

# Optimizing 3d Printability in NICE Bioinks through Compositional and Rheological Analysis

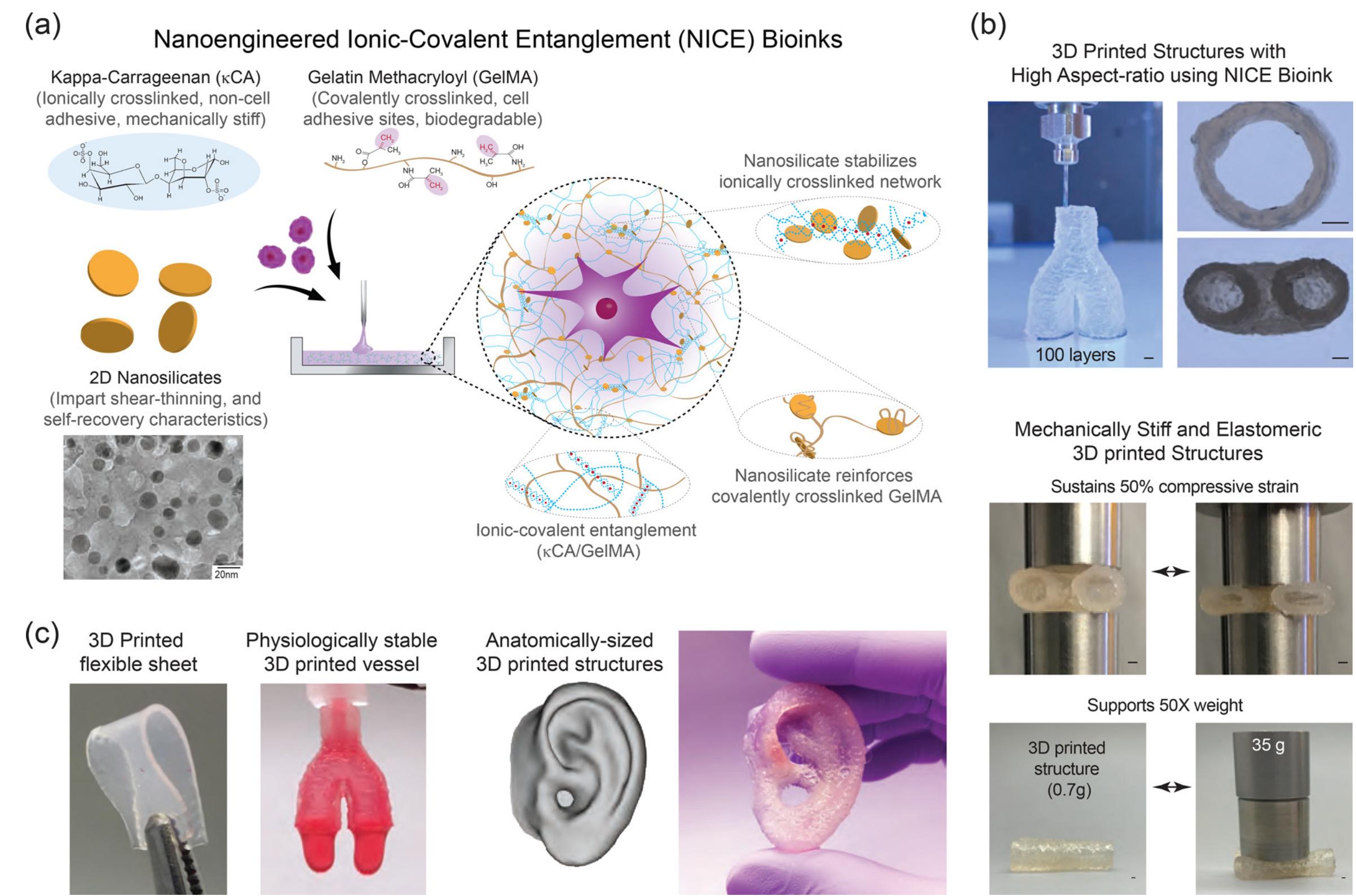
Logan Miller,<sup>1</sup> David Chimene,<sup>1</sup> Akhilesh K. Gaharwar<sup>1,2,3</sup>

