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Injectable Gelatin-Silk Fibroin Composite Hydrogels for *In Situ* Cell Encapsulation

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Abstract

Hydrogels are widely used tools for tissue engineering and regenerative medicine. Characterized as biofunctional, water-based polymer matrices with tunable mechanical properties, hydrogels have promising but limited applications in biomedical engineering, due to poor and static matrix strength. Here we plan to rectify this issue by introducing a new hydrogel made from a composite of gelatin and silk fibroin crosslinked by microbial transglutaminase (mTG) instantly and beta sheet formation gradually, respectively. This interpenetrating network (IPN) shows enhanced mechanical stiffness and strength compared to gelatin hydrogels, and is capable of encapsulating human cells with high viability demonstrated by the encapsulation of human dermal fibroblasts (hDFs). We also demonstrate that this hydrogel can encapsulate mesenchymal stem cells (MSC) as well as differentiate them into osteoblasts by dynamically stiffening the matrix over at 14-day period. These results indicate that this injectable hydrogel can be a great asset for tissue regeneration applications.

Introduction

Hydrogels are water-containing hydrophilic scaffolds widely used in regenerative medicine because of their comparability to the extracellular matrix[1]. Characteristics such as mechanical stiffness, biocompatibility, and injectability are important considerations when tuning the hydrogel and choosing the components to match the desired function. Hydrogels can be made from many hydrophilic polymers including collagen[2], polyethylene glycol (PEG)[3], gelatin[4], alginate[5], silk[6], or any combination. They are limited in mechanical stiffness due to their high-water content and usually need to be modified through crosslinking mechanisms to become applicable. Crosslinking connects the singular polymer chains together, physically or covalently, to stiffen the substrate as a whole[7]. Previous successfully fabricated hydrogels use a combination of gelatin and silk fibroin polymers with the crosslinking of each by ethanol and the enzyme microbial transglutaminase (mTG) respectively, to create what is called an interpenetrating network[8]. An interpenetrating network is formed when polymer chains are woven together with independent crosslinking mechanisms to create a rigid structure. A stiffer hydrogel is formed using this technique, however there are limitations to the injectable properties[8]. The ethanol-based crosslinking mechanism of silk fibroin results in a cytotoxic environment for cellular life, producing a mechanically stiff hydrogel that is unable to encapsulate cells or injected *in vivo*[8].

Gelatin is a product derived from collagen, which is what natural tissue, and the extracellular matrix consists, of. Hydrogels made from gelatin model as viable environments for cellular life *in situ* promoting successful proliferation[9]. For the covalent crosslinking of gelatin, the mTG-based process forms amide bonds between lysine and glutamine residues[10]. This ultimately allows for the gelatin hydrogel to adhere to the target tissue. Though gelatin chemically crosslinked with mTG creates a useful hydrogel, it is still not strong and stiff enough for many biomedical applications.

Silk fibroin is a protein extracted from the silkworm cocoon of the *Bompyx mori*, known for its biocompatibility and mechanical strength[11]. It is composed of anti- parallel, free moving beta sheets that, when crosslinked, connect together to form a stiffer structure. These beta sheets can be induced by the utilization of many different stimuli including temperature, pH, and mechanical perturbations[11]. A characteristic unique to silk fibroin is its ability to dynamically stiffen over time. Silk fibroin can be crosslinked slowly (~ over a 1-week time interval) by an incubation at 37°C[11] or rapidly (~ over a 5-minute time interval) if sonicated prior to 37°C

incubation[12]. If a cellular encapsulated hydrogel is stiffened too rapidly, the cells will be prohibited from spreading into the matrix and proliferating successfully. If the matrix remains mechanically weak, it will not be able to support cells that require a stiffer matrix, for example osteoblasts and chondrocytes[13]. This is a limitation found in many hydrogels.

Here an injectable hydrogel is created utilizing gelatin, covalently crosslinked with mTG, and silk fibroin, physically crosslinked by heat-induced beta-sheet formation, to create a dynamically stiffening hydrogel for mesenchymal stem cell (MSC) differentiation into osteoblasts. With the major impact that hydrogels have in the medical field, creation of an injectable artificial tissue consisting of a gelatin-silk fibroin composite hydrogel that includes high mechanical strength and cell friendly crosslinking mechanisms to perform *in situ* cell encapsulations is required. Utilizing properties of silk fibroin, the gelatin-silk fibroin composite injectable solution will become stiff gradually over one week allowing for the proliferation of MSCs to occur and then the differentiation into osteoblasts to take place over two weeks. This injectable scaffold will be useful in the biomedical engineering field for tissue engineering and regenerative medicine.

