Silk-Gelatin Composite Hydrogels for Tissue Engineering

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Abstract

We have previously made a mechanically tough hydrogel by creating an interpenetrating network (IPN) of gelatin and silk fibroin. The resulting hydrogel exhibited a very high stiffness (~2 MPa) and excellent biofunctionality, promoting cell adhesion and proliferation on its surface. However, inducing cellular growth inside the hydrogel was challenging. Here, we introduce a new method of creating an injectable gelatin-silk fibroin IPN hydrogel which can be used to encapsulate mammalian cells inside the hydrogel. Results from a confocal microscope imaging supports cell proliferation on all layers of the hydrogel. Gelatin-silk IPN hydrogel resulted in higher cellular proliferation than the gelatin only hydrogel. This makes for a great asset in tissue engineering and regenerative medicine.

Introduction and Previous Results

Hydrogels possess many good properties to interface human cells and tissues. However, most hydrogels are too brittle and weak, severely limiting their applications in medicine and biotechnology.

We developed a new method of producing a mechanically tough hydrogel by creating a multi-interpenetrating network (IPN) between silk fibroin and gelatin (Park *et al.* 2019). Silk fibroin adds to the rigidity of the IPN, and gelatin provides biofunctionality.

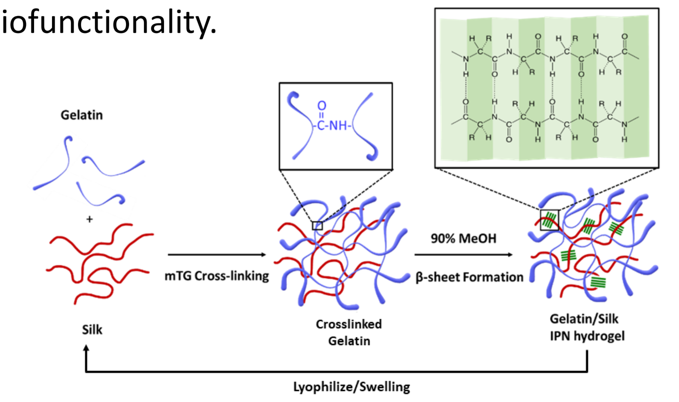


Figure 1. Schematic of the previous synthesis of the gelatin-silk fibroin IPN hydrogel. Gelatin was crosslinked by the action of an enzyme (mTG). Silk was crosslinked physically by the formation of β-sheets. This process of IPN formation was repeated on the same hydrogel.

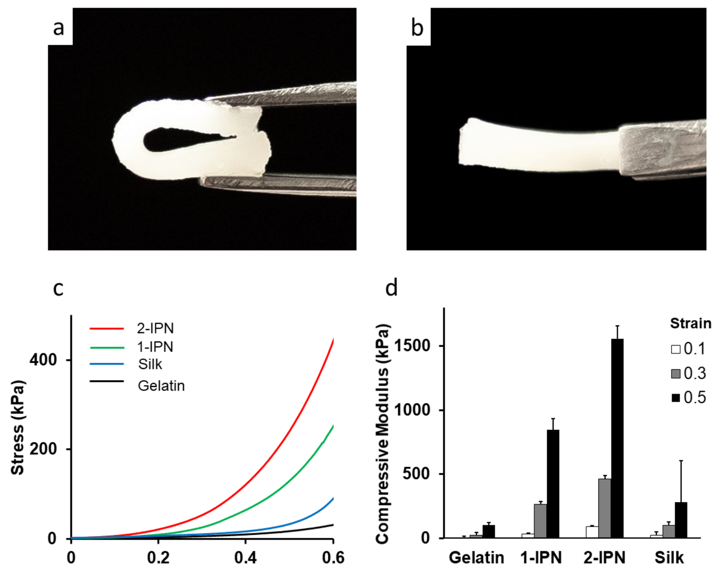
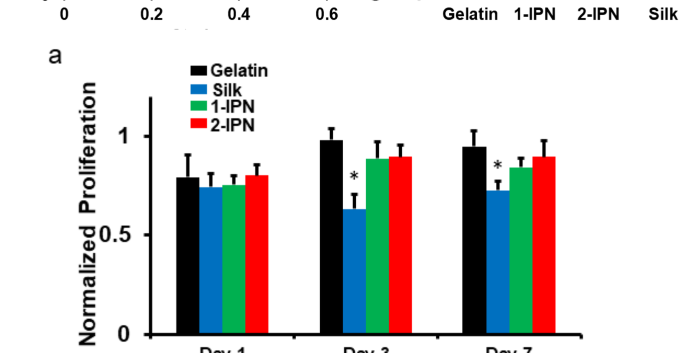


Figure 2. (a, b) Elasticity of the IPN hydrogel. The 2-IPN was completely bent, and retained its original shape when released[1]. (c) Compressive mechanical tests showing a strain-stress curve. (d) Compressive moduli of the hydrogel at 0.1, 0.3, and 0.5 strain.



