NANOCOMPOSITE BIOINK FOR ADDITIVE MANUFACTURING

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Nanocomposite Bioink for Additive Manufacturing

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In the medical field, there is a growing need for tissue replacements that are able to mimic the native structure and function of failing human tissues. 3D bioprinting is a manufacturing technique with the potential to fabricate patient-specific scaffolds for tissue engineering applications. Current bioinks consist of materials that lack shape-fidelity and modulatory abilities. These limitations need to be addressed in order to accurately mimic and sustain a functional human tissue. Bearing this in mind, there is a clinical need to develop scaffolds that are able to recapitulate the native properties of human tissues. Nanocomposite bioinks provide a tunable platform by altering concentrations and molecular weights of bioink components. An ink composed of gelatin methacrylate (GelMA), poly(ethylene glycol) diacrylate (PEGDA), and nanosilicates permits for control over scaffold swelling, compression, and degradation, therefore permitting the fabrication of scaffolds that mimic the patient tissue's innate structure. GelMA contains RGD domains that provide binding sites for cellular interactions, allowing for structure remodeling. Higher concentrations of GelMA in the bioink allow for increased cell interactions and limit overall swelling of the construct. PEGDA is a bioinert material that can modulate bioink mechanical properties through its molecular weight. Nanosilicates, through their unique structure, promote

shear-thinning and recoverability of the bioink throughout the printing process, permitting for the fabrication of high fidelity structures. As a result, this novel nanocomposite bioink is a promising solution to the current lack of a high-fidelity, modulatory bioink.

ACKNOWLEDGEMENTS

I would like to thank my research advisor, Dr. Gaharwar, for his guidance and support of undergraduate students in his lab and his encouragement of independent thinking. I also thank him for his financial support in attending research conferences. I would like to thank my graduate advisor, Karli Gold, for being my mentor. She has taught me everything I know about biomedical research practices and has inspired me to pursue an M.D./Ph.D. Thanks also to my fellow undergraduate researchers in the iNanoTE lab for talking through research set-backs and collaborating on new ideas. Finally, I would like to thank the other graduate students in the lab for their constant support and willingness to answer any questions I have.

NOMENCLATURE

GelMA	Gelatin Methacrylate
PEGDA	Poly(ethylene glycol) diacrylate
IRG	2-Hydroxy-4’-(2-hydroxyethoxy)-2-methylpropiophenone 98% (Irgacure)
NS	Nanosilicates
3DBP	3D bioprinting
PBS	Phosphate Buffer Solution
RO	Reverse Osmosis
DI	De-ionized

CHAPTER I

INTRODUCTION

Tissue engineering is an emerging field in biomedical engineering and medicine, aiming to help solve an array of clinical needs, such as tissue replacement and disease modeling[1]. Many diseases and trauma can lead to organ failure and tissue degeneration, and the current solution is organ replacement[2]. The gap between available organ donors and those on the organ wait list is rapidly increasing[3]. After donation, organ rejection and lifelong immune deficiency is a common occurrence[4]. A potential solution to organ donation is tissue engineering grafts or complete tissue engineered organs[5]. Currently in the medical field, attempts are being made to develop models to further understand disease, test drug delivery, and run physiologically relevant experiments. *In vivo* animal models and *in vitro* culture systems are the two most prevalent systems, but each contains inaccuracies that need improvement[6]. *In vivo* animal models are biologically relevant; however, variability and physiological differences between species limit predictability[7]. *In vitro* culture systems are highly controllable, yet are oversimplified and cannot exactly reproduce human physiology[8]. In this project, we aim to provide a platform to meet the clinical needs of an accurate tissue replacement system and a biologically relevant, predictive disease model. This will be done by 3D printing an arterial scaffold that is able to mimic the specific cellular and mechanical characteristics of a human artery. Our system should be able to reproduce tissue architecture, cell-cell interactions, cell-matrix interactions, complex flow, and dynamic pressure conditions as seen in native human arteries.

In order to do this, a bioink must be developed in order to 3D print a patient-specific arterial scaffold. 3D printing is a manufacturing technique that lends itself to creating anatomically

accurate models, medical devices, and scaffolds. 3D bioprinting is the fabrication of hydrogels with the direct incorporation of cells[9]. We will be starting with 3D printing, and once we develop the optimal bioink, we will incorporate cells and 3D bioprint. Our bioink must be optimized to have the desired mechanical and biological properties in order to withstand extrusion forces, maintain a high-fidelity print, and promote cell proliferation on the scaffold and remodel the matrix of the arterial graft. The ink must be biocompatible, allowing for cell proliferation and eventual decomposition at the same rate as regrowth of the extracellular matrix. We aim to recapitulate these properties by combining GelMA, PEGDA, and NS. Gelatin is a synthetic polymer, synthesized from a natural polymer – collagen, which is used to provide cytocompatibility to the system. This material provides cellular interactions through RGD-binding domains, allowing for cell proliferation[10]. Gelatin can be modified to contain methacrylate groups, producing GelMA, to enable for crosslinking into a stable matrix. However, as the sole polymer in solution, it is very difficult to modulate the mechanical properties of the hydrogel. In order to overcome this, poly(ethylene glycol) diacrylate (PEGDA) is used to create an easily tunable platform without affecting the ink's bioactivity[11]. PEGDA is a bioinert, synthetic polymer with distinct molecular weights that can be utilized to alter moduli and stiffnesses of the hydrogel. If PEGDA is the only polymer in solution, the hydrogel will not be able to interact with the body or allow cell proliferation. The combination of GelMA and PEGDA creates a network that exemplifies the mechanical properties of PEGDA and the bioactivity of GelMA. In order to 3D print this composite, the materials must also be able to recover from shear forces while withstanding extrusion-based forces. However, hydrogel precursor solutions, such as the composite of GelMA and PEGDA, consists of Newtonian rheological behavior. The incorporation of nanosilicates into the bioink allows for a shear-thinning behavior, while allowing rapid rebuilding of the material's

internal structure to recover from the high shear forces exposed to the material from the printing process[12]. The combination of GelMA, PEGDA, and nanosilicates composes a novel bioink that will meet the clinical need for a biocompatible, modulatory bioink. By varying bioink ratios and concentrations, we are able to optimize the bioink properties to produce an arterial scaffold that will mimic native arteries it will replace or respond to the same environmental conditions in physiological testing. An example 3D printed arterial model and construct can be seen in Figure 1.

