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# Injectable Nanocomposite Hydrogels for Cell Delivery and Bioprinting

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**ABSTRACT:** Injectable hydrogels are investigated for a range of biomedical applications including cell/therapeutic delivery and bioprinting applications. In this research, we develop nanoengineered hydrogels from polyethylene glycol diacrylate (PEGDA) and gelatin that is reinforced with two-dimensional (2D) nanosilicates to generate injectable hydrogels that can be used for cell delivery and bioprinting. PEGDA forms chemically crosslinked hydrogels, while gelatin provides cell adhesion sites. The composite hydrogels resulted in biomaterials with robust mechanical properties and high cell viability. Nanosilicates were added to the polymer mixtures to improve shear-thinning properties and enhance printability and injectability of the hydrogel. The addition of nanosilicate also resulted in a significant increase in the mechanical stiffness of hydrogels and supported cell growth and proliferation. Overall, these injectable hydrogels can be used for cell delivery and bioprinting applications.
KEYWORDS: *bioprinting, hydrogel, nanocomposites, injectable, nanosilicates*

## INTRODUCTION

Each year, an estimated 8,000 American people die from failure to receive timely organ transplants. There are opportunities in the field of material science and biology for these lives to be saved, prolonged, or provided better quality by tissue or organ implants. Tissue engineering, the science of replacing and regenerating tissues, hold promise to regenerate damage tissue and organ using biomaterials, cells and instructive cues. Injectable hydrogels can be used deliver a wide range of cells and therapeutic proteins to facilitate tissue regeneration. Hydrogels are water swollen polymer matrices such as alginate, chitosan, poly(ethylene glycol) (PEG), or polyacrylamide. In order for the hydrogel to be injectable, it must exhibit a viscosity suitable for syringe extrusion and direct entry into the site of injury. Due to high water content, most hydrogels are mechanically weak.[6] For example, alginate is mechanically weak and due to the chemical structures, fails to present cell binding sites. Whereas, chitosan exhibits low mechanical strength and reduced solubility in water, making it slow to biodegrade. Synthetic polymers such as polyacrylamide and poly(ethylene glycol) can form mechanically stable hydrogels, but have limited ability to direct cellular activity and are considered relatively non-degradable. The development of a tough and injectable hydrogel faces major obstacles, which include, but are not limited to, poor cell-matrix interactions, and weak mechanical stiffness.[7] To overcome these limitations, a combination of natural and synthetic polymers are used to generate mechanically robust, biodegradable, cytocompatible, and injectable hydrogels.

Recently, the addition of nanoparticles can be used to further modulate the physical and biological properties of hydrogels. These nanocomposite hydrogels (NC gels) can be used as drug delivery vehicles, bioactuators, biosensors and tissue engineered scaffolds. Shear-thinning NC gels can be used for injectability and bioprinting applications due to superior physical and chemical properties compared to

individual components. The nanoparticles (NPs) used to fabricate NC gels include carbon-based NPs, metal/metal oxides based NPs, and polymeric NPs. Among different NPs, two-dimensional (2D) NPs are extensively investigated to fabricate shear-thinning NC gels. Two-dimensional nanosilicates are disc shaped NPs with 30 nm in diameter and 1 nm in thickness. The edge of the nanosilicates is positively charged, while the surface is negatively charged. These characteristics of nanosilicates render enhanced electrostatic interactions with polymeric chains. Recent, studies have highlighted the use of nanosilicates to obtain injectable hydrogels. Other studies also report that nanosilicates are effective at transporting molecules, proteins, and drugs, and can be used for sustained and prolonged release of therapeutics.

Here, we present injectable nanoengineered hydrogels from poly(ethylene glycol) diacrylate (PEGDA) and gelatin, reinforced with 2D nanosilicates. We use poly(ethylene glycol) diacrylate (PEGDA) due to its ability to form covalently crosslinked hydrogel networks. Gelatin was added to the PEG hydrogels as it contains Arg-Gly-Asp polypeptide (RGD) sequence that support cells adhesion and proliferation. By combining PEG and gelatin network, we were able to modulate physical characteristics of hydrogel network. The addition of nanosilicates to composite network results in shear thinning characteristics and improve injectability that can be used for minimally invasive cell therapy. The shear-thinning hydrogels can be used for 3D bioprinting, which can be easily modulated by controlling the viscosities, and flow characteristics via nanosilicate addition.

