SIGNIFICANCE OF RESEARCH

Three-dimensional (3D) bioprinting uses additive manufacturing to construct highly-organized 3D structures using biocompatible or cell material.[1] Among these structures is the ability to make hydrogels that have similar mechanical properties to that of tissues within the body. This opens a host of possibilities that include creating scaffolds to house cell growth, create replacement tissue, and treat a myriad of patients needing tissue replacement. The cells can grow in a 3D environment more naturally to the cell, with many benefits (***Figure 1***).[2] **A major technical gap** that remains with hydrogel formation is the challenge to precise control over the porosity, which is essential for cell growth and nutrient exchange.[3]

The protein uniquely found in hagfish has unique bioinert properties that make them a fair candidate as a 3D printable bioink. Crosslinked hagfish proteins are uniquely soft and can make soft hydrogels suitable for cell growth and avoid abnormal phenotypes. This protein is secreted by the hagfish as a defense mechanism against predators, releasing the protein to mix with the water, crosslinking with the salt, and creating slime with intriguing mechanical properties.[4] Through biomimetic processing, this protein can be continuously crosslinked to form a cell-friendly hydrogel.[5] Porosity can be achieved through lyophilization, a cell-incompatible process. Herein I propose a novel combinatory approach, which re-processes the lyophilized hydrogel as an additive to the bioink. I **hypothesize** that subsequent bioprinting using the re-processed bioink will form the optimal printing viscosity and create a controlled porosity hydrogel.[6]

Objective 1: Characterize bioink's properties (before printing), namely viscosity, rheology, and crosslinking time optimal for bioprinting.

Objective 2: Characterize the hydrogel printout's properties (after printing), including stress/strain rates and hydrogel porosity.

PLAN TO MEET OBJECTIVES (*Figure 2*)

Approach for Objective 1: The viscosity of the hagfish solution will be measured at different shear rates to understand the viscosity profile using a variable shear rate viscometer (***Figure 3***). A portion of the crosslinked hagfish solution will be re-processed and mixed with a new free solution to control the bioink viscosity. Tests to examine crosslinking properties at different concentrations and time lengths will also be conducted by using a microplate reader and measuring hagfish proteins at different absorbances between crosslinked and non-cross-linked proteins.

Reasoning: To generate the re-processed crosslinked solution, the crushed lyophilized particles are mixed with a pre-crosslinked solution to increase the overall viscosity. When bioink is organized into a 3D structure, it must maintain its shape until the crosslinking process has finished. Understanding the time it takes to crosslink the solution and the optimal printing viscosity will ensure the highest printing resolution and accuracy of the models they are building.

Approach for Objective 2: Different parameters, including the printing temperature, printing speed, nozzle size, extruder pressure, crosslinking technique, crosslinking concentrations, and environmental conditions, will be tested and analyzed to understand optimal printing conditions for hydrogel pore density. A standard log-cabin-style print will be used (***Figure 4***).

Reasoning: Each parameter will affect the porosity of the printed hydrogel differently. We hypothesize that lowering the temperature makes the pores larger by slowing the nucleation. Printing speed and nozzle size affect the shear rate of the fluid, and a higher shear rate may also result in larger pore size.

Lab Facility and Equipment: The research will be completed in Dr. Huang's laboratory under his supervision. 3D printers, dehydrated hagfish proteins, *processor,* SEM, and confocal microscope will be used in the USU facility.

Timeline: This research will take the course of Spring 2023 – Spring 2024. Characterization of unprinted bioink will be completed in the Spring of 2023. Printing and characterization of printed hydrogels will be completed in the following two semesters (Fall 2023 to Spring 2024).

Personal Relationship to Project: I aim to research and develop new biomedical tools to benefit the community. Acquiring undergraduate research will help with my future goals to get into graduate school and further my career as a Biomedical Engineer. Specifically, this proposed research will advance my current study, by providing in-depth training in project planning and other invaluable skills in biomedical material engineering.