Dept. of Biomedical Engineering,<sup>1</sup> Dept. of Materials Science,<sup>2</sup> Center for Remote Health Technologies and Systems,<sup>3</sup> Texas A&M University

## Introduction

### 3d Bioprinting

3d bioprinting is a rapidly emerging field in tissue engineering that promises to enable custom fabrication of new tissues and organs. However, conventional bioinks have suffered from limited printability and cytocompatibility. We have recently developed a novel nanoengineered ionic covalent entanglement bioink that combines nanosilicate reinforcement and an interpenetrating ionically crosslinked network to significantly improve the printability and mechanical strength of a GelMA bioink, while maintaining a high level of cytocompatibility.



Range of Component Concentrations Analyzed				
GelMA	5%	7.5%	10%	12.5%
Nanosilicates	0%	1%	2%	3%
kCA	0%	0.5%	1%	1.5%

Standard formulation of NICE bioink consists of 1% Kappa-Carrageenan, 10% Gelatin Methacrylate and 2% Nanosilicates

Individual components of the bioink were altered

Corresponding effects on the ink characteristics can be discerned

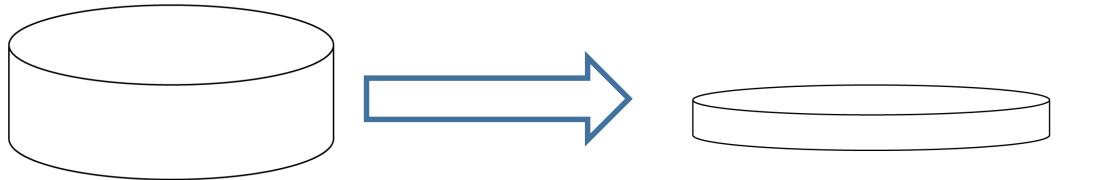
Fifteen unique component compositions of bioinks were produced.

