Characterization of Growth Factor Release and Bioactivity in Electrospun Polymer-Ceramic Composites

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Statement of Purpose: Recapitulating the structure and function of bone's extracellular matrix (ECM) using advances in tissue engineering is of importance in the regeneration of bone tissue. The ECM of bone is a composite consisting of organic and inorganic phases in the form of fibrillar Type I collagen and hydroxyapatite (HA) crystals respectively. Electrospinning is a polymer fabrication technique that generates non-woven fibrous scaffolds with fiber diameter sizes ranging on the order of nanometers to microns, thus resembling the structure of the ECM. The ECM also serves as a reservoir for growth factors such as bone morphogenetic protein-2 (BMP-2) and platelet derived growth factor-BB (PDGF-BB) which binds to receptors on mesenchymal stem cells (MSCs) to initiate the process of osteoinduction. In this study, PDGF-BB was incorporated in an electrospun polymer/ceramic composite consisting of polycaprolactone (PCL), and nanoparticles of biphasic ceramic, β-tricalcium phosphate (β-TCP) and hydroxyapatite (HA) (Patlolla, Collins et al. 2010) and evaluated for release and bioactivity as determined by the growth and osteogenic differentiation of human MSCs. PDGF-BB was incorporated using phase separation by the addition of polyethylene oxide (PEO) and by examining a novel technique of incorporating a cationic surfactant. PDGF-BB has an isoelectric point of 11.1 which would have an electrostatic attraction to the negative phosphate ions in the ceramic and may impede its release from the composite. Therefore, the cationic surfactant, hexadecyltrimethylammonium bromide (CTAB) was added to form a complex with the phosphate ions which would, in turn, reduce the electrostatic attraction between PDGF-BB and the biphasic ceramic, leading to the release of PDGF-BB from the electrospun scaffolds.

Methods: *Composite Fabrication:* PCL and PEO were dissolved in chloroform at a weight ratio of 30% PEO/70% PCL. The nanoparticles of 80/20 β-TCP/HA was suspended in chloroform and added to the PEO/PCL solutions at a concentration of 30% (w/w) with respect to polymer mass. For CTAB incorporated solutions, CTAB was added to the 80/20 β-TCP/HA suspension in chloroform at a molecular ratio of 1 CTAB to 6 phosphate (PO_4^{3-}) ions. The non-ionic surfactant Span® 80 was also added to the polymer/ceramic solutions before the incorporation of recombinant human PDGF-BB (Invitrogen). The solutions were emulsified using a probe ultrasonicator. The resulting emulsions were then electrospun using standard parameters (Patlolla, Collins et al. 2010). For controls, PDGF-BB incorporated PEO/PCL solutions without ceramic were electrospun. *PDGF-BB Release:* Electrospun mats were placed in a 24 well plate and incubated in phosphate buffered saline (PBS) at 37°C for designated time-points. A PDGF-BB ELISA (Peprotech) kit was used to quantify the amount of PDGF-BB released. *MSC Growth and Differentiation:* Electrospun mats were cut into 6 mm discs and placed in polypropylene 96 well plates. Human MSCs were seeded onto the scaffolds at 4000 cells/well in medium containing 5% fetal bovine serum (FBS) and osteoinductive factors: β-glycerophosphate, L-ascorbic acid phosphate and dexamethasone. At designated time-points, the cells were harvested for the Picogreen and Alkaline Phosphatase (AP) assays.

Results: The amount of PDGF-BB released from PEO/PCL+ ceramic was significantly less than the PDGF-BB released from PEO/PCL (Figure 1). By incorporating CTAB in 80/20 β-TCP/HA, the amount of PDGF-BB released increased significantly.

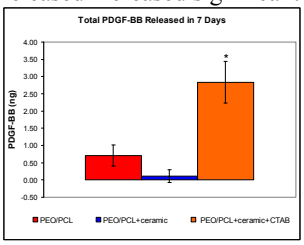

Figure 1. Total PDGF-BB released in 7 days. (*p<0.05)

The cell proliferation of MSCs on the CTAB incorporated scaffold with PDGF-BB was higher than the other electrospun scaffolds, although not statistically significant, at time-points (days 4-9) (Figure 2A).

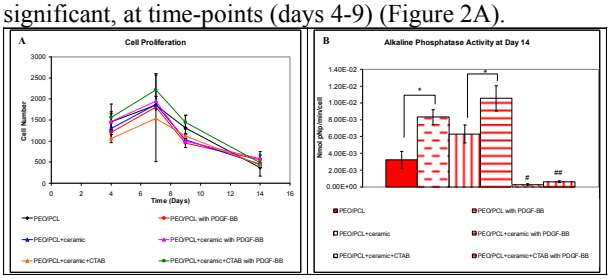

Figure 2. A)Cell proliferation. B)AP Activity at Day 14. (*p<0.05).

However, at day 14, there was statistically less AP activity for cells cultured on the CTAB containing electrospun scaffolds with and without PDGF-BB (Figure 2B). Interestingly, cells cultured on PDGF-BB incorporated scaffolds of PEO/PCL and PEO/PCL+ ceramic had statistically higher AP activity as compared to scaffolds without PDGF-BB indicating that the PDGF-BB is bioactive and promoting the osteogenic differentiation of MSCs.

Conclusions: The 80/20 β-TCP/HA reduces the amount of PDGF-BB released from the scaffold due to the electrostatic interactions. However, the incorporated PDGF-BB remains bioactive as indicated by the increased AP activity of the cells. CTAB had a positive effect on protein release and cell proliferation but not on AP activity which may be an adverse effect of CTAB. An alternative cationic agent may be warranted for further investigation.