3D Bioprinter Project

Ashley Hale, Nicole Finchum, Molly Kaufman, and Sam Luke
Advisor: Melanie G. Watson, Ph.D.
Trine University
One University Avenue, Angola, Indiana 46703

Trine University
Biomedical Engineering

Introduction:

Osteoarthritis (OA) is the most common chronic (long-lasting) joint condition and a leading cause of disability, affecting more than 30 million people in the United States. OA is a painful, degenerative joint disease affecting joint cartilage and underlying bone, common affected areas include hips, knees, hands, neck, and spine. Symptoms include stiffness, inflammation, decreased range of motion, and joint stability. Current treatment options include pain relievers, steroid injections, or joint replacement but often leave patients with constant pain and discomfort. The goal of the bioprinter project is to create a cartilage scaffold that encourages cell adhesion and growth to produce a therapeutic tissue. This project encompassed improving a bioink recipe and scaffold design to better mimic cartilage, creating and implementing testing methods including tensile, compression, chemical degradation, cell viability and adhesion, producing an aseptic printing method and running ANSYS trials to determine scaffold properties.

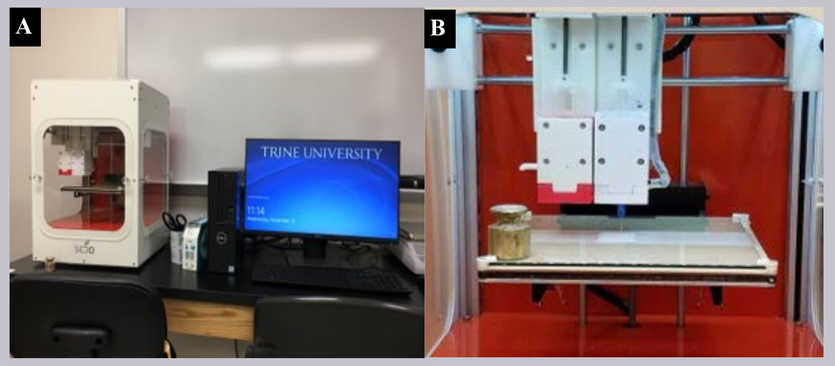

Figure 1: **(A)** Bioprinting setup **(B)** Close up of bioprinter system.

Materials and Methods:

Software programs used include Solidworks, Slic3r and Pronterface.

Bioink Final Formulation

- 5% laponite, 9% collagen, 0.5% hyaluronic acid, and 0.5% Sodium Alginate

Testing Methods

- Degradation testing performed by placing the scaffolds in DMEM at 37° C for 72 hours and recording scaffold weights.
- Tensile testing on an Instron machine fitted with pneumatic grips.
- Compression testing on an Instron machine using a 10 KN load cell and top and bottom compression plates.
- Cell viability testing performed using a hemocytometer and cells.
- Cellular adhesion testing performed using a 96 well plate, bioink, cells, media, Calcein-AM, 37° C 5% CO_2 incubator and plate reader.
- ANSYS workbench was used to create a finite element analysis of a honeycomb compression sample scaffold.

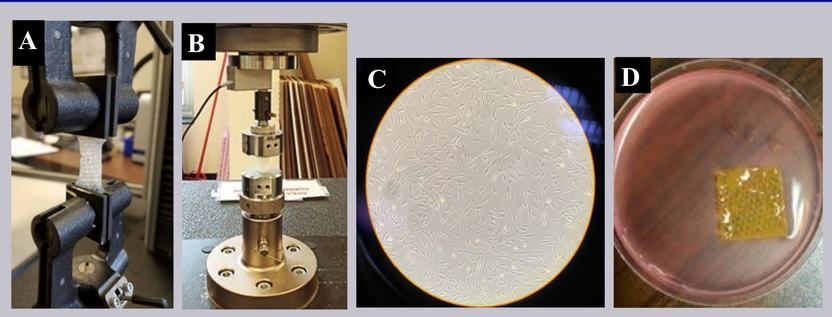

Figure 2: **(A)** Tensile testing set-up. **(B)** Compression testing set-up. **(C)** Cell culture used for cell viability and adhesion testing. **(D)** Chemical degradation testing setup.

