Primitive Architecture Fosters Cell Proliferation and Provides Mechanical Strength in 3-D Printed Polycaprolactone

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INTRODUCTION: Bone fractures occur in approximately 5.5 million people each year, with about 1 million requiring surgical reconstruction [1]. Depending on the severity of the fracture, surgical procedures may be necessary to allow a patient's bone to completely heal without permanent deformities. Current clinical outcomes have complications associated with short- and long-term implant failure, irritation, and cosmetic concerns. Bioresorbable bone grafts represent a solution to these problems. Polycaprolactone (PCL) is a biocompatible and biodegradable material, which has been used successfully to make fracture reconstruction devices [2]. Extrusion-based additive manufacturing (AM) of PCL provides new method for design and manufacture of scaffolds and allows for the introduction of complex infill architectures that may improve cellular growth. In particular, triply periodic minimal surface (TPMS) architectures have high surface-to-volume ratios with concave surfaces, which support cell proliferation after implantation [3]. When fabricating AM PCL implants, print times can be reduced by increasing layer thickness, but it is unknown if this change affects final geometry or influences cellular behavior. In this study we tested two TPMS architectures (gyroidal and primitive) and 2 print layer heights (0.1 and 0.2 mm). We hypothesized that gyroid implants printed with the smaller height would provide the best microenvironment for cellular adhesion and metabolic activity.

METHODS: 24 cylindrical test samples (15 mm diameter, 10 mm height) were fabricated using a fused deposition 3D printer (Creality Ender 3 Pro). 4 experimental groups (n=3) were created by varying TPMS architecture (gyroid and primitive) and print layer height (0.1mm and 0.2mm; **Fig. 1**). Half of the samples were mechanically tested at Day 0, while the rest were used for an 8-week cell culture experiment. All samples had a minimal pore diameter of ~300µm. Specimens were subjected to microcomputed tomography (µCT) scanning at a voxel size of 60 microns and an X-ray energy of 55 kV. To determine the accuracy of the 3D printed samples compared to the original software design, CAD-based and µCT-based 3D models were registered, and differences in overall geometry were quantified using the Hausdorff distance algorithm in MeshLab 2022.22.

For the cell culture model, PCL coupons were sterilized in 70% ethanol for 30 minutes and immersed in sterile PBS for 12 hours before seeding. At Day 0, passage 2 bovine bone marrow-derived mesenchymal stromal cells (bMSCs) were seeded at 6,666 cells/mm^2 on test coupons. Specimens were cultured using standard culture conditions (37°C, 5% CO2) on an orbital shaker. To characterize changes in metabolic activity, we performed Alamar Blue Assays (AB) using a 560-590 wavelength setting on a plate reader at 0, 2, 4, 6, and 8 weeks. To observe calcium presence, Von Kossa staining was performed at 8 weeks, specimens were photographed, and average image intensity was analyzed in ImageJ. Darker images represented increased mineralization within the scaffold. Specimens were then scanned again with µCT to determine the presence of interior calcium content. Finally, A quasi-static compression test as per ASTM standards [ASTM D-695] was performed to determine changes in mechanical properties at 8 weeks. Statistical analyses were not used due to the small sample size in the experiment.

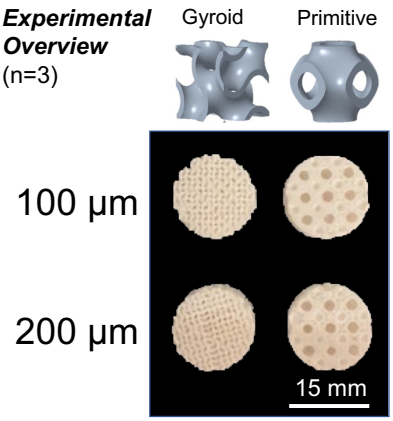

Fig. 1: Overview of the experiment, which tested 3-D printed PCL coupons designed with primitive and gyroidal infill architectures, printed at 2 different layer thicknesses.

RESULTS: *Print speed:* Gyroid samples with 100 µm and 200 µm layer heights took 90 min/sample and 48 min/sample to print, respectively. Primitive samples with 100 µm and 200 µm layer heights took 108 min/sample, and 62 min/sample, respectively. *Print accuracy:* The mean displacement of the vertices of the print from the CAD file are <0.1% for all groups. *Cellular behavior:* The Alamar Blue results showed proliferation on all samples, with significant increases in cell attachment in the Gyroid group at Day 0, but this difference in cell metabolism did not exist at 2 weeks and beyond (**Fig. 2A**). Von Kossa staining at 8 weeks resulted in changes in pixel intensity differences at 8 weeks, where the primitive scaffolds were darker in color than the gyroidal ones (**Fig. 2B**). Micro-CT analysis supported this finding with no measurable mineralization in the gyroidal samples, while primitive scaffolds had an average mineral volume of 26.5±6.1 and 23.9±7.4 mm^3 for P100 and 200, respectively. *Mechanical testing:* Primitive samples had higher initial stiffness (792.1±27.2 N/mm) and yield force (403.8±15.2 N) during compression testing than gyroid samples (584.7±17.7 N/mm and 259.1±6.8 N, respectively) (**Fig. 2C&D**). After 8 weeks, the gyroid samples lost some stiffness (414.2±77.4 N/mm), but there were no notable changes in mechanical properties of the primitive scaffolds.

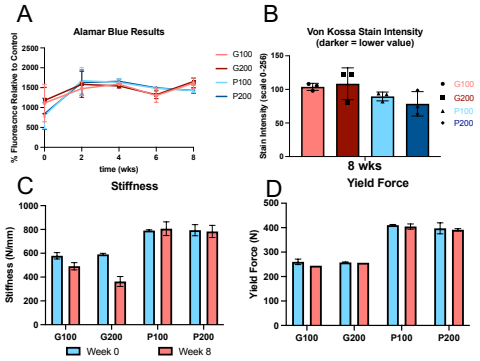

Fig. 2: Results from (A) Alamar Blue testing, (B) Von Kossa Staining, and (C,D) mechanical testing. Primitive infills generated more mineralization and were inherently stronger than gyroidal ones.

DISCUSSION: The results presented show that both gyroid and primitive TPMS-based scaffolds provided microenvironments that are conducive to cellular metabolism. Contrary to our hypothesis, the primitive geometries provided preferable increases in mineral production and mechanical properties. We expected the accuracy of the prints from larger layer heights to be significantly reduced, but this was not the case. The observed defects included abnormally shaped or discontinuous passageways, which would affect the infiltration of the bMSCs within the samples, but these were rare. Results suggest that the increase in layer height provides the ability to print faster without negative consequences. This is beneficial for a future goal of finding a quick and reliable method of 3D printing implants for orthopedic trauma. This cell culture study had several limitations, including a small sample size, a limited number of time points, and a lack of mechanical stress applied to the implants during incubation. Our future work will include the application of mechanical loads using bioreactors and an in vivo model.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides preliminary results to further guide the design and development of AM PCL implants. Findings show that the primitive TPMS structure printed with 0.2 mm layer thicknesses creates implants that are mechanically strong and create a microenvironment that may be beneficial to bone regeneration.

REFERENCES: [1] Cheung+ Clin Pod. Med Surg., 2005; [2] Hashimi+,*Materials Today Comm.*, 2022; [3] Lehder,+ Biomech Mod Mechanobiol, 2021.

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