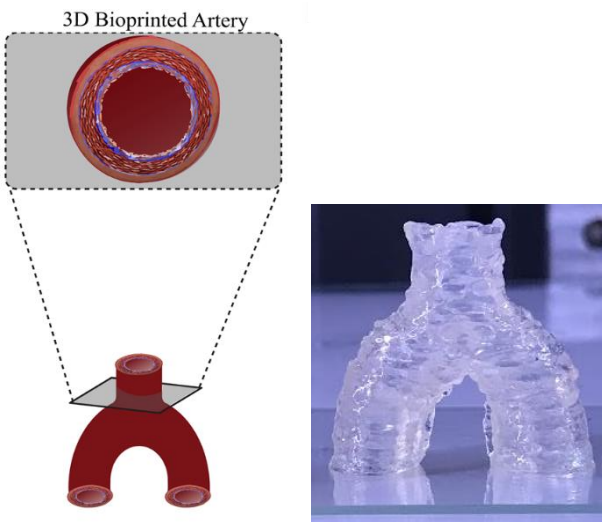


Figure 1. Example 3D Printed Artery

CHAPTER II

METHODS

Materials

Four materials compose our novel bioink: GelMA, PEGDA, NS, and IRG. We synthesize GelMA in the Gaharwar lab using type A gelatin from porcine skin. GelMA is a synthetic polymer, methacrylated gelatin. Gelatin is a natural polymer, which is just hydrolyzed collagen. The GelMA synthesis is described in depth in the following section. The structure of GelMA is shown in Figure 2. Because GelMA is derived from collagen, the most abundant protein found in the human body, it contains RGD binding sites which allow for cell proliferation[13]. RGD is an amino acid peptide, arginylglycylaspartic acid, which is responsible for cell adhesion to the extracellular matrix[14]. These binding sites promote integrins of the patient's primary cells to attach to our hydrogel scaffold. This means eventually the patient's cells will grow over the hydrogel scaffold, and the native extracellular matrix will replace the tissue engineered graft.

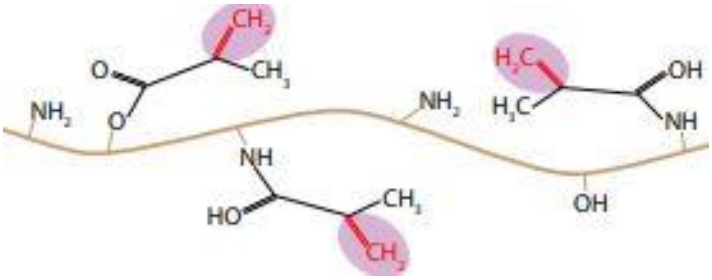


Figure 2. GelMA Structure

PEGDA is a synthetic polymer that was purchased from PolySciences with a number average molecular weight (Mn) of 10,000 grams per mole. PEGDA is known as the "stealthy" polymer because it is bioinert. As such, it will not cause a foreign body reaction in the patient, and the bioactivity of the GelMA will predominate. The structure of PEGDA is shown in Figure 3.

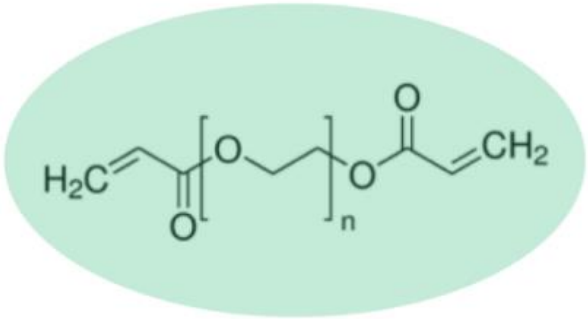


Figure 3. PEGDA Structure

Nanosilicates represent the most novel component of our bioink as they give printability properties, shear-thinning, and recoverability to our bioink. These properties are discussed more in following sections. NS are two-dimensional nanoparticles with a negative face charge and positive rim charge at pH's below 9[15]. Their structure can be seen in Figure 4. This unique charged structure allows the particles to build up into a "house-of-cards" structure, which gives diverse rheological properties.

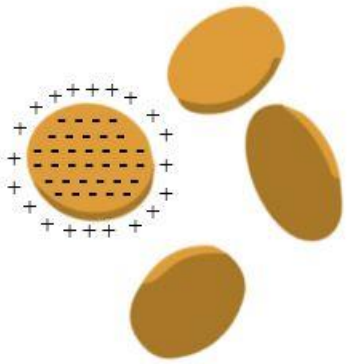


Figure 4. 2D Nanoparticle Structure

The final component of our bioink is a photo-initiator, Irgacure. We need a photo-initiator in our bioink in order to UV crosslink the hydrogel. Only a very small amount of IRG is necessary for crosslinking; for the bioink tested, 0.3 wt% IRG was used within the bioinks for photo-

initiation. Because every UV crosslinked hydrogel requires a photo-initiator, IRG is often not included in our description of the components of the bioink.

GelMA Synthesis

GelMA was synthesized as described: Two 1x (100mL and 400mL) dilute solutions of 10x phosphate buffer solution (PBS) were prepared. The 100 mL solution of 1x PBS was heated at 60 degrees Celsius on a hot / stir plate, monitored using a thermometer. The 400 mL solution of 1x PBS was covered with aluminum foil and heated at 40-50 degrees Celsius in a water bath. Ten grams of porcine gelatin (Type A) were weighed out and added to the 100 mL of PBS. The mixture was stirred and heated for one hour or until the gelatin was completely dissolved and the solution clear. In order to methacrylate the gelatin polymer 80%, 8 mL of methacrylic anhydride were added dropwise and allowed to react for three hours. The reaction was stopped by adding the 400 mL of 1x PBS. The solution was allowed to sit for 15 minutes while the dialysis filtration was prepared. Cellulose dialysis tubing was soaked in RO water, and it was knotted and clamped at one end. The GelMA solution was poured in, and the tubing was knotted and clamped at the other end. The filled tubing was placed in a 1000 mL beaker and filled with DI water. The by-products of the reaction – such as methacrylic acid and unreacted methacrylic anhydride – were allowed to filter out of the dialysis tubing, down the concentration gradient. This gradient was re-established three times a day for one week by changing out the DI water. The GelMA was then filtered through a Buchner funnel and filter paper with a vacuum in order to remove any foreign particulate. The solution was added to centrifuge tubes and freezed for 24 hours in order to form ice crystals. The frozen solution was lyophilized for three days to remove these ice crystals – freeze drying (sublimating) the GelMA. This GelMA was stored in the same centrifuge tubes at room temperature, away from direct sunlight in order to avoid premature photo-crosslinking.

