Injectable Gelatin-Silk Fibroin Composite Hydrogels for *in situ* Cell Encapsulation

Ryann Boudreau[1]*, Rachel Yee[2]* and Kyung Jae Jeong[1]

[1]*Department of Chemical Engineering, University of New Hampshire, Durham, NH 03824*

[2]*Department of Molecular, Cellular and Biomedical Sciences, University of New Hampshire, NH 03824*

Introduction

Hydrogels are ideal materials to interface human cells and tissues due to their high water-content. Hydrogels can be made injectable and used for *in situ* cell encapsulation. However, most of the hydrogels for *in situ* cell encapsulation are mechanically weak because of the restrictions of the crosslinking methods for cytocompatibility. This limits their applications in regeneration of mechanically stiff or load bearing tissues. Mechanically strong and injectable hydrogels that can be used for cell encapsulation are highly needed.

Here, we introduce a new method of creating gelatin-silk fibroin interpenetrating network (IPN) hydrogel which can be used for the encapsulation of human cells. The hydrogel is formed initially by the enzymatic crosslinking of gelatin (< 10 min) by microbial transglutaminase (mTG), and further stiffening of the hydrogel is achieved by gradual thermal crosslinking of silk fibroin at 37 °C (over a week), forming an IPN between gelatin and silk fibroin (**Fig 1**). Initial soft hydrogel allows for the spreading and proliferation of the encapsulated cells, and the gradual hardening improves the mechanical strength of the hydrogel.

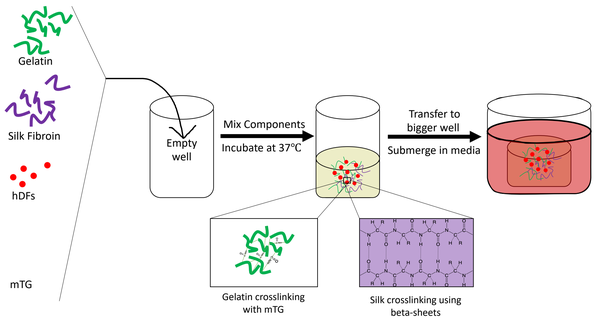

Figure 1. Schematic of synthesis of the injectable gelatin-silk fibroin IPN hydrogel. Gelatin is crosslinked with mTG, and the β-sheet formation of silk fibroin is induced thermally at 37 °C. Human dermal fibroblasts (hDFs) or human mesenchymal stem cells (hMSCs) were encapsulated within the hydrogels.

Hydrogel Compositions

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

Table 1. Composition of different hydrogels tested in this research. Concentrations are in weight per volume (w/v).

Rheology

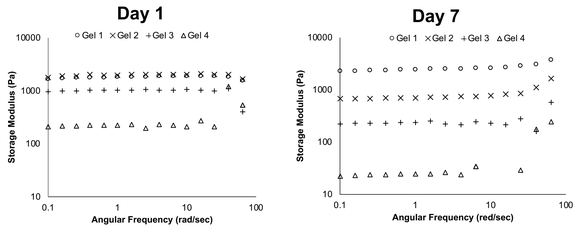

Figure 2. Frequency sweep of various hydrogels on day 1 and day 7. Storage modulus of Gel 1 increased over 1 week. Loss moduli were orders of magnitude smaller than storage moduli for all samples.

Mechanical Testing

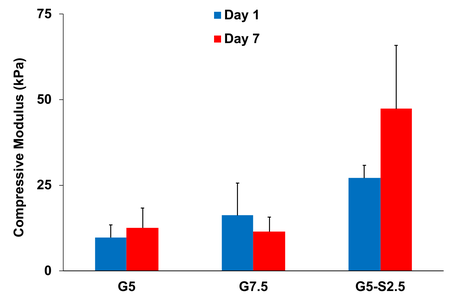

Figure 3. Compressive modulus. Addition of silk fibroin to gelatin hydrogel slightly increased the modulus on day 1, compared to gelatin-only hydrogels. The measurements were performed by compressing disc-shaped hydrogels (diameter = 15.5 mm) at 5 mm/min. There was a significant increase in compressive modulus of silk-containing hydrogel by day 7 due to the gradual thermal crosslinking of silk fibroin. Data are averages and standard deviations. (N = 3)

FT-IR

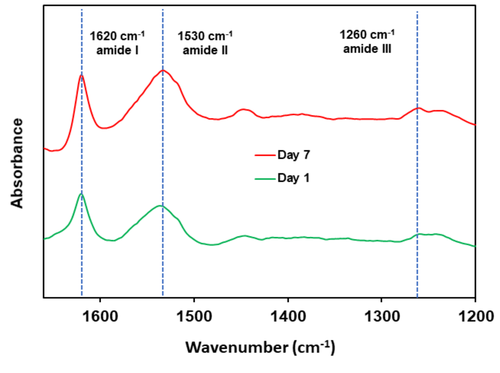

Figure 4. Fourier-transformed infrared spectroscopy (FT-IR). The spectra were obtained by subtracting the absorbance spectrum of Gel 4 from that of Gel 1. Both on day 1 and 7, the absorbance peaks corresponding to the beta sheets of silk fibroin are evident. The peak heights increased from day 1 to day 7, proving that the hydrogel was further crosslinked by thermally-induced beta sheets of silk fibroin.