Methods

Preparation of the Gelatin-Silk Fibroin Injectable Scaffold

Gelatin from porcine skin (300g bloom, Sigma-Aldrich, USA) was mixed with Phosphate Buffer Saline (PBS; pH 7.4; Sigma-Aldrich, USA) to create a 10% (w/v) stock solution and incubated to bring the temperature up to 45ºC. The gelatin solution was vacuum sterilized with a 0.22 µm filter to create a sterilized solution for use. 5% silk fibroin solution was obtained from Advanced BioMatrix and sterilized through a 0.22 µm filter. Care was taken to push the silk fibroin slowly through the filter as to minimize shear stress. MTG powder (Ajinomoto, Japan) was taken out of 4ºC and mixed with PBS to create a 30% (w/v) stock solution. The mTG solution was incubated to 45ºC and then filter sterilized through a 0.22 µm filter. The three solutions were then added to a 24 well plate accordance to the final concentrations in **Table 1**. The well plate was then incubated at 37ºC for 7 days. Careful consideration was taken to insure insignificant evaporation during this time. Once gelled, the hydrogels were removed from the well plate and tests were performed on day 1 and day 7 of the incubation period.

Table 1.

	Hydrogel 1	Hydrogel 2	Hydrogel 3	Hydrogel 4
Silk	2.5%	1.25%	0%	0%
Gelatin	5%	6.25%	7.5%	5%
mTG	5%	5%	5%	5%

Table 1. Final concentrations of each hydrogel solution created.

Rheological Characterization of the Hydrogels

Rheological analysis was implemented on the Texas Instruments AR 550 Rheometer. The hydrogel was placed on the stage and all measurements were taken with the geometry 20 mm stainless steel plate, an angular frequency of 0.1-100 (rad/sec), and an oscillation stress of 2. The storage and loss modulus were obtained for each hydrogel and compared.

Compressive Characterization of the Hydrogels

The Universal Mechanical Tester was used to perform compressive analysis of the hydrogels. All measurements were created by forming a load on the hydrogels, decreasing the distance between the stage and the plate by 5 mm/minute until a load of 50N was measured. The initial average thickness and diameter of the hydrogels were 3.19 mm and 15.27 mm respectively.

Beta Sheet Characterization

Beta-sheet formation was confirmed by the use of the Thermo Nicolet iS10 Fourier Transform Infrared Spectrophotometer. A resolution of 4 was used to take 128 scans.

Cell Culture and Encapsulation

Mesenchymal Stem Cells (MSCs) or Human Dermal Fibroblasts (hDFs) (Cell Applications, USA), depending on the experiment, were seeded on a T75 flask at 500,000 cells/cm^2. Cells were allowed to become 80% confluent, and then detached and added to the hydrogel solutions at a density of 600 cells/µL as follows. 5mL of PBS were added to the T75 flask and the flask was mixed for 2 minutes. The PBS was aspirated, and 3 mL of TrypLE Express solution (Gibco, USA) was added to the flask. PBS is used to rinse the cells before treatment of TrypLE Express

to remove any excess calcium from the cells. Calcium is needed to create proteins that aid the cells in adhering to the bottom of the flask. TrypLE Express is then added to cleave the proteins in the cells. It initially cleaves the proteins adhering the hDFs to the flask resuspending them in solution but then will start cleaving other proteins in the cells, which could kill them. Growth media is added later to neutralize the TrypLE Express by incorporating an abundance of proteins into the solution, keeping the enzyme occupied and away from the cell proteins.

The flask was incubated at 37ºC for 5 minutes, shaking of the flask occurred at 2.5 minutes. The cells were visualized under the microscope (EVOS Life Technologies) to confirm that they had detached from the flask and the 10 mL of growth media (Gibco, USA) were added. The mixture was transferred to a conical tube and the contents were centrifuged at 200xg for 5 minutes. The supernatant was aspirated, and growth media was added to resuspend the pellet of MSCs, or hDFs, to create the desired concentration.