## MATERIALS AND METHODS

Different combinations of gelatin and PEGDA were developed. Seven combinations of PEGDA/Gelatin (%wt/vol) concentrations were formulated: 0/10, 5/10, 10/10, 15/10, 15/0, 15/5, and 15/10. Hydrogels made with a single polymer at its highest concentration served as the controls.

### *Synthesis of diacrylated polyethylene glycol (PEGDA)*

Poly(ethylene glycol) (PEG) ($M_w$ ~ 35kDa, Sigma Aldrich (USA)) and gelatin were used to engineer the injectable hydrogels. Diacrylation of PEG was carried out by dissolving 10 grams of solid PEG in 30 mL of dichloromethane in a flask. The flask was purged and vented for 5 minutes with nitrogen gas to remove any water vapor. Triethylamine was added into the flask in 5 molar excess of end hydroxyl groups. Using an addition funnel, 5 molar excess of acryloyl chloride in dicholormethane was added drop wise to the flask. The flask was kept in an ice bath for the duration of the reaction. The solutions was washed and precipated into ice col diethyl ether and placed for drying in the vacuum oven.

### *Fabrication of single-crosslinked double polymer injectable hydrogels*

The prepolymer solutions were prepared in 2.5 ml falcon tubes. Half a milliliter of distilled water and half a milliliter of 0.6% Irgacure 2595 photo initiator solution (created through dissolving PI in water) were added to each tube. The outside of the tubes was covered in aluminum foil to block light entry and prevent undesirable, premature cross-linking. The appropriate combinations of PEG and gelatin were used to prepare the 1 ml solutions of each concentration. The modified PEG (PEGDA) and gelatin were added in powder form to each falcon tube. For example, in the 0/10 (% PEGDA/% Gelatin) tube, 0 grams of PEGDA and 0.10 grams of gelatin were mixed along with the water and photo initiator solution to form the prepolymer blend. These blends were immediately vortexed, sonicated, or put in the oven at 40 degrees Celsius to break up small particles and dissolve the polymers in solution. The same whole process was repeated for each concentration.

Approximately 250-400 microliters of the prepolymer solutions were pipetted onto a hydrophobic surface (a circular, drop-like structure was made). The surfaces with polymer were exposed to ultraviolet light for one minute at an intensity of 1 $W/cm^2$ to crosslink the hydrogel. After crosslinking, the hydrogels were carefully removed from the hydrophobic surface using a thin glass micro slide and transferred into a well plate with 1-2 milliliters of distilled water in each well. The hydrogel was allowed to swell in water for approximately 24 hours. The procedure was repeated four more times for each concentration for a total of 35 hydrogel samples. An alternate method for creating the hydrogels involved making them in sheets. A 5 mm diameter hole punch was used to form the circular gel shape in these hydrogels for mechanical testing. This method tended to produce more reliable results since the shape was consistent throughout all the hydrogels.

***Mechanical and Hydration Analysis***

To evaluate the biomechanical properties (strength and elasticity) of the hydrogels, a mechanical compression tester was used. The hydrogels were removed from 24 hour swelling in the well plates and loaded onto the mechanical tester. Each circular gel was compressed to 50% its height for one cycle (compress, decompress). The stress, strain, modulus, and toughness of the hydrogels were calculated through the data obtained from the compression tester. The gels were remade, lyophilized (freeze dried), and weighed to calculate their water content. Finally, the hydrogels were remade again and swollen in water for a total of 48 hours. Each gel was weighed at different time points to determine its water absorption over the span of two days. After each weighing, the gels were immediately put back in the well plate to continue swelling.