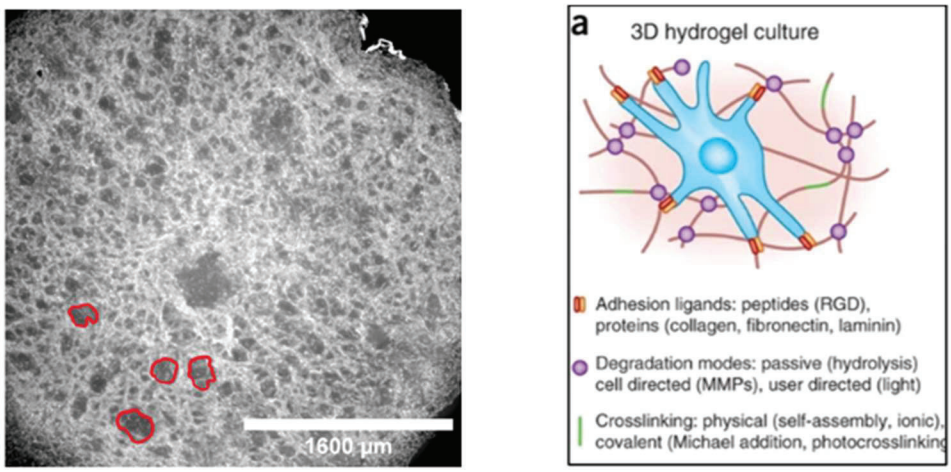

Figure 1: (Left) Preliminary Research Image of Pores (Highlighted) Formed in a Cross-Linked Hagfish Protein Ideal for Cell Growth and Nutrient Movement (Right) Model of 3D Hydrogel Cell Culture in Natural Shape (Caliari).

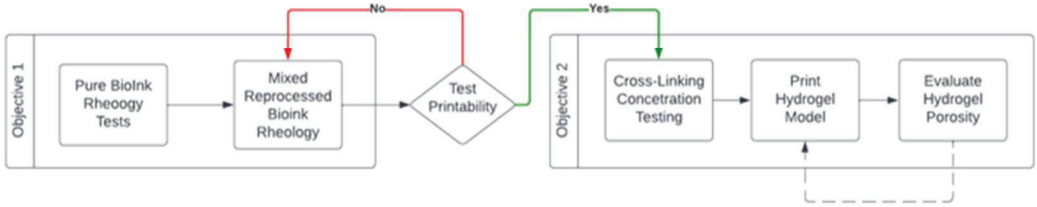

Figure 2: Flowchart of the Objective Timeline

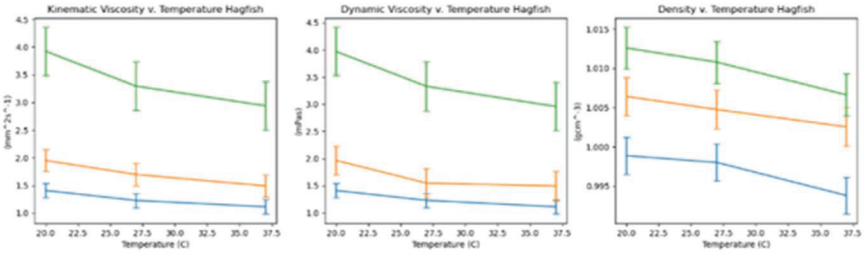

Figure 3: Viscosity Measurements Taken at Different Temperatures Showing Tunable Viscosity of Hagfish Bioink (Green 5% solution, Orange 3% solution, Blue 1% solution).

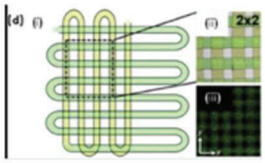

Figure 4: (Right) Diagram of Patterned Bioink Extruded into Different Height-Width Ratios Illustrating Printing Design for Hagfish Proteins (4)

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