The effect of pore architecture in calcium phosphate additively manufactured scaffolds

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Calcium phosphate-based scaffolds with porous structures similar to natural bone are frequently used in bone tissue engineering. The current challenge to further improve their performance is focusing on the optimisation of their 3D structure with additive manufacturing allowing to produce scaffolds with a high degree of local control on the internal pore architecture meeting. This allows balancing the biological requirements with the biomechanical ones, such as is the case for gradient porous scaffolds (GPS). GPSs are porous structures where the porosity changes in space with a specific gradient. To properly test the effectiveness of scaffold architecture on drawing in cells and guiding neotissue formation *in vitro*, an environment similar to the physiological conditions is needed. In spite of ample studies showing the importance of pore gradient in conventional *in vitro* models, much less research has been done on the performance of GPS biomaterials in a dynamic 3D culture environment.

Therefore, this study investigated the influence of pore architecture on cell proliferation and matrix deposition. Gradient and consistent porous scaffolds used in this study are triply periodic minimal surface (TPMS) scaffolds. TPMS structures have zero mean curvature and minimal surface. They offer several advantages including a high surface to volume ratio, less stress concentration and increased permeability compared to the traditional lattice structures, thereby aiding in better cell adhesion, migration, and proliferation.

Immortalized bone marrow mesenchymal stem cells (hTERT-BMMSCs) were seeded on additively manufactured calcium-phosphate-based scaffolds and cultured for up to 21 days in static and dynamic perfusion bioreactor cultures. Live/Dead staining, DNA quantification and metabolite concentration analyses were performed on different culture conditions. In addition, contrast-enhanced Nanofocus Computed Tomography (nanoCT) imaging was used to visualize the neotissue (cells + extracellular matrix) formed inside the scaffolds.

This study provides a quantitative insight into biological consequence of the scaffold's pore architecture and confirms the interest of a perfusion bioreactor system for the *in vitro* development of 3D cell–carrier constructs.