***3D Bioprinting the injectable hydrogels*.**

The PEG and gelatin prepolymer solutions were made in large amounts for work with the bioprinter (Hyrel System 30M). Approximately 10 milliliters of the higher viscosity bioink solutions (10/10 and 15/10 PEG/gelatin) were used for printing. Before printing, the inks were extruded from a plastic 20-milliliter syringe to test their viscosity and mechanical flow. If the materials exhibited shear-thinning properties (flowed when stress was applied, i.e., toothpaste) through extrusion, they were used with the bioprinter. The injectable hydrogels with 10% PEG and 10% gelatin had robust material properties, but failed to extrude as a gel from the bioprinter. To combat this problem, nanosilicates (Laponite B) were added to this concentration in the following amounts: 10% PEG/10% Gelatin/(0, 1, 2, 3, 4, 5)% Nanosilicate. The nanocomposite hydrogels were mechanically tested using the same procedures in *Mechanical and Hydration Analysis*. The nanosilicates were expected to aid in the extrusion and printing process. The hydrogels were extruded with the syringe and moved to bioprinting applications. The printer settings were adjusted to accommodate the viscosity and material properties of the bioink. A disk-like design was creating using solidworks software and sent to the printer, which constructed the disk. The process was repeated multiple times with different concentration bioinks in order to determine the best formulation for 3D bioprinting.

The methods for constructing and printing were slightly modified to accommodate higher concentrations of the biomaterial (more viscoelastic materials were heated, vortexed, and sonicated longer).

## RESULTS/DISCUSSION

### Polymer Synthesis and Hydrogel Fabrication

FTIR analysis of PEG-DA and Gelatin are shown in Figure 1. As observed, FTIR spectra of PEG presents the typical bands of C-H stretch ca 2900 and C-O stretch ca 1100. Gelatin exhibits characteristic amide absorption bands around ca 1600, 1700, 3200. Scaffolds were created according to Figure 2. In short, Gelatin and PEGDA were varied from 0-15% (wt./vol) while silicate nanoparticles were varied from 0-5% (wt./vol). The prehydrogel solution was dissolved in di$H_2O$ with IRGACURE 2959 (0.25% wt./vol).

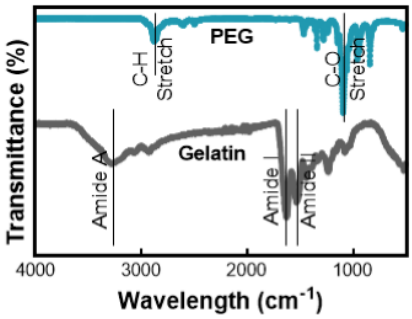
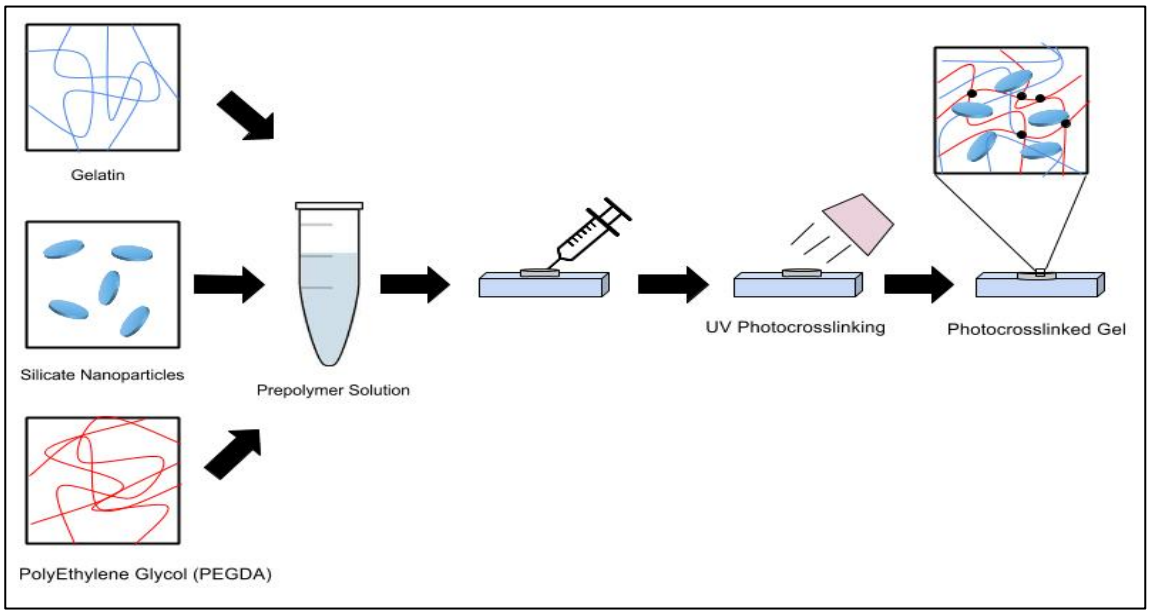



