endopeptidase trypsin. When encapsulated in sol- gels, Chd is stable over a larger temperature range (5 to 80 C) and has shown a potential for storage and reuse over a month-long period. Activity of these encapsulated enzymes has also been observed over a broad range of pH. These data show that encapsulated Chd may have applications for chlorothalonil degradation in drinking water.

Poster Number: 36

Investigation of Shear and Extensional Rheology of Silk Fibroin in Applications of Tissue Engineering

Laura Brunmaier, South Dakota School of Mines & Technology

Kara Huse

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South Dakota School of Mines & Technology

Dr. Tugba Ozdemir

Nanoscience and Biomedical Engineering Department

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South Dakota School of Mines & Technology

Dr. Travis Walker

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South Dakota School of Mines & Technology

Many tissues of the body rely on innate elastic properties to convey the proper function of the tissue. Loss of elasticity, especially in blood vessels, often leads to the onset of pathology. The tissue composition of blood vessels contain a high percentage of a protein that is called elastin. Elastin is largely responsible for the expansion and recoil of the vessel wall throughout the cardiac cycle. Elastin is notoriously challenging to process ex vivo while still retaining the same level of mechanical properties. Silk fibroin (SF) is an elastic protein, similar to elastin, that has impressive mechanical properties and biocompatibility, making it an attractive material in the development of tissue engineered blood vessels. Silk fibroin can be easily isolated from Bombyx mori silkworm cocoons however, the regenerated SF in water is an unstable solution delivering inhomogeneous results. The goal of this research is to control and characterize the SF at each step of the extraction-solubilization process to produce regenerated SF (rSF) in water. The resulting rSF solution is characterized via small amplitude oscillatory shear measurements and extensional rheology. The rSF is then processed using electrospinning or dip-coating to form a tube that is mechanically characterized via burst pressure and compliance measurements. Relationships can be formed between the rheological measurements and resulting mechanics of the tube. Furthermore, based on the rheological characterization of the solution, we can predict the successful application of a processing method and mechanical output of the final product.

Poster Number: 37

Design of Injectable and Adhesive Hydrogel Cardiac Patches

Morgan Riffe, University of Colorado-Boulder

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Myocardial infarction (MI), more commonly known as a heart attack, is one of the leading medical issues globally. The delivery of therapeutics such as drugs, cells, and biomaterials is being explored to mitigate the damage caused by MI. One delivery method of great interest is the application of a patch to the surface of the heart. Patches can be made of a multitude of materials and are typically implanted surgically and applied with an adhesive or sutures [1]. Unfortunately, this approach is not minimally-invasive and can cause trauma to the patient and tissue during the surgical procedure [2].

To address this concern, our goal is to develop a patch that can be injected into the pericardial space around the heart and that presents an adhesive on one-side for application to the heart. This is so that once injected, the patch only attaches to the heart muscle and not the pericardium lining. Our approach is to use hydrogel foams that are formed as a cryogel (reaction once frozen), that can be compressed for loading into a syringe and then expands once injected. Specifically, we are creating hydrogel foams from hydrogel precursors such as methacrylated-hyaluronic acid (MeHA) that crosslinks in the presence of a radical initiator once frozen, to effectively introduce macropores into the structure during crosslinking. This allows for dehydration, rehydration, and injectability of the patch. Various fabrication methods are currently being explored to spatially control adhesion and future work includes investigation of the therapeutic activity of the material once implanted.

[1] Mei et al., Recent developments in Therapeutic Cardiac Patches, Frontiers, 2020.

[2] Adu-Berchie et al., Adoptive T cell transfer and host antigen-presenting cell recruitment with cryogel scaffolds promotes long-term protection against solid tumors, Nat. Comm., 2023.

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2024 REGIONAL SYMPOSIA

September 19–20, 2024

FINAL PROGRAM

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2024 Regional Symposia

Northwest Symposium: University of Washington

September 19 - 20, 2024

Program Agenda

Thursday, September 19, 2024

7:45 AM - 8:25 AM	Registration / Check-in
8:25 AM - 8:30 AM	Welcome
8:30 AM - 9:45 AM	Plenary Session I: ***Shana Kelley, Northwestern University*** ***Joel Collier, Duke University*** *(2024 Clemson Award for Basic Research Recipient)*
9:45 AM - 11:00 AM	Session I: Tissue Engineering
11:00 AM - 11:15 AM	Break
11:15 AM - 12:30 PM	Session II: Engineering Cells and Their Microenvironments
12:30 PM - 1:15 PM	Lunch
1:15 PM - 2:30 PM	Plenary Session II: ***Sarah Stabenfeldt, Arizona State University*** ***Danielle Benoit, University of Oregon***
2:30 PM - 2:45 PM	Break

Program Agenda

Thursday, September 19, 2024 *(continued)*

2:45 PM - 4:15 PM	Session III: Tissue Specific Biomaterials ***Invited Speaker:*** ***Aidan Gilchrist, University of California, Davis***
4:15 PM - 4:30 PM	Break
4:30 PM - 5:30 PM	Panel Discussion: Establishing Effective Mentee/Mentor Relationships ***Panelists:*** ***Renee Desing, University of Washington*** ***Bill Mahoney, University of Washington*** ***Amanda Murphy, Western Washington University***
5:30 PM - 6:30 PM	Poster Session
6:30 PM - 9:30 PM	Social Event - Flatstick Pub South Lake Union *Sponsored by University of Washington Bioengineering*

Program Agenda

Friday, September 20, 2024

8:00 AM - 8:30 AM	Registration / Check-in
8:30 AM - 9:45 AM	Plenary Session III: ***Elazer Edelman, Massachusettes Institute of Technology*** *(2024 Founders Award Recipient)* ***Cynthia Reinhart-King, Rice University***
9:45 AM - 11:00 AM	Session IV: Drug Delivery
11:00 AM - 11:15 AM	Break
11:15 AM - 12:30 PM	Session V: Nanomaterials: Surface Characterization ***Invited Speakers:*** ***Anna Blakney, University of British Columbia*** ***AJ Mellott, Ronawk***
12:30 PM - 1:30 PM	Lunch
1:30 PM - 2:30 PM	Concurrent Session VI: Rapid Fire Talks ***Invited Speaker:*** ***Buddy Ratner, University of Washington***
2:30 PM - 3:45 PM	Poster Session
3:45 PM - 4:00 PM	Awards and Closing

2024 Regional Symposia Chair:

Anita Shukla, PhD. Brown University

Northwest Regional Symposium Co-Chairs:

Cole DeForest, PhD, University of Washington

Jenny Robinson, PhD, University of Washington

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Brian Aguado, PhD, University of California San Diego

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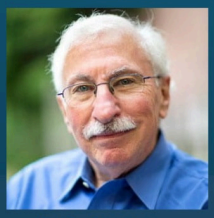
Ngan Huang, PhD, Stanford University

2024 Society for Biomaterials (SFB) Northwest Symposia, U. Washington, Seattle, WA

Plenary Speaker

Dr. Danielle Benoit
University of Oregon

Invited Speaker



Dr. Buddy Ratner
U. Washington

Invited Speaker

Dr. Anna Blakney
U. British Columbia

Invited Speaker

Dr. Aidan Gilchrist
U. California Davis

Invited Speaker

Dr. A.J. Mellot
Ronawk

Invited Panelist

Dr. Bill Mahoney
U. Washington

Invited Panelist

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PLENARY SESSION I
Thursday, September 19

Dr. Shana Kelley
Northwestern University

PLENARY SESSION I
Thursday, September 19

Dr. Joel Collier
Duke University
(2024 Clemson Award for Basic Research)

PLENARY SESSION II
Thursday, September 19

Dr. Sarah Stabenfeldt
Arizona State University

PLENARY SESSION II
Thursday, September 19

Dr. Danielle Benoit
University of Oregon

PLENARY SESSION III
Friday, September 20

Dr. Elazer Edelman
Massachusetts Institute of Technology
(2024 Founders Award)

PLENARY SESSION III
Friday, September 20

Dr. Cynthia Reinhart-King
Rice University

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2024 Regional Symposia

September 19th - 20th

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- **Southwest: University of Texas at Austin**
- **Western: University of Colorado, Denver | Anschutz Medical Campus**
- **Northwest: University of Washington**

SESSION 1: TISSUE ENGINEERING

9:45 AM - 10:00 AM

Leveraging Focused Ultrasound for Remote Genetic Manipulation of Cells and Spheroids in 3D Bioprinted Tissue Constructs

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Controlling when and where cell signaling proteins present in 3D tissue constructs is critical for coordinating biological processes and modeling disease states, including cancer. 3D bioprinting techniques have great value for in-vitro cancer modeling as they can be utilized to recapitulate tissue architectures and biophysical parameters characteristic of the tumor microenvironment. Genetic manipulation of targeted cells at specific times and locations within these bioprinted tissues is vital for modeling dynamic changes in cell signaling, but current strategies are limited in spatiotemporal control due to the diffusional barriers created by thick 3D scaffolds. To address this challenge, we have developed an ultrasound-responsive bioprinting platform for noninvasive, spatiotemporally-controlled genetic manipulation of cells in 3D-bioprinted tissue constructs. In this platform, focused ultrasound interacts with integrated lipid-based ultrasound-responsive microbubbles in our bioprinted constructs to locally deliver nucleic acids to cells. Alginate hydrogel filaments containing ultrasound-responsive gene delivery microbubbles and either HEK293T cells or breast epithelial (MCF10A) spheroids were bioprinted via extrusion, with over 80% viability observed in both cell types. By varying extrusion needle size, microbubble-containing filaments were reproducibly printed with diameters of 200-850 μm with microbubbles remaining intact and well-dispersed post-printing. In response to focused ultrasound, the gene-delivery microbubbles ruptured only in the region of ultrasound exposure, leaving surrounding particles intact. At 48hr post-ultrasound, GFP transgene-expressing cells were observed only within the region of ultrasound application. By varying the number of focused ultrasound pulses and the concentration of gene delivery microbubbles, we demonstrated reproducible modulation of the number of transgene-expressing cells and the size of the gene delivery region within the print. In bioprinted constructs containing breast epithelial spheroids, cells and cell clusters within spheroids in the ultrasound focal zone were seen to overexpress GFP transgene. Additionally, HER2 oncogene-coupled microbubbles were incorporated in breast epithelial spheroid bioprints, and ultrasound-activated HER2 overexpression was observed in targeted spheroid cells within the focal zone. These results demonstrate the first platform for facilitating ultrasound-responsive gene delivery in a 3D-bioprinted construct, allowing for spatiotemporal control of genetic manipulation. Future studies will leverage this platform to interrogate cell behavior and signaling in the 3D context upon induced oncogene overexpression.

10:00 AM - 10:15 AM

Protein-Based Shape Memory Polymer Metamaterials with Strain-Induced Remodeling

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Mechanical deformation of a polymer network is transferred from the macroscale to nanoscale to cause molecular-level motions and bond scission that ultimately lead to material failure. Understanding how to mitigate polymer disentanglement and bond scission is a significant challenge, especially in the development of active and shape-morphing materials. We report the additive manufacturing of hierarchically designed mechanical metamaterial lattices made with a protein-based polymer network that undergoes a unique strain learning behavior that combines mechanical remodeling with shape memory. At the molecular level, protein mechanophores unfold in the presence of a mechanical force to release its “stored length”, thereby stiffening in the direction of applied load after undergoing a healing cycle. Incomplete refolding of proteins during shape recovery affords a network with enhanced stiffness. At the macroscale, architected lattices distribute stress across a 3D printed structure to mitigate damage and enable complete shape recovery, and the efficiency of this process varies with the lattice architecture. The combined hierarchical responses cause a mechano-activated remodeling of folded proteins in the network to afford up to a 2 to 3-fold improvement in the mechanical properties. These bio-inspired materials offer a unique opportunity to develop novel materials that can autonomously remodel under an arbitrary applied load.

10:15 AM - 10:30 AM

Development of a 3D-printed microfluidic model to investigate endothelial crosstalk in breast cancer progression

Holly Day, Oregon Health and Science University

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1Biomedical Engineering Department, Oregon Health and Science University, Portland, OR 97201, USA

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The growth and progression of triple-negative breast cancer can be impacted by crosstalk with nearby vascular endothelial cells. However, there are limited studies that assess how spatial relationships affect this crosstalk in 3D culture. The range over which a tumor can influence endothelial phenotype can be investigated using a bioprinted microfluidic model, which can control the placement of tumor spheroids in relation to a printed perfusable vessel. To develop this bioprinted model, bioink formulations including three gelatin/alginate blends (4%/1%, 4%/4%, and 1%/4%) and two GelMA-based bioinks (5% GelMA with and without 0.5% collagen) underwent rheological, mechanical, and biocompatibility studies to investigate bioink suitability for extrusion bioprinting and modeling breast cancer-endothelial crosstalk. Rheological shear rate sweeps of uncrosslinked bioinks revealed that three bioinks (4%/1% and 4%/4% alginate/gelatin and 5%/0.5% GelMA/collagen) exhibited shear-thinning properties. Compression testing of crosslinked bioinks found that alginate/gelatin bioinks exhibited Young's moduli of 16-38kPa, whereas GelMA bioinks exhibited moduli closer to normal breast tissue stiffness (2-4kPa). Extrusion bioprinted lattice constructs were used to assess print fidelity of alginate/gelatin bioinks through the comparison of filament length, width, and intersectional widths to the original CAD design values, with 1%/4% alginate/gelatin showing highest print fidelity. Cell viability staining of endothelial (HUVEC) and triple-negative breast epithelial (MDA-MB-231) cells was performed for all bioink formulations. To simulate how HUVECs and MDA-MB-231s will be cultured in the bioprinted model, HUVECs were cultured on the crosslinked bioink surface while MDA-MB-231s were embedded within bioinks. After culturing for 24hr, HUVECs exhibited greater cell spreading in addition to high viability (>80%) in the GelMA bioinks, while MDA-MB-231 showed high cell viability (>80%) in both alginate/gelatin and GelMA bioinks. These bioink characterization studies suggest that the GelMA bioinks may be better suited for a bioprinted microfluidic model designed to investigate triple-negative breast cancer-endothelial interactions. GelMA bioink formulations were further utilized to fabricate perfusable channels that supported endothelial cell adhesion, as visualized with confocal imaging. Ongoing work will characterize 3D endothelial phenotype under flow conditions and utilize 3D bioprinting to control breast spheroid placement to study how tumor spatial arrangement can affect endothelial states that fuel breast cancer progression.