Swelling Analysis

Swelling experiments were performed on biopsy-punched crosslinked ink in Eppendorf tubes. The bioink solution was pipetted in between two glass slides and pressed closed with binder clips. It was then crosslinked using an Omnicure UV lamp at 5mW/cm² for two minutes on each side in order to crosslink the hydrogel. Biopsy punches were used to punch out circular disks. These disks were then weighed in the Eppendorf tube. Swelling experiments were performed multiple times over several months in order to ensure accuracy and precision in the experiment. Four different solutions were used to soak the hydrogel: DI water, non-sterile media, 1 unit/mL collagenase, and 0.5 unit/mL collagenase. The initial weights of the hydrogels were taken, and then subsequent measurements were made over hours, days, and weeks. The solution was removed before weighing the swollen hydrogel by pipetting the solution out of the Eppendorf, without cracking or sucking up the hydrogel into the pipette tip. The swollen hydrogel was then weighed inside the Eppendorf tube, and its weight was subtracted out for each calculation. New solution was then pipetted into the tube until the next time point. The swelling ratio was found by calculating the mass of the swollen hydrogel divided by the mass of the initial weight of the hydrogel.

Hydrogel Degradation

Simultaneously with swelling experiments, degradation was performed on the hydrogels. The amount of mass decreasing over time was used to create a degradation profile for each sample and solution. The only solutions in which the hydrogels are susceptible to degradation are the two concentrations of collagenase. This is due to the enzymatic degradation mechanism that acts on the GelMA within the ink. Collagenase is an enzyme that breaks down the peptide bonds in collagen. Because GelMA is synthesized from gelatin, which is hydrolyzed collagen, the

collagenase also acts on GelMA. PEGDA is susceptible to oxidative degradation, but none of the solutions contain reactive oxygen species. Neither polymer is susceptible to hydrolytic degradation, so the hydrogels do not degrade in water or media. Because of this fact, once the samples in collagenase degraded completely, the measurements of the samples in water and media were stopped, as they otherwise would continue indefinitely without degrading. The method for measuring degradation is the same as described above for the swelling study.

Mechanical Testing

Compression tests were performed on a mechanical tester. The bioink solution was pipetted in between two glass slides and pressed closed with binder clips. It was then crosslinked using an Omnicure UV lamp at 5mW/cm² for two minutes on each side in order to crosslink the hydrogel. Biopsy punches were used to punch out 1 inch circular disks. These were placed in between the bed and transducer. The specimen was then placed under cyclic compression, and the deformation was measured. Compressive strain and stress were graphed, in which the elastic modulus was measured by the slope of the linear region. The same tests were performed before and after swelling of the hydrogel. Figures were created to reflect the compressive moduli. Tensile testing cannot be performed on hydrogels as they are too slick to grip in the machine's clamps.

Rheological Testing

A controlled stress single head rheometer was used to perform a variety of rheological tests. A shear-stress sweep was performed on the precursor solution to calculate the yield point, storage modulus, and loss modulus, and to analyze the different components of the ink. A shear-rate sweep was performed to determine the fluidic character of the ink and flow behavior index. A peak-hold test was performed in order to determine the recoverability properties and viscosity of the ink. All of these rheological tests helped to map the diverse fluidic properties of the ink and exemplify its

3D-printability. In most analytical testing for this project, NS are not included because they do not affect the properties and are expensive to waste, but for rheology testing they are the most important component. The inclusion of NS in the ink induce diverse rheological properties that transform the ink from a Newtonian solution, into a shear-thinning material – one that can be 3D printed.

Hydrogel Preparation

In order to develop a useful ink for 3DBP constructs, the method of ink fabrication needed to be optimized. The method should produce little to no bubbles, as bubbles in an ink would be printed into the construct. These bubbles would act as stress concentrators, which would cause the scaffold to crack and affect degradation. The ink must also be completely homogenous. If pockets of the different components formed in the solution, they would not form an entangled network necessary to utilize the needed properties of the GelMA, PEGDA, IRG, and NS. The process would also be more efficient if exfoliation of the NS could happen immediately, instead of a time lag existing between fabricating the ink and printing it.

Previous attempts to fabricate the bioink were made, however homogeneity posed an issue. First, all three components (GelMA, PEGDA, and NS) were mixed together and dissolved in water. This produced a nonhomogeneous ink, specifically with regard to separation of the hydrogel components and the NS. In order to produce overcome this, a different approach was made by doubling the concentrations of PEGDA and GelMA followed by diluting the solution with exfoliated NS. However, this method was not easily manipulated due to the difficulty in using such a high concentration of exfoliated NS with its thick viscosity. To overcome such limitations, another attempt was made by adding a GelMA solution to powdered components (NS,

PEGDA). Although this method produced a homogeneous ink able to be printed, the constructs contained pockets of air that were difficult to eliminate due to the ink's high viscosity.

The most promising method of ink fabrication that was tested was continuous mixing in a beaker on a hot plate. First, the desired amount of water was heated to a constant temperature of around 37 degrees Celsius. Then the desired amount of GelMA was added to dissolve. The continuous mixing process decreased the amount of bubbles that formed due to the minimization of air incorporation. This can be compared to adding GelMA to a falcon tube, heating it in the oven, vortexing, and leaving in the oven when bubbles were highly prevalent. Then the PEGDA and IRG were added to dissolve completely. The continuous mixing allowed for a homogenized solution instead of the previous pockets of PEGDA or IRG in the GelMA solution. Finally, the NS were added slowly to the stirring solution so as not to form clumps. The NS innately want to clump together due to their unique charge and house-of-cards structure. Because of the continuous mixing on the hot plate, the NS are exfoliated over the time it takes the whole solution to dissolve homogenously. The previous method was to add all of the components to a Falcon tube to allow the NS to exfoliate in the oven for three days. This method of continuous mixing is optimal because of the instantaneous exfoliation of NS. From here, the ink can be immediately printed. This optimized method will be used for the duration of the project to print the tissue engineering constructs. As can be seen in Figure 5, there is a marked difference in bubble concentration between the traditional and continuous-stirring method of hydrogel preparation.

