Hierarchical Macro/Microporous 3D Scaffolds Support the Growth of MC3T3-E1 Osteoblastic Cells

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Statement of Purpose: Organ failure or loss of tissue is one of the most devastating and costly burden on human healthcare. Tissue engineering applies the knowledge of biology, cell transplantation, materials science, and bioengineering to construct biological substitutes that can restore and maintain normal function in injured and diseased tissues. This work proposes a hybrid solid freeform fabrication (SFF)/thermally induced phase separation (TIPS) technique. The morphology of the scaffolds produced by the TIPS technique can be closely controlled by manipulating the process parameters, including the polymer concentration, ceramic content, quenching temperature and rate. In this study we have tested the hypothesis that orthogonally-interconnected channels produced by the SFF technique, surrounded by a microporous matrix generated by the TIPS method, can provide an ideal environment to guide bone ingrowth.

Methods: The TIPS technique allowed producing micropores within the hierarchical scaffolds. Maintaining adequate mechanical properties was desirable for these constructs. Therefore, small pore size range for the micropores was targeted in this study (< 50 μm), whereas the size of the macro-channels produced by the SFF technique was greater than 250 μm. Poly(lactic-co-glycolic acid) (Resomer LG 824S) was mixed with 1,4-dioxane (Sigma) at a concentration of 7% w/v. Some mixtures also contained 20% w/w hydroxyapatite nanoparticles (nHA) with a grain size of <200 nm (Sigma) to enhance the mechanical properties and osteoconductivity. The mixture was stirred at 60°C for 2 hours and sonicated for 15 min. The solution was quenched to a temperature of –20°C and maintained at this temperature for 3 hours to solidify the solvent and induce phase separation. Freeze-extraction of dioxane by ethanol for 48 hrs produced the microporous matrix. During the scaffold fabrication process, the macro-channels were generated using the SFF technique. The scaffolds were dried in room temperature for 24 hours and punched into disks of 5-mm diameter for mechanical testing and *in vitro* trials. BOSE ElectroForce 3200 mechanical tester was used to measure the compressive modulus of the scaffolds at 1mm/min displacement rate. The scaffolds for *in vitro* trials were sterilized in 70% ethanol and seeded with MC3T3-E1 osteoblastic cells (Sigma). The cells were cultured in alpha modified minimum essential medium (α-MEM) (Gibco) containing 10% fetal bovine serum (FBS, Gemini) and 1% Penicillin/ Streptomycin (Gibco) at 37 °C in a 5% CO_2 incubator. The DNA content for the scaffolds was evaluated using a CyQUANT cell proliferation assay kit (Life Technologies) for up to 21 days in culture (n=3). Scanning electron microscopy (SEM) was performed on the scaffolds using Zeiss Supra 35VP at 10 kV.

Results: The SEM micrographs revealed a microporous structure for the scaffolds made by the TIPS method (Fig. 1a), while showing a bimodal pore size distribution for the macro/microporous scaffolds fabricated by the hybrid SFF-TIPS technique (Fig. 1b). The micropore size for the hierarchical scaffolds ranged between 10 μm to 50 μm, whereas the diameter of the macro-channels resulting from SFF technique was greater than 250 μm. Figure 2 shows the SEM micrographs of the PLGA scaffolds after 7 days of *in vitro* culture. The cells adhered to the scaffold surface and remained viable during the 21-day *in vitro* trial. Most of the cells were located at the interior regions of the macro-channels, as can be seen in Fig. 2. Cells were also observed on the surface of the scaffolds. The high density of cell attachment to the hierarchical scaffolds on day zero demonstrated a surface topography favorable for cell adhesion.

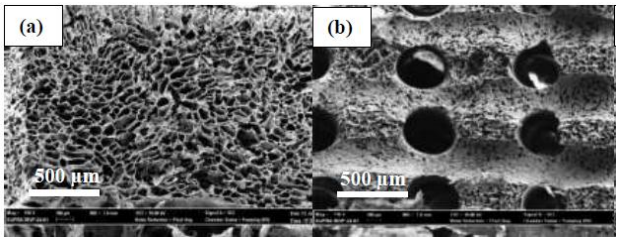

Figure 1. SEM micrographs of the PLGA scaffolds fabricated by (a) TIPS, (b) hybrid SFF-TIPS technique.

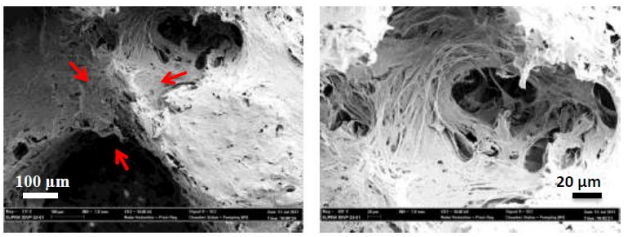

Figure 2. SEM micrographs of the PLGA scaffolds fabricated by the hybrid SFF-TIPS technique after 7 days of *in vitro* culture (two different magnifications).

Conclusions: The hybrid SFF-TIPS technique is a promising approach to fabricating scaffolds with a highly controlled internal architecture, a porosity level greater than 90%, and a bimodal pore size distribution (5-50 μm and > 250 μm). The scaffolds showed a great potential in supporting three-dimensional cell adhesion and growth, and demonstrated cell penetration into the macro-channels as early as 7 days of *in vitro* culture. The results of the proliferation assay showed that 50% of the initial seeded cells were retained, and the cells remained viable for the duration of the *in vitro* trials (21 days).

References: (1) Langer R, Vacanti J P. Tissue Eng Sci. 1993; 260: 920–926. (2) Wei G, Ma PX. Biomat. 2004; 25: 4749–4757.