SESSION II: ENGINEERING CELLS AND THEIR MICROENVIRONMENTS

11:15 AM - 11:30 AM

Tunable and modular viscoelastic matrices to study morphogenesis and invasion of mammary epithelium

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Basement membrane proteins are required for proper epithelial morphogenesis. Reconstituted basement membrane (rBM) products, like Matrigel, are commonly used as extracellular matrix (ECM) surrogates for in vitro 3D culture of epithelial tissues, organoids, spheroids and cancer models. Despite widespread use, rBM has several inherent disadvantages, including inconsistencies between batches, lack of mechanical and biochemical tunability, and recently, chronic shortages.

To overcome these limitations, we sought to develop a 3D engineered basement membrane matrix by conjugating ECM adhesion peptide motifs (IKVAV, YIGSR, RGD) to a mechanically-tunable alginate hydrogel network. We found a combination of mechanical and adhesion cues that can recapitulate mammary acinus morphogenesis from MCF10A cells with similar morphology, apico-basal polarity, and growth rate to acini grown in rBM. To further demonstrate the utility of our platform, we obtained patient-derived mammary tumor organoids, for which rBM is considered required for expansion. Compared to rBM cultures, these organoids in our engineered matrices had similar growth rates and morphologies.

We then utilized the mechanical control of our platform to investigate the independent contributions of stiffness and stress relaxation rate on driving acinar versus tumorigenic phenotypes. MCF10As encapsulated in IKVAV-modified matrices develop into polarized, acinar structures when the matrix is either soft (200 Pa) or fast-relaxing ($\tau 1/2$ = 100 s). Only when the matrix is both stiff (2000 Pa) and slow relaxing ($\tau 1/2$ = 1000 s) do the clusters become invasive, losing polarity. In contrast, MCF10As encapsulated in RGD-modified matrices fail to form polarized acinar structures regardless of the stress relaxation or stiffness properties of the matrix, and remain as single cells or develop into invasive clusters. We also found differences in hemidesmosome formation and YAP nuclear translocation in response to peptide type, stress relaxation, and stiffness.

Our results demonstrate that reductionist matrices sufficiently induce 3D mammary acinar morphogenesis and are an excellent platform to decipher how cell-matrix interactions lead to phenotypic outcomes. We show that both peptide and gel mechanics influence downstream signaling pathways and phenotype. This modular and tunable platform enables both basic biological investigations into microenvironmentally-guided morphogenesis and more translational applications in disease modeling and development of engineered replacement tissues.

11:30 AM - 11:45 AM

Development and validation of healthy endometrium and endometriosis models for high- throughput phenotypic screening

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All co-authors are from Oregon State University

Endometriosis affects 10-15% of women and is characterized by the presence of endometrial-like tissue outside of the uterus, leading to chronic pelvic pain, infertility, and diminished quality of life. Current diagnostic methods, including imaging and laparoscopy, often fail to detect early-stage and microscopic lesions, critical for effective intervention and management. This diagnostic inadequacy, coupled with a limited understanding of the disease's pathophysiology, contributes to delayed treatments and suboptimal patient outcomes. Furthermore, the lack of targeted therapies underscores a pressing need for innovative approaches to identify and evaluate novel therapeutic targets and diagnostic tools.

To address this need, our study focused on the development and application of two distinct three-dimensional (3D) in vitro models designed to emulate the physiological and pathological conditions of the endometrium in both healthy states and in endometriosis. Employing Design of Experiments (DOE) with a central composite face design, we analyzed the effects of six variables across 47 unique hydrogel formulations to study their impact on endometrial thickness, microvessel formation, epithelial invasion, and fibroblast activation. Cultured in IBIDI angiogenesis plates, the models underwent hormonal treatment with estrogen, progesterone, and luteinizing hormone gradients for 72 hours, mimicking the hormonal flux during ovulation that maximizes endometrial lining thickness. Models were were assessed through 85 µm z-stack imaging every three hours and cell dynamics were evaluated using our automated image analysis software.

All of the input variables evaluated (collagen I, collagen IV, fibronectin, laminin, fibroblasts, estrogen, and progesterone) significantly influenced all of the cell responses ($p \leq 0.05$). By tuning the concentrations of these components we successfully replicated the complex layered architecture and cellular interactions characteristic of both healthy and endometriotic endometrium. These models, compatible with high-throughput screening and automatic evaluation, offer promising tools for in-depth mechanistic studies on endometriosis's onset and progression, potentially leading to novel diagnostic and therapeutic strategies.

11:45 AM - 12:00 PM

Probing Cellular Mechanical Memory within Dynamically Softened Biomaterials

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Biomechanical contributions of the ECM play a crucial role in governing cell growth and proliferation, differentiation, signal transduction, and other decisions of fate. As such, biomaterials that change mechanical properties in situ and that enable spatial control over their stiffness profiles over time are extremely valuable for studying these mechanobiological phenomena. Herein, we sought to design a poly(ethylene glycol) (PEG)-based hydrogel model consisting of two interpenetrating polymeric networks that can be independently formed via bioorthogonal chemistries and sequentially degraded upon treatment with bacterial transpeptidases, with mechanical property customization across stiffnesses spanning healthy and diseased soft tissues (e.g., 500 Pa – 6 kPa) and terminal cell recovery for pooled and/or single-cell analysis in a near “biologically invisible” manner. 4D spatial control over the gelation was achieved within preformed hydrogel substrates via mask-based and two-photon lithography; these patterned stiff regions could be subsequently returned to their initial soft state via sortase degradation with no effect on the supporting network. We investigated the effects of spatiotemporal changes in network mechanics on human mesenchymal stem cell (hMSC) morphology and Caco-2 colorectal cancer cell mechanomemory at the global transcriptome level via RNAseq. We expect this platform to be of broad utility for studying and directing mechanobiological phenomena, patterned cell fate, as well as disease resolution in softer matrices.

12:00 PM - 12:15 PM

Low-Cost, High-Resolution 3D Printing of Microfluidics for Self-Sustaining Hydration in Engineered Living Materials

Aileen Y. Sun, University of Washington

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Engineered living materials (ELMs) sit at the convergence of synthetic biology and polymeric materials, where living, engineered cells are embedded within polymer matrices. To sustain viability of the engineered cells and deploy these emerging materials into real-world settings, hydration and nourishment on a system-level become crucial considerations in the design and fabrication of ELMs. Capillary microfluidics utilize surface tension forces from the interplay between microfluidic channel geometry and surface chemistry for the controlled and autonomous delivery of liquids. Incorporation of capillary microfluidics with ELMs would thus facilitate the regulation of fluids without the need for external instrumentation and opens the potential for scale-up of ELMs to self-sustaining engineered living systems (ELiS). Although gold standard digital light processing stereolithography (DLP-SLA) 3D printing offers high-resolution fabrication of microfluidic structures, DLP-SLA 3D printers are expensive (≥$USD 10,000), limiting their accessibility. Our team recently demonstrated the 3D-printing of high-resolution capillary microfluidics using low-cost (≤$USD 500) masked stereolithography (mSLA) 3D printers with commercial resins. In a similar approach, we formulated custom poly(ethylene glycol) diacrylate (PEGDA)- and bovine serum albumin (BSA)-based hydrogel resins for the high-resolution 3D-printing of ELMs on an mSLA printer. PEGDA and BSA have anti-fouling properties and are biocompatible matrix materials suitable for the manufacturing of ELMs. We optimized print parameters such as light exposure intensity and time, lift/retract speed and build plate distance, and light-off time between each polymerized layer. We obtained microchannels with measured dimensions within 10% of designed values and coefficients of variation (CVs) less than 5%. The measured open-channel flow rates of fluids in the 3D-printed open microchannels were over 600 µm/sec, showcasing the potential for prompt hydration of ELiS. This research not only validated our prior demonstration of achieving high-resolution capillary microfluidic channels on low-cost mSLA 3D-printers but also expanded the library of resins into custom biocompatible hydrogel resins designed to sustain microbial viability of ELMs.

SESSION III: TISSUE SPECIFIC BIOMATERIALS

2:45 PM – 3:15 PM INVITED SPEAKER: AIDAN GILCHRIST, UC-DAVIS

3:15 PM - 3:30 PM

Engineering Polymers for the Resuscitation of Trauma Patients

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Hemorrhagic shock is the leading cause of death across civilian and military populations, with 150,000 trauma-related deaths annually in the United States alone. Patient rescue is achieved by resuscitation, which reintroduces fluid into the bloodstream to prevent hypovolemia and to recover blood pressure and tissue oxygenation. Traditional resuscitants operate on the principle of aggressive fluid replacement, a strategy which can interfere with coagulation, is impractical in the prehospital setting. However, recent efforts have demonstrated the powerful oncotic resuscitation profiles of high molecular weight, hydrophilic polymers such as PEG-20k. We are developing a suite of novel oncotic polymers for emergency resuscitation applications and investigating the effects of polymer composition, architecture, and branching density upon oncotic profiles. Our research aims to develop next-generation low-volume resuscitants for the treatment of traumatic injury.

Through analysis of our suite of oncotic polymers, we are now able to understand the relationship between polymer architecture/composition and desirable oncotic properties for resuscitation. Through our in vivo model, we have demonstrated that a 10% blood volume dose of oncotic polymer recovers mean arterial pressure (MAP) and achieves resuscitation and survival after severe hemorrhages in which 60-70% blood volume was lost. Our oncotic polymers are highly competitive with PEG-20k, meaning that resuscitation is achieved with dramatic improvement over the current standard of care. Furthermore, we demonstrate for the first time an oncotic polymer capable of achieving resuscitation without interfering with coagulation, an imperative but historically ignored criterion for trauma therapies. The performance of our polymers was evaluated for survival, blood pressure recovery, and metabolic correction in a rat model of lethal hemorrhagic shock.

3:30 PM - 3:45 PM

Multiscale patterning of 3D microvascular scaffolds via image stack-guided photodegradation

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Vasculature is essential in every organ system, playing critical roles in tissue regeneration and cellular responses. To fully understand physiological cellular function and engineer tissue for transplantation, recapitulating such vasculature features in vitro, especially at the capillary scale, is in great need. While several manufacturing methods have been explored to generate vascular constructs in vitro using different polymeric hydrogel materials and printing techniques, existing efforts have largely failed to replicate the essential size ranges and complexity of the vascular networks, particularly of smallest capillaries (<10 μm diameter) across large distances (>mm). In this work, we exploit image stack-guided two-photon (2P) lithography in conjunction with photodegradable hydrogel materials to create large-scale, physiologically relevant microvasculature networks in vitro. Using a 2x2x2 mm^3 3D vasculature image stack acquired from a neonatal mouse brain, microvascular channels with various diameters (3 - 100 μm) were rapidly patterned (~1 hr) at native complexity within photodegradable hydrogels. Channels were demonstrated to be patent and could be subsequently seeded with living endothelial cells. The "subtractive manufacturing" technique on highly 2P sensitive hydrogel materials enables natively complex microvoids to be optically sculpted within otherwise solid biomaterials at unprecedented resolutions and at high speed. We expect these hydrogel scaffolds to find great utility for studying vascularized systems in vitro, including 3D disease modeling, therapeutic screening, and many other applications that can accelerate current clinical trials.

3:45 PM - 4:00 PM

Mannosylated STING Agonist Drugamers For Dendritic Cell-Mediated Cancer Immunotherapy

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The Stimulator of Interferon Genes (STING) pathway is a promising target for cancer immunotherapy. Despite recent advances, therapies targeting the STING pathway are often limited by routes of administration, sub-optimal STING activation or off-target toxicity. Here, we report a dendritic cell (DC)-targeted polymeric prodrug platform (polySTING) that is designed to optimize intracellular delivery of a diamidobenzimidazole (diABZI) small molecule STING agonist, while minimizing off-target toxicity after parenteral administration. PolySTING incorporates mannose targeting ligands as a comonomer, which facilitates its uptake in CD206+/mannose receptor+ professional antigen-presenting cells (APCs) in the tumor microenvironment (TME). The STING agonist is conjugated through a cathepsin B-cleavable Valine-Alanine (VA) linker for selective intracellular drug release after receptor-mediated endocytosis. When administered intravenously in tumor-bearing mice, polySTING selectively targeted CD206+/mannose receptor+ APCs in the TME, resulting in increased cross-presenting CD8+ DCs, infiltrating CD8+ T cells in the TME, as well as maturation across multiple DC subtypes in the tumor-draining lymph node (TDLN). Systemic administration of polySTING slowed tumor growth in a B16-F10 murine melanoma model, as well as a 4T1 murine breast cancer model with an acceptable safety profile. Thus, we demonstrate that polySTING delivers STING agonists to professional APCs after systemic administration, generating an efficacious DC-driven anti-tumor immunity with minimal side effects. This new polymeric prodrug platform may offer new opportunities for combining efficient targeted STING agonist delivery with other selective tumor therapeutic strategies.

4:00 PM - 4:15 PM

Bone-targeted nanoparticles for accelerating non-union fracture healing

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100,000 non-union fractures occur in the United States annually, resulting in medical expenditures exceeding $2.5 billion annually. Despite clinical management including revision surgery, autografts, and/or fixation, 5-10% of non-unions persist, highlighting the need to develop minimally invasive options. To address this need, we have developed bone-targeted nanoparticles (NPs) composed of poly(styrene-alt-maleic anhydride-b-poly(styrene) (PSMA-b-PS). NPs are functionalized with a tartrate-resistant acid phosphatase (TRAP)-binding peptide (TBP). TBP-NPs loaded with a GSK-3 inhibitor (AR28) (TBP-NPAR28) exhibited enhanced accumulation (~3-fold) at conventional fractures site versus untargeted NPs and resulted in 1.8-fold greater callus volume versus control and resumption of biomechanical properties, successfully healing fractures without additional surgical intervention in 8-week-old mice. Further analysis of the fracture site revealed a shift to greater M2:M1 macrophage ratio underpinning TBP-NPAR28-mediated healing. Given the promise of this therapeutic approach, off-target effects on other bone proximal cells (like osteoclasts), as well as its potential efficacy in clinically relevant models, such as age-related effects in non-unions and delayed unions, is our current focus. Our data indicate that while osteoclasts can take up TBP-NPs, they are non-toxic. Furthermore, TBP-NP showed ~2-fold greater accumulation at conventional fracture s in aged mice (17-18 months old) versus non-targeted NPs. Current efforts include assessing the effect of the NPs on osteoclasts through cytokine analysis and gene expression studies and TBP-NP targeting efficacy and regenerative potential in critical size defects in middle-aged and aged mice.

