Optimizing 3D printability in Hydrogel Bioinks through compositional and rheological analysis

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Statement of Purpose: Engineering complex tissues that can mimic, augment or replace native tissue functions holds enormous promise for treating organ failures resulting from injuries, aging and disease. Bioprinting, which uses modified 3D printers to fabricate structures out of cell-friendly hydrogels, is quickly emerging as a popular strategy for creating complex distributions of cell populations and biomaterials. However, available bioinks cannot effectively replicate larger 3D tissue structures due to their weak mechanical and rheological properties - structures quickly collapse under their own weight. This prevents fabrication of human-scale 3D structures that are needed to replicate biological tissues. This need for mechanically resilient, 3D printable, and bioresponsive inks to create 3D tissue structures is a pressing demand in tissue engineering.(Chimene D. Ann Biomed Eng 2016, 44: 2090). This project addresses a key question in tissue engineering- what material properties are essential for the highly printable bioinks neccessary for for the next generation of bioprinted structures?

Methods: We set up our experiment using a recently developed class of multicomponent bioinks that demostrate excellent 3D printability: Nanoengineered Ionic-Covalent Entanglement (NICE) bioinks. Next, the bioink's composition(GelMa, Kappa-carrageenan, and Nanosilicates) ratios are varied to create inks that are empirically printable and not printable according to a series of quantitative tests**(Fig. 1)**. These tests include 2D tests like line width and consistency, as well as 3D tests measuring max printed height and shape fidelity of a standardized structure. Next, rheology and mechanical tests will be run to compare printable and non-printable inks and identify ideal properties for developing highly printable bioinks, as well as ideal storage conditions. Cells will be encapsulated and bioprinted to ensure that final bioink designs maintain the high cell viability and bioactivity of previously described NICE bioinks.

Results: In this study, we quantified the effects of mechanical and rheological properties of the printability of series of Nanoengineered Ionic Covalent Entanglement (NICE) bioinks in order develop our understanding of the relationship between materials properties and printability. Bioink structures were printed as freestanding, high aspect ratio structures to 30mm and 150 layers tall and photographed and measured to quantify max height and 3D shape fidelity. Different ranges of component ratios were found for maximizing printability**(Fig. 2)**, and the effects of varying components on mechanical properties were also quantified**(Fig. 2)**. Mechanical data shows c Rheological testing through storage modulus, strain sweeps, and peak-hold experiments are underway. Meanwhile, bioprinted cells maintained high viability(~90%), attachment, and spreading, consistent with reports from earlier NICE bioinks. We will use rheological data to create Herschel-Bulkley fluid model to simulate bioink behavior and predict behavior of bioinks during printing.

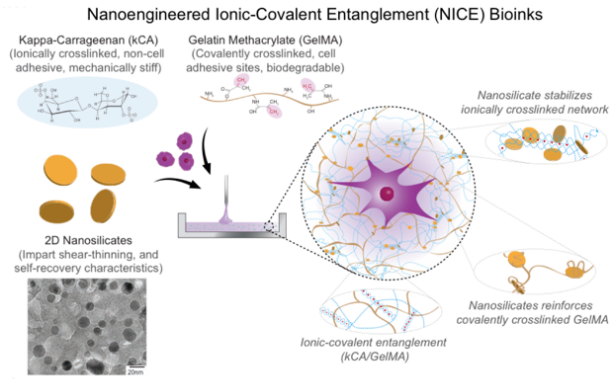

Figure 1. Conceptual model of NICE bioinks showing the contributions of bioink components that were varied in this experiment

Conclusions: The overarching goal of 3D bioprinting is to quickly generate cell-laden structures that can be used to recreate functional tissues. However, existing bioinks lack the necessary 3D printability, mechanical properties, and/or bioactivity to recreate 3 dimensional tissue structures. In this study, we studied a range of bioinks in the NICE family to examine the relationship between component ratios and important bioinks characteristics, particularly 3D printability and mechanical properties. The identification of key rheological parametes associated with high printability will allow for high throughput testing and optimization of future bioinks. We expect this development will accelerate development of future highly 3D printable bioinks.

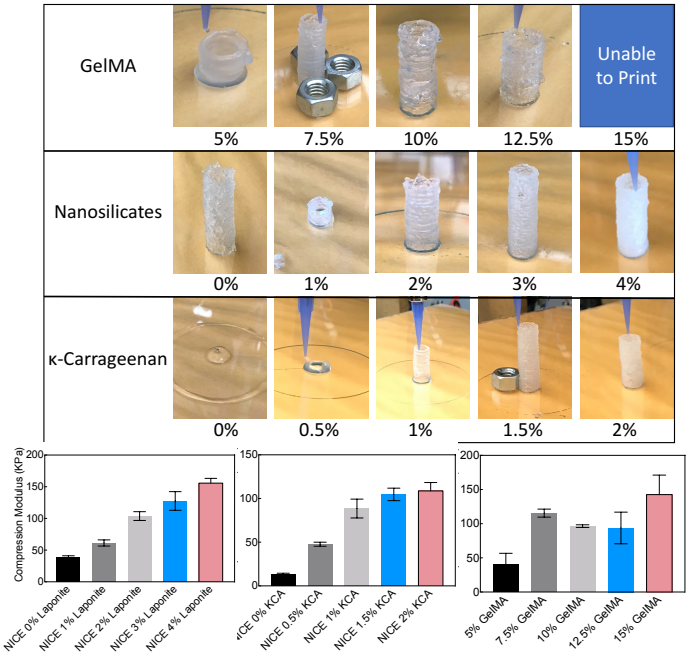

Figure 2: 3D Printability and Mechanical properties of Different formulas of NICE reinforced bioinks