Gelatin was mixed with growth media to create a 10% (w/v) stock solution and incubated to 45ºC. The gelatin solution was vacuum sterilized with a 0.22 µm filter to create a sterilized solution for use. 5% silk fibroin solution was sterilized through a 0.22 µm filter. MTG powder was taken out of 4ºC and mixed with growth media to create a 30% (w/v) stock solution. The mTG solution was incubated to 45ºC and then filter sterilized through a 0.22 µm filter. The three solutions were then added to a 24 well plate accordance to the final concentrations in **Table 1**.

The solution of cells was added to the prepared hydrogel solution to create a final concentration of 600 cells/µL in each well. The hydrogels were then incubated at 37ºC for 60 minutes, until gelled, and removed from the 24 well plate. The hydrogels were placed in a 12 well plate and 1 mL of growth media was added to submerge the hydrogel. The hydrogels were then incubated at 37ºC for 7 days, with a change in media every 24 hours. After day 1 incubation of the MSCs, the media was switched to osteoblast differentiation media (Gibco, USA). The hDFs remained in growth media throughout the experiment.

Cellular Viability Characterization

Cellular viability was measured by performing a Live/Dead Viability/ Cytotoxicity Kit (Invitrogen; ThermoFisher Scientific, USA) and visualizing the results with confocal microscopy. Calcein-AM and Ethidium Homodimer were removed from -20ºC and defrosted. Special care was taken to keep the fluorescent solutions away from the light. 5 mL of Dulbecco's

Phosphate Buffer Saline (DPBS; Gibco, USA), 5 µL of Calcein-Am, and 20 µL of Ethidium Homodimer were combined and made into a homogenous solution. The hDF media was aspirated out of the wells and 1 mL of this solution was added to each well to submerge the hydrogels. The well plate was incubated for 60 minutes, agitating every 10 minutes to incorporate the solution into the hydrogels. Special care was taken to keep the hydrogels away from the light. The well plate was then wrapped in foil and the hydrogels were visualized under the confocal microscope. The Nikon A1R laser scanning Confocal Fluorescence Microscope was used to visualize the fluorescence. The Z stack captured the location and viability of the cellular life in the 3D hydrogel.

Differentiation Characterization

The alizarin red assay was used to measure the osteoblast differentiation of the MSCs in the hydrogel. 2% (w/v) Alizarin Red S solution (Sigma-Aldrich, USA) was prepared. MSC differentiation media was removed from the wells containing hydrogels and they were rinsed twice with PBS. The hydrogels were then fixed with 4% (v/v) paraformaldehyde for 24 hours and then rinsed twice with PBS. 2 mL of Alizarin Red S solution was added to each well and incubated at 25ºC for 30 minutes. The solution was then removed, and the wells were rinsed with PBS until the control group, a hydrogel without MSCs, was clear. The hydrogels were then imaged.

Results and Discussion

The hydrogels without cells were prepared as described in the methods section and rheological testing was performed to test their stiffness over various angular frequencies to confirm the desired viscoelastic properties. The output value, storage modulus, is represented by the symbol G' and the greater this value is, the stiffer the hydrogel is. Rheology was performed on day 1 and day 7 to measure the dynamic stiffening properties of the slowly crosslinked silk fibroin. Since the beta sheet formation within silk fibroin does not occur immediately, it was hypothesized that the hydrogels would have a bigger G' value on day 7 than on day 1. As shown in **Figure 1**, the hydrogels containing gelatin and silk (hydrogel 1 from **Table 1**) became stiffer as time increased, starting at a G' value of 1800 Pa on day 1 and ending with a G' value of 2500 Pa on day 7 for angular frequencies from 0.1 rad/sec to 100 rad/sec. No significant increase in stiffness was

found in hydrogels 2, 3, and 4. Degradation of gelatin overtime can cause the storage modulus to decrease in hydrogels containing low concentrations of silk fibroin.