Ink compositions were 3d printed to test the printability of the various inks

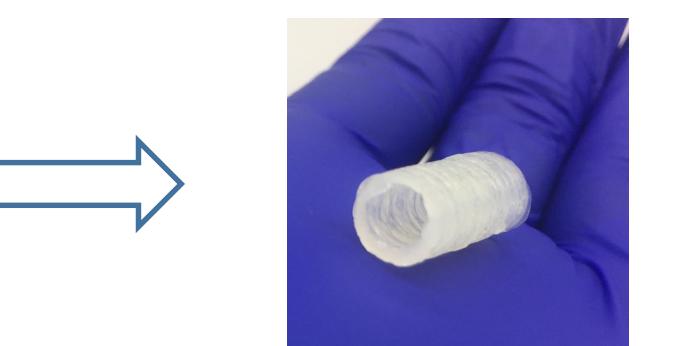
## Methodology

### Percent Composition Manipulation

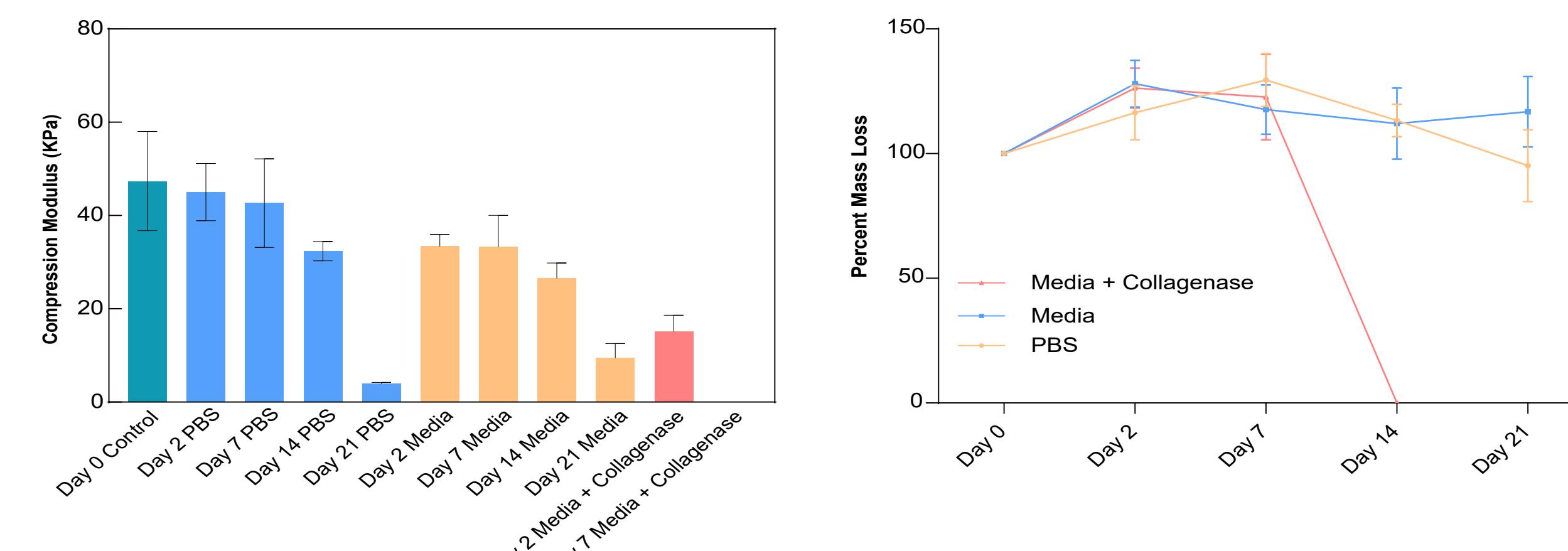
Mechanical unconstrained compression testing procedure utilized compression testing with a max strain of 30% original height used to calculate compression modulus and toughness