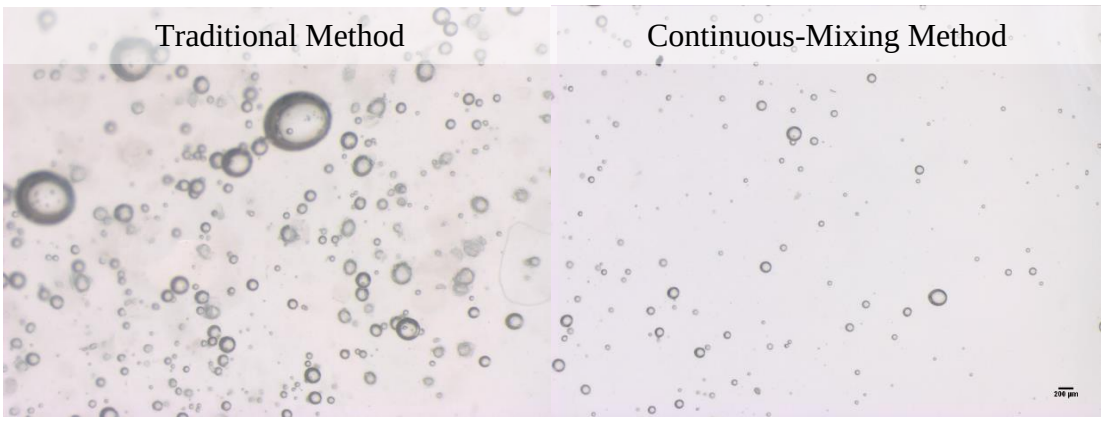


Figure 5. Bubble Concentration of Hydrogel Preparation Methods

As shown in Figure 6, the continuous-stirring method does not significantly affect the shear-thinning rheological properties of the solution. This was a previous concern as the NS can crash out of solution with too high of shear forces. The NS, however, exfoliate on-point and then maintain their rheological properties throughout the hydrogel preparation and printing process. After the ink is properly fabricated, it is stored in centrifuge tubes in an oven regulated at 37 degrees Celsius to mimic body temperature.

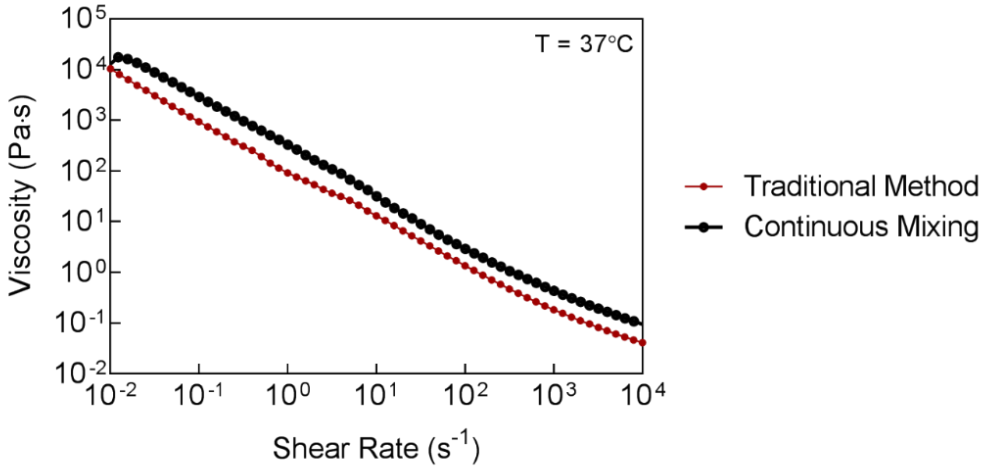


Figure 6. Shear-Rate Sweep of Hydrogel Preparation Methods

3D Printing

A Hyrel 3D printer was used to print all constructs. We used an extrusion based, layer by layer deposition approach. The extruder was pre-heated to 37 degrees Celsius to mimic body temperature, and then the ink was loaded into the screw extruder with a spatula. The extruder was loaded into the printer, and a motor turns the screw of the extruder. Different needle gauges were tested until the optimal printing was found to occur with a 23 mm gauge needle tip. Constructs were designed using SolidWorks and uploaded into the Slic3r software to control printing parameters. Once modified in Slic3r, the file was exported into a .STL file, translating into instructions for the 3D printer to follow and deposit the ink. After optimizing the bioink, it prints smoothly into a high fidelity scaffold. The bottom-up printing approach can lead to spreading of the material toward the bottom of the structure, especially when a tall structure is printed. In the future, print fidelity testing will be performed to quantify the spreading error. The ink is resistant to delamination upon crosslinking the structure under UV. An example of the 3D printer extruding the ink into a hollow cylinder structure can be seen in Figure 7.