SESSION IV: DRUG DELIVERY

9:45 AM - 10:00 AM

An injectable recombinant protein-based hydrogel affords local immunotherapy for treatment of pediatric brain tumor

Yusuke Suita, Seattle Children's Research Institute

Ken Brasel, Seattle Children's Research Institute,

Shelli Morris, Seattle Children's Research Institute,

Nicole Gregorio, University of Washington,

Jason Price, Seattle Children's Research Institute,

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Pediatric brain tumors (PBTs) are the most common solid pediatric tumor. Recurrent high-grade glioma (HGG) is nearly universally fatal despite exhaustive standard treatments (surgical removal, chemotherapy, and radiotherapy). Thus, a new therapy such as immunotherapy (IT) warrants attention. However, IT trials have shown little benefit for PBTs, likely because of challenges presented by blood-brain barrier (BBB) exclusion, immunosuppressive tumor microenvironments (TMEs), poor antigen presentation, and target heterogeneity. To address these challenges, we propose a local IT platform: a shear-thinning and self-healing recombinant protein-based hydrogel implanted in the tumor resection cavity or peritumoral tissue. This hydrogel releases two protein therapeutics 1) IFNg over a period of days in order to convert the TME into a pro-inflammatory state, recruit immune cells, and upregulate PD-L1 expression on cancer cells; and 2) PD-L1:CD3 Bi-specific T-cell Engager (BTE) over a period of 4-6 weeks in order to redirect T cells to kill PD-L1-positive cancer cells, proliferate, and release more IFNg in a feed-forward circuit. In essence, this BTE approach converts PD-L1 upregulation from a cancer defense against immune surveillance into a vulnerability that makes the cancer highly susceptible to T cell redirecting therapy.

We showed that the hydrogel can slowly release IFNg and PD-L1:CD3 BTE and perform dual-release of those molecules. We showed that IFNg exposure dramatically induces PD-L1 across entire populations of PBT cells, making it one of the most ubiquitously expressed proteins on the surface of PBTs. We have shown that our PD-L1:CD3 BTE engages both PD-L1 on cancer cells and CD3 on T cells enabling the T cells to efficiently kill cancer cells, and moreover, the combination of IFNg and PD-L1:CD3 BTE kills cancer cells better. This local immunotherapy has the potential to incorporate other protein-based therapies and to be used to treat brain tumors, as well as solid tumors such as sarcomas.

10:00 AM - 10:15 AM

Fully synthetic injectable drugamer depot with low volume and tunable pharmacokinetics for long-acting drug delivery

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Long-acting injectable and implant products are playing important roles in improving drug dosing adherence for therapy and pre-exposure prophylaxis. However, current long-acting injectable depot platforms are not ideal as they require multiple milliliter injections and are generally challenged by undesirable drug release profiles at the early and late phases. For the comfort of the recipient, high drug content in a small volume and the prevention of a burst release at both early and late stages are required in order to decrease injection frequency and be able to maintain a controlled release, respectively. We report here a long-acting depot platform consisting of a single polymeric prodrug component that can be engineered to control the drug release properties. The model drug was islatravir (ISL), which is a first-in-class nucleoside reverse transcriptase translocation inhibitor. The prodrug monomers were obtained as methacrylate monomers with different linker structures or different spacer lengths between the linker and the methacrylate group. The obtained monomers were polymerized by Reversible Addition Fragmentation chain Transfer (RAFT) polymerization. To control the drug release, the prodrug monomers were copolymerized with a hydrophilic methacrylate monomer, 2-(methylsulfinyl) ethyl methacrylate (MSEMA). The polymeric prodrug termed “Drugamer” was formulated in a small amount of DMSO (110 or 158 µL), and then subcutaneously injected to the right flank of a rat. ISL concentration in plasma was determined with an analytical liquid chromatography method, coupled with tandem triple quadrupole mass spectrometry (LC-MS/MS). It was found that the ISL release from the drugamer was controlled by the ethylene glycol (EG) spacer between the linker and methacrylate group. The drugamer with a long EG spacer showed a high ISL release while the one with a short EG spacer achieved a longer sustained release which had a duration of almost a year. Also, the ISL release was controlled by the linker chemistry, the monomer composition (ISL prodrug monomer vs MSEMA), and the drugamer molecular weight. This ISL drugamer is the first long-acting depot well-controlled by rational molecular design, and achieves the low injection volume, long and designable working durations, and simple formulation.

10:15 AM - 10:30 AM

Dendrimer-microbubble complexes as ultrasound-activated nanocarriers for precision cancer gene therapy

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Precision delivery of therapeutics is a promising strategy to improve cancer treatment outcomes in the early stages of tumor growth while minimizing off-target effects resulting from non-specific cargo interactions. Cancer gene therapy employs vectors to deliver therapeutic genes to tumor cells to modulate disease-related gene expression. Branched polymeric dendrimer particles can efficiently deliver genes with high transfection efficacy because of their high loading capacity, endosomal escape capability, and reduced immunogenicity compared to viral vectors. However, achieving site-specific delivery after systemic administration remains challenging. To address this challenge, we are developing a novel, dendrimer-microbubble complex as an ultrasound-activated nanocarrier for spatially-localized gene therapy. In our particle design, dendrimers are functionalized with a linker and coupled with plasmid DNA via electrostatic attraction, forming dendriplexes. Dendriplexes are then bound to the surface of functionalized lipid microbubbles. Upon application of low-intensity focused ultrasound, microbubbles will collapse, releasing attached nanoparticles for localized delivery. Focused ultrasound is a powerful stimulus for precision medicine as it is non-invasive, has high penetration depth, can be focused to a tight spatial volume, and is deemed clinically safe at low intensities. Ultrasound-stimulated microbubble cavitation also induces transient pores in nearby cell membranes, which can facilitate enhanced nanoparticle uptake. In our results, we have demonstrated the successful functionalization of dendrimers with linker groups for microbubble coupling via NMR spectroscopy. DNA-loaded dendriplexes showed diameters in the 100-200 nm size range suitable for endocytosis. The dendriplexes exhibited a decrease in zeta potential with increasing DNA loading across different weight ratios supporting successful loading of plasmid DNA. Functionalized microbubbles were fabricated in the 1-5 μm diameter range, which is desirable for ultrasound responsivity at our applied frequency, and showed successful complexation with the dendriplexes. Upon ultrasound application at 2.25 MHz, the microbubble-dendriplex complexes were observed to collapse within the 1 mm ultrasound focal zone. Ongoing work will evaluate the efficiency of these new ultrasound-activatable dendrimer complexes for spatially-localized gene delivery to cancer cells. Overall, these microbubble-dendrimer complexes offer promise as a new stimuli-responsive gene delivery strategy for precision cancer therapy.

10:30 AM - 10:45 AM

Self-Illuminating Biomaterials for On-Demand Localized Drug Delivery

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Stimuli-responsive biomaterials offer substantial promise in controlled small molecule and protein therapeutic delivery, alleviating challenges of systemic drug administration through suppressed off-targeted interactions, immunoprotection, and prolonged cargo activity. Engineered platforms that respond to exogenous cues (e.g., light, temperature, ultrasound) can afford user-defined release; the extent of active therapeutic release from these systems can be advantageously specified in time and space. Light-responsive biomaterials have found substantial use for in vitro study, however, light's comparatively poor penetrance through tissue dramatically limits its in vivo utility; only optically accessible regions typically <1 cm from the light source can be manipulated. Ongoing efforts in the community seek to create materials that respond to low-energy light (e.g., visible, infrared) that better penetrates tissue. Seeking to develop a photoresponsive material platform that could be theoretically controlled anywhere in the body, we employed bioluminescence resonance energy transfer (BRET) – an energy transfer process between a light-emitting luciferase and a photosensitive acceptor molecule – to drive biomolecule release from hydrogel biomaterials.

In this work, we introduce a modular, synthetically accessible system that enables rapid development of new biomaterial platforms for the release of various cargos at size scales ranging from small molecules to full proteins. In addition, the simple chemistry employed in our group is materials agnostic and is demonstrated in both synthetic and natural polymer biomaterials. This promising new BRET-based bioluminolysis method is effective for the release of various cargos from hydrogel depots, suggesting a powerful new platform for on-demand, localized drug delivery based on systemic administration of a small molecule.

10:45 AM - 11:00 AM

Recapitulating angiogenesis using affinity-controlled protein delivery

Marian Hettiaratchi, University of Oregon

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Angiogenesis is a carefully coordinated process during which the temporally regulated presentation of many signaling proteins stimulates vascular network expansion. Disruptions in this sequence of protein presentation caused by injury and disease can result in poor healing and impaired tissue function. The objective of our work is to develop a tunable delivery system capable of phased therapeutic protein delivery to enhance angiogenesis. To tackle this challenge, we have developed a "designer affinity" approach in which we control protein release from biomaterials via affinity interactions with tethered protein domains (affibodies) identified using directed evolution and rational protein design. Directed evolution of a yeast surface display library enables the identification of affibodies with high specificity and a wide range of affinities, facilitating controlled protein release at vastly different rates. We have identified unique, specific affibodies for multiple well-characterized proteins involved in angiogenesis, including vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), and platelet-derived growth factor (PDGF), with dissociation constants ranging between 1E-5 M and 1E-10 M. We further tuned the dissociation constants of these affibodies by using Rosetta-based rational protein design to identify and mutate key residues involved in protein-affibody binding. Conjugation of different affibodies to polyethylene glycol hydrogels enabled tunable, independent control over the rate and final amount of VEGF, FGF-2 and PDGF released, between 10-100% of protein loaded. Importantly, these affibodies are highly specific, displaying affinity only for the target protein, and demonstrating that this strategy can be used to independently tune the release of multiple therapeutics from a single hydrogel. We are currently investigating the use of this platform to stimulate vascular network formation of rodent microvascular fragments (MVFs) containing endothelial cells, pericytes, and other stromal cells. We have demonstrated that sequential delivery of VEGF followed by FGF-2 and PDGF increased MVF network length compared to simultaneous delivery of these proteins. We are currently tuning the amounts and types of affibodies in our biomaterials to mimic this sequence of delivery. Ultimately, this versatile platform will allow us to ask and answer numerous questions regarding the timing and extent of protein presentation required to recapitulate native mechanisms of angiogenesis.

SESSION V: NANOMATERIALS: SURFACE CHARACTERIZATION

11:15 AM – 11:45 AM INVITED SPEAKER ANNA BLAKNEY, UNIVERSITY OF BRITISH COLUMBIA

11:45 AM - 12:00 PM

Complementary peptide coassemblies for driving the structural order of thermochromic and phototransducer nanomaterials

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The hierarchical organization of natural peptides can be utilized as a synthetic tool for enabling properties in soft biomaterials that are highly reliant on molecular ordering and the formation of nanostructured networks. In this presentation, I will be discussing the utility of peptides as side chains with tunable noncovalent interactions that can modulate the properties of chromogenic and optoelectronic conjugated polymers, as well as the impact of the resulting bioscaffolds on morphology and behavior of excitable cells such as cardiomyocytes. First, I will present how electrostatically driven peptide coassemblies direct the conformational dependent structural order and thermochromic behavior of peptide-functionalized chromogenic polymers (polydiacetylenes, PDAs) under neutral, aqueous environments. A suite of spectroscopic characterization unravels the inherent structural order of model peptide-PDAs that control the chromatic phases that exist during thermochromic cycles. The positively charged peptide-PDA formed a β-sheet-like assembly with higher structural order than its disordered, negatively charged peptide-PDA. The equimolar coassembly resulted in a polymer with a more ordered structure than negatively charged peptide-PDA that still meets the geometric requirement for topochemical polymerization. Although all samples demonstrated thermochromicity, the coassembly experienced the least hysteresis in the aqueous state, and stabilization of the coexistence of blue and red phase chains was observed in the film state. In the latter half of this talk, I will discuss the utility of a sequence pair with charge complementarity for directing the order of energy donor and acceptor units to create photocurrent-generating bioscaffolds compatible with cardiac tissues. The properties of these coassemblies provide important insights on the role of coassembly-driven photoinduced energy transport processes for this biomaterial. Importantly, our coassembled scaffolds can generate photocurrents both as dry films (~2 nA) and under aqueous environments (~12 nA) upon 415 nm illumination. Lastly, these complementary peptide coassemblies were interfaced with cardiomyocytes, whereby their contractility indicates that the cardiac beating rate could follow the frequency of light pulses even without any optogenetic modification on the cells. Ultimately, with these stimulatory peptide-based nanomaterials, we hope to address long-standing

challenges in the fidelity of human cardiac tissue models used for high-throughput drug screening or disease modeling.

12:00 PM – 12:30 PM INVITED SPEAKER: AJ MELLOT, RONAWK

SESSION VI: RAPID FIRE TALKS

1:30 PM – 2:00 PM INVITED SPEAKER: BUDDY RATNER, UNIVERSITY OF WASHINGTON

RAPID FIRE SESSION I: TISSUE ENGINEERING

10:30 AM - 10:35 AM

An engineered collagen-binding fusion protein to improve localized delivery of bone morphogenetic protein-2 for bone regeneration

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Robust bone repair relies on dynamic interactions between numerous cells, proteins, and surrounding extracellular matrix molecules (ECM) such as collagen. In critically-sized bone fractures, which do not heal without intervention, the dynamic reciprocity between cells and the ECM is disturbed, leading to inhibited osteogenic protein expression, including the expression of bone morphogenetic protein-2 (BMP-2). BMP-2 is critical for bone regeneration, as it mediates the osteogenic differentiation of mesenchymal stem cells, which actively participate in bone formation by depositing new mineral. While clinical treatment strategies exist to deliver exogenous BMP-2 to large bone defects, these treatments have limited efficacy due to the use of a collagen sponge delivery vehicle that cannot sustain BMP-2 localization within the injury site due to the weak affinity between collagen and BMP-2.

To tackle this clinical limitation, we developed a fusion protein with a collagen-binding domain and an affibody engineered to bind specifically to BMP-2 as a method to localize BMP-2 within collagen sponges. An amino acid sequence specific to type I collagen anchors the fusion protein to the clinical collagen sponge, while the affibody controls BMP-2 release from the implantation site. We used AlphaFold2 to visualize the fusion protein structure with both rigid and flexible linkers between the two binding domains. We expressed the fusion protein in E. coli with a C-terminal hexahistidine tag to enable protein purification using immobilized metal affinity chromatography. The fusion protein size was verified to be ~9 kDa by sodium dodecyl-sulfate polyacrylamide gel electrophoresis. We used biolayer interferometry (BLI) to measure the affinities between the fusion protein, collagen, and BMP-2. BLI revealed a dissociation constant of 163±7.4nM for the BMP-2 and fusion protein interaction and 16.4±6.9nM for the collagen and the collagen-binding domain interaction. These binding affinities suggest a desirable dual-affinity interaction platform where the fusion protein binds more strongly to the collagen and less strongly to BMP-2, enabling local BMP-2 release and eventual bone regeneration. In future work, we will investigate the ability of our fusion protein to control BMP-2 release from the clinical collagen sponge in vitro and in vivo.