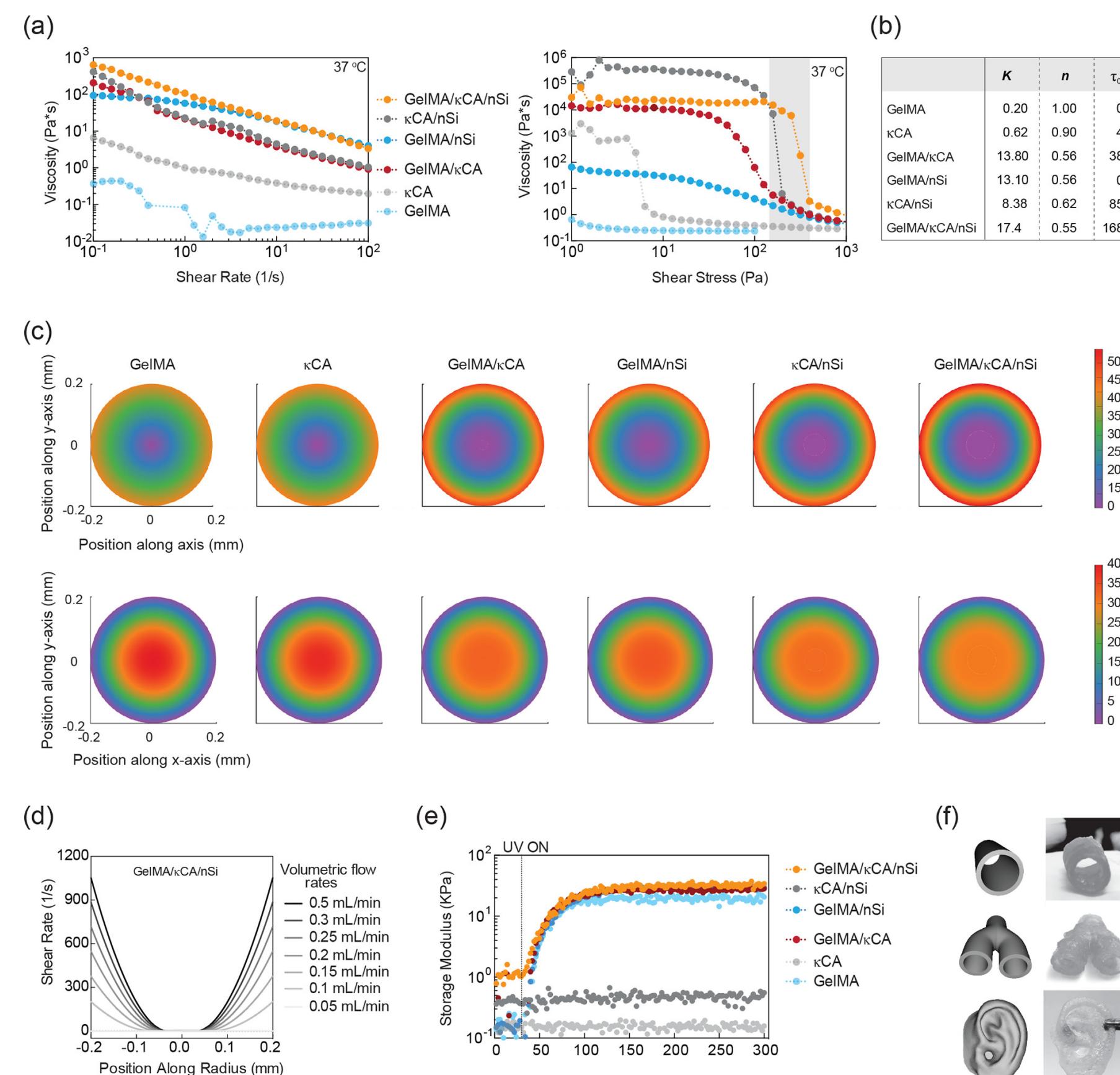
Printability testing was performed using a 3 cm tall hollow cylinder with 8 mm internal diameter and 1 mm wall, which allow for accurate demonstration of self-recovery and print fidelity