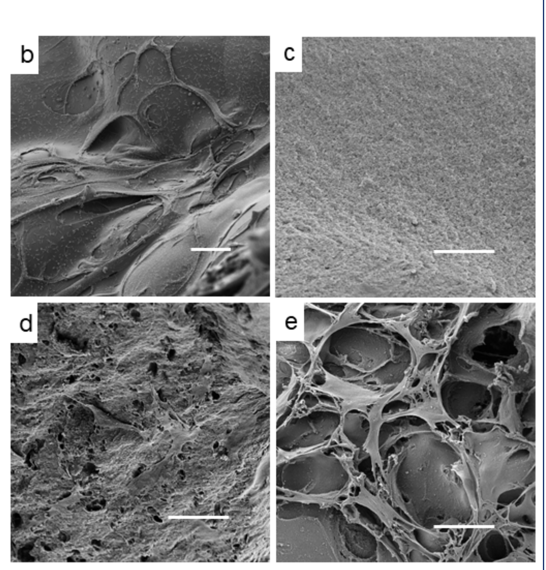


Figure 3. Proliferation of human dermal fibroblasts (hDFs) on hydrogels. The results were normalized to the cells on tissue culture polystyrene (TCPS).
* denotes $p < 0.05$ compared to the gelatin group ($n = 4$).
(b–e) SEM images on day 3. (b) gelatin only hydrogel. (c) silk-only hydrogel. (d) 1-IPN; (e) 2-IPN
Scale bar = 50 μm.

Limitations:

- Crosslinking of gelatin and silk fibroin involved harsh conditions for the cells (high temperature (50°C) and ethanol treatment).
- Cells could grow only on the surface of the hydrogels.
- Encapsulation of the cells within the hydrogel could not be achieved.

A new approach:
Create a mechanically tough IPN hydrogel between gelatin and silk using cell friendly crosslinking mechanisms, which will allow cell encapsulation for tissue engineering.

Silk-Gelatin Hydrogel for Cell Encapsulation

Hypothesis: Long-term incubation of mTG crosslinked gelatin-silk fibroin mixture at 37 °C will result in a mechanically tough IPN hydrogel.