10:35 AM - 10:40 AM

Organotypic Whole Hemisphere Brain Slice Model of Hypoxic Ischemic Brain Injury in Low-and Middle-Income Countries

Sydney Floryanzia, University of Washington

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The incidence of hypoxic-ischemic encephalopathy (HIE) brain injury after perinatal (≥ 36 weeks gestation) oxygen deprivation is around 1-4 per 1,000 live births in the Western world and 2-3 times higher in Low- and Middle-Income Countries (LMIC) contributing to 8% of childhood deaths worldwide. The recent phase-III HELIX (hypothermia for moderate or severe neonatal encephalopathy in low- and middle-income countries) trial showed that HIE treatment used in high-resource areas is not beneficial in LMICs and may, in fact, result in increased deaths. This is largely due to a different pattern of HIE injury in LMIC populations characterized by an intermittent insult, and growth-restriction indicating nutrient insufficiency. A few clinical and animal studies have shown that intermittent oxygen deprivation results in a similar injury pattern. However, to our knowledge, no in vitro models fully mimic this complex injury process which are needed for accurate screening of neurotherapeutics. Here, we present a tunable organotypic whole hemisphere brain slice model to investigate stimuli-responsiveness and replicate the injury pattern seen in HIE in LMICs. We first expose slices to oxygen-glucose deprivation (OGD) conditions to model in vivo ischemic conditions as is extensively done in ex vivo models. To induce the additional intermittent injury, we alternated OGD exposure with periods of reoxygenation (OGDR). To induce injury resulting from nutritional deficiency, we cultured slices with media containing 2.5% serum compared to the standard 5% serum concentration. OGDR was applied with 3 and 5 cycles on slices cultured in 2.5% and 5% serum. We evaluated global cell death via LDH assay and region-specific differences in the striatum, thalamus, and cortex via live-dead staining and confocal imaging. We showed lower cell viability corresponding to the slices cultured with lower serum and increased cell death with increased OGD time. Vessels were stained with Occludin and cell death was seen distinctly in areas further from large vessels also indicating successful recapitulation of LMIC HI-like injury. Future work will use this model to screen combinatorial therapeutics with promise for translation and use in LMIC HIE cohorts.

10:40 AM- 10:45 AM

Leveraging an Open-Source Melt Electrowriting System to Fabricate High-Resolution Scaffolds from Rapidly Degrading Polymers

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Melt Electrowriting (MEW) is an innovative additive manufacturing method that uses high voltage and molten polymer to create high-porosity scaffolds from microfibers, applicable across various fields. Poly-ε-caprolactone (PCL), used as a biomaterial, is the "gold standard" material used for MEW scaffolds; however, its degradation time of two years limits its application as a biomaterial for shorter-term tissue regeneration and healing challenges.

The development of the MEWron, an open-source MEW platform, has made cost-effective filament-based MEW possible. This filament approach, as opposed to traditional syringe approach, reduces the time for which a polymer is molten, allowing continuous scaffold production from polydioxanone (PDO), a polymer widely used in sutures and meshes that hydrolytically degrades within six months.

Initial MEWron working parameters for printing PDO filament were established by varying melt temperatures (125-150°C), extrusion rates (6-100 µL/hr), collector distances (1-3 mm), applied voltage (1.7 kV/mm), and collector temperatures (50-80°C). Further optimization included vacuum drying the filament and testing longer printing periods.

While previous efforts to MEW PDO scaffolds have been limited by severe thermal degradation within short print times (<2 hours), we found stable MEW printing possible at lower temperatures (125-127°C), where thermal degradation remains minimal while the polymer is molten. To prevent premature solidification of the melt, the intensity of the heat gradient can be decreased by keeping a small collector distance (≤1 mm) and heating the print bed (≥50°C). After initial circulation of the polymer melt, PDO fibers with uniform diameter and limited thermal degradation can be printed indefinitely.

While lower temperatures enable MEW printing for longer than previously established, the more viscous melts require high extrusion rates (30-100 µL/hr) for stable fiber production, resulting in larger fibers (>30 µm). Vacuum drying the filament enables stable printing at higher temperatures (135°C) with low extrusion rates (6 µL/hr) for the duration of the polymer's melt residence, resulting in smaller fibers (10-20 µm). While exact working parameters may vary across filament MEW systems, this work demonstrates that through meticulous optimization and drying techniques, it is feasible to fabricate high-resolution scaffolds that degrade rapidly, offering promising solutions for biomaterial and tissue engineering challenges.

10:45 AM - 10:50 AM

In Cellulo Photoactivation of Diverse Protein Targets Enables 4D Spatiotemporal Control of Cell Behavior and Activity

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Proteins play an essential role in many biological processes, including regulating cell behavior, metabolism, and maintaining phenotype. Controlling protein activity spatiotemporally provides a direct route to manipulate biological processes in 4D, including for gene editing and transdifferentiation, all of which are necessary for the creation of engineered tissue. Towards this goal, we have created a versatile workflow to stably endow cell lines with the ability to inducibly express photoactivatable proteins of interest. Via the installation of non-canonical amino acids containing a photolabile chemical modification at catalytic residues, protein activity can be effectively “caged” until light exposure. These cell lines were shown to be photoactivatable on 2D surfaces via mask-based patterning and in 3D culture via multiphoton-based lithography. We anticipate that this pipeline will be readily extendable to photoactivate many functionally diverse proteins of interest, including Cas9 and its derivatives to enable 4D control over base editing, indel generation, and CRISPR activation/inhibition.

10:50 AM - 10:55 AM

Optimization of Applied Strain on Electrospun Fibrous Scaffolds for the Development of an In vitro Meniscus Injury Model

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Tissues like the meniscus, a wedge-shaped pad of connective tissue found in the knee, are fibrous with highly aligned collagen architecture. This fibrocartilaginous tissue also undergoes active mechanical stimulation which modifies cell signaling and tissue health. Unfortunately, regions of this tissue do not regenerate after injury likely based on the loss of aligned structure which modifies the microenvironmental cues required for optimal regeneration. We previously used the J1 Mechanoculture bioreactor to apply strain on an unaligned electrospun fibrous polymer scaffold laden with human primary meniscus cells and observed nonsignificant variances between testing groups with mock injury vs. no injury. The applied strain (10%) was modeled after what the meniscus experiences physiologically. Based on prior data showing minimal changes in cell behavior in mock injury samples, it is likely that the applied strain did not induce plastic deformation in the synthetic scaffold comparable to that experienced post injury within the native meniscus. We hypothesize that increasing strain to achieve plastic deformation within the electrospun samples will create a fibrotic and apoptotic response like that shown to develop in other fibrous connective tissues after loss of residual strain from disruption of the highly aligned matrix organization.

Ongoing work focuses on analyzing how the bioreactor will interact with unaligned electrospun PCL samples with no cells present. This will demonstrate the necessary applied strain to instigate a significant material response to create an effective mock injury model. By inducing significant changes to scaffold material properties and underlying structure, we believe this will aid in inducing the fibrotic and apoptotic cell response in vitro mimicking immediate cell reactions to meniscal injuries in vivo. These markers will be assessed via immunofluorescence assays to semi-quantify αSMA activation (fibrosis) and caspase-3 activation (apoptosis). On the conclusion of this study, we expect that greater tensile strain will cause more plastic deformation within the polymer scaffold. This can be applied to an in vitro meniscus injury model to better understand the response of primary meniscal cells to strain in an environment with disrupted mechanics.

10:55 AM - 11:00 AM

Hyaluronic Acid-Coated Melt-Electrowritten Scaffolds Promote Alignment and Differentiation of Skeletal Myoblasts

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Volumetric muscle loss poses a significant challenge due to the limited regenerative capacity of muscle tissue. Tissue-engineered scaffolds can provide structured support and enhance functional muscle repair by guiding myoblast alignment through biophysical and biochemical cues. We developed hyaluronic acid (HA)-coated polycaprolactone scaffolds to guide myoblast alignment and enhance muscle tissue repair. Our HA platform can additionally be modified with functional groups that enable the conjugation of proteins which can aid in the regeneration of functional tissue. Scaffolds were fabricated using melt electrowriting (MEW), an additive manufacturing technique that combines melt extrusion and electrohydrodynamics to deposit individual fibers and produces highly porous scaffolds. We hypothesized that coating MEW scaffolds with HA hydrogels would increase cellular attachment and alignment. HA was modified with adipic acid dihydrazide functional groups or oxidized to reveal aldehydes to produce a hydrazone crosslinked HA hydrogel. We then embedded scaffolds between equal volumes of modified polymer solutions to create HA-coated scaffolds. We seeded C2C12 murine skeletal myoblasts on top of the scaffolds to evaluate their attachment. Cells proliferated in high serum medium (10% fetal bovine serum) for 24 hours before exchanging media to low serum medium (1% fetal bovine serum) for 48 hours to promote the early phase of myoblast differentiation and myotube formation. Cells were then fixed and stained with phalloidin to label F-actin and DAPI to label nuclei for confocal microscopy. We observed a significant increase in cellular attachment on HA-coated scaffolds compared to non-coated scaffolds. We are currently investigating skeletal myoblast differentiation by exposing the cells to differentiation medium containing 2% horse serum. Cells will be stained for myosin heavy chain and DAPI to evaluate the extent of differentiation. Preliminary results show that our platform successfully supports myoblast differentiation. We have also successfully incorporated functional groups into our HA hydrogel that will enable the tethering of RGD peptides to further promote myoblast attachment and differentiation. In future work, we will vary the fiber spacing to improve anisotropic alignment. Ultimately, this biomaterial could provide a novel platform to accelerate functional muscle tissue repair through the unique combination of tunable biophysical and biochemical cues.

RAPID FIRE SESSION II: ENGINEERING CELLS AND THEIR MICROENVIRONMENTS

12:00 PM - 12:05 PM

Protein sensors for real time monitoring of reactive oxygen species, calcium and opioids.

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Genetically encoded sensors, combined with fluorescent microscopy, have revolutionized real-time monitoring of dynamic physiological processes. However, the number of high-performing sensors that meet experimental needs is still limited, while there are hundreds of biological compounds for which no sensors exist. The Berndt lab develops sensors by integrating state-of-the-art approaches such as high-throughput screening, machine learning, and predictive protein engineering. These combined efforts aim to address the intrinsic complexity of protein sensors which effectively hinders the fast deployment of new tools. We engineered novel sensors for reactive oxygen species, calcium, dopamine, and opioids. One highlight is calcium indicators, predicted by machine learning ensemble models, with increased dynamic range without sacrificing calcium sensitivity and fast kinetics.

Furthermore, we focus on optimizing multiplexed approaches, such as combining the readout of ROS and calcium from the same cells. Monitoring these processes could reveal critical insights into the pathophysiology of cardiovascular and neurodegenerative diseases and neuromodulation in motivated behaviors. Our sensors have been optimized and validated in various host systems such as mammalian cell cultures, IPSC-derived neurons and cardiomyocytes, brain slices, and behaving animals. Our future efforts aim to build custom-designed tools that meet cardiovascular and neuroscience research

requirements, such as high ligand selectivity, affinity, large response amplitudes, low toxicity, fast kinetics, and cell-type and cell-type and subcellular targeting.

12:05 PM - 12:10 PM

Tuning Viscoelasticity in a Hyaluronic Acid-Based Hydrogel System to Investigate Meniscal Fibrochondrocyte Mechanotransduction.

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Knee meniscus tears are common musculoskeletal injuries that have difficulty healing on their own but required surgical interventions often fail to restore the function of damaged tissue resulting in the need to develop effective therapies. To do so, it is necessary to develop in vitro models that further investigate mechanisms of regeneration in the meniscus. The extracellular matrix, a major component of the meniscus, contains macromolecules that provide structural support and biochemical and mechanical cues to the resident cells. Its mechanical properties are known to affect crucial regeneration processes such as growth, proliferation, and differentiation. To model the mechanical environment of the meniscus, our lab developed a pentanoate-functionalized hyaluronic acid (PHA) hydrogel. Our lab has previously shown that modulating the degrees of substitution (DoS) of the reactive -ene groups on the polymer backbone controls compressive modulus and, subsequently, meniscal fibrochondrocyte (MFC) morphology. Preliminary data indicated possible control of viscous responses which is important to further investigate because of the meniscus' viscoelastic rather than purely elastic nature. The objective is to further develop the PHA hydrogel system to control viscoelastic properties, including stress relaxation time, independently of compressive modulus. The overall hypothesis is that viscoelastic properties, such as stress relaxation time, can be controlled by systematically varying the ratio of crosslinked to un-crosslinked HA in the hydrogel system. Keeping the total hydrogel weight percent (wt%) at 8%, the gels will have 0, 0.5, 1, or 2 wt% uncrosslinked HA. Stress relaxation tests will be performed on the gels using a dynamic mechanical analysis system and time constants will be calculated. Uniaxial compression at a constant strain rate will also be performed to ensure elastic compressive modulus is constant while stress relaxation time varies. It is expected that increased amounts of uncrosslinked HA will increase stress relaxation time. This data will confirm control over viscous response independent of elastic response and the resulting hydrogel system can be used for further in vitro studies investigating the effect of viscoelasticity on MFC behavior. Ultimately, the knowledge of viscoelastic properties' impact on MFC behavior will contribute to the development of regenerative therapies for meniscal tears.

12:10 PM - 12:15 PM

Sustainable release of retinoic acid by porous silicon microparticles enhances the functional maturation of in vitro motor neurons derived from human induced pluripotent stem cells

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Human induced pluripotent stem cells (iPSCs) have enabled significant advances in the study of rare neuromuscular diseases. However, the inability to promote the development of adult phenotypes in iPSC-derived neurons remains a challenge. This problem is, in part, due an inability to accurately replicate the spatiotemporal gradient of morphogens that exist during embryogenesis in vitro. In particular, retinoic acid (RA) plays an essential role in motor neuron differentiation from iPSCs as it drives adoption of a caudal identity in differentiating neuronal progenitors. However, RA suffers from a short half-life (~6 hours) when added to conventional culture media. This results in significant variations in RA concentration in differentiating cultures over 24 hours. It is unclear what effect this has on the resulting neuronal population. Porous silicon microparticles (pSiMPs) are an attractive candidate for RA delivery carriers as their biocompatibility and surface modifications help to prolong RA half-life in culture. In this study, we utilized biodegradable pSiMPs to maintain intact RA concentrations during differentiation of motor neurons from iPSCs and investigated their impact on functional maturation.

