Optimizing Three-Dimensional Bioprinting for Cell Culture Scaffolds

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INTRODUCTION

Leukemia

- A type of blood cancer that originates within bone marrow
- About 40% of Leukemia patients experience relapse after bone marrow transplant treatment, which has a high mortality rate[1]

Trabecular Bone

- Spongy bone that houses the bone marrow
- Red blood cells, white blood cells, and platelets are formed[2]
- Difficult to study *in vivo* due to the location and type of tissue

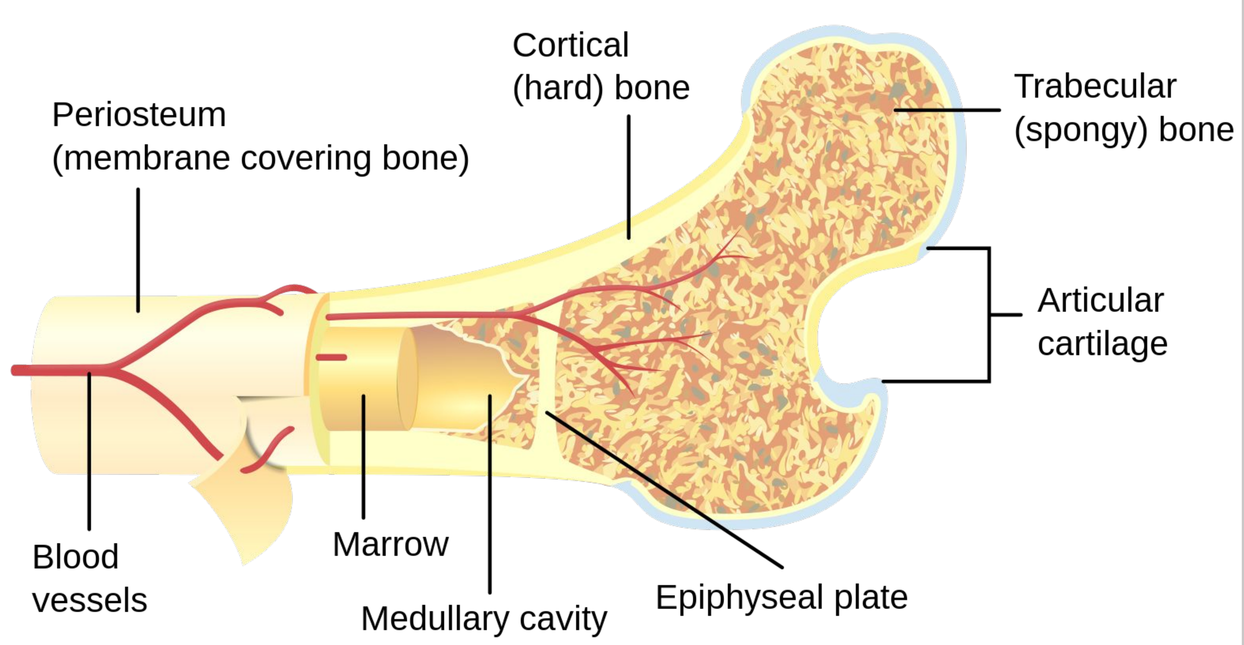

Figure 1. Trabecular Bone Diagram[3]

OBJECTIVE

Optimize and print a biomimetic trabecular bone scaffold to study cell interactions for improving leukemia treatment

METHODS

Bioprinting

- Cellink BioX printer & bioinks
- .stl from MAL

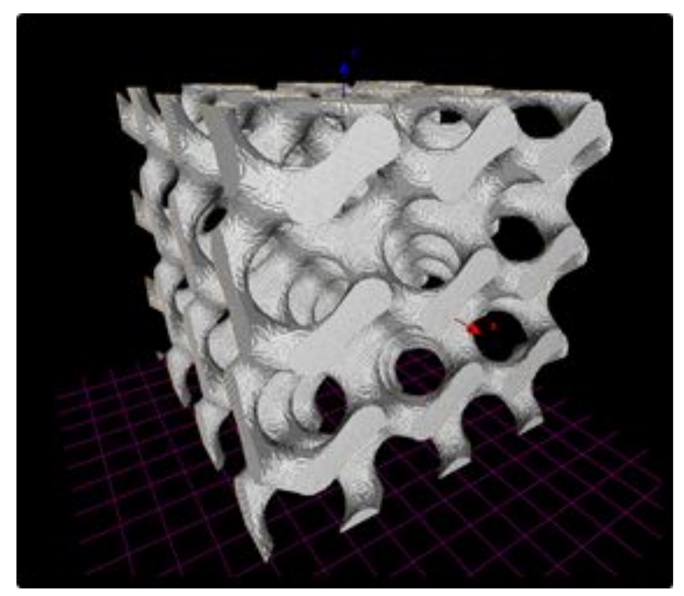

Figure 2. Trabecular Bone Gyroid Structure .stl File

- Print parameters changed:
  - speed, nozzle gauge, layer height, extrusion pressure, infill density
- G-Code
- Ink Dilution and Culture Conditions (media, PBS, incubation)

Testing and Characterization

- Ink testing: filament test, stack test, layer height test[4]
- Z-stack pore size measurements
  - 300-600 µm pore size for healthy trabecular bone[5]