Degradation study of mass loss and mechanical properties



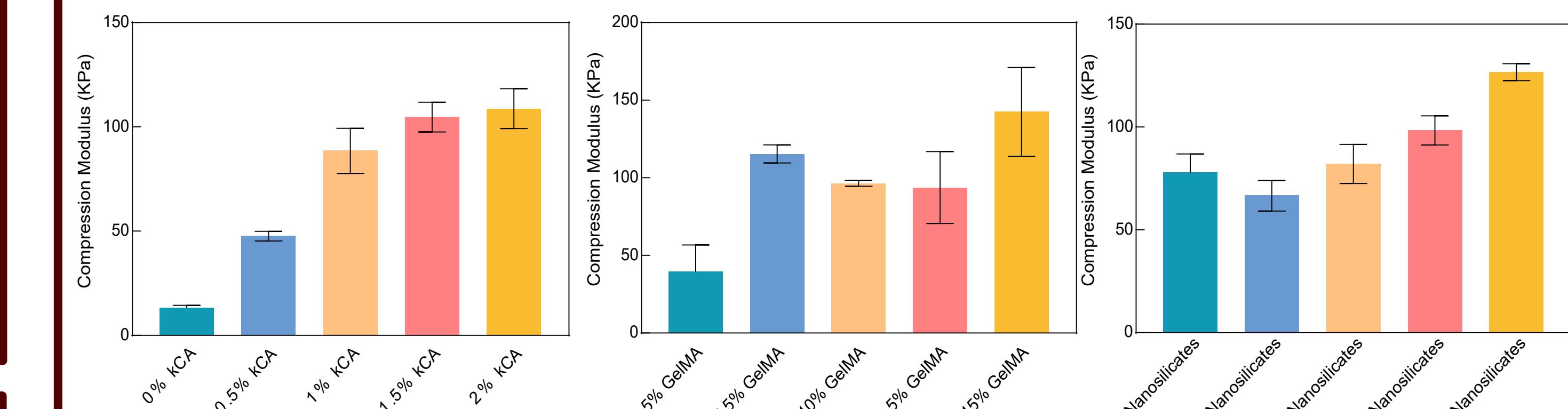
## Methodology



Based on previous rheological data, peak-hold tests were designed to stimulate the shear rates and temperature changes the bioinks experience during bioprinting to find the optimal component concentrations