Figure 1

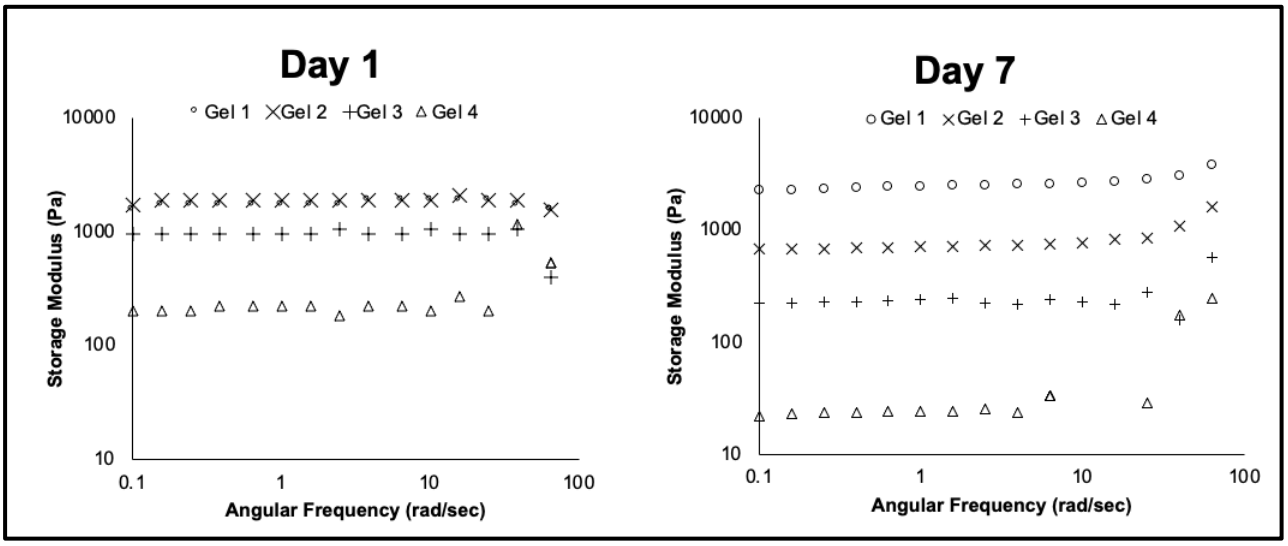

Figure 1. Rheological data of hydrogels including four different concentration combinations of silk fibroin and gelatin. On day 1, gel 1 and gel 2 have the greatest G' value. These gels have concentrations of silk fibroin. Gel 4 has the smallest G' value and has the lowest concentration of polymer solution. On day 7, gel 1 has the greatest G' value and the greatest concentration of silk fibroin whereas gel 4 still has the smallest G' value.

Compressive testing was performed on the hydrogels utilizing the Universal Mechanical Tester. The Universal Mechanical Tester is a machine that measures the compressive strength of a substance by applying a load to the material until a predetermined normal force is reached. It then measures how far it had to move to achieve that load. The amount of force required to change the thickness of the hydrogel can be found and used to find the relationship between the stress and strain. Young's modulus, also known as the compressive modulus, can be obtained from the initial linear stress-strain relationship. The stiffer the material is, the greater Young's modulus is. **Figure 2** depicts the

Figure 2

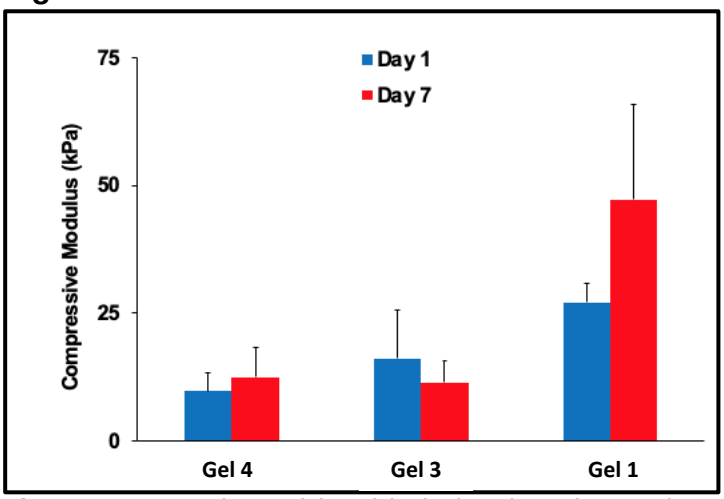

Figure 2. Compressive modulus of the hydrogels on day 1 and day 7 of 37ºC incubation. There is no statistical significance between the hydrogels on day 1 of incubation, confirming that they are initially soft. There is statistical significance between gel 3 and gel 1 on day 7 confirming that the silk-fibroin gradually stiffens over time compared to the control hydrogel.