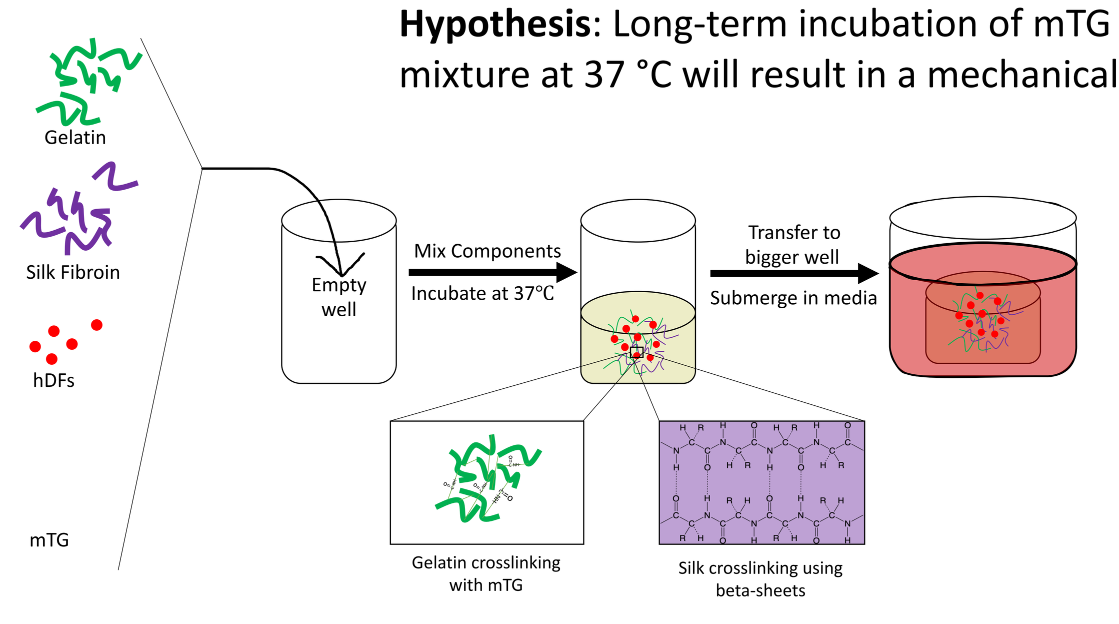
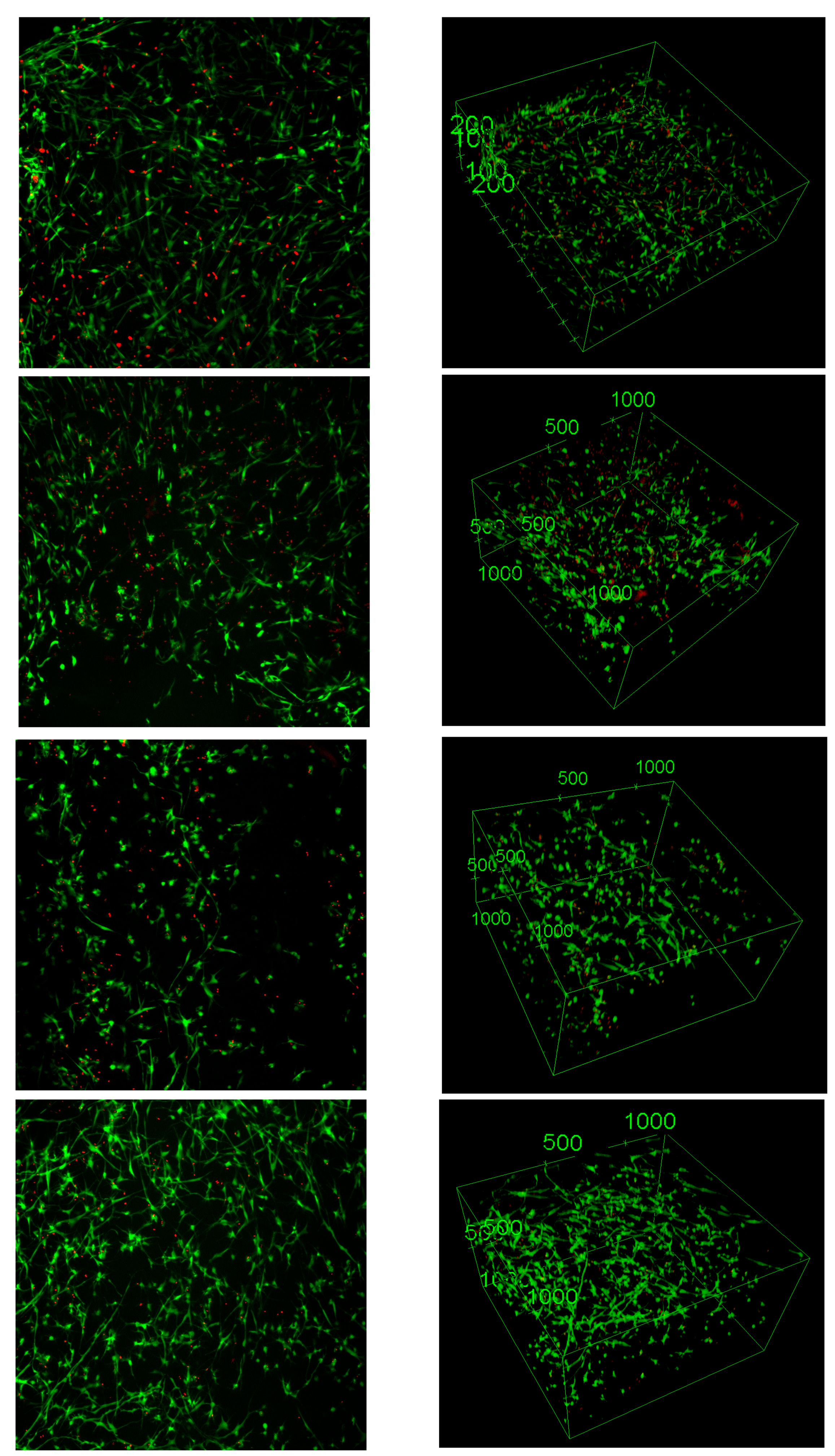


Figure 4. Schematic of synthesis of the injectable gelatin-silk fibroin IPN hydrogel. Gelatin was crosslinked with mTG and the silk fibroin was crosslinked by the formation of β-sheet structures. Human dermal fibroblasts (hDFs) were encapsulated within the hydrogels and grown in media.

	Gel 1	Gel 2	Gel 3	Gel 4	Gel 5*
Gelatin	5%	5%	4%	2.5%	0%
Silk	0%	2%	2%	2%	5%
mTG	5%	5%	5%	5%	0%

Table 1. Composition of different hydrogels tested in this research. *Confocal images were not done for Gel 5.

Live/Dead Assay Using Confocal Microscopy



Gel 1 (5% Gelatin)

Gel 2 (5% Gelatin+ 2% Silk)

Gel 3 (4% Gelatin+ 2% Silk)

Gel 4 (2.5% Gelatin+ 2% Silk)

Summary and Conclusions

- The new method of crosslinking resulted in stable silk-gelatin hydrogels, and hDFs were successfully encapsulated in these composite hydrogels.
- Proliferation was higher in the hydrogels containing a higher concentration of gelatin, while the addition of silk fibroin seemed to slightly hinder the growth of the hDFs.
- More tests are needed to draw a definite conclusion.
- The composite hydrogels introduced here are injectable and can be used as a vehicle for cell delivery and regenerative medicine.

Future Work

- Rheological characterization
  - Viscoelastic properties
- Mechanical testing
  - Stress-strain curves (both tensile and compressive)
- Encapsulation of mesenchymal stem cells (MSCs) and MSC differentiation into chondrocytes and osteoblasts.

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References

S. Park, S. Edwards, S. Hou, R. Boudreau, R. Yee and K.J. Jeong, "A multi-interpenetrating network (IPN) hydrogel with gelatin and silk fibroin" *Biomat. Sci.* (2019) 7, 1276-1280