Figure 1. Fourier Transmission Infrared (FTIR) Spectroscopy of Poly(ethylene) Glycol and Gelatin

Solutions were pipetted and UV cured for ca 2 min. A depiction of internal hydrogel structure is shown in Figure 2 (far right). Since gelatin has not been modified it is unable to covalently crosslink within the network. Rather, it is suspected that an interpenetrating network is formed where PEG-DA bond have formed a covalent network and Gelatin is interacting with the nanoparticle.



## Mechanical Characterization

Compression modulus was examined by two by two experimental designs: (1) concentration of PEG-DA was fixed while Gelatin concentration increased and (2) Gelatin was fixed with increasing PEG-DA concentration. Ultimately, 10% PEG/10% Gelatin was chosen to add silicate nanoparticles to due to a significant difference in the stress at 50% strain Additionally, since the 10/10 composition represents a "middle of the road" formulation it is easier to draw conclusions about other compositions should silicates

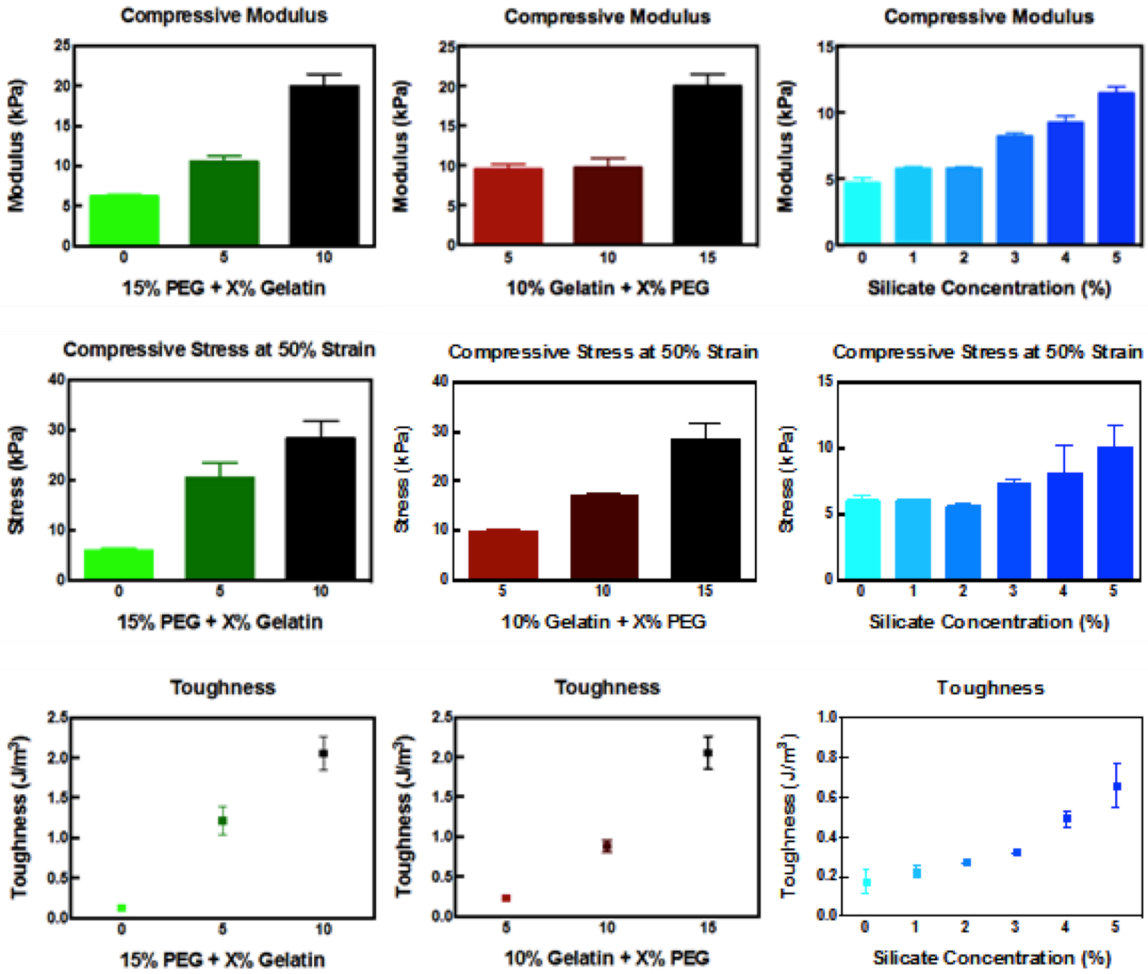



Figure 3. Mechanical Data for injectable nanocomposite hydrogels. (a) Compressive Moduli (kPa) of the injectable hydrogels with increasing Gelatin concentration, increasing PEG concentration, and increasing nanosilicate concentration with 10% PEG and 10% Gelatin respectively. (b) Compressive Stress (kPa) at 50% Strain of the injectable hydrogels with increasing Gelatin concentration, increasing PEG concentration, and increasing nanosilicate concentration with 10% PEG and 10% Gelatin. (c) Toughness ($J/m^3$) of the injectable hydrogels with increasing Gelatin concentration, increasing PEG concentration, and increasing nanosilicate concentration with 10% PEG and 10% Gelatin.

