Bioink Optimization and Effects of Microgravity on 3D Printed Cell Laden Constructs

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Statement of Purpose: It is known that space flight can lead to altered endothelial cell (EC) morphology and senescence, likely in part due to microgravity (µg). However, the results from simulated µg on ECs and angiogenesis are not clear [1]. For example, many studies have shown that simulated µg leads to increased nitric oxide (NO) expression and EC proliferation, while others have shown the opposite [1, 2]. The goal of this study is to investigate the impact of µg on cellular function in 3-D bioprinted constructs, which to our knowledge has not been performed previously. We hypothesize that the cells' interaction with 3-D biomaterials can impact their response to microgravity. These bioprinted 3-D constructs can potentially serve as *in vitro* tools for the study of cell interactions as well as therapeutic replacements of human tissues. Here, we optimized the composition of sodium alginate, gelatin, and fibrinogen bioinks for 3D printing of EC-laden constructs with good print fidelity and high cell viability. Next, we exposed constructs to controlled µg and assessed the effects of µg on EC cell response.

Methods: Hydrogels were prepared using alginate, gelatin, and fibrinogen to make 5% and 10% (w/v) solutions with different alginate to gelatin ratios (1:9 and 2:3). The bioink also contained 2.5 mg/ml fibrinogen and 1.25 mM $CaCl_2$ for initial crosslinking. Human umbilical vein endothelial cells (HUVECs) were either embedded within printed grid structures (at $8x10^6$ cells/mL) or seeded into template channels to allow for different cell interactions with the material. For template channels, Pluronic F127 was used as a sacrificial material to form 2.5 mm diameter channels. Constructs were printed using syringe-based extrusion and then secondary crosslinked with 0.5M $CaCl_2$. Construct shape fidelity, porosity, and integrity over time in culture media were characterized with light microscopy and SEM images. Rheology, compression, and biocompatibility testing was performed. For µg, 3D bioprinted constructs were placed in a 3-D clinostat (Gravite microgravity simulator system) at NASA KSC at a speed of 3 rpm for 24 h. Post microgravity exposure, tissue constructs were embedded in OCT and sectioned (5 mm thick) for analysis of NO and reactive oxygen species (ROS). ANOVA with Tukey's HSD was performed ($p < 0.05$).

Results: Line width and void area were impacted by bioink composition, with 10% 1:9 showing the best shape fidelity, comparable to the original model geometry. The lower hydrogel concentration (5%) with a higher ratio of alginate (2:3) showed the lowest print integrity **(Fig 1A).** Differences were also seen in the surface morphology via SEM, where the incorporation of more gelatin (1:9) resulted in a higher density of pores, but still exhibited stability after 7 days in culture with the higher 10% concentration (**Fig 1B**). These pores are created by the non-crosslinked gelatin leaving the hydrogel. Biocompatibility tests via the live-dead assay further confirmed the benefit of the 10% 1:9 condition, with the highest cell percent cell viability **(Fig 2)**. This is likely due to the higher number of pores for the embedded cells.

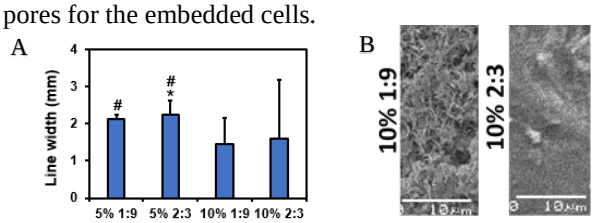

Fig.1. (A) 3D constructs line width measurements. *significant from 10% 1:9, # significant from 10% 2:3. (B) SEM images of hydrogel concentrations of gelatin to alginate ratios after day 7. Scale bar:10µm.

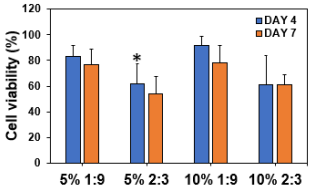

Fig.2. Cell viability with live-dead assay for different hydrogel compositions. *significant from 10% 1:9 Day 4.

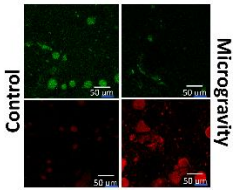

Fig.3. Fluorescence microscopy images of ECs for NO, stained green with DAF-FM and ROS, stained red with DHE. Scale bar:50µm.

When exposed to simulated µg, the 3D bioprinted constructs exhibited an increase in ROS production and a decrease in NO bioavailability compared to static control samples ($p<0.05$) **(Fig 3)**. These results indicate an increase in oxidative stress with µg, suggesting endothelial dysfunction. This has been demonstrated both with ECs embedded within a grid structure and ECs post-seeded in a template channel. We are currently investigating a co-culture system for the template channels architecture relevant for tissue vascularization. In particular, the co-culture system includes normal human dermal fibroblasts (NHDFs) embedded in the bulk hydrogel and HUVECs seeded within the channel.

Conclusions: The alginate, gelatin, and fibrinogen bioink system used in this study allowed for porosity and cell attachment sites that provided cell viability. Using this system, we demonstrated that the exposure to microgravity affected the ECs by decreasing production of NO and increasing the level of ROS. This result is similar to what has been found with space flight [1]; interestingly, it is different than many µg simulation studies. While it is likely that the method of microgravity simulation (e.g., duration) will impact the results of different studies, our results suggest that interaction with a 3D biomaterial will also likely impact the cells' response to µg. This 3D bioprinted system has the potential to be used to better mimic µg and has relevance for tissue vascularization.

References and Support:

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Support: NASA Florida Space Grant Research Program