Triply Periodic Minimal Surface Architectures Improve In Vitro Bone Growth in PCL Scaffolds

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INTRODUCTION: Globally there are about 178 million fractures a year worldwide and critical-sized bone defect injuries occur more than 100,000 times annually. [1,2] Treatment of critical defects typically requires bone grafting, a procedure that has been increasingly improved with tissue engineered scaffolds and bone grafting techniques [3]. In particular, PCL is a biocompatible and biodegradable material which has been used successfully to make a spectrum of fracture reconstruction devices. [4] Extrusion-based additive manufacturing (AM) of PCL provides a new method for design and manufacture of scaffolds and allows for the introduction of complex infill architectures that may improve cellular growth. Triply periodic minimal surface (TPMS) architectures have been identified as a potential breakthrough for bone regeneration because they have completely interconnected pore networks, high surface-to-volume ratios, and concave surfaces—all of which support cell proliferation after implantation. There is still a lot that is unknown about TPMS PCL devices, including the relationships between printing parameters, mechanical properties, and biological function. To better understand the relationships between bone regeneration and infill architectures in PCL scaffolds, we tested cylindrical scaffolds in an in vitro cell culture experiment. In this model, the outer cylinder consisted of 3D printed PCL and the hole was stuffed with bovine morselized cancellous bone and bone marrow as graft material (**Fig. 1**). We tested 4 unique scaffold designs by making alterations to infill architectures (TPMS gyroid and honeycomb) and infill percentages (40% and 60%). We hypothesized that 60% gyroid (G60) architectures would lead to improved and accelerated bone healing responses.

METHODS: A total of 72 cylindrical test samples (n=18/group) with an outer diameter of 15 mm, an inner diameter of 5 mm, and a height of 10 mm were fabricated using a fused deposition 3D printer. All scaffolds were printed at 8 mm/s and an extrusion temperature of 90°C. From each group, 6 of the samples were mechanically tested at Day 0. The remaining PCL scaffolds were sterilized in 70% ethanol for 30 minutes and immersed in sterile PBS for 12 hours before being packed bone graft material extracted from juvenile bovine tibiae. The filled PCL scaffolds were transferred to a 12-well plate containing alpha-MEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin-fungizone. Specimens were cultured using standard culture conditions (37°C, 5% CO2) on an orbital shaker. The basal medium was exchanged every 3–4 days. To characterize changes in metabolic activity, we performed Alamar Blue Assays using a 560-590 wavelength setting on a plate reader at 0, 1, 2, 3, and 4 weeks. To observe calcium presence, Von Kossa staining was performed at 4 weeks. Specimens were photographed, and average image intensity was analyzed in ImageJ. Darker images represented increased mineralization within the scaffold. Specimens were then scanned with micro-CT to determine the volume and spread of calcium content within the scaffolds. Scans were performed at a voxel size of 60 microns and an X-ray energy of 55 kV. Finally, quasi-static compression tests [ASTM D-695] were performed to determine changes in mechanical properties.

RESULTS: Cellular behavior: Alamar Blue assays showed that all constructs were able to maintain a high level of cell viability during the 4-week incubation period. However, scaffolds with Gyroid architecture, especially the 40% Gyroid group, had higher amounts of metabolic activity compared to honeycomb architectures (**Fig. 2A**). Von Kossa staining at 4 weeks, especially in the higher porosity (40% infill) showed that G40 scaffolds were darker in color (49.60±8.57) than the H40 specimens (68.42±7.90, p =0.015) (**Fig. 2B**). For both structures, the lower porosity groups (G60, H60) showed decreased stain intensity, suggesting higher levels of mineral deposition in these scaffolds. Micro-CT analysis showed that, G40 (58.5±5.0 mm³) developed significantly more bone than H40 (26.9±2.9 mm³, $p < 0.0001$). The same behavior was observed when comparing G60 (58.1±5.4 mm³) to H60 (26.3±7.4 mm³, $p < 0.0001$; **Fig. 2C**). Mechanical testing: Honeycomb samples had higher stiffness and yield force during compression testing compared to gyroid samples. Specifically, at 40% porosity, H40 stiffness was 1175.44 ± 115.11 N/mm compared to 711.84 ± 93.24 N/mm for G40 ($p<0.0001$). At 60% porosity, H60 stiffness was 533.79 ± 74.47 N/mm, while G60 stiffness was 423.58 ± 15.22 N/mm ($p>0.05$, **Fig. 2D**). At week 4, all samples demonstrated a statistically significant increase in their mechanical properties which can be attributed to bone tissue growth and integration within the scaffold structure.