## Results and Discussion

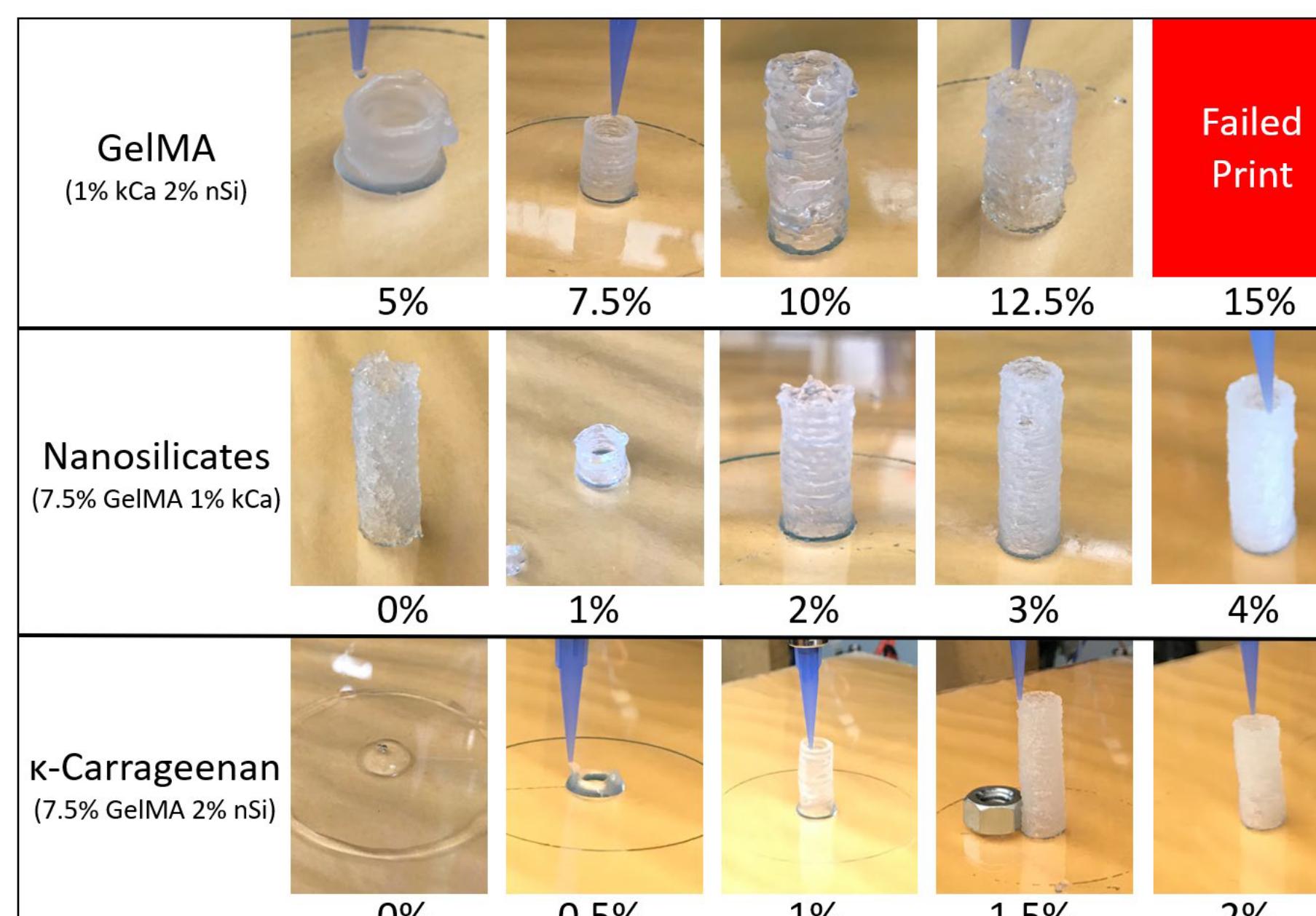
### Compression Testing



Increase in the compression modulus and energy dissipated of the sample hydrogel disks as the ink component concentrations increased

Increasing the concentration of different ink components would have a favorable outcome on the mechanical properties of the bioink and allow the bioink greater resistance to deformation