be added. With the addition of silicates we observe an increase in the compressive modulus. This increase is attributed to several synergistic factors. One factor is the close packed nature of adding in more silicates. Much like a filler, a higher concentration of silicates will pack in the same volume therefore leading to a more solid-like material. With an increase of silicate, it is expected that there are more polymer-silicate interactions which could also be leading to an increase in compressive modulus (Figure 3XXX). Toughness was characterized as a measure of the amount of energy the hydrogel samples can absorb before fracture. Here we observe an increase of toughness with an increase in polymer and silicates concentration (Figure 3XXX). An increase in polymer concentration causes an increase in toughness simply due to there being a higher amount of polymer. We speculate that per polymer chain the toughness should not change. However, it is interesting to note that there is a large increase from 10 to 15% PEG when added to 10% Gelatin. This is attributed to chain entanglements.

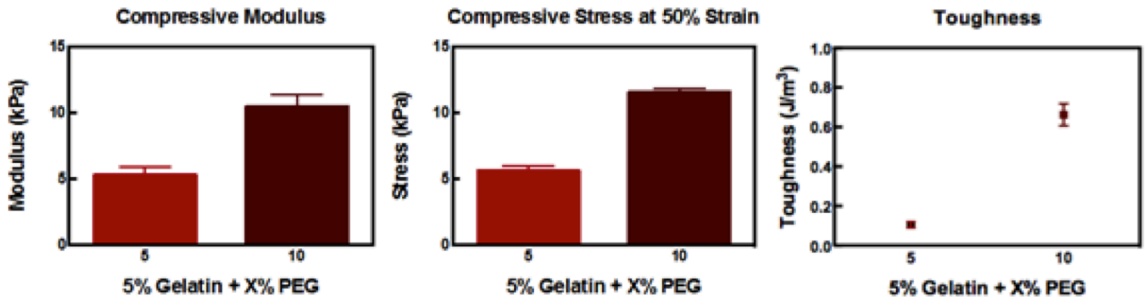



Figure 4. Mechanical Data for injectable hydrogels with low gelatin concentration. (a) Compressive Moduli (kPa), Compressive Stress at 50% Strain, and Toughness ($J/m^3$) of the injectable hydrogels with increasing PEG concentration

Hydration degree and swelling profiles were examined to determine how the addition of a fluid changes the hydrogel. While all previous experiments were carried out on as prepared samples, the importance of swelling and hydration degree will be determined by the materials material properties.

