Design and Characterization of Fully Osteoconductive Scaffolds for Homogeneous and Enhanced Bone Regeneration

Ami Amini[a], Cato Laurencin[a,b], Syam Nukavarapu[a,b]
[a]Orthopaedic Surgery, [b]Chemical, Materials & Biomolecular Engineering, University of Connecticut, Farmington, CT

Statement of Purpose: Fully osteoconductive scaffolds are able to achieve mineralization and bone tissue formation throughout the entire graft, including the periphery and interior. As there have been no such scaffolds fabricated thus far, literature has largely reported surface-limited cell survival and bone formation[1], mainly attributed to constricted pore sizes and the resulting poor transport of oxygen and nutrients to the scaffold's interior[2]. In this study, we addressed the issue of limited osteoconductivity by developing PLGA microsphere scaffolds with increased pore sizes and interconnectivity, referred to as macro-porous (i.e., MP) scaffolds. Such scaffolds are expected to achieve full osteoconductivity and vascular in-growth by overcoming the diffusional constraints, and yet, retain mechanical compatibility for effective bone regeneration.

Methods: Macro-porous scaffolds were fabricated by packing PLGA microspheres (425–600 μ m) and a porogen, NaCl crystals (200-300 μm), together in a steel mold, heat sintering at 100°C for 1 hour, and subsequently, leaching out the porogen in water. The control scaffolds were fabricated similarly, except no porogen was used. Pore connectivity and accessible pore volume were accessed via MicroCT (Scanco μCT40). We seeded MC3T3-E1 pre-osteoblastic cells on the scaffolds, and cultured in mineralization media up to 28 days at 37°C, 5% CO_2 and 95% humidified air. We examined mineralization (Alizarin Red staining), cell survival (Live/Dead assay), and cellular expression (immunohistochemistry (IHC)).

Results: Thermal sintering combined with porogen leaching provided macro-porus (MP) scaffolds (Fig. 1). MP scaffolds display significantly higher pore sizes (i.e., 200-800 μm) and interconnectivity than control scaffolds, two aspects required for enhanced osteogenesis and angiogenesis in BTE. Fig. 2 illustrates all scaffold pores accessible from the outside at a cut-off dimension of 200 μm for control and MP scaffolds, whereby grey area represents accessible pore space.

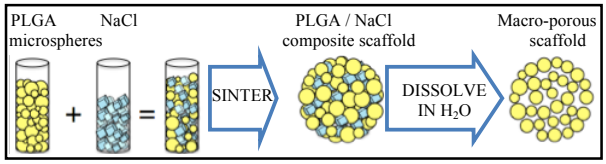

Figure 1. Macro-porous (MP) scaffold fabrication process.

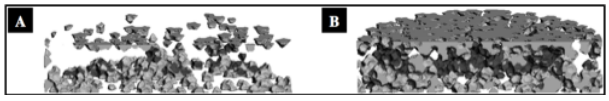

Figure 2. MicroCT interconnectivity analysis.

We confirmed the ability of MP scaffolds to significantly enhance cell survival throughout the entire scaffold, respective of control scaffolds. Fig. 3A and B are representative images of the center of the cell-scaffold constructs after 14 days of culturing. In addition, we confirmed that MP scaffolds support cell expression of established bone markers (i.e., osteocalcin, osteopontin (OPN), Collagen I) throughout the scaffold. IHC staining for OPN displayed its expression on the surface and even in the center of the MP scaffolds (Fig. 3D). In contrast, control scaffolds showed OPN expression only on the scaffold surface, but not in the scaffold interior (Fig. 3C).

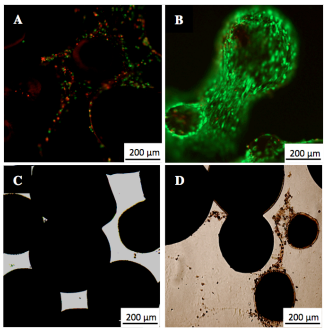

Figure 3. Osteoblast survival in the center of (A) control and (B) macro-porus (MP) scaffolds (red = death, green = survival). OPN expression in center of (C) control and (D) MP scaffolds.

MP scaffolds displayed superior mineralization potential in respect to control (Fig. 4). Control scaffolds displayed Alizarin Red mineralization staining mostly limited to the scaffold surface (Fig. 4B), whereas MP scaffolds displayed mineralization throughout the scaffold (i.e. top, center and bottom, Fig. 4C). Furthermore, to determine the extent of mineralization occurring only at the scaffold's center, we removed the top and bottom 2 mm of the scaffolds (5mm diameter, 10mm height). Significantly higher mineralization was observed in MP scaffolds compared to control, in respect to the whole construct, as well as the center. Specifically, >52% of the mineralization occurred on the surface of control scaffolds, whereas only 37% occurred on the surface of MP scaffolds. Approximately two-thirds of the mineralization of MP scaffolds occurred within the scaffold, not the surface.

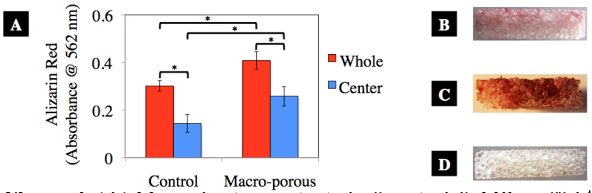

Figure 4. (A) Mineralization potential of control & MP scaffolds. Cross-section images of (B) control and (C) MP scaffolds stained with Alizarin Red post 28 day culture with MC3T3 cells, and (D) control scaffolds without cells. * signifies $p < 0.05$.

Conclusions: We have developed a biodegradable scaffold with increased porosity, and human bone mechanical compatiblity for bone defect repair. The macro-porus scaffolds have demonstrated their fully osteoconductive nature by displaying osteoblast survival and mineralization throughout the entire scaffold. Provided the osteoinductive environment, macro-porus scaffolds may promote homogeneous and enhanced bone regeneration throughout the scaffold. Thus, the fully osteoconductive scaffolds may address a significant challenge in scaffold-based bone tissue engineering approach for clinical bone repair/regeneration.

References: [1]Jiang *et al.* 2010. J Biomed Mater ResA. 93:1193-208.
[2]Karageorgiou *et al*. 2005. Biomaterials. 26: 5474-5491.