Bioprinting of Hagfish Keratin Proteins

Nathan F. Harward [1], Bailey J. McFarland[1,2], Dr. Justin Jones[2] and Dr. Yu Huang[1] ✉
Yu.Huang@usu.edu
1. Microbrain Laboratory, Department of Biological Engineering 2. Spider Silk Laboratory, Department of Biology, USU

Goals

- Characterize and control the porosity and mechanical properties of hagfish protein hydrogels.
- Explore potential uses of mechanically and structurally tunable hydrogels.

Background

Recombinant hagfish protein hydrogels:

- Biocompatible
- Potentially bioactive
- Extremely low stiffness
- Can be bioprinted
- Easily processed
- Contains porous structures resembling the native ECM.
- Cost-effective to produce.

Bioink Preparation:

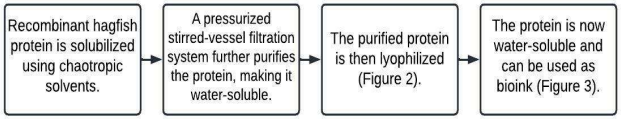


Figure 1. *Process flow diagram of the hagfish protein bioink preparation process used to ensure the bioink is water soluble and processable.*

Bioprinting and Cell Culture:

- A 3D computer generated model of the application-specific tissue scaffold is created.
- The bioink is patterned using a CELLINK Inkredible+ bioprinter.
- The hydrogels are then seeded with cells.

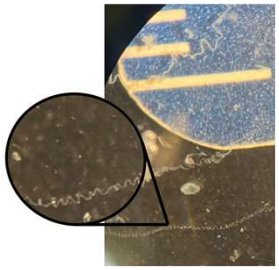
https://www.cellink.com/wp-content/uploads//2019/12/INKREDIBLE-s.png

Bioink and Bioprinting:

Figure 2. *Purified and lyophilized hagfish protein.*

Figure 3. *Purified hagfish protein solubilized in water.*

- Hagfish bioink is stable and easily tailored to a variety of applications.
- Bioink can be extruded with resolution in the single micron range (below).



Mechanical Properties

- Stiffness is measured using the CellScale Micro Tester, which compresses the samples and outputs force and deflection data to be used in stiffness calculations (Figure 3).

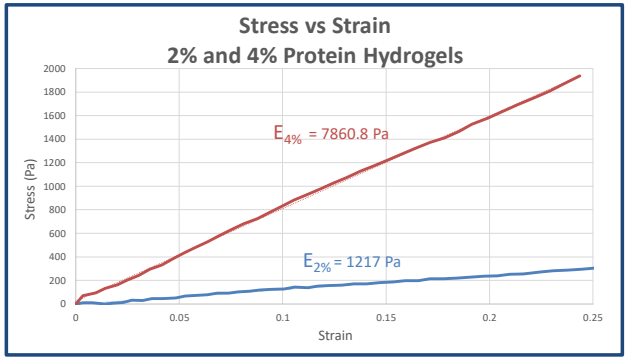


Figure 3. *Preliminary mechanical testing shows that hagfish protein hydrogels are extremely soft, with protein concentration being positively correlated with stiffness. Further testing of more replicants is required to confirm these findings and to enhance understanding of methods useful to the tunability of hagfish hydrogel stiffness.*

Porosity

- Controlling the porosity of hydrogels is crucial for cell culture as each cell type requires specific porosities.
- A computer vision program was adapted to identify and quantify pores in both fluorescent and brightfield images (Figure 4). This is an adaption of a protocol from Jamshidi et al. (https://doi.org/10.12688/f1000research.27372.2)

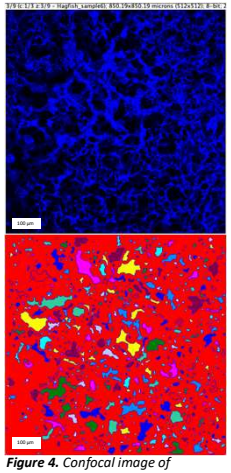


Figure 4. *Confocal image of hydrogel (top) and quantified porosity render (bottom).*

Conclusions and Future Work

- Bioprinting hagfish protein bioink allows for case-specific tissue scaffolds.
- Adding bioink additives and changing crosslinking and/or processing methods can tailor the mechanical properties and porosity of hydrogels to specific applications.
- Additional methods are being explored and developed to further the customizability of hagfish bioinks for hydrogel tissue scaffolds.
- It is desired to develop cell-laden bioinks to increase the efficacy of tissue scaffolds.

Acknowledgments

Nathan Harward is partially supported by USU EURP. Bailey McFarland is partially supported by NASA-NSTGRO, USU EURP program, and NIH (1 R15 GM132877-01) Dr. Huang is partially supported by NIH (R15 GM132877-01 and R35GM143194-01).