RA-loaded pSiMPs were applied to differentiating neuronal progenitors using a transwell system to enable sustainable release of RA molecules while minimizing direct contact between pSiMPs and cells. We found that our differentiating cells could be cultured with pSiMPs for over 24 hours without changes in cell viability or differentiation efficiency, and that RA in these cultures was maintained at a constant concentration during this period. Neurons differentiated using sustained RA release showed improved electrophysiological function, not only in individual neuron activity but also in neuronal network development. Furthermore, at the transcriptome level, sustained RA release led to increased expression of axon genesis and neurofilament bundle assembly genes.

The biodegradable sealing chemistry of pSiMPs provides sustained release of RA in its active form within in vitro culture systems to prevent rapid deactivation of RA. Consistent supplement of RA in culture using pSiMPs enhances iPSC-derived neuronal function without negative effects on cell viability or differentiation efficiency. As a release platform, we suggest that pSiMPs are therefore a promising tool for enhancing in vitro neuronal differentiation from iPSCs.

SESSION VI: RAPID FIRE TALKS

1:45 PM - 1:50 PM

Improving Bio-inductive Properties of Affibody-Conjugated Hydrogels to Control Osteogenic Protein Delivery for Promoting Localized Bone Repair

Payton Jefferis, University of Oregon | Knight Campus

Bone morphogenetic protein-2 (BMP-2), an osteogenic protein, is used clinically to promote bone regeneration. However, the current method of delivering BMP-2 from an absorbable collagen sponge can result in uncontrolled BMP-2 release and poor localization, leading to adverse effects such as ectopic bone formation. Therefore, there is a clear need to develop a delivery vehicle that can control the release rate of BMP-2. Affibodies, which are small, antibody-mimicking proteins, can alter the rate of protein release through variable affinity binding. Our lab has identified several affibodies specific to BMP-2 from a yeast surface display library. BMP-2 release rates were tuned from affibody-conjugated polyethylene glycol-maleimide (PEG-Mal) hydrogels regulated by the equilibrium dissociation constant (KD) of the protein-to-affibody interaction. However, PEG-based hydrogels do not effectively promote cellular adhesion, potentially limiting their use in vivo for tissue regeneration. To overcome this challenge, we introduce RGD, a major integrin-binding domain in the extracellular matrix into the hydrogel. The objective of this study was to characterize hydrogel physicochemical properties with the addition of affibody and RGD and determine whether RGD affects the BMP-2-affibody interactions.

Hydrogel porosity with and without the affibody was determined through the Equilibrium Swelling Theory, which relates the mass of the relaxed and the swelled hydrogel. The mesh size was not altered by the addition of affibody. PEG-Mal hydrogels were conjugated with affibody and/or RGD, through a Micheal-thiol addition, and loaded with BMP-2. Protein encapsulation and release were assessed by enzyme-linked immunosorbent assay, indicating that RGD did not significantly alter BMP-2 release over 2 weeks.

BMP-2-specific-affibody-conjugated hydrogels with and without RGD were implanted subcutaneously into the backs of Sprague Dawley rats. After one week, the hydrogels were excised and cryosectioned. Sections were stained with DAPI or hematoxylin and eosin (H&E) to stain cell nuclei. Cell counts and penetration depth indicated that integrating RGD into the hydrogels promoted cell infiltration.

The addition of RGD into BMP-2 affibody-conjugated PEG-mal hydrogels improved cellular infiltration in vivo without affecting BMP-2 release in vitro. Taken together, this work demonstrates significant progress toward improving the cellular interaction of a hydrogel delivery vehicle for localized bone repair.

1:50 PM - 1:55 PM

Modulating Macrophage Polarization Using Surface Roughness on Electrospun Fibers to Promote Healing in Soft Connective Tissue Injuries

Aidan Alemifar, University of Washington

Purpose/Objectives: Injuries to fibrous connective tissues suffer from poor healing, leading to over 17 million doctor's visits and 1.4 million surgeries annually in the US. Even if the tissue does heal, the resultant tissue often has inferior mechanical properties. Treatments often lead to pro-inflammatory activation of immune cells, precipitating osteoarthritis. Tissue engineering solutions have been proposed to regenerate functional tissue after injury. Research has focused on designing biomaterials capable of modulating macrophage polarization to control the transition from a pro-inflammatory (M1) state to a pro-regenerative (M2) state. Implants with increasing SR led to increased inflammation in vivo. However, there have been no studies investigating the role that SR fibers that mimic collagen structure and are used for tissue engineering plays in controlling macrophage phenotype. The objective of this study is to utilize a tunable, electrospun fiber system to study the impact of SR on macrophage polarization. We hypothesize that a material with a SR on the same length-scale as the d-band of collagen (67 nm), the major source of SR on the collagen fiber, will minimize inflammation and promote regeneration.

Preliminary Data/Results: Relative humidity was used to modulate poly(caprolactone) electospun mesh to tune SR (Ra = 9.6 ± 4.5, 32.9 ± 16.6, 58.9 ± 12.9 nm). Primary macrophages, isolated from the blood from healthy volunteers (n =3, male, age 20-35), were seeded on this mesh (60,000 cells/mesh) and allowed to culture for five days. M0 macrophages cultured on the high SR mesh showed decreased expression of pro- and anti-inflammatory cytokines from a Luminex assay and an increase in the M2:M1 ratio via immunofluorescence staining. In MacGreen (GFP-labeled CSFR-1) mice, high SR group produced an increase in the ratio of M2:M1 phenotype via immunofluorescence surface markers.

Conclusion/Significance: Macrophage polarization can be controlled by modulationg electrospun fiber SR both in vitro and in vivo. A better understanding of this response and how to control it would allow for the design of biomaterials capable of controlling the polarization of macrophages, and thus controlling crosstalk with meniscal cells, to promote the regeneration of functional tissue after fibrous connective tissue injuries.

1:55 PM - 2:00 PM

SMART BIOINK WITH BIOACTIVE MOLECULES TO HELP PROMOTING HEALING PROCESS IN CHRONIC WOUNDS

GISELLE DIAZ, University of Victoria

Chronic wounds, resulting from disrupted skin integrity, cause pain, limit mobility, and degrade quality of life, affecting 1-2% of the population in developed countries. Consequently, wound care costs have escalated, posing a significant financial burden on healthcare systems, amounting to billions of dollars. Traditional wound care approaches have primarily focused on repair rather than restoration, often failing to achieve complete healing and functional recovery.

Recent advances in wound care emphasize innovative treatments that promote restoration. One such advancement is 3D bioprinting, a cutting-edge technology that utilizes biomaterials-based bioinks containing living cells to create tissue scaffolds. These scaffolds provide the ideal architecture for cellular survival, growth, and maturation, mimicking the natural extracellular matrix. Despite its potential, current bioprinting technology lacks the ability to efficiently support microbiome recovery, a critical factor for complete skin regeneration and functionality.

To address this limitation, we are developing a smart bioink incorporated with bioactive molecules that interacts with the external environment, such as pH and O2 levels. Additionally, it stimulates the secretion of therapeutic proteins, enhancing the restoration of the microbiome during the wound healing process. This targeted approach reduces the probability of dysbiosis, which can lead to inefficient skin cell proliferation and opportunistic infections.

By integrating bioactive molecules, this smart bioink offers a promising solution to enhance the healing process in chronic wounds. It provides a comprehensive strategy that not only supports cellular activities but also ensures the recovery of the microbiome, thereby promoting effective skin regeneration and reducing the risk of complications.

This innovative approach represents a significant advancement in wound care management, with the potential to alleviate the financial burden on healthcare systems and improve the quality of life for patients with chronic wounds.

2:05 PM - 2:10 PM

Enhancement of Schwann cell migration using YIGSR-functionalized aligned nanofiber scaffolds for peripheral nerve reconstruction

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Despite optimal peripheral nerve reconstruction for nerve defects, return of sensory and motor function is often slow and inconsistent. The current standard-of-care for critically-sized transection injuries (greater than 3 cm in humans) is autologous grafting, which faces many difficulties such as undesirable reliance on donor nerve availability, sacrifice of donor function, and size and structural compatibility. Consequently, a new methodology allowing for in-situ endogenous repair of the severed nerve would advance patient prognosis compared to autologous grafts. Key to the regenerative process is the behavior of Schwann cells (SCs) to impart biomolecular and topographical cues for regrowing axons. Axonal regeneration only proceeds to the extent of which SCs are able to migrate from the proximally intact nerve. By applying concentration gradients of peptides known to influence the migration of SCs to nanofiber scaffolds, SC migration can be directionally guided from proximal to distal ends. Using a novel touch-spinning fabrication method, functional poly(caprolactone) (PCL) aligned nanofiber conduits with well-defined diameters (1.65 ± 0.25, 1.18 ± 0.25, and 0.87 ± 0.27 μm) were produced. Peptide concentration gradients were covalently bound to the nanofibers via a thiol-ene click reaction. On non-functionalized fibers, seeded SCs exhibited elongated morphology when compared to plates without fibers, indicating enhanced migratory behaviors. Further migratory studies found that SCs specifically migrate in the x-axis (defined as the direction of aligned fibers) on a 2D scaffold in the presence of nanofibers. SCs on nanofibers moved at speeds of 0.24, 0.25, and 0.22 μm/min along the x-axis for diameters of 1.65, 1.18, and 0.87 μm respectively, demonstrating higher rates than that of SCs on no fibers (0.19 μm/min). Near-zero speeds were recorded for SC migration on nanofibers in the y-direction. Future enhancement of SC behavior in the regenerative process was done by applying concentration profiles of relevant peptides to the scaffolds as biochemical cues. As such, the use of synthetic, nanofibrous scaffolds improved the directionality and rate of migration of SCs in vitro. Efforts to implement such nanofibrous scaffolds during nerve reconstruction may enhance the efficiency and efficacy of nerve repair through directionally-guiding SCs in the immediate postoperative regenerative period.

2:10 PM - 2:15 PM

Hepatocyte-Targeted Polymer Micelles Improve Endosomal Release of mRNA Delivered through a Lipid Nanoparticle

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MessengerRNA (mRNA) delivery is an area of research for its potential in protein therapeutics and vaccines due to cell-free synthesis, no risk of integration into the host genome, and rapid product development. In recent COVID-19 vaccines, mRNA has been delivered in Lipid Nanoparticles (LNPs). However, one hurdle to mRNA translation is escape from the endosome, where nucleic acids would otherwise be degraded. To address these concerns, the co-administration of a RAFT-synthesized diblock polymer micelle and a model LNP mRNA delivery vehicle will be applied to improve protein translation. Targeting hepatocytes is done through N-acetylgalactosamine (GalNAc), which targets the asialoglycoprotein receptor (ASGPR). Previous works have used a terminal GalNAc group on the chain transfer agent and poly(ethylene glycol) [PEG] as a hydrophilic monomer. However, GalNAc's targeting efficacy improves with interaction valency and PEG has been shown to prompt an immune response in some patients. This polymer's first block will be composed of monomeric GalNAc, that will serve as the primary hydrophilic component and a multivalent targeting agent for hepatocytes, and hexyl methacrylate (HMA) to aid micelle stability through hydrophobic anchoring between chains. The polymer's second, primarily hydrophobic, block ["PDB"] will incorporate 2-propyl acrylic acid (PAA), 2-(dimethyl)aminoethyl methacrylate (DMAEMA), and butyl methacrylate (BMA). PAA is a pH-responsive monomer that protonates as the endosome acidifies, becoming hydrophobic and disrupting the endosomal membranes. The GalNAc-PDB micelles were ~30nm in diameter in formulation buffer. These polymers will be evaluated for cytotoxicity and improvement of transfection in vivo via luciferase expression, via luminescence readouts through IVIS, after a tail vein co-administration of GalNAc-PDB micelles and mRNA:LNP complexes.

2:15 PM - 2:20 PM

Continuous whole blood dialysis based on regenerated dialysate utilizing TiO2 nanowire photo-electrochemical oxidation and protective forward osmotic membrane

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While a traditional 4-hour dialysis treatment uses up to 120L of fresh dialysate per session, a full-scale bench-top system was developed to remove clinically relevant amounts of uremic toxins in whole blood using only 0.5L of commercial dialysate solution over a 24-hour continuous dialysis process. Urea was removed by photoelectrochemical method through a UV/TiO2 nanowires array device connected to a Forward Osmosis (FO) membrane in a protected close loop, while other non-urea toxins were removed by Activated Carbon (AC) in the dialysate loop. Here we demonstrate a daily urea removal rate of 14.2g using 0.4m2 of hemodialyzer membrane and 2.3m2 FO membrane with 0.18m2 TiO2 nanowires array in Photoelectrochemical Oxidative Urea Removal (POUR) unit. Other non-urea toxins represented by creatinine and phenylacetic acid with a daily removal rate of nearly 7g were achieved by 70g of AC absorbent in the dialysate loop. Urea, creatinine and phenylacetic acid were constantly infused to the saline solution with the speed of 15g/24h, 5.6g/24h and 1.4g/24h to simulate actual human toxins level. Urea is continuously removed in a 0.15M NaCl saline solution by the photo-oxidation reaction occurring on TiO2 nanowire surface with the presence of UV source and applied bias to enhance the electron-hole pair separation within the single crystal TiO2 nanowire. The POUR loop was also protected from the potential oxidative species generated from the photooxidation of urea by inline AC absorbent with real-time ORP and pH sensors, showing non-cytotoxicity to the blood loop. The FO membrane is also a key component to prevent small solute molecules from dialysate entering the POUR loop to lower its efficiency as well as forming harmful oxidative byproducts. Our results also showed a wide variety of treatment modes (ie. 4, 8, 12 hr daily times) by coupling different membrane components. More importantly, by eliminating the need for the external water source, our system offers possibilities of a variety of portable kidney dialysis treatment options for home, work and travel.

2:20 PM - 2:25 PM

Harnessing Nano- and Micro-fiber Alignment for Enhanced Peripheral Nerve Growth

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Peripheral nerve injury (PNI) can result from trauma or neurological diseases and affects approximately 20 million individuals every year. Many of these injuries are crushes, therefore a critical gap defect injury model addresses the repair of some of the most challenging injuries. While peripheral nerves possess regenerative capabilities, the natural repair process often falls short of achieving full functional recovery, leading to sensory, motor, and autonomic deficits that can drastically reduce a patient's quality of life. The incomplete repair is largely due to the distance across the gap defect and to the quality of the autograft bridge formed.

In neural tissue engineering, aligned fibers have bridged greater critical gaps after PNI than empty nerve guides. Both nanofibers and microfibers are shown to guide repair. Typically, solution electrospinning (SES) produces nanofibers alignment perpendicular to the rotating mandrel. In this study, melt electrowriting (MEW) microfibers deposited along the long axis of the mandrel results in an unexpected alignment of the nanofibers along this long axis, in the same direction as the MEW fibers. This method for unorthodox SES fiber alignment facilitates guided alignment in tubular scaffolds.