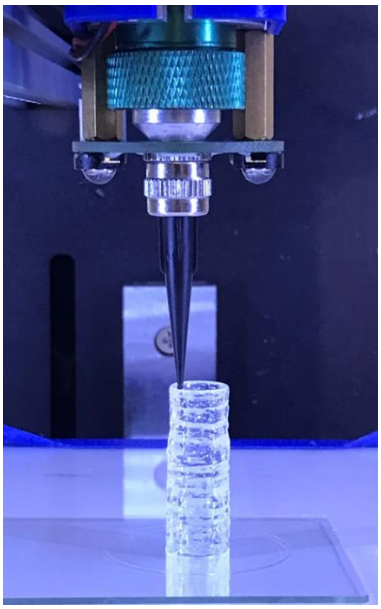


Figure 7. 3D-Printing Nanocomposite Ink

CHAPTER III

RESULTS

Swelling Properties

As described in the Methods section, swelling experiments were completed several times over 24 hours, and the data was averaged. The ratio of PEGDA was altered, increasing the percentage of PEGDA at set intervals to a constant 10% GelMA ratio, 7.5% GelMA ratio, and 5% GelMA ratio. The data can be seen below in Figure 8. Increasing the PEGDA concentration increased the swelling ratio. This is because PEGDA is a hydrophilic polymer that tends to uptake water. The swelling ratio (Q) was calculated as wet mass divided by the initial mass of the hydrogel after crosslinking. As the TEVG will be implanted into the body, the bioink must be able to withstand the physiological environment with minimal swelling, therefore preventing deviation from the intended print. Thus, we want to use the minimum amount of PEGDA possible, while still eliciting its mechanical properties in the bioink. NS were not included in swelling experiments as the flow properties were not necessary to utilize, and ions do not uptake any water or contribute to swelling.

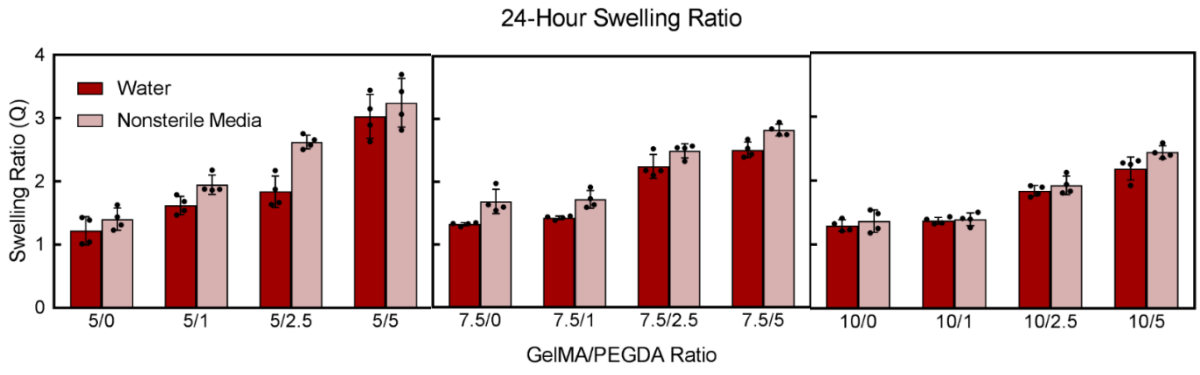


Figure 8. Swelling Experiment Data

Degradation Profiles

Two degradation profiles were compiled from the degradation studies performed. The concentration of GelMA was set at a constant 7.5% with increasing concentrations of PEGDA at set intervals. Once again NS were not included as they do not contribute to the degradation mechanism. None of the samples degraded in water or media, as the polymers used are not susceptible to hydrolytic degradation. The two solutions used for degradation were 0.5 units per milliliter collagenase and 1.0 unit per milliliter collagenase. As can be seen in Figure 13, the samples tested in 1 unit/mL collagenase degraded the fastest - about one third faster than in 0.5 units/mL collagenase. The ratios that degraded most quickly were the samples with the least concentration of polymer in the ink solution. The ratio that degraded the least was the 7.5% GelMA/5% PEGDA. The fact that the lower concentration polymer solutions degrade the fastest is due to the increased availability of the enzyme solution to attack the polymer. The polymeric network is less tightly packed, so the enzyme can more easily break down the polymer bonds.

