Scaffold-Directed Stem Cell Behavior: The Effect of Polymer Ceramic Composites on Bone Repair

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Statement of Purpose: Currently available bone grafts present certain limitations, donor-site morbidity for autografts and risk of disease transmission for allografts to name a few, which suggest a need for alternatives. Bone graft substitutes have been made using polymers, ceramics, and composites of both to form scaffolds suitable for bone tissue engineering. The use of calcium phosphates (CaP) has a widespread appeal due to its osteoconductivity and osteogenicity. We have developed a biodegradable scaffold for bone tissue engineering based on poly(lactide-*co*-glycolide)/calcium phosphate composite microspheres in which a low crystallinity calcium phosphate is synthesized within the forming microspheres [1]. The focus of the present work was to analyze the effectiveness of this composite scaffold as in osteoinductive scaffold for bone repair. Toward this end, human adipose-derived stem cells seeded on composite scaffolds were evaluated for osteoblast phenotype markers and mineralization.

Methods: Microsphere based matrices were formed using published methods [1]. Briefly, poly(lactide-co-glycolide) (PLAGA) was mixed with methylene chloride and separate solutions of calcium nitrate tetrahydrate and ammoniumhydrogen phosphate. The mixture was vortexed and added dropwise to a stirring solution of 1% polyvinyl alcohol (PVA) held at 4°C. Solution pH was maintained at pH 10 using a pHSTAT (Metrohm, Herisau, Switzerland) and mixed for 24 hours, during which time hydroxyapatite (HA) was precipitated and encapsulated within the microspheres. After mixing the composite microspheres were rinsed with DH_2O and isolated via vacuum filtration. Two ratios of polymer:ceramic microspheres were synthesized; low HA (17% HA) and high HA (27% HA). Microspheres were placed into a stainless steel mold and heated at 90ºC for 90 minutes to form 3-dimensional porous scaffolds with an interconnected pore structure. Human adipose-derived mesenchymal stem cells were seeded onto scaffolds and cultured in Dulbecco's modified eagles medium containing 10% fetal bovine serum and 1% antibiotics50 μM ascorbic acid, 10 μM β-glycerophosphate, and 10 nM vitamin D_3. Cells on scaffolds (n=3) were evaluated after 3, 7, 14, and 21 days for proliferation using Quant-iT PicoGreen dsDNA Reagent Kit (Molecular Probes, Invitrogen, Eugene, OR) and differentiation using an alkaline phosphatase reagent kit (Bio-Rad, Hercules CA), osteocalcin ELISA (Biomedical Technologies Inc. Stoughton MA), and an alizarin red mineralization quantification assay. Two-way ANOVA was used to evaluate statistical significance between groups ($p<0.05$).

Results: Cellular proliferation on the composite scaffolds indicated no significant differences on day 3, 7, and 21 but cell numbers were slightly higher on pure polymeric scaffolds than low HA content scaffolds after 14 days. Overall trends showed increases in cell number on both forms of composite scaffold and a peak in proliferation after 14 days on pure polymeric scaffolds. Similar trends were noted for cellular alkaline phosphatase expression,

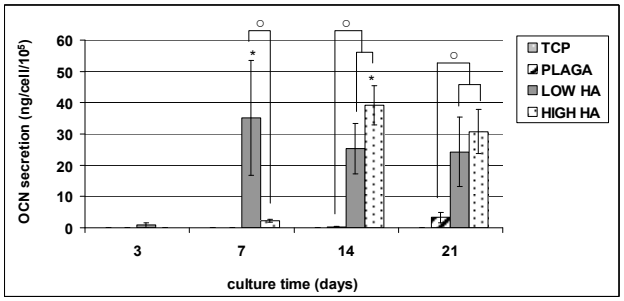

Figure 1. Osteocalcin secretion by cells seeded onto scaffolds after 3, 7, 14, and 21 days of culture. Scaffolds containing HA show elevated levels of secreted osteocalcin.

while measurable amounts were noted there was no clear trend over time related to scaffold content. Osteocalcin expression, however, was noted to be significantly higher after 14 and 21 days of culture in cells seeded on composite scaffolds as compared to cells seeded on either tissue culture polystyrene or pure polymeric scaffolds (see figure 1). Alizarin red staining was noted to be evident on both seeded and unseeded scaffolds, but was notably higher on seeded scaffolds. Interestingly, scaffolds containing no HA showed little to no positive alizarin red staining, suggesting no mineral deposition by seeded cells.

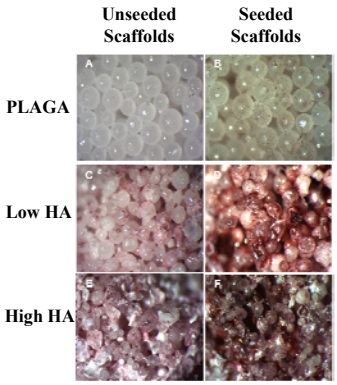

Figure 2. Alizarin red stained mineral formation on scaffolds without (A,B) and with (C-F) HA. Staining is visible on unseeded (A,C,E) but is more abundant on seeded scaffolds (B,D,F).

Conclusions: The results presented here show osteocalcin production and positive alizarin red staining on scaffolds seeded with adipose-derived mesenchymal stem cells, suggesting osteoinductive potential of the composite scaffolds. Degradation studies have shown calcium ion release from the composite scaffolds within hours of submersion into an aqueous milieu [2], which may be in part responsible for the osteoblast-like behavior seen here. Additional studies have shown the composite scaffolds to have much greater capacity to bind and retain proteins than pure polymeric scaffolds [3], suggesting that the addition of HA may make important proteins and factors available to adipose-derived stem cells. The potential osteoinductivity of this scaffold suggests a new tool in the field of scaffold-based bone repair.

References: [1]Ambrosio et al. J Biomed Mater Res 58(3), 295 (2001). [2]Khan Y et al. J Mat Sci 42 4183 (2007). [3] Cushnie et al. J Biomed Mater Res. IN PRESS