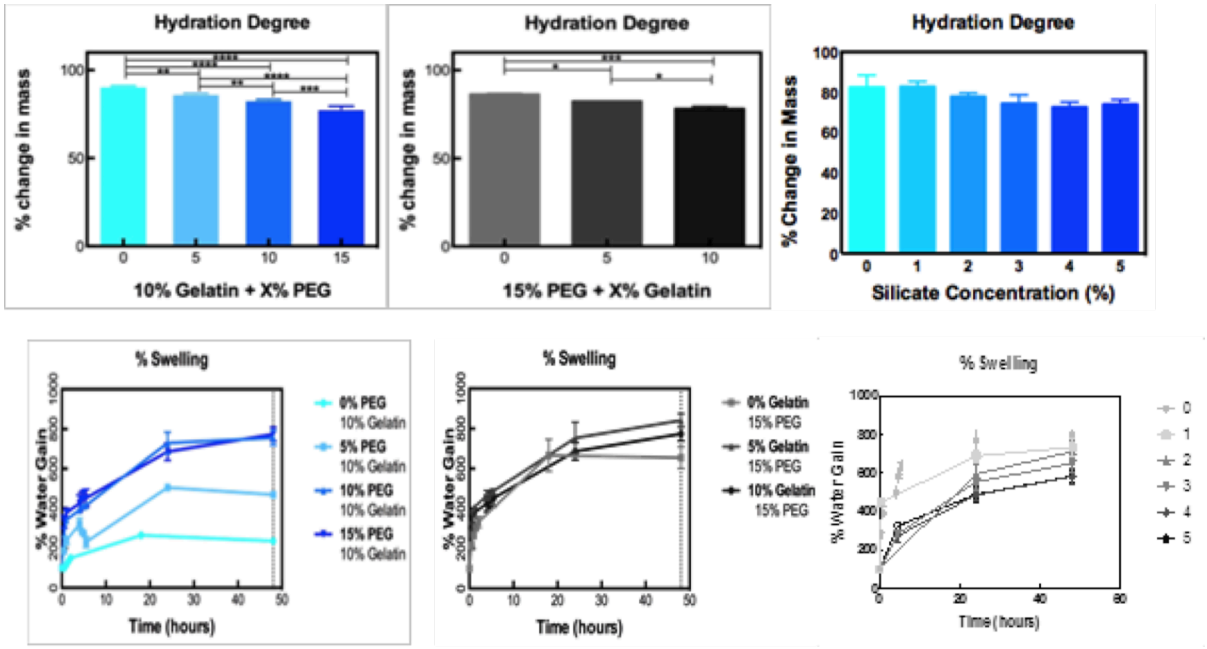



Figure 5. Hydration Data for injectable nanocomposite hydrogels. (a) Hydration Degree (% change in mass after lyophilization) of the injectable hydrogels with increasing Gelatin concentration, increasing PEG concentration, and increasing nanosilicate concentration with 10% PEG and 10% Gelatin respectively. (b) Percent water gain of the injectable hydrogels with increasing Gelatin concentration, increasing PEG concentration, and increasing nanosilicate concentration with 10% PEG and 10% Gelatin.

## CONCLUSION

The results demonstrate the desirable properties of injectable nanocomposite hydrogels that were set out to achieve and test. First, the hydrogels exhibit mechanical properties such as enhanced toughness and elasticity. Second, the biomaterial has sustainable properties that allow it to remain effective as an implantable and injectable biomaterial. Specifically, the degradation property of this material provides a temporary solution to tissue and organ failure, and facilitates its quick and smooth exit from the human body after implantation. Third, the injectable hydrogels exhibit shear-thinning properties, which are useful for injectable treatments.[15] Lastly, the hydrogels ability to change shape is vital to accommodate the various tissues and cells present in the body. These injectable hydrogels promote the growth of stronger tissues by relieving the burden of the damaged tissue or organ. Thus, this bioink overcomes the hurdles that have confronted prior researchers in this area.