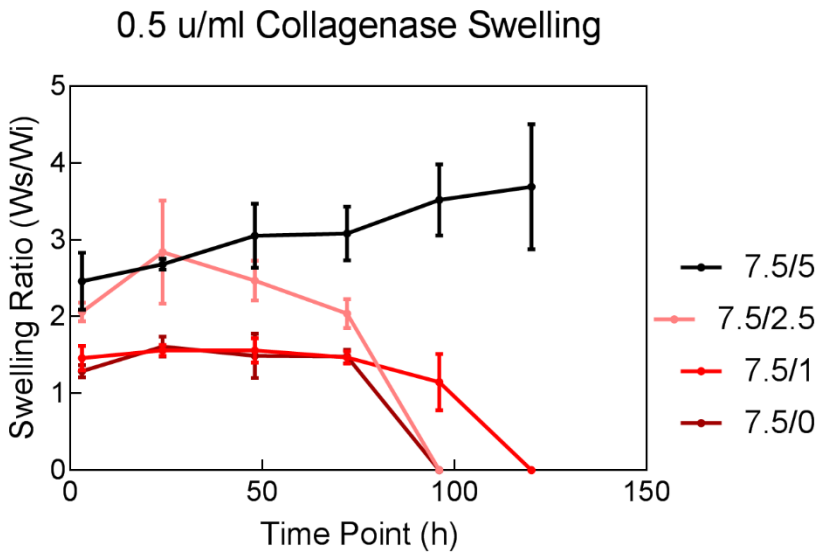


Figure 13. Degradation Profiles

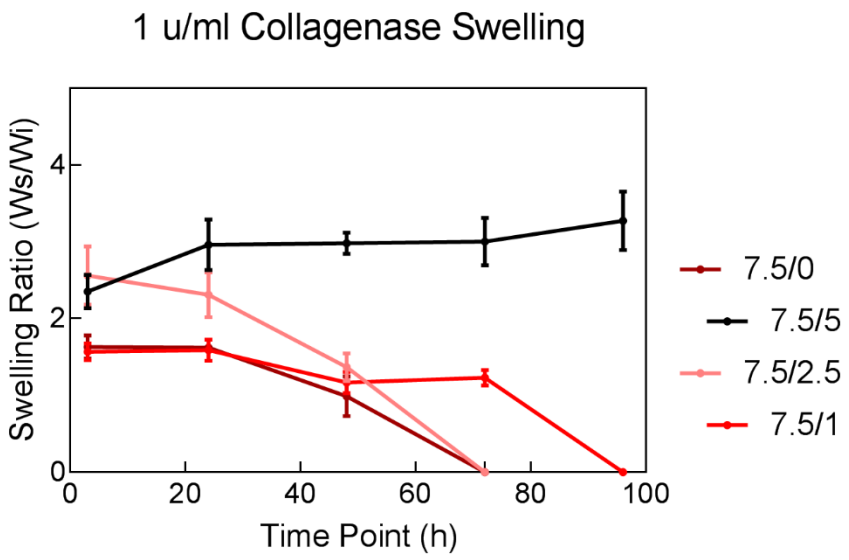


Figure 13 Cont. Degradation Profiles

Mechanical Properties

Hydrogel mechanical properties were tested with compression testing. Different ratios of polymer concentration were tested before and after hydrogel swelling, as can be seen in Figure 9. First, the ratio of PEGDA was altered, increasing the percentage of PEGDA at set intervals to a constant 10% GelMA ratio, 7.5% GelMA ratio, and 5% GelMA ratio. Increasing the overall polymer concentration increased the compressive modulus. Post-swelling, the modulus was lower due to softening of the hydrogel and water-uptake into its crosslinked network. As the percentage of PEGDA increases, the GelMA and PEGDA start to form an entangled network, contributing their own increased mechanical properties. The effects of this can be seen as the amount of change of modulus increases with increasing concentration of PEGDA. We want the TEVG to have a relatively high modulus, but it must be balanced with the amount of swelling that occurs. Taking both the swelling and compression experiments into account, we determined that an overall 10% polymer concentration was optimal, with 7.5% GelMA (to contribute bioactivity) and 2.5% PEGDA (to contribute mechanical properties but minimize swelling).

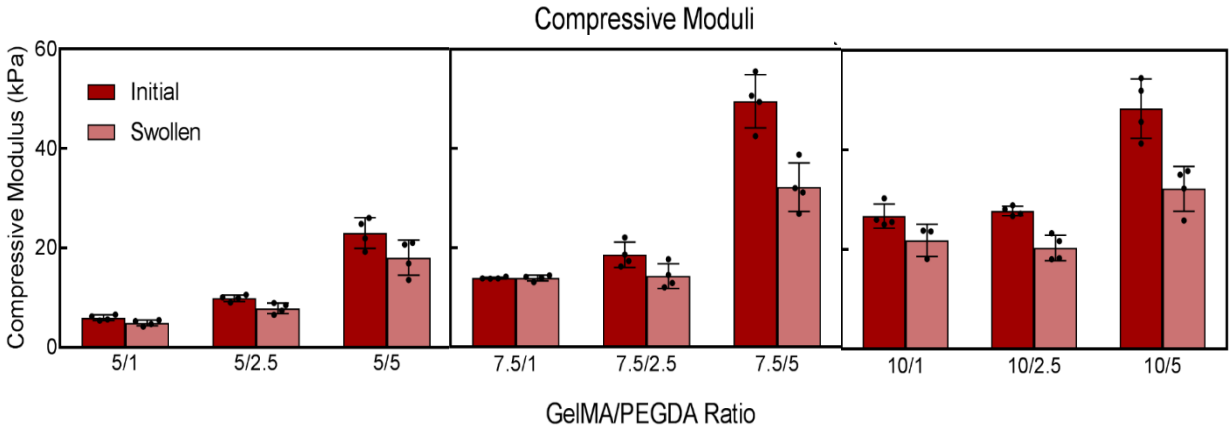


Figure 9. GelMA/PEGDA Compression Tests

Rheological Properties

Once the ideal polymer concentration was determined to be 10%, the NS concentration was determined. These tests were easy and qualitative: adding too much NS made the bioink so viscous it could not be printed. Adding too little NS did not induce enough rheological properties, and the ink was too thin to recover or hold its shape post-printing. Thus, the optimal ratio of our NS was found to be 4% in our novel bioink. Using this concentration, we were able to complete our rheological profiling of the bioink. Several shear-stress sweep tests were performed on the rheometer in order to show that the addition of nanosilicates causes a yield point in the ink. This can be seen in the Figure 10 graphs with NS. The storage modulus (G') represents the ink in a more solid state, while the loss modulus (G") represents the ink in a more liquid state. Although it appears in some of the graphs lacking NS that there is a yield point, the actual numerical values of the moduli are so close that there is no discernible point the solid deforms into a liquid. This is because the ink without nanosilicates has no solid state – it is essentially a Newtonian fluid. The addition of NS allows the ink to be viscous, which is a desirable property because we do not want

an implanted scaffold to be fluid in the body. The yield point offered by NS requires a higher stress for the bioink to flow.

The shear-stress sweeps also show that nanosilicates increase the modulus of the bioink. As can be seen in Figure 10, each component of the ink was tested individually, and then nanosilicates were added and the test was repeated. GelMA, PEGDA, and GelMA + PEGDA all have moduli around the order of 0.01 pascals, while NS alone has a modulus around 1000-10,000 pascals. After nanosilicates were added to any of the polymer components, the modulus increased by a factor of at least 10,000.