hydrogels' Young's modulus on day 1 and day 7. There is statistical significance between the day 1 compressive modulus of gel 4 and gel 1. This is understandable because gel 4 has a lower polymer concentration than gel 1. There is no statistical significance between gel 3 and gel 1 on day 1, confirming the hydrogels containing the same concentration of polymer solution are initially the same stiffness, soft. On day 7, gel 4 is expected to have the smallest compressive modulus because it has the lowest polymer concentration. gel 4 and gel 3 do not contain silk fibroin therefore it was hypothesized that they would not become stiffer as time increased. There is statistical significance between gel 3 and gel 1 on day 7. The hydrogel containing silk fibroin increased significantly over the 7-day time interval whereas the control group did not, confirming the hypothesis that the gelatin- silk fibroin composite hydrogel will initially be soft but become stiffer overtime.

Figure 3

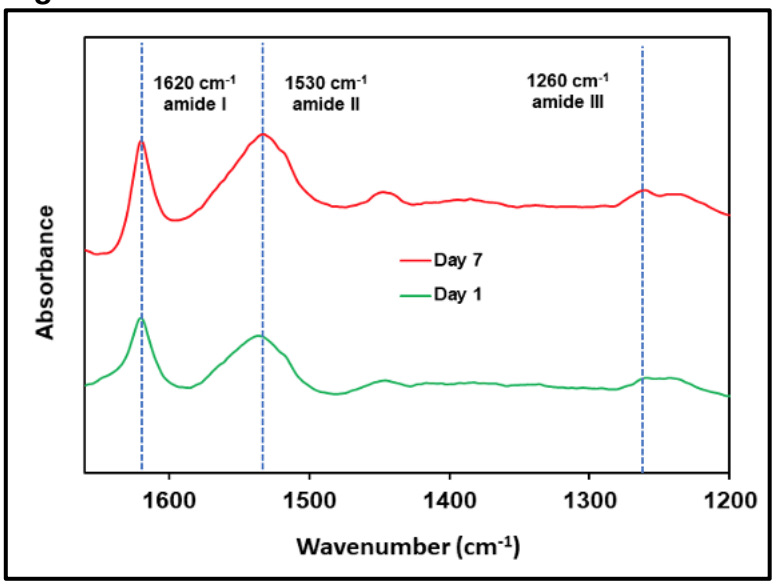

Figure 3. Absorbance of infrared light through hydrogel 1 on day 1 and day 7 of incubation at 37ºC. The amide functional regions are highlighted with the blue dotted line showing a slight increase in absorbance at those locations.

Fourier Transform Infrared Spectrophotometry (FTIR) was performed to visualize the formation of beta-sheets in the successfully crosslinked silk-fibroin of the hydrogels on day 1 and day 7. FTIR excites the vibrational energy of bonds in molecules utilizing IR, which allows for the identification of certain chemical functional groups within a material. When the light hits the crosslinked silk fibroin, the newly formed amide bonds vibrate differently than the control group, as shown in **Figure 3** for hydrogel 1. It was hypothesized that day 1 would not have beta-sheet formation and day 7 would have beta-sheet formation since the crosslinking of silk fibroin occurred over the 7-day time period at 37ºC. There is slight increase in the absorbance of the amide bond regions on day 7 compared to day 1 confirming beta sheet formation in the hydrogel.

After the matrix properties were analyzed, cellular encapsulation was performed with hDFs to visualize cell viability and proliferation in the injectable hydrogel scaffold. Confocal microscopy was used to visualize the cell viability in the hydrogels. Lasers of different wavelengths are used to fluorescently image individual layers of the hydrogel and stack them on top of each other (Z stack) to create a 3D composite image of the hydrogel. In order to observe the cells, they were stained with florescent dyes from the Live/Dead Viability/ Cytotoxicity Kit. Calcein-AM stains the living cells green and ethidium homodimer stains the dead cells red. Calcein-AM is a hydrophobic dye, allowing it to penetrate through the hydrophobic lipid bilayer cell membrane of living cells. It can also penetrate through dead cell membranes because those are "leaky" and open to the outside environment. Once inside a living cell, the enzyme esterase cleaves the bond between calcein and AM separating the molecules. Calcein is a hydrophilic molecule with green fluorescence preventing it from leaving the living cells and ultimately staining them green. Ethidium homodimer is a hydrophilic molecule so it cannot penetrate living hydrophobic membranes[14]. It enters dead cells and fluoresces the cell nucleus red.