Live/Dead Assay

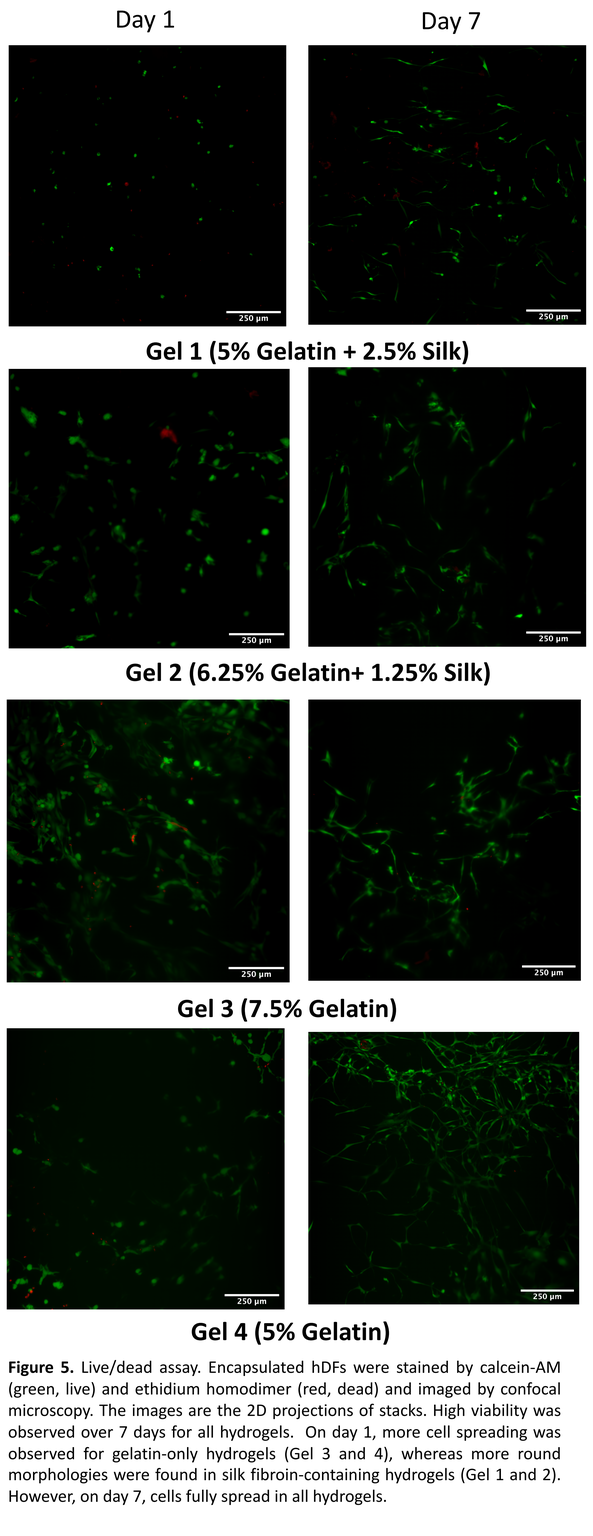

Figure 5. Live/dead assay. Encapsulated hDFs were stained by calcein-AM (green, live) and ethidium homodimer (red, dead) and imaged by confocal microscopy. The images are the 2D projections of stacks. High viability was observed over 7 days for all hydrogels. On day 1, more cell spreading was observed for gelatin-only hydrogels (Gel 3 and 4), whereas more round morphologies were found in silk fibroin-containing hydrogels (Gel 1 and 2). However, on day 7, cells fully spread in all hydrogels.

Osteogenic Differentiation of hMSCs

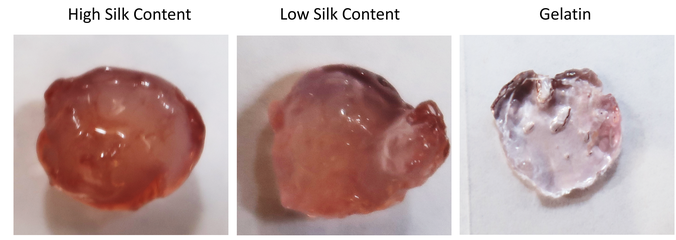

Figure 6. Alizarin Red staining. hMSCs were encapsulated in the hydrogels and cultured for 1 week in a growth medium, followed by the culture in the osteogenic medium for 2 weeks. More calcium staining was observed as silk content increased from 0 to 2.5%, indicating that there was more osteogenic differentiation of hMSCs. We attribute this to the unique micro-environment that gelatin-silk IPN hydrogel provides: the encapsulated cells can spread at early time points and the hydrogel stiffens over time providing an ideal environment for osteogenic differentiation.

Summary and Conclusions

- Gelatin-silk fibroin composite hydrogels were formed by dual crosslinking mechanisms: (1) mTG-based covalent crosslinking of gelatin and (2) thermal induction of beta sheet formation of silk fibroin. Both mechanism are cell-friendly.
- Time-wise progression of beta-sheet formation was confirmed by FT-IR. This led to significant stiffening of the hydrogels over 1 week.
- hDFs were encapsulated in these hydrogels with high viability. Although the cells exhibited more round morphologies as silk concentration increased, cells fully spread within the hydrogels within 1 week.
- More osteogenic differentiation of hMSCs was observed in the hydrogels with higher silk concentrations.
- The composite hydrogels introduced here are injectable and can be used as a vehicle for cell delivery and regenerative medicine.

Acknowledgements

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