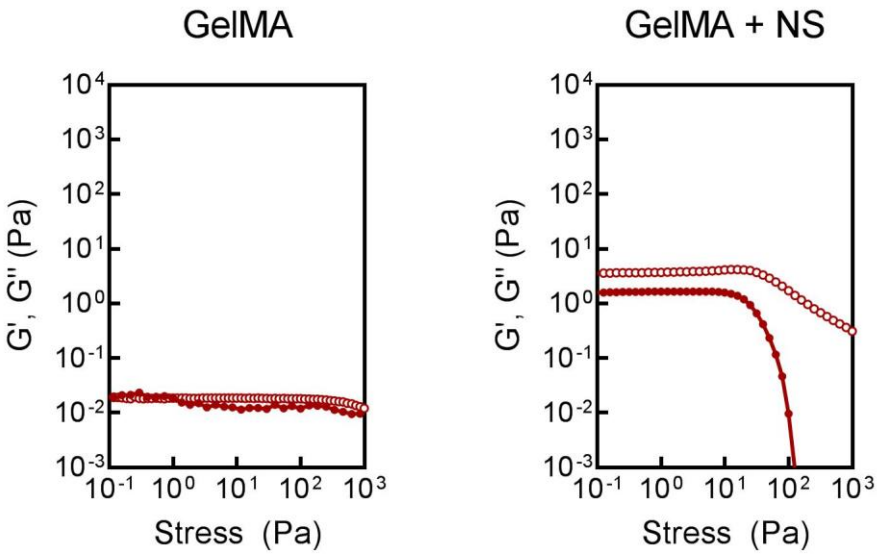


Figure 10. Shear-Stress Sweep Tests

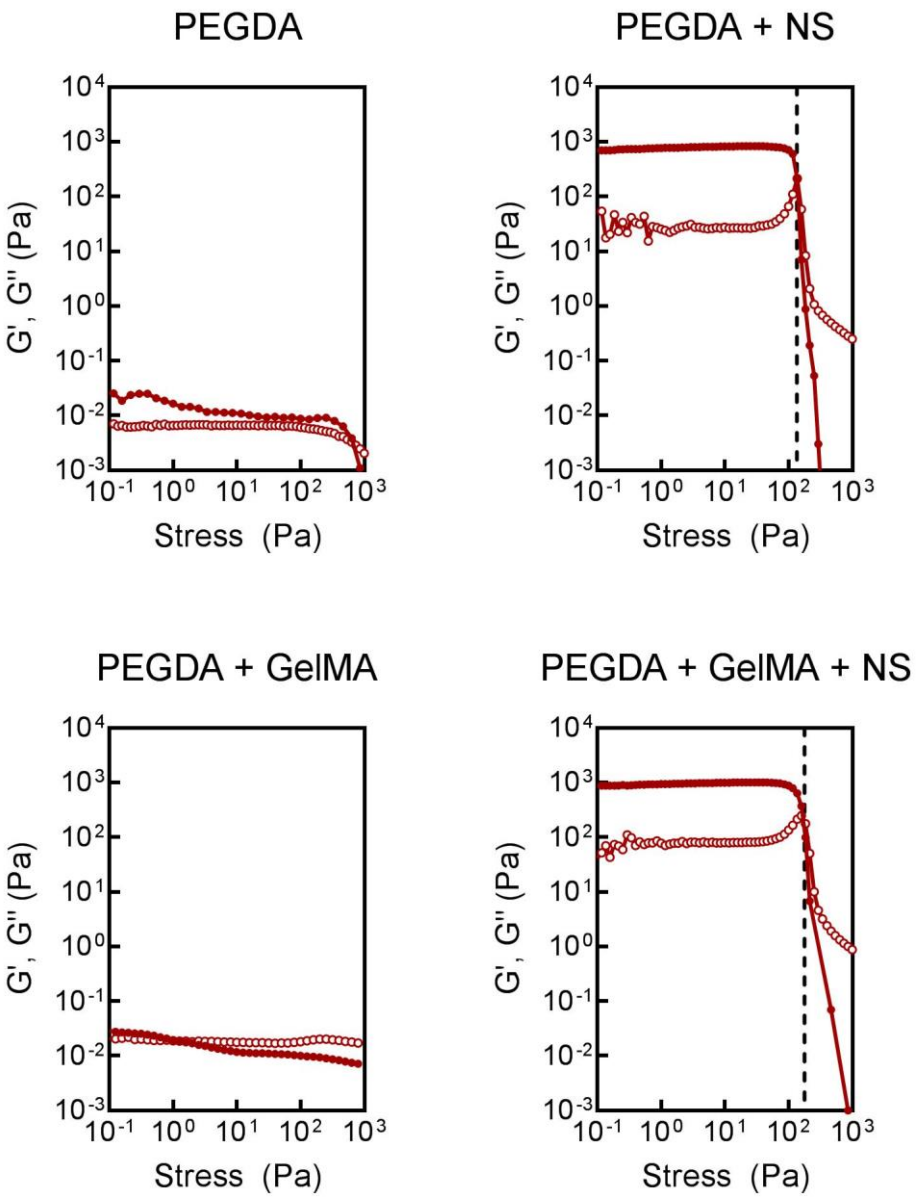


Figure 10 Cont. Shear-Stress Sweep Tests

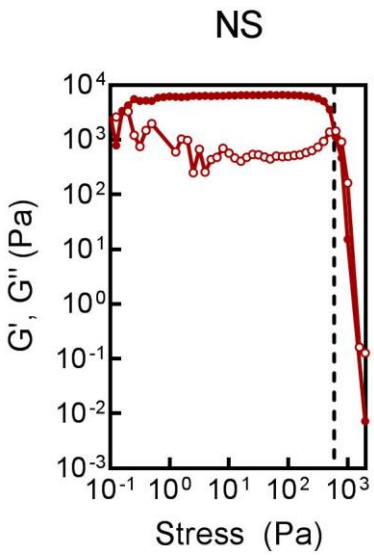


Figure 10 Cont. Shear-Stress Sweep Tests

The next test performed on the rheometer was a shear-rate sweep, as can be seen in Figure 11. This is one of the most important rheological tests as it offers a fluidic profile of the bioink which predicts a material's ability to extrude. As shown on the graph, with low shear rates applied, the ink is highly viscous; while with high shear rates applied, the ink decreases in viscosity. This phenomenon is called shear-thinning (High shear = low viscosity. Low shear = high viscosity). This is the desired pattern for 3D printing because in the needle, high shear rates are being applied to extrude the ink out. At this point, the ink should be fluid enough to exit the needle smoothly. Then, after printing onto the bed, the ink should be viscous enough to hold its shape. Therefore, a shear-thinning ink is required for 3D printing. As can be seen when comparing the other material components of the ink in the shear-rate sweep, it is the NS which induce the shear-thinning properties. The 2D nanoparticle has a unique structure of a negative face charge and a positive rim charge. This allows them to build up into a house-of-cards structure upon exfoliation and under low shear. This structure gives the ink its high modulus and viscosity. Under high shear, this house-of-cards structure is broken down, and the ink becomes fluid.