### Printability Testing

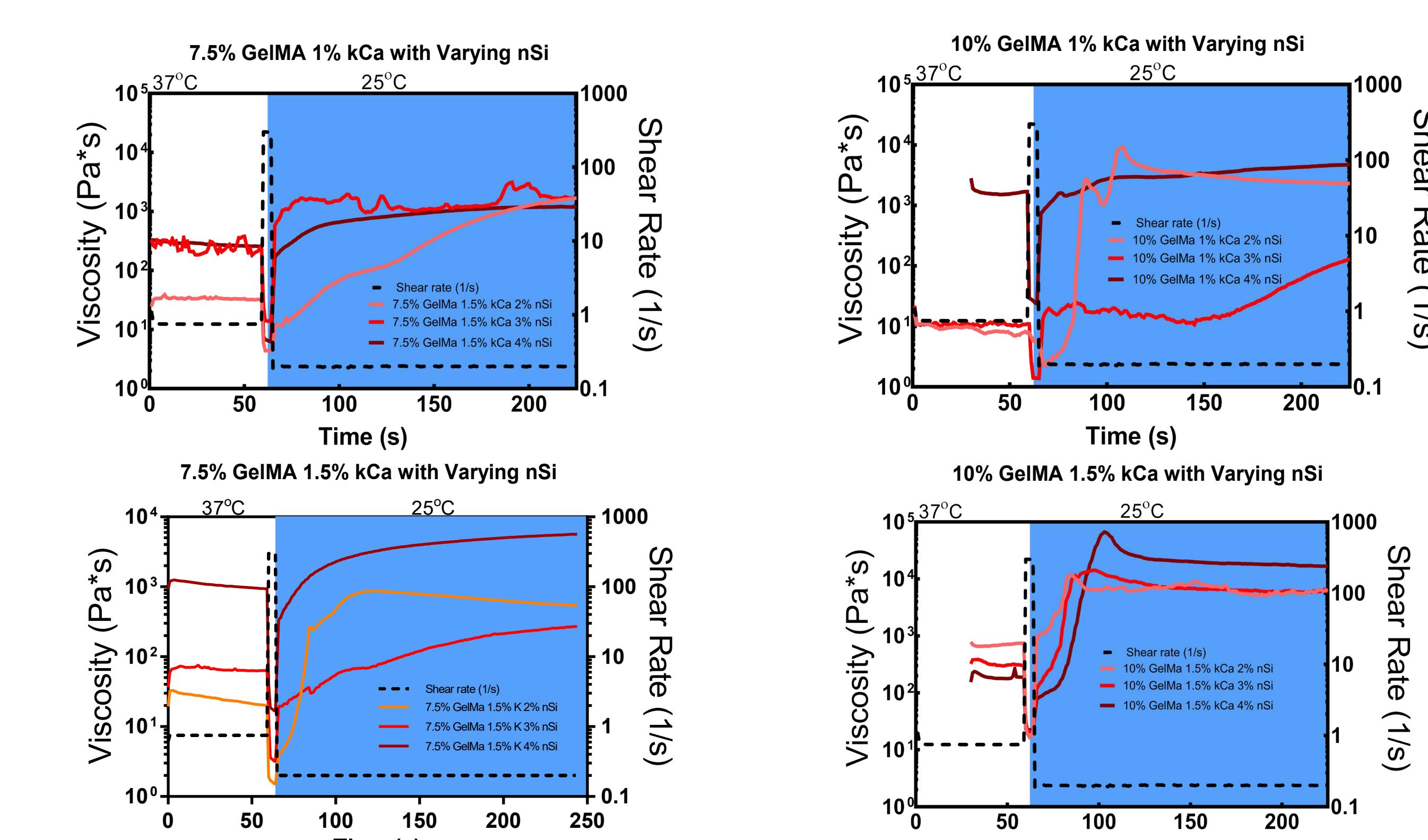


Increases in mechanical properties does not directly correlate with increase in printability for each component utilized in bioink

With increasing component concentration, the number of print inconsistencies was observed to increase as well

