PDGF-BB and Tyrosine-derived Polycarbonate with Calcium Phosphate Scaffolds Induce Osteogenic Differentiation

Pedro Alvarez-Urena1, Jinku Kim1, Maria Hanshella R. Magno2, Reva Street1, Aniq Darr2, Carlos Maria Olábarri-Santos3, Joachim Kohn2 and Jeffrey O. Hollinger1

1Bone Tissue Engineering Center, Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213, 2New Jersey Center for Biomaterials, Rutgers, The State University of New Jersey, Piscataway, NJ 08854

Introduction
There is a compelling need to develop synthetic bone substitutes that are biodegradable, biocompatible, and biomechanically matched to bone.(1) Further, a bone substitute should promote osteoblast cell attachment, proliferation, differentiation and mineralization. Based on these performance criteria, tyrosine-derived polycarbonates (Tyr-PC) are excellent bone substitute candidates. Tyr-PC are biocompatible, biodegradable, have mechanical properties suitable for bone, and can deliver biologicals.(2-3) Platelet derived growth factor (PDGF), a potent mitogen and chemoattractant, promotes wound healing and bone formation.(4, 5) Therefore, in this study, we determined the in vitro release kinetics, as well as the in vitro bioactivity of recombinant human (rh) PDGF-BB released from three-dimensional (3D) Tyr-PC scaffolds.

Materials and Methods
The porous scaffolds were synthesized from Tyr-PC terpolymers made from desaminotyrosyl tyrosine alkyl ester (DTR), desaminotyrosyl tyrosine (DT) and poly(ethylene glycol) (PEG) (Fig. 1). Selected scaffolds were coated through precipitation with calcium phosphate (CP), referred as Tyr-PC+CP. SEM was used determine Tyr-PC+CP porosity and in vitro biocompatibility was assessed with human mesenchymal stem cells (hMSCs). The dose of rhPDGF-BB added to the scaffolds was 2.5 µg. The release media was composed of incomplete Mesenchymal Stem Cell Growth Medium (MSCGM) supplemented with 1 % Bovine Serum Albumin (BSA) and 1 % antibiotic/antimycotic. The releasates were collected everyday until day 10 and thereafter every other day up to day 21. The rhPDGF-BB concentration was measured by Enzyme-linked Immunosorbent Assay (ELISA). Bioactivity of rhPDGF-BB releasates was determined with hMSCs and DNA concentration, alkaline phosphatase (ALP) activity and calcium content were measured for proliferation, osteoblast differentiation, and mineralization, respectively.

Results
Cumulative rhPDGF-BB release from Tyr-PC+CP scaffolds was greater than Tyr-PC, but not greater than a commercially available bone scaffold β-Tricalcium phosphate/ Collagen (β-TCP/ Collagen) (Fig. 2). Released rhPDGF-BB from scaffolds had similar bioactivity to exogenous rhPDGF-BB (added directly to the cell culture) and significantly increased DNA concentration, ALP activity and calcium content compared to scaffolds without rhPDGF-BB. The data suggest that rhPDGF releasates were biologically active and induced osteogenic differentiation and mineralization of hMSCs.

Discussion and Conclusions
Calcium phosphate incorporation into Tyr-PC scaffolds produced a sustained, calibrated release of rhPDGF-BB that was biologically active. The rhPDGF-BB releasates promoted osteogenic differentiation of hMSCs in vitro. Taken together, these data strongly suggest Tyr-PC+CP in combination with rhPDGF-BB may have a profound positive role in stimulating the osseous wound healing cascade and thus support bone regeneration.

References

Acknowledgment
This research was funded under AFIRM (Armed Forces Institute of Regenerative Medicine) by DoD activity contract # W81XWH-08-2-0034. This work was also supported by the BTEC at CMU and the NJCBM at Rutgers University and rhPDGF-BB was generously donated by BioMimetic Therapeutics, Inc. (Brentwood, TN).

Disclosures
The authors have nothing to disclose, except for J. Kohn who is the Chair of the Scientific Advisory Board of Trident Biomedical, Inc. Trident is commercializing this technology and J.Kohn is a stockholder of Trident and could receive royalty payments in the future.

Figure 1. General chemical structure of Tyr-PC polycarbonate terpolymers where XX and YY are %mol fractions of DT and PEG, respectively.

Figure 2. Cumulative rhPDGF-BB release from 3D scaffolds for 21 days. Tyr-PC is blue, Tyr-PC+CP is pink and β-TCP/Collagen is yellow. Data are reported as means (n=4) +/- standard deviations.