Figure 4

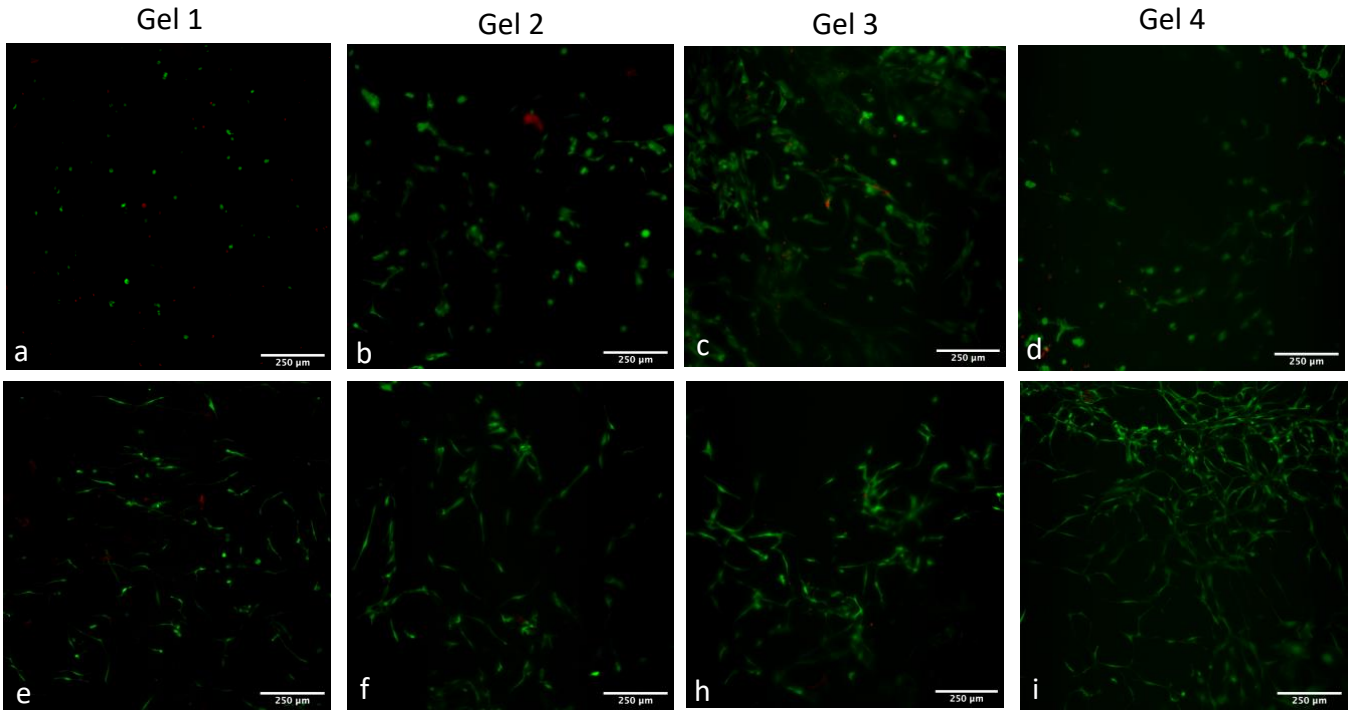

Figure 4. Confocal images utilizing the Live/ Dead assay to visualize the cell proliferation and viability of hDFs in the hydrogel matrix. (**a-d**) Day 1 hydrogels for each hydrogel showed good cell proliferation. Hydrogel 1 and 2 (**a**, **b**) showed round cell morphology whereas hydrogel 3 and 4 (**c**, **d**) were observed to have good, spread out cell morphology. (**e**-**i**) Day 7 hydrogels also showed good cell proliferation for each hydrogel. Hydrogel 1 and 2 (**e**, **f**) showed better cell morphology than on day 1. Scale bar: 250 µm