Schwann cells play a crucial role in peripheral nerve regeneration following PNI. They are an important cell in stimulating nerve regrowth due to their secretion of neurotrophic factors, production of myelin, and guidance of axons to bridge the injury site. Thus, ongoing studies utilize primary rat Schwann cells isolated from the sciatic nerve, which are a well-establish model for the study of regeneration in vitro. Cells are seeded on our MEW fibers within SES membrane and in vitro regenerative capacity is predicted based on Schwann cell phenotypic changes, proliferation and migration.

Overall, we describe a novel approach combining MEW-produced microfibers and SES nanofibers to create aligned tubular scaffolds with robust mechanical stability for peripheral nerve regeneration. Aligned fibers enclosed in membrane form a nerve guidance conduit aimed at addressing PNI.

POSTERS

Poster Number: 2

3D-printed Precision-Porous Implants Modulate Healing and Foreign Body Reaction

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When implantable biomedical devices are introduced into a biological system an inflammatory reaction (the foreign body response, FBR) is triggered leading to an avascular, fibrotic outcome., Thus the need to develop new biomaterials to improve healing and vascularization. Porous materials enable tissue to migrate, grow, and integrate within the implants. Biomaterials with uniform, interconnected 40 µm spherical porous structures have significantly improved FBR outcomes and promote vascular growth. Here, with a commercially available resin material, we present a 3D-printing method to develop precision-controlled 40 µm cubical porous materials. We hypothesized that these materials with cubical pores vascularize, reduce fibrosis, form a healthy extracellular matrix, and ultimately behave in a pro-healing manner in vivo. We implanted non-porous, 40 µm porous spherical, and 3-D printed 40 µm porous cubical structures into mice subcutaneously for analyzing tissue-implant interactions. Post-implantation, we evaluated the implants histologically with H&E, Masson trichrome, and Immunohistochemistry for foreign body capsule formation, endothelization (blood vessels), and biological integration. This research aims to deepen our understanding of pore geometry effects on the FBR to achieve improved integration of implantable biomedical devices.

Poster Number: 3

Smart Dura: Transparent microelectrode array as a large-scale multimodal neural interface for non-human primates

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Non-human primates (NHPs) are valuable animal models in neuroscience research by providing relevant translational insights with their high cognitive and behavioral complexity. Various neural interfaces have been developed to understand brain function. In particular, advanced neurotechnology has been demonstrated through multimodal interfaces that can provide both electrical and optical modalities. However, most are limited in their application to small animal models, such as rodents, being unlikely to be translated to NHPs. Here, we developed a micro-electrocorticography (μECoG) electrode array for large-scale neural recordings in NHPs, namely 'Smart Dura'. The Smart Dura has 32 or 64 gold/platinum electrodes, which are 20 – 40 μm in diameter, for stimulating and recording neuronal activity on the surface of the NHP brain, over or under the dura. These microelectrodes are patterned on the flexible and transparent polydimethylsiloxane (PDMS) substrate with the adhesion and insulation layers of Parylene C or SU8. Such optical access allows the Smart Dura to function as a multimodal interface that enables optical stimulation and imaging. In addition, due to the biocompatibility of the materials and minimal invasiveness of their implantation, the Smart Dura facilitates chronic application. With our Smart Dura, we demonstrated successful ECoG recordings from macaques, not only their spontaneous activity but also evoked responses induced by electrical, optical, and tactile stimulation and in correlation with behavior. We also validated the optical transparency by imaging fluorescent samples placed under the Smart Dura with multiphoton microscopy. As a large-scale multimodal tool for NHPs, our Smart Dura will help obtain significant translational information and further promote neurotechnology applicable to humans.

Poster Number: 4

Surfactant Modified Electrospun Fibrous Scaffolds for Use in In vitro Tissue Models

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Methods to promote regeneration in connective tissues with highly aligned, collagen dense matrix like the meniscus are unknown. After a meniscal injury, the organized collagen structure is disrupted which impacts the ability of endogenous meniscus cells to migrate to the injury site and receive adequate regenerative cues. In vitro systems offer the ability to ask specific questions about singular system modifications of complex tissues without confounding factors present in explant or animal models. Establishing a reliable system for creating synthetic models of structural extracellular matrix components is key to understanding cell-material interactions and dependence of cell morphology on varied material properties.

Electrospinning offers an easily modified, cheap, and well characterized system for mimicking collagen fibers. Additives like surfactants enable further manipulation of final fiber and scaffold properties like hydrophilicity, surface roughness, and tensile strength. The objective of this study is to establish replicable production of aligned polymer scaffolds with and without surfactant and define any differences in meniscal fibrochondrocyte (MFC) adhesion and alignment in these samples.

Base solutions consisted of 20%(w/v) polycaprolactone (PCL) in chloroform ($CHCl_3$) and 30%(w/w) Span80 in surfactant samples. Collection conditions consisted of low relative humidity, rotating collector speed of 1010±17RPM, 1.0mL/hr flow rate, 30cm collection distance, and 15kV applied voltage.

Mean fiber diameter of aligned surfactant fibers significantly increased compared to control (4.8±0.8µm, 4.4±0.4µm). However, mean fiber diameter difference was minimal (0.4±.09µm). Fiber fraction significantly increased in surfactant samples with minimal change (0.54±0.07µm vs. 0.50±0.04µm) and fiber alignment was not significantly different. Therefore, samples are relatively comparable in all aspects beyond added surfactant which resulted in lower contact angle (27±4 vs. 87±15) and based on preliminary mechanical testing increased ductility.

Preliminary fluorescent imaging of cultured native meniscus cells showed no noticeable differences in MFC density or ability to align on fibers with and without surfactant demonstrating the ability to incorporate surfactant in electrospun fibers to modify material properties without negatively affecting cell behavior. Future work will examine how surfactant in these fibers impacts synovial fluid protein adsorption to further elucidate the connection between fiber surface properties, protein adsorption, and the formation of focal adhesion complexes.

Poster Number: 6

Visualizing fiber path and generating G-code for melt electrowriting of tubular scaffolds using Grasshopper software

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Melt electrowriting (MEW) is an additive manufacturing technique that uses applied voltages to deposit molten polymers as micrometric fibers onto a collector. Typically, MEW creates planar structures, however mandrel collectors have yielded success in creating 3D, porous MEW tubes. Since MEW is a continuous fiber technology, designing and actualizing tubular configurations is inherently harder than creating their planar counterparts. Furthermore, the MEW workflow has not yet adopted common practices seen in other 3D printing, most notably lacking slicing software commonly seen in fusion deposition modeling that allows users to easily convert 3D visualizable designs to printing computer language. Without such practices, creating consistent MEW tubes can be excessively difficult, and inaccessible to new users. This research offers a new approach to tubular MEW scaffold design using Grasshopper, a software extension of the CAD program, Rhinoceros. A custom design workflow allows users to view a digital preview of the tube design, in addition to generating corresponding G-codes, streamlining the programming process and expediting printing iterations. Users can input design parameters such as fiber spacing, crosshatch angle, scaffold length, and number of layers into the program and receive G-code in a script compatible with the Aerotech axis printer, the system used to render tubes in this study. Additionally, the G-code includes built-in experimental information like adjusted printing parameters such as nozzle temperature, pressure, angular speed and linear speed that all must change to represent different design iterations and fiber diameters. This approach streamlines the process of manufacturing tubes as complex biomaterial structures. Overall, the presented platform focuses on predicting fiber path, offering users easy control of design parameters, and validating tube design given specific parameters. Grasshopper, commonly used in architectural communities, excels at generating quick visual ideations using parametric design. Merging two disciplines, architecture and bioengineering, for combined visual and mathematical design highlights the importance of collaboration as we pursue novelty in the world of bioengineering. As the design space for MEW tubes increases with growing applications, this research becomes more and more pertinent for the biomedical field.

Poster Number: 7

WITHDRAWN

Poster Number: 8

Rapid FTIR-ATR spectroscopic imaging for analysis of the dentin/adhesive interface

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Hydrolytically-stable acrylamides can preserve the integrity of the bonded resin/dentin interface in 2-step, total etch adhesives (Fugolin et al., 2019). Advancements in Fourier transform infrared (FTIR) microscopy coupled with attenuated total reflectance (micro-ATR) have enabled rapid chemical mapping alongside high-resolution surface imaging. This work evaluated the fidelity of FTIR-ATR for characterizing the adhesive and hybrid layer regions in dental restorations containing either traditional methacrylate-based or acrylamide-based adhesives.

Primers were comprised of 45wt% N,N-Diethyl-1,3-bis(acrylamido)propane – DEBAAP or 2-hydroxyethyl methacrylate – HEMA, 10wt% 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate) and 45wt% glycerol dimethacrylate – GDMA. Adhesives contained 45wt% DEBAAP or HEMA, 10 wt% 10-MDP and 45wt% urethane dimethacrylate (UDMA). Stick specimens were prepared using human dentin, commercial composite and 5 primer/bond combinations: CC (Clearfil Primer and Bond), HH (HEMA/HEMA), DD (DEBAAP/DEBAAP), HD (HEMA/DEBAAP) and DH (DEBAAP/HEMA). A Nicolet RaptIR FTIR microscope equipped with a germanium-tip ATR was used to collect 300x20µm specimen maps spanning the composite, adhesive/hybrid layer and dentin. Spectral data was collected in absorbance (64 sample scans, 16cm-1 resolution) between 4000-400cm-1. Contour fill plots and 3D colormaps were generated using Origin and were compared with confocal laser scanning microscopy (CLSM) images of similar interfacial regions.

Spectral features corresponding to dentin, adhesive and composite were identified in all groups. Amide and phosphate regions associated with collagen were apparent in dentin regions (amide 1 – 1647cm-1, amide 2 – 1553cm-1, PO43- – 1013cm-1), while the adhesive layer displayed increased absorbance in carbonyl (1720cm-1) and C-O (1165cm-1) vibrational stretches, consistent with methacrylate/acrylamide monomers. IR-mapped region of the adhesive layer varied in thickness among the groups, with HH displaying the thickest layer at approximately 90µm, while DH and HD had thicknesses of approximately 40µm. By contrast, CLSM showed HH had the thinnest adhesive layer, but a greater depth of penetration into the dentin. Regions of overlap were more difficult to elucidate using FTIR-ATR microscopy.

FTIR-ATR microscopy is a powerful tool for rapid measurement and visualization of compositional differences in the resin-dentin bonded interface. Additional studies are underway to optimize adhesive formulations with the goal of maximizing bond strength through the addition of acrylamides.

Poster Number: 9

Reproducible 3D bioprinting of Streptococcus mutans to create a novel oral biofilm model in vitro.

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Dental caries prevalence is around 50% of teeth, making it one of the most common diseases worldwide. Dental caries is initiated by the development of biofilms composed of bacteria that produce lactic acid, causing demineralization, also known as dental caries. Novel approaches are needed to study relationships between biofilm bacterial strains, enable three-dimensional biofilm deposition, and hasten the rigor and pace of biofilm experimentation. In prior work, 3D-bioprinters have been leveraged to deposit spatially patterned biofilms onto soft sugar-rich agar surfaces to study how microbial spatial organization impacts biofilm persistence and virulence. Herein, we have developed a new 3D-bioprinting method using biomimetic solid mineral substrates (hydroxyapatite) submerged in aqueous solution. The aim is creating a new automated methodology using the 3D printing technology for study oral biofilms in vitro, making it faster, reliable, and less costly. Streptococcus mutans UA159 was used to compare standard in vitro biofilm development with our new 3D-printed bio-ink hydrogels on hydroxyapatite discs, which mimic tooth surfaces. Biofilms formed using the bio-ink methodology showed no statistical difference in most of virulence factors, including environmental pH, biomass, and cell density, compared to biofilms formed using the standard in vitro methodology. However, the bio-ink technique resulted in twice higher water-polysaccharide content ($p \leq 0.0001$), and 50% higher in alkali-soluble polysaccharides ($p \leq 0.01$), a key virulence factor for biofilm cohesion and protection. However, the alginate-based hydrogel concentration used in this study impacted on the microbial distribution of the printable compared to the standard method. The 3-dimensional microbial distributions differ from the regular distribution founded in the standard control and in vivo. Our newly developed technique produces 3D-printable model biofilms that match the virulence benchmarks of the standard method, opening possibilities to print biofilms onto a mineral substrate and a new way to study multidimensional oral biofilm dynamics. Current efforts are focusing on improving the hydrogel density to allow a similar microbial spatial organization that better represents all virulence factors founded in vitro and in vivo biofilms models.

Poster Number: 10

Salivary Gland Tissue Chip for the Study of Irradiation-Mediated Dysfunction

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Xerostomia, or chronic dry mouth, afflicts approximately 80% of head and neck cancer patients undergoing radiotherapy, resulting in deterioration of the oral cavity, increased dental caries, and severely diminished quality-of-life. Radiation-mediated damage exacts short-term and long-term complications, including decreased saliva production and loss of acinar cell secretory function. While immune cells, such as macrophages and B- and T-cells, have been implicated in immune-mediated repair processes following radiotherapy, their pathophysiological role within the salivary gland remains elusive. This stems from difficulties studying the salivary gland, as in vivo models poorly recapitulate human gland structure and function, and in vitro culture of primary salivary gland cells results in abrupt loss of secretory acinar function. To overcome these challenges, we developed a salivary gland mimetic chip (SGm) composed of salivary gland acinar cell clusters and intercalated ducts encapsulated within a matrix metalloproteinase-degradable poly(ethylene glycol) hydrogel matrix. Microbubble arrays consisting of 15 nL microsphere wells molded in polydimethylsiloxane recapitulate the salivary gland acinar compartment. These microbubbles constrain the clusters within physiologically relevant niches, preserving 90% cell viability and secretory function (Aqp5, Nkcc1,and Mist1 expression) over 14 days. The SGm enables mechanistic investigations of salivary gland dysfunction and invaluable insights into the underlying cell-cell communications underpinning radiation-damage-associated salivary gland dysfunction. For instance, following irradiation, the loss of salivary gland macrophages diminishes salivary gland repair processes. Our current mimetic excludes immune cells, thereby only allowing us to isolate epithelial and acinar cell responses. However, to elucidate the interplay between the salivary gland and macrophages, we will incorporate immune cells to the SGm and assess their role in salivary gland dysfunction prior-to and post-radiation. Notably, with this inclusion of macrophages, we will identify key interactions between the salivary gland and macrophages with the overarching goal to identify strategies for mitigating radiation-induced dysfunction and reducing xerostomia.