Results and Discussion:

The bioprinter team had some promising results through testing:

- Compression testing showed collagen concentration had no effect on Young's modulus (6.25%-0.050 MPa, 9%-0.051 MPa) and extended crosslinking had a lower Young's modulus (0.037 MPa).
- Tensile testing was conducted and showed that the first recipe of bioink had a Young's modulus of 0.188, but the second formulation had a higher Young's modulus
- Chemical degradation testing indicated the scaffold experienced a 10.4% decrease in weight over three days, which falls below the ideal value of 21% decrease (scaffold fully degrade in two weeks).
- Cell viability results showed that cell cultures on average were 94% viable, within the accepted value range 85–100% viable.
- Cellular adhesion preliminary results validate the assay; 1st recipe had adherence average of 1496.2 cells/ well and 74.81% . The 2nd recipe adherence average was 1457.7 cells/ well and **72.89%.**

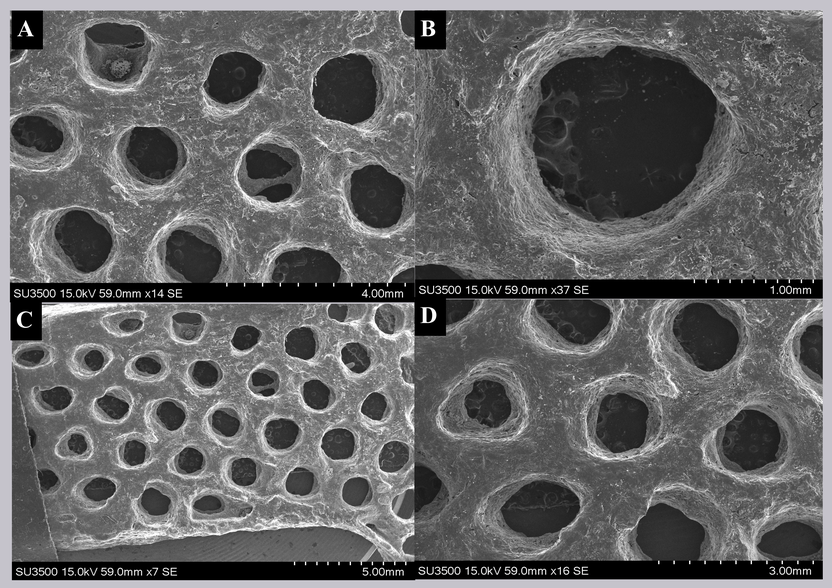

Figure 3:. Scanning electron microscope imaging of 3D bioprinted scaffolds taken at 15.0kV & 59.0 mm **(A)** Image at 14 SE **(B)** Image at 35 SE **(C)** Image at 7 SE **(D)** Image at 16 SE

Table 1: Comparison of the testing results for two recipes (1) 5% laponite, 6.25% collagen, 0.5% hyaluronic acid, and 0.5% Sodium Alginate (2) 5% laponite, 9% collagen, 0.5% hyaluronic acid, and 0.5% Sodium Alginate

Recipe Trial	Compression Young's Modulus (MPa)	Chemical Degradation (% wt. change)	Cellular Adhesion
One	0.0496	15%	1496.2 cells per well
Two	0.0510	5.85%	1457.7 cells per well

Conclusion:

The bioprinter team's goal was to design an articular cartilage-inspired scaffold. A three patterned infill was selected as the scaffold design, with a rectilinear bottom layer, gyroid middle section, and honeycomb top layer. Honeycomb scaffolds were printed with different variations in bioink recipe and were tested tensile strength, compression strength, chemical degradation, and pore size. The final bioink recipe was 5% laponite, 9% collagen, 0.5% hyaluronic acid, and 0.5% sodium alginate. The team used HUVECs as a proof of concept to validate cellular adhesion protocols then repeated testing with chondrocytes. The scaffold design, bioink recipe, and cells would have combined to make a cartilaginous scaffold seeded with cells.

Future Work:

The following are ideas to further improve the 3D Bioprinter project:

- Sterilizing scaffolds to be able to adhere human chondrocytes to them
- Adding chitosan to the bioink formulation to be able to increase thermal resistivity properties
- Create a three-layer scaffold design to better imitate human cartilage.

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