Polymer-Ceramic Composite Induces Osteogenic Differentiation of Human Mesenchymal Stem Cells

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Statement of Purpose: Bone is the most commonly replaced organ of the body[1]. Due to limitations associated with autografts, there is significant interest in a tissue-engineered substitute for bone repair. Previously, we have demonstrated *in vitro* that a composite scaffold of polylactide-*co*-glycolide (PLGA) and 45S5 bioactive glass (BG) is osteoconductive and osteoinductive[2]. To mimic the mineral phase of bone, we have fabricated a composite scaffold of PLGA and hydroxyapatite (HA).

The objectives of this study are to compare the response of human mesenchymal stem cells (hMSCs) cultured on PLGA-BG and PLGA-HA composites, and to test the hypothesis that ion dissolution from the polymer-ceramic composites mediates the osteoinduction of hMSCs. It is anticipated that the PLGA-BG composite will support hMSC osteogenic differentiation, and this induction is facilitated by solution ions.

Methods: Microspheres of PLGA, 85:15 and composite microspheres (PLGA-BG, PLGA-HA) were fabricated via a water-in-oil emulsion method[2]. The scaffolds were then formed by sintering the microspheres above polymer T*g* for 6 hours[2]. Mercury porosimetry and scanning electron microscopy (SEM) were used to determine average porosity, average pore size and surface characteristics of fabricated scaffolds.

Human mesenchymal stem cells (hMSCs, Cambrex, Herndon, VA) were seeded on each substrate (PLGA, PLGA-BG, PLGA-HA) at a density of 3,100 cells/cm^2 and cultured in DMEM+10% FBS, 1% antibiotics, and 1% non-essential amino acid over four weeks. Additionally, monolayer hMSCs were treated with conditioned media from PLGA, PLGA-HA and PLGA-BG scaffolds in order to evaluate the effects of ion concentration on cell differentiation. At each time point, the samples were collected and evaluated for total DNA content, alkaline phosphatase (ALP) activity and gene expression of osteocalcin and osteopontin.

Results: *Scaffold Characterization*– The as-fabricated microsphere scaffolds measured an average diameter of 6.5mm and height of 2mm. The average porosity and pore diameter were found to be 42 ± 2% and 109 ± 5 µm respectively.

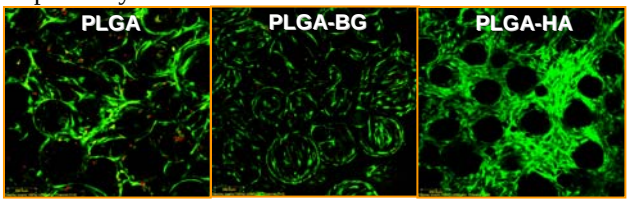

Figure 1: Cells was evenly distributed and viable after 28 days. Extensive matrix deposition was observed. Qualitatively, fewest cells were observed on PLGA-BG scaffolds.

Cellular Response – Cells remained viable and proliferated on the microsphere scaffolds for the duration of the experiment (Fig. 1). It was found that when compared to PLGA, PLGA-HA and monolayer controls, hMSCs grown on PLGA-BG composite scaffolds expressed the highest level of osteogenic markers such as osteocalcin and osteopontin in the absence of osteogenic media (Fig. 2). Also, ALP activity was significantly higher on the composite than on the PLGA scaffolds (Fig. 3A). Similar results were found in the conditioned media study (Fig. 3B).

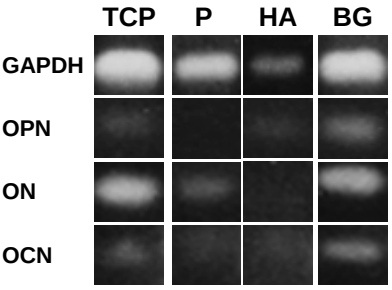

Figure 2: Gene expression for osteopontin (OPN), osteonectin (ON), and osteocalcin (OCN) by hMSCs grown on tissue culture polystyrene (TCP), PLGA (P), and PLGA-BG (BG), Day 7.

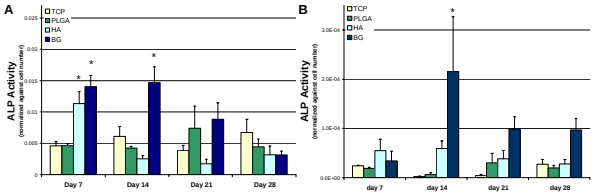

Figure 3: Normalized ALP activity for hMSCs cultured on TCP, PLGA, PLGA-HA and PLGA-BG.A) hMSCs seeded on microsphere scaffolds. B) hMSCs treated with conditioned media from microsphere scaffolds.

Our results suggest that substrate composition plays a significant role in modulating the osteogenic differentiation of hMSCs. Stem cells grown on PLGA-BG expressed osteogenic markers while none were found PLGA-HA or PLGA scaffolds. Moreover, osteoinduction of hMSCs on PLGA-BG was independent of stimulation with osteogenic media, suggesting that the observe responses were mediated by the scaffold alone.

Several studies have reported on the osteoinductive potential of BG[3-6], and our results suggest that PLGA-BG composite is also osteoinductive. The mechanism for this induction is however not known. We found here that conditioned media from PLGA-BG promoted hMSC ALP activity, suggesting that solution ions (e.g. Si, Ca or phosphate) may be important for osteoinduction.

Conclusions: Stem cells grown on PLGA-BG scaffolds exhibited greater osteogenic potential than those of PLGA-HA or PLGA alone. The observe responses were mediated by the scaffold, independent of stimulation by osteogenic media. The findings of these studies collectively demonstrate the superior osteoinductivity of the PLGA-BG composite and suggest that induction is mediate by soluble ions released during surface transformation of the PLGA-BG scaffold.

References: [1] Delacure, Otolaryngol Clin North Am. 1994. [2]Lu *et al.*, J Biomed Mater Res 2003. [3] Matsuda and Davies, Biomaterials 1987. [4]Yuan *et al.*, J Biomed Mater Res 2001 [5]Gatti et al., Biomaterials. 1994. [6] Ohgushi *et al.*, J. Biomed. Mater. Res. 1996. [7] Bosetti and Cannas, Biomaterials. 2005. [8] Yao *et al.*, J Biomed Mater Res 2005. **Acknowledgement:** This study was supported by NIH-NIAMS and the Wallace H. Coulter Foundation.