Poster Number: 11

3D models of vaginal mucosal epithelium: the influence of age, hormones, and inflammation on barrier function and drug delivery

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Understanding the dynamic interactions at the vaginal mucosal barrier is critical for developing therapeutic interventions and enhancing patient defenses against infections. The vaginal mucosal epithelium, a target site for drug delivery and a critical barrier to pathogen entry, is influenced by age, hormonal cycles, and inflammation. Despite clinical evidence linking these factors to disease susceptibility, their effects on mucosal permeability, drug diffusion, and pathogen resistance remain poorly understood. To bridge this knowledge gap, we are developing three-dimensional (3D) models of the vaginal mucosal epithelium to study the impacts of these factors on permeability and apparent diffusivity.

We first analyzed the diffusion of a synthetic dye through collagen I hydrogels at concentrations of 2.5 and 6 mg/mL, comparing the resultant apparent diffusion coefficients with those in a phosphate-buffered saline (PBS) solution. We observed an inverse correlation between collagen density and apparent diffusivity, with a 3.5-fold reduction in the diffusion coefficient at 6 mg/mL of collagen compared to PBS alone. This finding underscored the significant influence of ECM density on diffusion properties, validating our experimental approach for future investigations into drug diffusion dynamics.

Expanding on these insights, we developed a multi-layer, multicellular model of the vaginal epithelium incorporating gelatin methacryloyl (GelMA) and ECM components reflective of vaginal tissue, such as collagen I, collagen III, elastin, heparin, hyaluronic acid, and versican, together with primary human fibroblasts, microvascular, and vaginal epithelial cells. This model mimics the stratified architecture of the vaginal epithelium and is compatible with high-throughput, high-content screening. Future work will involve simulating physiological changes due to age, hormonal cycles, and inflammation using cells from donors of varied ages, hormonal treatments, and the inclusion of activated macrophages. Through measurements of apparent diffusivity and transepithelial resistance, we aim to elucidate how physiological changes affect mucosal permeability. This comprehensive approach not only promises to deepen our understanding of the mucosal barrier function but also opens avenues for the development of novel therapies tailored for the vaginal epithelium and the identification of new therapeutic targets to enhance mucosal defense mechanisms against infection.

Poster Number: 12

Evaluating Sex-Based Variances in Extracellular Vesicle Therapy Within a Neonatal Hypoxic-Ischemic Injury Model

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Hypoxic-Ischemic Encephalopathy (HIE) resulting from a lack of blood and oxygen to the brain is the leading cause of mortality and lifelong neurological impairments in term newborns. We have shown extracellular vesicles (EVs) applied on models after hypoxic ischemia (HI) injury alleviate inflammation from injury, likely due to their ability in serving as critical transporters of biomolecules between cells. In our prior studies, therapeutic efficacy of EVs has only been evaluated in males, as males are more susceptible to worse outcomes following HIE injury, yet knowledge about EVs and their behavior when administered to females is still needed. In this study, we aimed to address this knowledge gap by systematically comparing the efficacy of male and female neonatal brain-derived EVs (mEVs, fEVs, respectively) applied on male and female neonatal rat ex vivo brain slices. We first confirmed the purity of isolated EVs with protein assays and immunoblots. We then utilized an ex vivo oxygen-glucose deprivation (OGD) model of HI injury, applying fEVs and mEVs to sex-matched OGD-exposed brain slices. We evaluated cell viability after 24h of EV exposure with propidium iodide (PI) staining and ImageJ analysis, and our results have shown that fEVs decrease inflammation and cytotoxicity in OGD models. When compared to previous results using mEV treatment, our results show females have a more robust anti-inflammatory response system to injury. Ongoing work to better understand the therapeutic effect of EVs involves further observing morphological shifts in microglia through confocal imaging. Microglia are homeostatic-mediating cells in the brain, and fEV application will likely result in microglia shifting towards anti-inflammatory phenotypes, similar to what was previously observed after mEV application. We are also quantifying expression levels of various inflammatory and reparative genes in ex vivo brain slices through reverse transcription quantitative polymerase chain reactions (RT-qPCR) after application of EVs. Overall, we have demonstrated in these pilot studies that fEVs have a different therapeutic effect in OGD injury compared to mEVs. This research is intended to open up pathways for more personalized sex-based treatments for various injuries and therapeutics in the future.

Poster Number: 13

Genetically Encoded Interpenetrating Networks as Injectable Biomaterials for Controlled Protein Therapeutic Delivery

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Over the past several decades, there have been growing applications for recombinant protein-based therapeutics. Due to their low oral bioavailability, these new modalities need to be delivered intravenously, which leads to off-target effects and short serum half-lives, severely compromising therapeutic index. As an increasing number of protein therapeutics receive FDA approval each year, there is a pressing need to develop novel delivery systems. Therefore, we sought to design the first reported injectable, fully recombinant protein-based interpenetrating network (IPN) hydrogel to be an ideal material for protein therapeutic delivery. Previously reported IPNs are composed of covalently crosslinked synthetic polymer networks sometimes interlaced with a naturally harvested protein network (e.g., collagen, fibrin). These materials are tunable and easy to manufacture, but lack injectability, biocompatibility, and often the ability to be degraded by the body. Recombinant protein-based polymers are advantageous due to their bioactivity, intrinsic biocompatibility, tunability, superior resorption, and highly-defined properties. The biomaterial is composed of two networks made from orthogonal self-assembling coiled-coil domains (Q or A domains) connected by a hydrophilic unstructured protein linker. These crosslinks allow for rapid shear-thinning and self-healing behaviors, important for material injectability. The linker is bisected by two orthogonal peptide-protein covalent complexations to enable modular network customization. Protein drugs can be covalently ligated to each network, eliminating the burst release profile commonly associated with drug-loaded hydrogels. The IPNs, regardless of crosslink compositions, can be made into a homogeneously mixed material, allowing for even drug elution through surface erosion. The combination of two protein networks allows for modulation of mechanical properties based on crosslink composition while maintaining erosion rates and injectability. The material stiffness is inversely related to the cross over point of material liquefaction, indicating that stiffer IPNs are less ductile. The ability to modulate the mechanical properties while maintaining erosion rates allows for user-defined properties per desired application while controlling drug release. Genetically encoded IPNs are ideal biomaterials for multi-protein drug delivery or research applications including tissue engineering.

Poster Number: 14

WITHDRAWN

Poster Number: 15

WITHDRAWN

Poster Number: 16

Polymeric Prodrugs Forming Single-Chain Nanoparticles

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Nanomedicine research is directed towards improving treatment efficacies and reduce side effects. Polymeric nanoparticles are highly modular in nature and designed to introduce targeted delivery of therapeutics in order to enhance the local drug concentration. Single-chain nanoparticles (SCNPs) are unique in this application due to ease of preparation in a sub-15 nm size range.1 Size and dispersity of SCNPs are highly dependent on their precursor polymers, which are obtained by utilizing controlled polymerization. RAFT polymerization allows to incorporate polymeric prodrugs targeting molecules such as N-acetylgalactosamine (GalNAc), which is used for targeting the asialoglycoprotein (ASGPR) receptor of hepatocytes.2 Polymeric prodrugs consist of a therapeutic which is conjugated to a polymerizable group via a degradable linker. Previous work has reported a valine-citrulline peptide sequence which is cleaved by the intracellular enzyme cathepsin and thereby increases blood stability of produgs.3 In this work, we focus on the infectious disease of malaria and the species of Plasmodium vivax, which has the ability to form a dormant state in hepatocytes and leads to the recurrence of the disease. Tafenoquine (TQ) eliminates the liver-stage hypnozoites, however, TQ causes hemolytic anemia in G6PD-deficient humans.4 Therefore, targeted delivery of TQ is needed to avert side effects and provide the therapeutic safely to patients. Polymers containing prodrugs, GalNAc for targeting and pentafluorophenyl (PFP) moieties for crosslinking were synthesized. Intramolecular crosslinking using PEG diamines yielded sub-15 nm particles with high drug loading.

Poster Number: 17

Tuning nanoparticle properties for siRNA delivery to chondrogenic cells in vitro

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Osteoarthritis (OA) is a degenerative joint disease characterized by pathological tissue remodeling in the joint. OA afflicts over 500 million people worldwide. Yet, there are no FDA-approved disease-modifying OA drugs. RNA-based therapeutics offer an innovative approach to OA drug development by enabling precise targeting of biological pathways associated with OA. Specifically, short interfering RNA (siRNA) can silence specific genes of interest by facilitating mRNA degradation. Although siRNA therapeutics are promising, delivery has presented a barrier to clinical translation since siRNA requires cytosolic localization to function. Due to the high molecular weight and negative charge of siRNA, as well as its vulnerability to nuclease degradation, intracellular delivery has presented a formidable challenge. Previous work has shown nanoparticle-mediated siRNA delivery is nanoparticle composition- and cell type-specific. This study generated a library of nanoparticles to investigate the effects of tunable nanoparticle properties on siRNA delivery to ATDC5 cells, a mouse chondrogenic cell line. These nanoparticles are self-assembled from amphiphilic diblock copolymers. The cationic poly(dimethylaminoethyl methacrylate) corona permits siRNA-loaded nanoparticles to associate with the cell membrane by electrostatic interactions and modulates endosomal uptake. The pH-responsive poly(dimethylaminoethyl methacrylate-co-butyl methacrylate-co-propylacrylic acid) core facilitates intracellular siRNA release. Diblock copolymers were synthesized by reversible addition-fragmentation chain transfer polymerization. To generate the library of nanoparticles, the degrees of polymerization and butyl methacrylate feed ratio were varied based on design of experiments. These variations resulted in diblock copolymer formulations that varied in molecular weight from 17.2 kDa to 68.0 kDa. The butyl methacrylate feed ratio in the second block ranged from 50% to 90%. Nanoparticles varied in size from 20.4 nm to 86.2 nm and in zeta potential from 13.9 mV to 22.03 mV. Critical charge ratio and critical micelle concentration were experimentally determined to confirm loading capacity and stability. To quantify siRNA delivery, siRNA uptake and gene silencing were measured by flow cytometry and qRT-PCR. Initial results in a representative formulation show that nanoparticles enable siRNA uptake and facilitate siRNA-mediated gene silencing of GAPDH. These results will be further strengthened by measuring siRNA uptake and gene silencing in all nanoparticle formulations to determine how nanoparticle properties influence siRNA delivery.

Poster Number: 19

Investigation of meniscal cell traction forces on substrates of varied stiffness

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Knee meniscus tears are among the most common musculoskeletal injuries. The meniscus is a mechanically active tissue that is crucial to health and stability of the joint but is largely incapable of self-healing. Regenerative cell behaviors such as migration, proliferation, and extracellular matrix (ECM) deposition are driven by mechanical signaling between cells and their surrounding microenvironment. To develop necessary therapies for meniscal repair, it is important to understand and control this mechanical signaling, or mechanotransduction. Traction forces (TFs), or the forces cells exert through focal adhesion complexes on their ECM, are important components of mechanotransduction in the meniscus. Our lab has previously illustrated the dependence of meniscal cell morphology on substrate compressive modulus using a mechanically tunable hyaluronic acid-based hydrogel system, motivating further characterization of meniscal cell-ECM mechanotransduction. The objective of this study is to further investigate these results by quantifying meniscal cell traction forces on substrates of varying stiffness. The overall hypothesis is that meniscal cell traction forces will increase with increasing substrate stiffness. PDMS substrates of varying elastic modulus were patterned with a fluorescent grid containing an array of black dots. Then, primary human meniscal cells from six donors (3 male and 3 female) were seeded on these substrates (~2500 cells/cm2) and cultured for 24 hours. Cells were fixed, permeabilized, and stained to visualize nuclei and actin cytoskeleton. Cells were then imaged and total traction force for each individual cell was calculated using the displacement of the black dots and the elastic modulus of the substrate. To further characterize meniscal cell-ECM mechanotransduction, immunofluorescence staining of focal adhesion support proteins (paxillin, vinculin, and talin) and ECM components (collagen type I and II) was also performed. The analysis of this data is currently underway. In conclusion, quantifying traction forces exerted by meniscal cells on their microenvironments and determining the effect of substrate stiffness on traction force contributes to overall understanding of mechanotransduction in the meniscus. This knowledge will facilitate development of therapies for meniscus tears that provide the mechanical cues necessary for regeneration of the tissue.

Poster Number: 20

Light Responsive Hydrogel with Polypyridyl Ruthenium Crosslinker for Tissue Engineering

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Behaviors of cells are highly regulated by the external environment. Changes in the physical properties of the extracellular matrix (ECM) can transmit signals via mechanotransduction that influence tumor development, fibroblast activation, and other disease states. Conventional cell culturing procedure on hard plastic platforms is easy to perform but rarely provides accurate representation of native tissue leading to poorly translatable results. Responsive biomaterials with tunable properties can mimic the complexity of native ECM and facilitate dynamic cell-ECM interactions, thus have become more prevalent as an alternative platform for studying cell behavior. In this study, we report the development of a light responsive hydrogel system using a polypyridyl ruthenium crosslinker (RuXlinker) that absorbs low energy light (< 530 nm). The ruthenium complex functionalized with aldehydes facilitates the formation of hyaluronic acid hydrogels via a hydrazone crosslink. Upon visible light irradiation, the hydrogel softens following the cleavage of the RuXlinker. The ability to modulate the stiffness of the ECM allows us to not only trigger cellular processes and disease states but also probe the mechanical reversibility of these events. This material design is expected to become a powerful tool in tissue engineering and enable the discovery of novel therapeutic targets.

Poster Number: 27

Exploring the Mesoscale Framework of Bacterial Cellulose: Insights into 3D Network Formation during Biosynthesis

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Materials derived from renewable sources and produced under mild conditions offer advantages in sustainability compared to their petroleum-based counterparts, which are notorious for their contribution to pollution and health hazards. Bacterial cellulose (BC) is composed of elongated fibrils that assemble into fibers, constructing intricate 3D networks influenced by growth conditions. BC pellicles, cultivated under static conditions and commonly utilized in research and practical applications, exhibit a stratified structure at the mesoscale. These layers emerge as successive interwoven fiber networks are externally deposited during BC growth. This layered configuration distinguishes BC pellicles and plays a pivotal role in defining their exceptional mechanical properties. The growth conditions governing BC fiber synthesis profoundly impact the alignment, crystallinity, and 3D morphology of the resulting network. Yet, there remains a gap in comprehending the underlying principles governing the aggregation of cellulose fibers into network structures. A profound grasp of how nanoscale fibrils bundle, intertwine, and form larger constructs at the mesoscale is imperative for tailoring BC properties for diverse applications ranging from biotechnology to materials science. Our investigation explores the intricate structural metamorphosis of BC, spanning from the nanoscale to the mesoscale, with a specific emphasis on unraveling entanglement phenomena. Additionally, our study probes the significance of fibril entanglement in BC networks, shedding light on the intricate mechanism through which individual nanofibrils interlace to construct the broader mesoscale architecture. This research aims to provide a comprehensive understanding of the structural development of BC.