DISCUSSION: The results presented support our hypothesis that additively manufactured PCL scaffolds with TPMS architecture can create favorable microenvironments that enhance cellular metabolic activity and bone growth. Importantly, the bone formation occurred without osteogenic media, meaning that the scaffold design itself was responsible for the differences in bone growth. Micro-CT analysis demonstrated that gyroid scaffolds led to double the bone formation compared to honeycomb scaffolds at week 4, highlighting the effectiveness of the gyroid design. At the same time, the mechanical properties of the honeycomb scaffolds were superior to those of the gyroid test coupons. Improvements in mechanical properties of honeycomb specimens were larger over time compared to gyroid, suggesting that honeycomb architectures may be more amenable to mechanical reinforcement from developing bone. This represents a tradeoff between optimizing scaffold designs, as initial strength of scaffolds must be considered, depending on the application. This cell culture study had several limitations. It was conducted in vitro, which does not fully mimic the complexity of in vivo conditions, such as vascularization, immune responses, and mechanical loading. While PCL offers favorable mechanical properties and is easy to fabricate, its hydrophobicity limits cell attachment, and its slow degradation may delay bone remodeling. Our future work will focus on optimizing the relationships between printable biomaterials, printing parameters, scaffold strength, and biological performance, with the goal of developing implants for testing in large animal models and clinical trials.

SIGNIFICANCE/CLINICAL RELEVANCE: This study highlights the potential of using additively manufactured PCL implants. Findings indicate that the gyroid TPMS architectures create implants that are both mechanically strong and conducive to bone regeneration.

REFERENCES: [1] Wu+, *Lancet Healthy Longev.*, 2021; [2] Huang+, *Bioeng. Basel*, 2022; [3] Lewandrowski+, *Biomaterials*, 2000; [4] Dwivedi+, *J. Oral Biol. Craniofacial Res.*, 2019.

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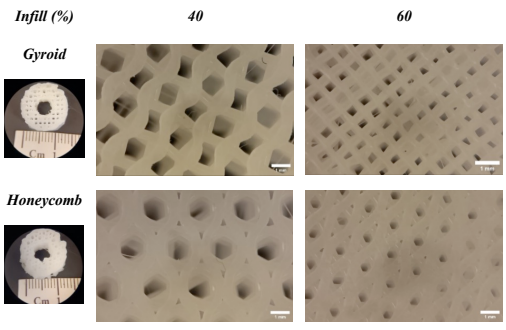

Figure 1 *Overview of the experiment testing 3D-printed PCL scaffolds designed with TPMS gyroid and honeycomb lattices architectures, each with two different infill percentages.*

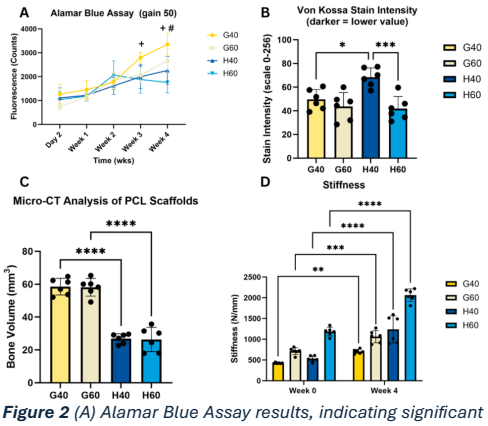

Figure 2 *(A) Alamar Blue Assay results, indicating significant differences (+ represents a significant difference between G40 and H40; # represents a significant difference between G60 and H60). (B) Von Kossa staining. (C) Micro-CT analysis. (D) Mechanical testing outcomes.*