Previous research in this area focused on creating tough and durable hydrogels, but strayed from 3D printing, as it jeopardized the material properties.[16, 17] The new polymer blends created by this project photocrosslink through extrusion, which allow for tunable mechanical properties. Other methods for crosslinking injectable hydrogels include temperature (thermal), ionic, and enzymatic.[18, 19] However, thermal crosslinking requires lengthy gelation times and ionic post-crosslinking results in fragile constructs. The nanosilicates in the injectable hydrogels hold the hydrogel network together through 3D printing, retaining the desirable mechanical properties. The gelatin in the hydrogel scaffold contributes to biocompatibility and biodegradability. Additionally, the polymers used in this research do not require support materials and gelate within minutes. This research utilizes the advantages of the polymer chemistry behind hydrogels and the mechanics of bioprinting to generate robust and complex biomaterials for long-term medical applications. Thus, this research significantly extends our understanding and application of injectable nanocomposite hydrogels.

The enhanced understanding of biomaterials that enable 3D bioprinting of living tissues and organs can reveal important conclusions about the properties of different injectable hydrogels. Some classes of hydrogel bioinks may have common features; others may have differing features. However, the viability of the injectable nanocomposite hydrogel developed in this research is an important initial step in moving forward to a superior understanding of bioinks and bioprinting in the future.

A minor limitation to this hydrogel design is the usage of ultraviolet (UV) light, which could pose a threat to future cells seeding, similar to some other biomaterials.[20] Another drawback is the use of a synthetic material that may risk rejection by the human body. Viscosity of the injectable hydrogels is an important parameter in the bioink's design that allows ease of interaction with 3D printers. The bioink viscosities can be modulated through nanosilicate and gelatin concentrations. Additional research is needed to develop an improved injectable hydrogel bioink that resists potentially harmful exposure to UV radiation.

## FUTURE IMPACT AND APPLICATIONS

Although new injectable nanocomposite hydrogels with the desirable toughness and biocompatible properties were successfully created and tested in the laboratory, this research is still in progress. In the next phase, this research will continue to focus on material characterization before moving onto cell studies on the specific material. In the future, we hope to 3D print more complex bioink constructs and test their compatibility with cells from the human body.

My newly developed hydrogel has important future applications in medicine. This hydrogel bioink can be used to print custom-made implants that fit directly into a patient's body. The injectable hydrogels offer immediate access to scaffolds that the body may use in reconstruction. It will likely have an immediate significant impact in the areas of biomedical devices, wound and surgical sealants, drug delivery, and microfluidics as these areas are ripe for the utilization of such a material.[21]

Bioprinting will also likely lead to a convergence of disciplines and professionals. Doctors, engineers, and scientists across many disciplines are increasingly learning to manipulate living tissues at the cellular level. Such efforts can lead to extension of all forms of life.

The usefulness of these injectable nanocomposite hydrogels extends beyond tissue and organ implants. The material and methodology are generalizable to other contexts and can be used in areas such as sensor and actuator technologies, robotics, lithography, and batteries. Technologies in these areas are at a point where a different methodology such as one using a cell-compatible resilient material.[22]

Injectable hydrogels and 3D bioprinting will also have significant effects on related areas like bioadditive manufacturing, biofabrication, biomimetic printing, and even 4D bioprinting. 4D bioprinting goes beyond 3D bioprinting through pre-programmed and anticipatory changes to the bioprinted parts even after printing. These changes may include shape-morphing, cellular differentiation, and tissue pattern formation, reflecting a level of adaptability that mimics common biological systems. Such a development is somewhat far away given that 3D bioprinting is not yet fully commercial. However, such forward thinking is critical in the continued improvement of injectable nanocomposite hydrogels and the advancement of 3D bioprinting.

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