Poster Number: 30

Advanced 3D Cell Culture Systems Leveraging Suspended Fibers fabricated by Melt Electrowriting

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Advanced cell culture systems employing melt electrowritten (MEW) scaffolds have evolved through the inclusion of topographical cues, influencing cell positioning, orientation, process extension, and cell-cell interactions. However, conventional MEW scaffolds designed with a 0/90º X/Y deposition pattern exhibit one continuous pore along the Z-axis, limiting porosity in other directions, between adjacent pores. To address this, we aim to introduce an alternative MEW scaffold design of suspended fiber (SF) arrays for directed neurite guidance and cell spreading along semicontinuous pores in the XY plane.

An in-house established, custom MEW printer was used to fabricate scaffolds with suspended polycaprolactone fibers and adjustable porosity in XY direction. Parameters including temperature, collector speed, and nozzle voltage were optimized to achieve desired scaffold structures (collector speed 9000 mm min-1, SF diameter 2.5 µm ± 5 µm, SF distance 36 µm). In vitro experiments were performed to evaluate cell behavior on different scaffold types, demonstrating the interactions between cells and suspended fiber architectures. Human glioblastoma cells (U-87 MG, ATCC HTB-14) were pelleted prior to seeding onto SF MEW scaffolds. The seeded scaffolds were cultured for 3 days and characterized using cytochemical staining for nuclear and actin filamentous structures.

The custom MEW printer allows for the precise placement of suspended fibers, contributing to the fabrication of scaffold architectures with enhanced control over scaffold porosity in an XY directionality. This study introduces a suspended fiber array that behaves as a topographical feature for MEW scaffolds, specifically for the study of neural cell outgrowth. Cell experiments are ongoing to characterize the effects of scaffold and fiber architectures on neural tissue growth. By harnessing the topographical cues of suspended fibers, the research will highlight the potential of MEW-printed microfibers in guiding neural cell behavior and provide new opportunities to study 3D cell-substrate interactions.

Poster Number: 32

Magnetic Microgels for Composite Musculoskeletal Tissue Regeneration

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The regenerative capacity of musculoskeletal tissues is restricted, such that healing often culminates in scarring. To this end, we utilize murine digits to interrogate the potential for mammalian limb regeneration. Previous studies show that delivering bone morphogenetic protein-2 (BMP-2) and BMP-9 to amputated digits induces skeletal and joint regeneration. Our objective was to develop an injectable platform to instruct the sequential and spatial formation of bone followed by articular cartilage. To direct protein release towards specific regional compartments, we engineered micron-sized hydrogels ('microgels') that can be guided using a magnetic field. Microgels were fabricated using 8-arm 40 kDa poly(ethylene glycol)-norbonene (PEG-NB, 5% w/v), PEG-dithiol (3.8 mM), Biotin-PEG-thiol (2 mM), and photoinitiator (Lithium phenyl-2,4,6-trimethylbenzoylphosphinate, 2.5 mM), which were dissolved in a water-in-oil emulsion and photopolymerized (λ: 365 nm) after agitation. Magnetically responsive microgels were generated by encapsulating iron oxide nanoparticles (FeO2) at Low (23.3 mM) and High (221.4 M) concentrations. Microgels were imaged with bright field microscopy and the average diameter, circularity, and opacity were quantified with ImageJ. Low FeO2 microgels were injected into a polydimethylsiloxane channel with or without exposure to a magnet to quantify magnetic responsivity, where microgel velocity was determined by tracking the centroid in PBS over 10 seconds. To determine whether the microgel surface could also be functionalized, superparamagnetic beads coupled with streptavidin (Dynabeads) were incubated with Control microgels and assessed by bright field microscopy. Significance was assessed by 2-tailed Student's t-test or 1-way ANOVA with Tukey's post-hoc test ($p<0.05$). Magnetically responsive microgels were successfully fabricated by encapsulating FeO2 nanoparticles. The average diameter and circularity of both Low and High FeO2 microgels were significantly less than the Control values. Microgel opacity increased with FeO2 concentration. High FeO2 microgels exhibited the greatest morphometric heterogeneity, indicating that there is a limit to nanoparticle encapsulation. Low FeO2 microgels moved in a linear direction at approximately 0.2 mm/s in the presence of a magnet and remained stationary without magnetic stimulation. Similarly, Control microgels with surface-conjugated Dynabeads were magnetically responsive. In summary, magnetic PEG-NB microgels represent a versatile platform that may be adapted for spatiotemporally controlled drug delivery to direct musculoskeletal regeneration.

Poster Number: 33

Optimizing Hydrophilic Properties of Capillary Microfluidic Devices Produced on LCD 3D Printers

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Capillary microfluidics capitalize on surface tension effects encoded in microchannel geometry and chemistry to transfer liquids without external instruments, making them a user-friendly technology for point-of-care tests. For most applications, hydrophilic surfaces (contact angle < 90˚) are necessary to induce surface tension driven flow. Currently, this is achieved with vacuum plasma chambers that alter surface chemistry. Unfortunately, the hydrophilic properties made with plasma processing are temporary and unstable. Alternatively, an inherently stable hydrophilic 3D-printing resin containing polyethylene glycol diacrylate (PEGDA) and acrylic acid (AA) was recently developed for capillary microfluidics [1]. However, this hydrophilic resin has not been thoroughly validated for inexpensive (<$300) liquid crystal display (LCD) printers. Our objective is to optimize and validate 3D-printing parameters including exposure time, UV power, layer thickness, and lift/retract speed using this hydrophilic PEGDA-AA resin with three LCD 3D printers (AnyCubic Photon Mono X 6K, AnyCubic Photon Mono M5s Pro, and Phrozen Sonic Mini 8K). Our analysis includes measuring wettability with contact angle, printed channels dimensional fidelity relative to design specifications, and verification of flow rates as a function of microchannel cross-sectional dimensions. Our proof-of-concept prints on the Mono X 6K printer had average contact angle measurements of 42.8° +/- 8.77. The percent differences between designed and printed channel lengths, widths, and depths were 31.5 +/- 0.23%, 28.9 +/- 3.41%, and 2.40 +/- 13.9% respectively. By optimizing the print parameters of cost-effective 3D printers with the inherently stable hydrophilic resin, we enable capillary microfluidic technologies for users in low income/resource settings who may not have access to vacuum plasma chambers. Future work will explore spatial patterning of hydrophilicity and protein immobilization in microchips. Patterned surface-energy introduces flexibility in 3D-printed microfluidic design to control flow, such as with stop valves, for ease of automation. Protein immobilization increases the detection limits, stability, and analytical specificity of immunoassays by reducing protein interference.

[1]V. Karamzadeh, A. S. Kashani, M. Shen, and D. Juncker, “Digital Manufacturing of Functional Ready-to-Use Microfluidic Systems,” Advanced Materials, vol. 35, no. 47

Poster Number: 34

Enhanced stem cell cultivation in 3D chitosan scaffolds

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Advancements in stem cell therapies critically reply on the development of cultivation techniques that efficiently support cell growth and differentiation while meeting stringent clinical standards. Our research demonstrates chitosan, a biopolymer with adjustable degrees of deacetylation (DD), as an excellent candidate for cultivating human neural stem cells (hNSCs) and human induced pluripotent stem cells (hiPSCs). We started with investigating the effect of varying DD on the physicochemical properties of chitosan films. Our findings revealed that a higher DD significantly improved cell adhesion, proliferation, and the preservation of multipotency in hNSCs. Expanding on these findings, we engineered 3D porous scaffolds from medical-grade chitosan with high DD and determined that scaffolds fabricated from higher concentrations of chitosan markedly enhanced hNSC renewal and the maintenance of their multipotent status.

Applying this approach to hiPSC culture, we optimized the 3D chitosan scaffolds using conditioned medium (CM) to enhance cell penetration and distribution throughout the scaffold matrix. This modification led to improved uniformity in cell dispersion and an upregulation of pluripotency marker expression, fostering a more primitive and undifferentiated state of hiPSCs compared to traditional 2D cultures.

Our findings collectively suggest that by fine-tuning chitosan's physical properties, specifically the DD and concentration, in conjunction with advanced 3D culture techniques, we can create a more conductive environment for culturing hNSCs and hiPSCs, resulting in significantly enhanced culture efficiency. This research holds significant implications for the large-scale production of stem cells, offering cost-effective solutions aligned with regulatory standards for clinical applications. The potential applications of this technology are extensive, ranging from regenerative medicine and disease modeling to the discovery of innovative drug therapies. Our studies establish chitosan as a valuable component in stem cell culture platforms.

Poster Number: 35

High-Resolution Bioprinting of Soft Hydrogel Biomaterials

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3D printing has revolutionized tissue engineering through rapid customization of tissue scaffolds from a variety of biomaterials. Despite these advances, ongoing challenges lie in the dichotomy between printing resolution and material biocompatibility: stiffer printed materials afford higher structural resolution with sustained spatial architecture while softer materials promote greater proliferation, remodeling, and self-organization of living cells embedded in the printed tissues. To address this challenge, we have developed and characterized a new biomaterial platform that prints “stiff” cellularized materials at high resolution but can be softened on-demand using cytocompatible proteases.

We have established a protease-degradable copolymer hydrogel system and have shown that this method can completely degrade with vast tunability and control while remaining viable for 3D cell culture. This copolymer system consists of a photocrosslinked network of both a natural polymer component, Gelatin Methacrylate (GelMA) and synthetic polymer component, Poly(ethylene glycol) Diacrylate (PEGDA). Treating the hydrogel with a cytocompatible protease specifically targets only the GelMA portion of the network leaving the PEGDA component intact, softening the gel by several fold.

We found altering both the ratio of GelMA to PEGDA and the total weight percent of acryloyl crosslinks allows us to access a range of storage moduli from 2kPa - 82kPa then further degrade to a storage modulus of 0.17kPa. By optimizing this range of moduli for high-resolution 3D printing and maximal cytocompatibility, we hope for incorporation and retention of large-scale vasculature networks in the hydrogel construct. We are forming vascular networks using anatomical maps of blood vessels in human tissues sourced from datasets of cleared, immunostained, and computationally reconstructed organs. The bioprinted hydrogel will access physiologic scale architecture to produce vessels <50 µm in diameter with high resolution that is maintained after softening. This will enable us to seed endothelial cells in these networks and further determine if these vessels maintain perfusion and viable endothelial lining.

This new approach to create bioprinted hydrogel materials with tunable mechanical properties will yield high resolution, cytocompatible, and implantable biomaterials to treat injured liver tissue as a bridge or alternative to transplantation. This work will be transformative for future tissue engineering applications.

Poster Number: 36

Revolutionizing In Vivo Screening: A High-Throughput 3D-Printed Platform for Investigating Bioink Materials and Cell Formulations

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Background and Purpose:

Three-dimensional (3D) printed tissues hold tremendous potential for revolutionizing tissue engineering and regenerative medicine. However, their development often stalls at pre-clinical animal studies due to the low-throughput nature of in vivo investigation. To address this challenge, we introduce a high-throughput in vivo screening array platform, called Parallelized Host Apposition for Screening Tissues (PHAST), which allows for parallelized in vivo screening of 43 unique three-dimensional microtissues within one single 3D printed array device implanted in one single animal.

Methods:

A variety of bioink formulations composed of hydrogel materials (e.g. gelatin methacrylate, fibrin, collagen, matrigel) and different cells (e.g. human umbilical vein endothelial cells, human dermal fibroblasts, mesenchymal stem cells, dental pulp stem cells, human hepatocytes) were fabricated and added to the PHAST array and crosslinked in situ to form 43 microtissues. The array carrying 43 microtissues was then implanted on the abdominal area of an athymic mice. Through immunofluorescent staining and confocal microscopy, we identify formulations that support critical aspects of tissue engineering, including vascular self-assembly, host integration and tissue function.

Results:

Our studies yield invaluable insights into the role of the choice of bioink solution material and encapsulating cell type in influencing tissue vascularization and physiological function. Notably, our findings highlighted the increase of weight percent of bioink solution hinder the vascular self-assembly and cell survival within these microtissues. The incorporation of stromal or stem cells can rescue the vessel formation at a higher weight percent up to 10%.

Conclusion:

Utilizing PHAST method, we examined more than 10 different bioink solutions and 7 cell types and their various combinations with a significant reduced use of animals and resources. PHAST has the potential to significantly accelerate pre-clinical progress across diverse medical applications, spanning from tissue therapy to regenerative medicine.

Poster Number: 37

Modular Shape-Controllable Engineered Tissues for Muscle Tissue Engineering

Ngan Huang, Stanford University

Purpose/Objectives:

Volumetric muscle loss (VML) is characterized by the loss of a significant portion of skeletal muscle due to traumatic injury, leading to irreversibly impaired muscle function and regeneration. Tissue engineering strategies to regenerate traumatically injured muscle are promising but are limited in the ability to customize the tissue geometry to match that of the muscle defect. The objective was to develop lego-inspired custom-shaped muscle units for bioprinting-assisting engineering of custom-shaped muscle units to treat VML.

Methodology:

We developed shape-customizable building blocks by taking advantage of sacrificial molds whose geometries were designed by computer aided design (CAD) and bioprinted using polylactic acid (PLA). The PLA molds were immersed in agarose and then cooled to 4°C to induce agarose solidification. Third, upon solidification, the mold was removed, leaving behind a negative mold. Fourth, to create a high-density scaffold-free muscle lego, a muscle myoblast cell suspension was filled into the mold to allow for cell compaction into the shape of the mold. We could fabricate modular lego units in diverse sizes and geometries, including star, hexagon, donut, and alphabet letters. In vitro characterization of cell survival was performed, along with therapeutic testing in a murine model of VML, in which aspiration-assisted bioprinting was used for precision placement into the ablated muscle.

Results:

We could fabricate modular lego units in diverse sizes and geometries, including star, hexagon, donut, and alphabet letters. The shape of the tissues showed high fidelity to that of the mold. Live/Dead viability analysis and subsequent confocal imaging through the thickness of the scaffold showed cell viability >80%. The rectangular solid-shaped lego was implanted into the tibialis anterior muscle after a 40% muscle ablation. After 3 weeks, histological analysis showed 5 times higher muscle regeneration area, compared to animals with no treatment.

Conclusion/Significance:

These data demonstrate that scaffold-free modular muscle units can be geometrically shaped with high viability and then shown to induce muscle regeneration in a murine VML model with the assistance of aspiration bioprinting.