The lasers of the confocal microscope went through the layers of the hydrogel matrix, detecting the green and red fluorescence and imaging the cell morphology and viability. It was important to image the entire matrix to confirm cellular life throughout in order to replicate a native human tissue environment. As shown in **Figure 4**, on day 1 (**a**-**d**) of the confocal microscope testing, cells showed a round morphology in hydrogels 1 and 2 (**a**, **b**) and more of a "spread-out" shape in the hydrogels 3 and 4 (**c**, **d**). It is expected for this outcome because the silk fibroin is bioinert and does not promote cellular growth whereas gelatin does. There were many viable cells in all hydrogels. Day 7 samples (**e**-**i**) showed more dead cells, however, the cells that were living in the hydrogels 1 and 2 (**e**, **f**) had a more promising morphology than on day 1.

Once the cells were confirmed to successfully grow and proliferate inside the hydrogel matrix, their ability to differentiate MSCs into osteoblasts was observed. MSCs are important cells in tissue engineering because if their ability to differentiate into many cell types including adipocytes, chondrocytes, and osteoblasts. One of the factors that stimulates osteoblast formation is a stiff matrix. The stiffer the matrix, the more likely the MSCs will differentiate into the osteoblast cell lineage rather than the other lineages[15].

Figure 5

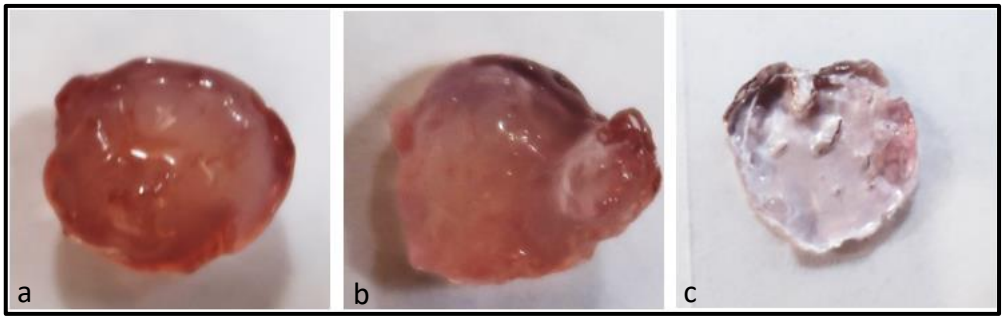

Figure 5. Images of the hydrogels stained with Alizarin Red S stain. (**a**) Hydrogel 1 contains the most calcium suggesting that is also contains the most osteoblasts. (**b**) Hydrogel 2 is also red confirming that the stiffening properties of the silk is creating a matrix habitable for osteoblasts. (**c**) Hydrogel 3 does not have any silk and therefore is not stiff enough to different ate the MSCs into osteoblasts.

Alizarin Red S stain was used to qualitatively measure osteogenic differentiation in the hydrogels. Alizarin Red has a binding affinity towards calcium, which is produced by osteoblasts. The more calcium present in the hydrogels, the redder in color the result. As shown in **Figure 5**, after two weeks of culture in the osteogenic differentiation medium, more osteogenic differentiation was observed. As the silk concentration increased in the hydrogel confirming that the gelatin- silk fibroin composite injectable hydrogel created a suitable mechanical environment for osteogenic differentiation.

Conclusion

Here, an injectable gelatin-silk fibroin composite hydrogel for *in situ* cell encapsulation is described. The hydrogel was formed using dual crosslinking mechanisms; mTG covalently crosslinking gelatin through amide bond creation and thermal induction physically crosslinking silk fibroin through beta-sheet formation. These crosslinking mechanisms are cell-friendly allowing for the cells to be introduced to the matrix in solution form resulting in an injectable hydrogel substance. The formation of beta-sheets was confirmed by the use of FT-IR, which led to significant stiffening over the 1-week time interval, as shown with rheological analysis and mechanical testing. The gradual dynamic stiffening of the matrix allows for cellular life to grow and proliferate inside the soft hydrogel before differentiating into osteoblasts as it stiffens. hDFs showed high viability and spread cell morphologies inside the gelatin- silk fibroin matrix supported by confocal microscopy Z stack images. More significant osteogenic differentiation of MSCs was observed in the hydrogels containing silk than the gelatin only hydrogels, by providing a stiffer environment which is known to be more conducive for osteogenic differentiation. Future studies can involve Computed Tomography (CT) quantitative analysis of the differentiation supplimenting the Alizarin Red S stain which measures the differentiation qualitatively. This injectable scaffold will be useful for tissue engineering and regenerative medicine.