## Results and Discussion

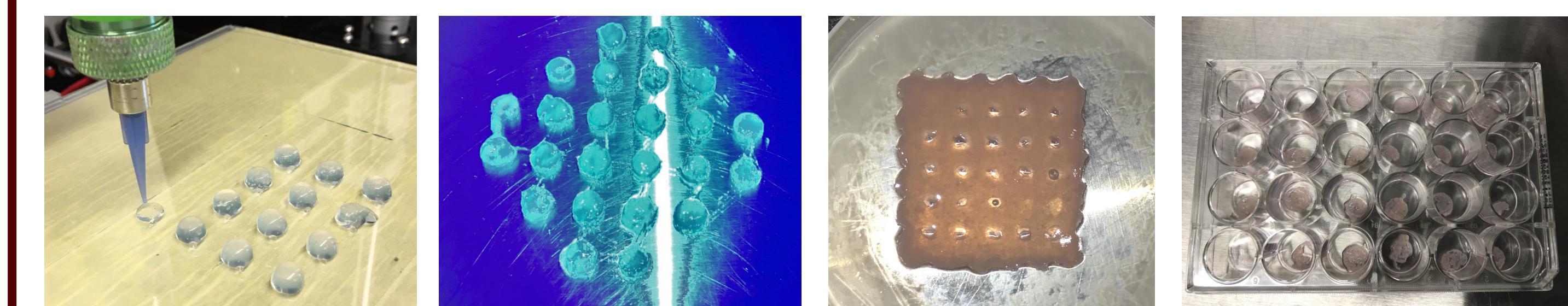
### Rheology Testing



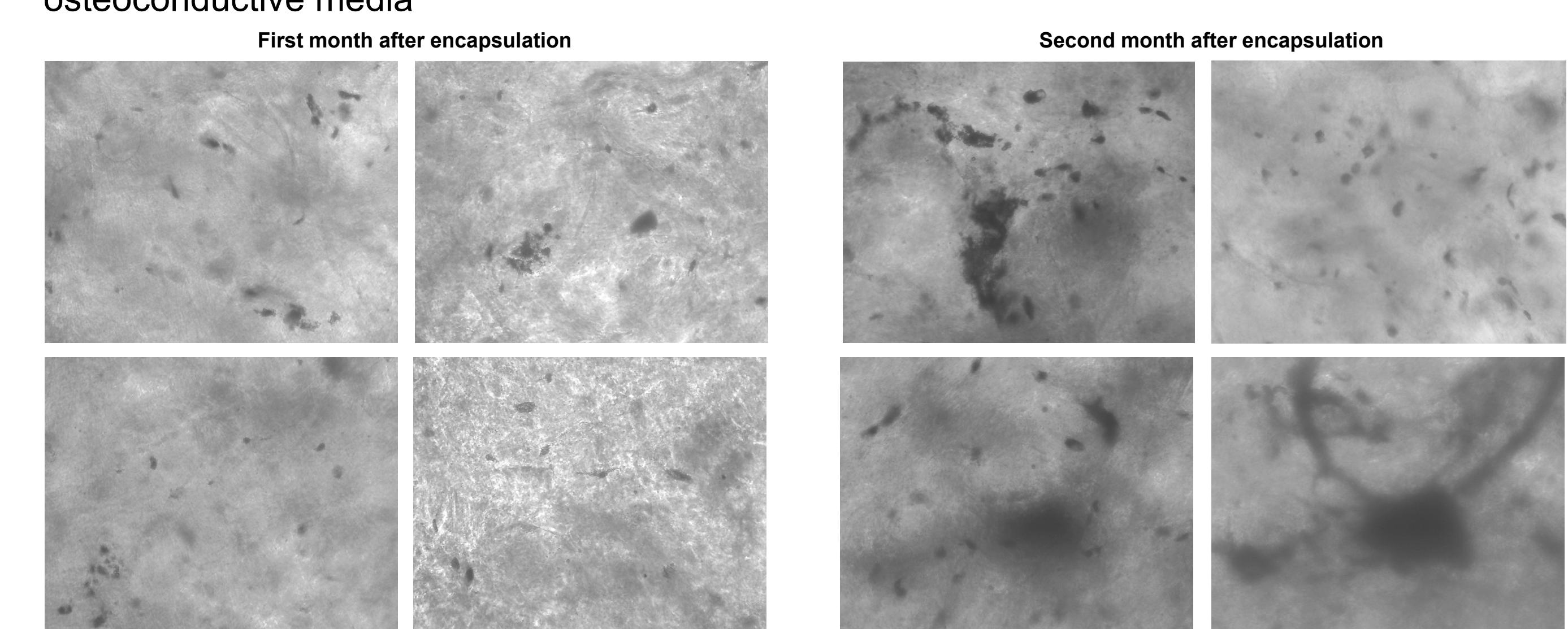
Initial rheology tests displays shear-thinning and recoverability properties of various bioink compositions

Component concentrations were shown to effect rate recoverability

### Analysis of hMSC Differentiation and Bone Matrix



3d printing of encapsulated hMSCs in NICE bioink followed by UV crosslinking and culturing in osteoconductive media



hMSC clumping was observed, which are early signs of bone matrix formation.  
At one month, calcium quantitative analysis detected 0.08mg of calcium in a 6.5 mg sample  
At two months, cell density and grouping has increased, and opaque white nodules of bone have begun to form

## Conclusion & Future Work

Our results show that all 3 bioink components contribute to improved mechanical properties, including increasing compression modulus and energy dissipated  
However, increasing component concentration does not always improve print fidelity, indicating that there is an ideal range of components that maximizes printability  
Rheology data shows that recoverability and apparent viscosity depend on component concentrations  
Further cellular studies will be performed with a focus on gene expression through RNA sequencing to compare 2d seeded scaffolds to 3d encapsulated scaffolds  
The calcified cell encapsulated samples will be mechanically tested and compared to reference samples

## References

- D Chimene, CW Peak, J Gentry, JK Carrow, LM Cross, E Mondragon, GBC Cardoso, R Kaunas, AK Gaharwar. Nanoengineered Ionic-Covalent Entanglement (NICE) Bioinks for 3D Bioprinting. *ACS applied materials & interfaces* 10 (12), 9957–9968
- D Chimene, KK Lennox, RR Kaunas, AK Gaharwar. Advanced Bioinks for 3D Printing: A Materials Science Perspective. *Annals of Biomedical Engineering* 44 (6), 2090-2102
- D Chimene, DL Alge, AK Gaharwar. Two-Dimensional Nanomaterials for Biomedical Applications: Emerging Trends and Future Prospects. *Advanced Materials* 27 (45), 7261-7284

## Acknowledgements

Research reported in this publication was supported by the National Science Foundation (NSF) under Award Number CBET 1705852 (AKG).