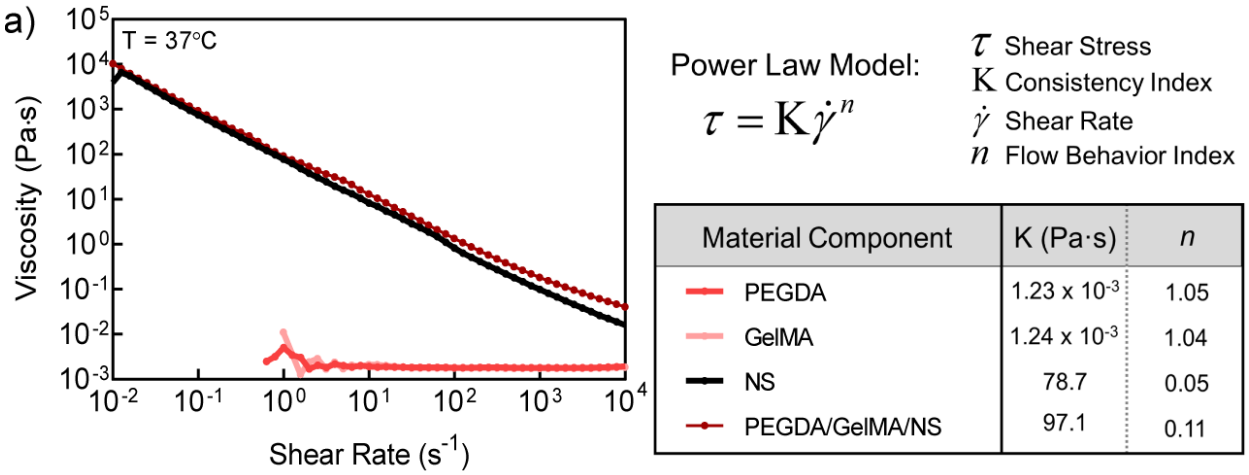
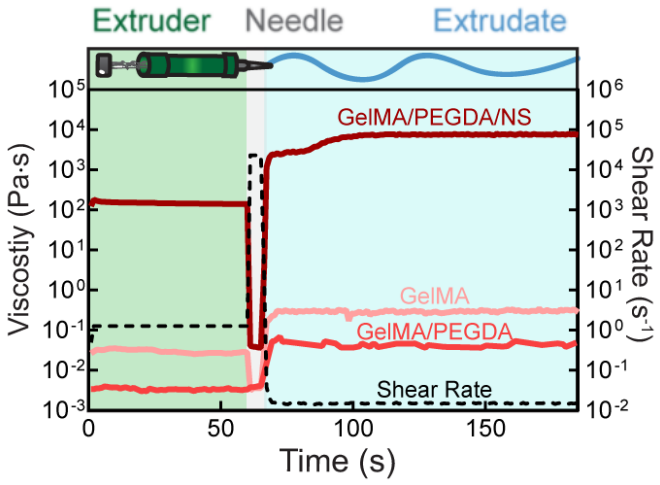


Figure 11. Shear-Rate Sweep Testing

In order for an ink to be 3D-printable, it must not only be shear-thinning, but also recoverable. Recoverability means that after the ink is shear-thinned, it does not just withhold its low viscosity. It increases again to its original viscosity so it can hold its shape post-printing. This is shown below in Figure 12. A peak-hold test was performed in order to test recoverability of the ink. It showed that the nanosilicates greatly increase the recoverability of the ink. Again, this property is derived from their unique structure. The test showed that the nanosilicates can reform their house-of-cards structure after a high shear rate is applied; they do not permanently deform. This characteristic allows the ink to be printed as a fluid and then regain its viscosity on the printer bed, recovering its modulus as well. Recoverability is an essential property of an ink that is able to have high fidelity printing. The figure shows three distinct phases of printing: In the extruder, the shear rate is low and viscosity is high. In the needle, shear rate is high and viscosity drops drastically. On the bed, the extrudate increases rapidly back up to its original viscosity (and even higher) at low shear rate. Once again, comparing among the other material components shows that it is NS which induce recoverability of the bioink.



Rabinowitsch Equation:

$$\dot{\gamma} = \frac{3n+1}{4n} \times \frac{4Q}{\pi R}$$

Flow Rate: Q = 1/15 (mL/min)
Flow Behavior Index: n = 0.11

Extruder: 37°C, R = 25 mm, $\dot{\gamma}$
Needle: 37°C, R = 0.603 mm, $\dot{\gamma}$
Extrudate: 25°C, R = ∞, $\dot{\gamma}$

Figure 12. Peak-hold Test

CHAPTER IV

CONCLUSION

Ideal Composition of Nanocomposite Ink

After performing swelling studies and mechanical testing, the ideal composition of the nanocomposite ink was found to be 7.5% GelMA, 2.5% PEGDA, and 4% NS. This composition maintains the total polymer concentration at 10%, maximizes PEGDA modulatory abilities, minimizing swelling caused by PEGDA, and maximizes cellular interactions through GelMA's RGD binding sites. The shear-thinning and recoverability properties offered by NS allow the ink to be 3D printable. The easily tuned molecular weight of PEGDA will allow simple modulation of mechanical properties. This bioink provides a biocompatible, robust ink with a simple fabrication process. Overall, this bioink development project is a platform that many diverse research projects can build on, using our novel ink. It is highly suitable for additive manufacturing and has the potential to influence the tissue engineering field's current perspective on bioink.

Future Work

This project could have many potential future directions due to its wide applicability. One possible direction is 3D bioprinting a tissue engineered vascular graft. Our novel bioink is a viable option for this application because it is highly modulatory. In the future, we will be altering the molecular weights of PEGDA below and above the current 10k weight in order to modulate the bioink's mechanical properties for different applications. An atherosclerosis model could be created modifying the mechanical properties to mimic the innate vasculature structure. The modulus can be changed by altering the molecular weight of PEGDA, while holding the other

components constant. This atherosclerotic TEVG could be an accurate disease model wherein disease properties could be tested.

After fabricating different hydrogel scaffolds, the next step is to complete cell work. Human smooth muscle cells will be encapsulated in the ink and 3D bioprinted into a scaffold. After applying the shear forces from printing, cell viability will be tested. If a vascular graft is printed, endothelial cells will be flowed through it to model the interior endothelial lining of arteries. Different assays will be used to quantify cell viability and phenotype on the TEVG. The goal is for the rate of extracellular matrix regrowth to match the rate of degradation of the scaffold. This would allow for complete resorption and resolution of the vascular injury. After completion of cellular testing, more degradation analysis will need to be performed in order to accurately match the rate of cell proliferation onto the scaffold.

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