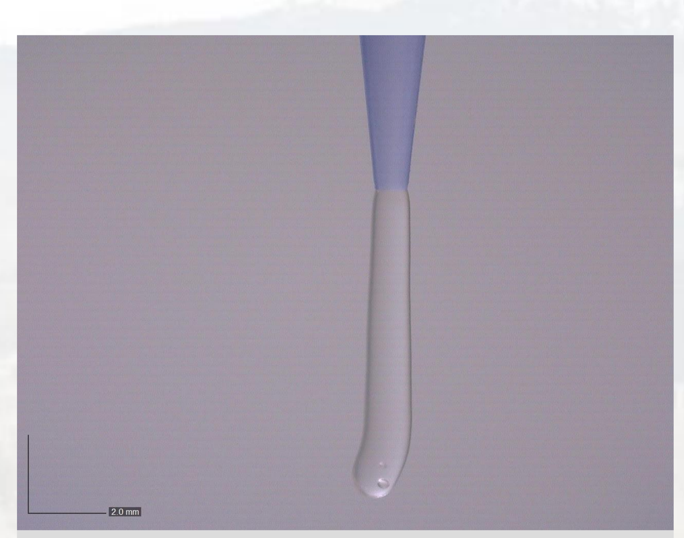

Figure 3. Bioink Filament Test (2mm scale bar)

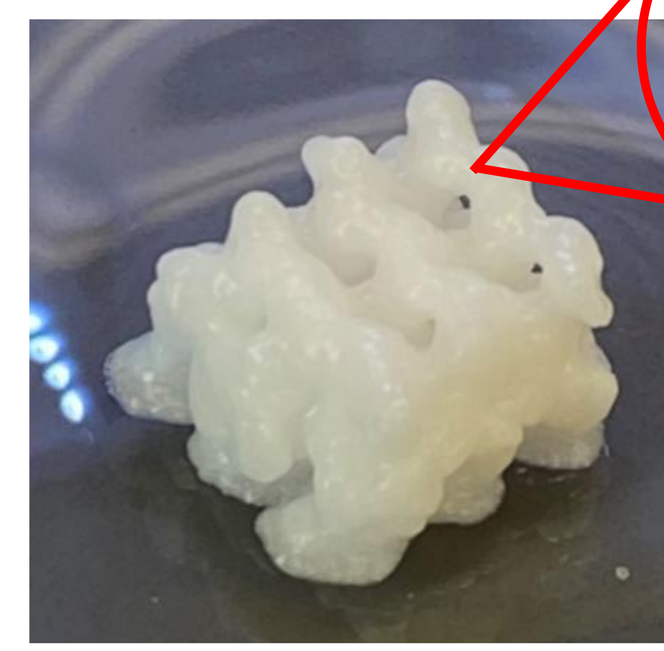

Figure 4. 3D Printed Bone Scaffold

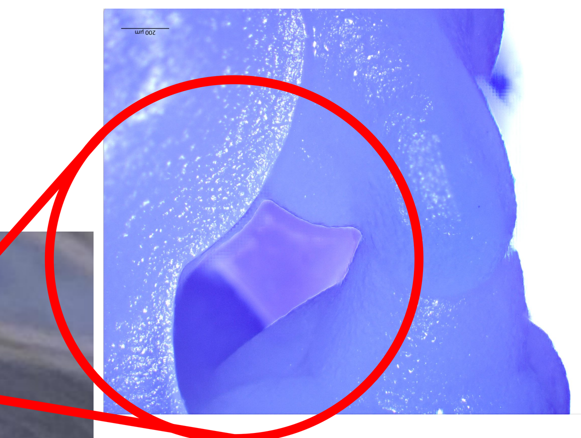

Figure 5. Z-stack Image of Scaffold Pore (top left scale bar = 200 µm)

RESULTS

- Average short diameter of pores: 821.33 µm
- Average long diameter of pores: 1299.01 µm
- PBS destroys chemical cross linked prints*

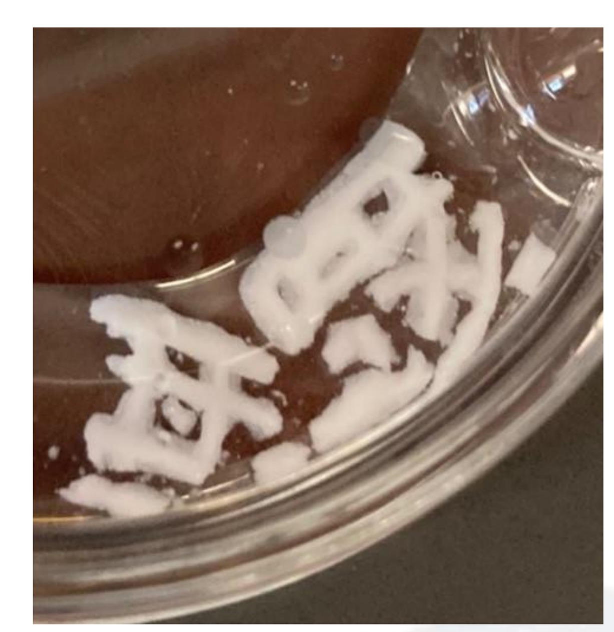

Figure 6. Dissolved Scaffold in PBS

- Best resolution with low pressure and low speed
- Pore sizes are 135.6% bigger than actual*
- Diluted inks survived PBS soak, undiluted survived media soak

CONCLUSIONS

From the work done, the following conclusions were made:

1. The pore sizes measured may be closer to the appropriate size than it seems due to the structure of the scaffold and the measurement method.
2. The ion exchange that occurs between the crosslinking agent and the PBS may only occur at higher temperatures.
3. In order to increase resolution, smaller nozzle size may be necessary.
4. Incorporating supports and printing directly into the crosslinking agent has the possibility to provide a better structure.

FUTURE WORK

- Incorporating additives
- Mixing in cells
- Improving optimization protocols
- Ink testing
- Observing structural integrity for long-term culture conditions

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