Conflicts of Interest

There are no conflicts of interest.

Acknowledgements

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References

1. Lee, J. H., & Kim, H. W. (2018). Emerging properties of hydrogels in tissue engineering. Journal of tissue engineering, 9, 2041731418768285.
2. Helary, C., Bataille, I., Abed, A., Illoul, C., Anglo, A., Louedec, L., Meddahi-Pellé, A., & Giraud-Guille, M. M. (2010). Concentrated collagen hydrogels as dermal substitutes. Biomaterials, 31(3), 481-490.

3. Lin, C. C., & Anseth, K. S. (2009). PEG hydrogels for the controlled release of biomolecules in regenerative medicine. Pharmaceutical research, 26(3), 631-643.
4. Zhao, X., Lang, Q., Yildirimer, L., Lin, Z. Y., Cui, W., Annabi, N., Ng, K. W., Dokmeci, M. R., Ghaemmaghami, A. M., & Khademhosseini, A. (2016). Photocrosslinkable gelatin hydrogel for epidermal tissue engineering. Advanced healthcare materials, 5(1), 108-118.
5. Novikova, L. N., Mosahebi, A., Wiberg, M., Terenghi, G., Kellerth, J. O., & Novikov, L. N. (2006). Alginate hydrogel and matrigel as potential cell carriers for neurotransplantation. Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 77(2), 242-252.
6. Chao, P. H. G., Yodmuang, S., Wang, X., Sun, L., Kaplan, D. L., & Vunjak-Novakovic, G. (2010). Silk hydrogel for cartilage tissue engineering. Journal of biomedical materials research part B: applied biomaterials, 95(1), 84-90.
7. Hennink, W. E., & van Nostrum, C. F. (2012). Novel crosslinking methods to design hydrogels. Advanced drug delivery reviews, 64, 223-236.
8. Park, S., Edwards, S., Hou, S., Boudreau, R., Yee, R., & Jeong, K. J. (2019). A multi-interpenetrating network (IPN) hydrogel with gelatin and silk fibroin. Biomaterials science, 7(4), 1276-1280.
9. Gupta, A. K., Gupta, M., Yarwood, S. J., & Curtis, A. S. (2004). Effect of cellular uptake of gelatin nanoparticles on adhesion, morphology and cytoskeleton organisation of human fibroblasts. Journal of Controlled Release, 95(2), 197-207.
10. McDermott, M. K., Chen, T., Williams, C. M., Markley, K. M., & Payne, G. F. (2004). Mechanical properties of biomimetic tissue adhesive based on the microbial transglutaminase-catalyzed crosslinking of gelatin. Biomacromolecules, 5(4), 1270-1279.
11. Matsumoto, A., Chen, J., Collette, A. L., Kim, U. J., Altman, G. H., Cebe, P., & Kaplan, D. L. (2006). Mechanisms of silk fibroin sol− gel transitions. The journal of physical chemistry B, 110(43), 21630-21638.
12. Wang, X., Kluge, J. A., Leisk, G. G., & Kaplan, D. L. (2008). Sonication-induced gelation of silk fibroin for cell encapsulation. Biomaterials, 29(8), 1054-1064.
13. Oh, S. H., An, D. B., Kim, T. H., & Lee, J. H. (2016). Wide-range stiffness gradient PVA/HA hydrogel to investigate stem cell differentiation behavior. Acta biomaterialia, 35, 23-31.
14. Decherchi, P., Cochard, P., & Gauthier, P. (1997). Dual staining assessment of Schwann cell viability within whole peripheral nerves using calcein-AM and ethidium homodimer. Journal of Neuroscience Methods, 71(2), 205–213. doi: 10.1016/s0165-0270(96)00146-x
15. Zhang, T., Lin, S., Shao, X., Zhang, Q., Xue, C., Zhang, S., Yunfeng, L., Bofeng, Z., & Cai, X. (2017). Effect of matrix stiffness on osteoblast functionalization. Cell Proliferation, 50(3), e12338.