

Exploring the Impact of Spatial Pore Architecture on the Biological Functionality of Calcium Phosphate Bone Scaffolds: *In Vitro* and *In Vivo* Evaluations

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Tissue engineering represents a highly interdisciplinary field, combining expertise from biology, materials science, engineering, and clinical medicine to develop innovative solutions for regenerating damaged or diseased tissues. In the context of bone tissue engineering, Calcium phosphate (CaP)-based scaffolds are continuously studied. This study aimed to assess how spatial pore architecture and gradients influence the biological functionality of scaffolds. To this end, four scaffold designs based on the Triply Periodic Minimal Surfaces (TPMS) family were additively manufactured using hydroxyapatite (HAp). These scaffolds were then evaluated for their biological potential through comprehensive *in vitro* and *in vivo* testing. *In vitro*, the scaffolds were seeded with immortalized bone marrow-derived stromal cells (hTERT-BMMSCs) and cultured under both static and dynamic conditions. The subsequent assessments included cell viability, DNA content, gene expression, and neo-tissue formation. Overall, the dynamic culture conditions demonstrated superior biological performance. Although no significant differences were observed among the geometries, the Primitive design showed a slight advantage over the Gyroid and Diamond designs, particularly in promoting rapid neo-tissue growth. Several scaffold designs were implanted ectopically in nude mice to assess their *in vivo* bone formation potential after being loaded with Bone Morphogenic Protein (BMP)-2 and/or human Periosteum-derived Derived Cells (hPDCs). The *in vivo* experiments showed bone formation in all tested conditions containing BMP-2. These findings contribute to the body of work focusing on the optimization of the design of CaP-based scaffolds for bone tissue engineering by emphasizing the significant influence of the scaffold pore architecture on the *in vitro* neotissue and *in vivo* bone formation.