Development of Tyrosine-Derived Polycarbonates as Bone Tissue Engineering Scaffolds

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Statement of Purpose: There is a compelling need to regenerate tissue lost as a consequence of trauma. Currently, treatment options include grafts, metallic implants, ceramics, biodegradable and non-biodegradable synthetic polymers.[1] One of the desirable characteristics of biomaterials for regeneration of tissues is their tunability of properties via modification of their structure, composition and architecture.[2] Such class of biomaterials includes the tyrosine-derived polycarbonates. Tyrosine-derived polycarbonates will be utilized for the purpose of designing and developing therapeutics for bone regeneration. The objective of the current study is to design and develop a tyrosine-derived polycarbonate scaffold for bone tissue engineering that is osteoconductive, biodegradable and functionalized as a carrier for biologics and cells.

Methods: Tyrosine-derived polycarbonates were synthesized using triphosgene in dichloromethane with pyridine. Scaffolds were fabricated using a combination of solvent casting, phase separation and particulate leaching techniques. Biological performance of the scaffolds was determined *in vitro* using MC3T3-E1 pre-osteoblast cells by measuring cell proliferation, alkaline phosphatase (ALP) activity, osteocalcin (OCN), and calcium content produced by the cells grown on the scaffolds up to 21 days. RhBMP-2 release profiles from the scaffolds were determined by ELISA and bioactivity of the releasates was determined by measuring proliferation, ALP activity of MC3T3-E1 cells and compared to exogenous rhBMP-2. For histology analysis, the samples were stained with Sanderson's Rapid Bone Stain and counterstained with van Gieson's picrofuchsin,

Results: Tyrosine-derived polycarbonates were synthesized and their chemical, physical and biological properties were evaluated. The chemical structure (Fig. 1) was confirmed by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The architecture of porous scaffolds was assessed by scanning electron microscopy (SEM). The scaffolds displayed (1) a bimodal pore distribution with micropores of less than 100 μm and macropores between 200 – 400 μm, (2) a highly interconnected and open pore architecture, and (3) micropores that are oriented and aligned along the sides of the macropores. *In vitro* biocompatibility was assessed in the presence of polymers up to 14 days by measuring metabolic activity of pre-osteoblasts and showed a similar response to the control. Pre-osteoblast attachment on the highly porous (90% porosity) tyrosine-derived polycarbonate scaffolds was examined by confocal microscopy as well as SEM and showed robust cell attachment throughout the scaffolds, including individual pores (Fig. 2).

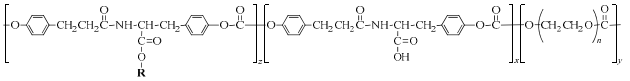

Figure 1. Chemical structure of poly(DTR-co-x%DT-co-y%PEG$_{MW}$ carbonate), where *x* and *y* are mole fractions of DT and PEG, respectively, where *z*, mole fraction of DTR, equals 1- x-y and *n* is the number of ethylene glycol repeat units in PEG.

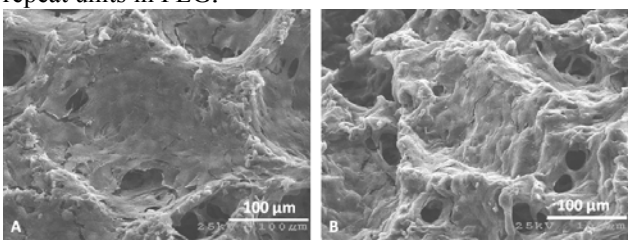

Figure 2. SEM images of MC3T3 E1 cells cultured on different tyrosine-derived polycarbonate scaffolds (A and B) at 24 hrs.

Histological assessment of *in vivo* biocompatibility of the scaffolds using a critical sized defect (CSD) in a rabbit calvaria model showed minimal inflammatory responses after 12 weeks of implantation. Further, 3D polymeric scaffolds significantly promoted osteogenic differentiation and mineralization of pre-osteoblasts, compared to 2D controls (2D film and tissue culture plate) likely due to their unique pore architecture. In addition, rhBMP-2 release profiles from the 3D scaffolds showed sustained release over a 15 day period and the bioactivity of the releasates revealed similar bioactivity as compared with exogenous rhBMP-2.

Conclusions: Tyrosine-derived polycarbonates were synthesized and fabricated into 3D highly porous scaffolds with bimodal pore distribution. Degradation rate is tunable and is dependent on the polymer composition. These polymeric scaffolds appeared to be biocompatible both *in vitro* and *in vivo*. Cell attachment on the tyrosine-derived polycarbonate scaffolds revealed robust cell attachment throughout the scaffolds. These 3D scaffolds significantly promoted osteogenic differentiation and mineralization as compared with 2D controls. These scaffolds also showed a potential as rhBMP-2 delivery carrier with controlled release profiles. *In vivo* studies are currently underway to determine bone regeneration in a CSD in the rabbit calvaria using rhBMP-2 treated tyrosine derived polycarbonate scaffolds.

References:

1. Langer R, Vacanti J. Science. 1993;260:920-6.
2. Griffith LG, Naughton G. Science. 2002;295:1009-1014.