Potential of Poly(ε-caprolactone fumarate) as a Bone Tissue Engineering Scaffold

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Statement of Purpose: There is a compelling need to regenerate bone tissue lost as a consequence of trauma. Currently, treatment options include grafts, metallic implants, ceramics, and biodegradable or non-biodegradable synthetic polymers.[1] One desirable characteristic of biomaterials for tissue regeneration is the ability to tune properties via modification of structure, composition and architecture.[2] Consequently, we are developing therapeutics for bon regeneration. Poly(ε-caprolactone fumarate (PCLF) based materials will be exploited for the purpose of designing and developing regenerative therapeutics. The objective of the current study was to design and develop PCLF formulations as bone tissue engineering scaffolds that are osteoconductive, biodegradable and function as carriers for biologics and cells. In vitro biological performance of the PCLF scaffolds was assessed.

Methods: PCLF were synthesized as described previously[3]. 3D PCLF scaffolds were fabricated using a salt porogen leaching technique. To increase eventual bone formation, 30% (by weight) of hydroxyl apatite (HA) nanoparticles were incorporated into PCLF before crosslinking. Biological performance of the scaffolds was determined *in vitro* using MC3T3-E1 pre-osteoblast cells by measuring cell attachment, cell proliferation, alkaline phosphatase (ALP) activity, osteocalcin (OCN), and calcium content produced by the cells grown on PCLF scaffolds for up to 21 days. Recombinant human bone morphogenetic protein-2 (RhBMP-2) release profiles from the scaffolds were determined by ELISA and bioactivity of the releasates was determined by measuring proliferation and ALP activity of MC3T3-E1 cells in the presence of releasate compared to exogenous rhBMP-2.

Results: PCLF was synthesized and its chemical, physical and biological properties were evaluated. Chemical structure (Fig. 1) was confirmed by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The architecture of 3D porous scaffolds (75% porosity) was assessed by scanning electron microscopy (SEM), revealing irregular cubical pore shapes and poor inter-connectivity. *In vitro* biocompatibility was assessed in the presence of PCLF polymers for up to 14 days by measurement of proliferation of pre-osteoblasts (MC3T3-E1) using a Live/Dead stain. Results showed a similar response to the control (tissue culture plate). Pre-osteoblast attachment on 2D PCLF surfaces showed robust cell attachment regardless of HA incorporation (Fig. 2A &B). The cell attachment on 3D PCLF scaffolds with and without HA also showed robust cell attachment on the surface. Cells cultured on PCLF scaffold with HA showed uneven distribution in comparison to cells on PCLF scaffold without HA, likely due to the difference in surface morphology. Further

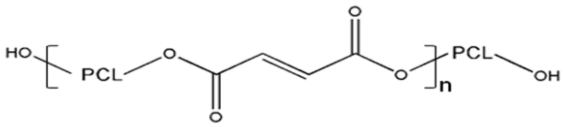

Figure 1. Chemical structure of poly(ε-caprolactone fumarate) (PCLF); PCL: poly(ε-caprolactone).

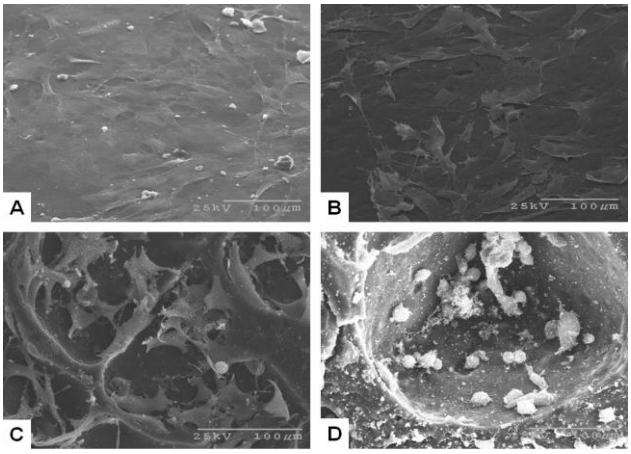

Figure 2. SEM images of MC3T3-E1 cells cultured on A) 2D PCLF, B) 2D PCLF/30% HA, C) 3D PCLF, and D) 3D PCLF/30% HA) at 24 hrs.

studies, including the determination of osteogenic differentiation and mineralization on the scaffolds will reveal more details of biological performance of 3D PCLF scaffolds in response to scaffold architecture as well as the effect of HA incorporation. In addition, rhBMP-2 release profiles from the 3D scaffolds were determined and showed sustained release over 21 days. The bioactivity of the releasates were also determined and revealed similar bioactivity as exogenous rhBMP-2.

Conclusions: PCLF formulations were synthesized and fabricated into 3D porous scaffolds. Chemical and physical properties depend on polymer backbone composition and incorporated HA. These polymeric scaffolds appear to be biocompatible. Cell attachment on the scaffolds revealed robust cell attachment throughout the surface of the scaffolds. In vitro and in vivo studies are currently underway to determine osteogenic differentiation and bone regeneration in a rabbit calvarial model using PCLF scaffolds incorporating rhBMP-2.

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