Synergistic effect of sustained release growth factors from PLGA microspheres and dynamic bioreactor flow on hMSC osteogenic differentiation in alginate scaffolds

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Statement of Purpose: Microparticles have been utilized as delivery vehicles of soluble factors, such as growth factors, to enhance tissue engineering regeneration by modifying cellular behavior. When incorporated into 3D systems, they can provide both geometrical and temporal controlled release of bioactive agents to cells (Oliveria MB Biotech Prog. 2011;27(4):897-912). Current cultures of cells and growth factors in scaffolds however, have limitations such as inadequate exposure and inaccurate delivery of growth factors. In addition, existing combinations of microparticles in dynamic flow conditions have been unsuccessful. This study investigates the effect of growth factor release from PLGA microspheres on the osteogenic differentiation of human mesenchymal stem cells (hMSCs) encapsulated in alginate bead scaffolds when cultured in a dynamic tubular perfusion (TPS) bioreactor system. We hypothesize that the addition of sustained-released growth factors to a dynamic flow environment will results in a synergistic effect on osteogenic differentiation of hMSCs.

Methods: Growth-factor-loaded PLGA microspheres were fabricated using the novel continuous super-emulsion extraction (SEE) method, averaging 2-3µm in diameter (Della Porta G. Biotech & Bioeng 2011;208(3):676-686). They were loaded with bone morphogenic protein-2 (BMP-2), vein endothelial growth factor (VEGF), or a 50:50 mix of both. Unloaded microspheres were used as controls. The microspheres and hMSCs were homogenously incorporated into a 2% alginate solution and added drop-wise to a calcium chloride solution using a syringe and 16½G needle creating alginate beads scaffolds (Figure 1A). Approximately 100,000 hMSCs and 10mg of growth factor were encapsulated into each bead. SEM images of flash-frozen alginate beads show distribution of the cells and microspheres (Figure 1B). The cell- and microsphere-loaded scaffold was then cultured in the TPS bioreactor as previously described (Yeatts AB. Biotech & Bioeng. 2012;109(9):2381-2391) for 21 days. Each experimental group was placed into individual growth chambers and cultured with osteogenic hMSC media at 2.5mL/min. Beads cultured in static conditions were used as controls. On days 0, 7, and 21, beads were removed from each experimental group for histological analysis. Slices were immunostained for osteogenic markers including alkaline phosphatase (ALP), osteocalcin (OCN), and osteopontin (OPN). Cell viability was determined using ethidium homodimer and calcein AM stains. In addition, the release of growth factors from the SEE-fabricated microspheres in media at physiological conditions was studied over a 21-day period and monitored using an ELISA assay. All tests were completed with triplicates.

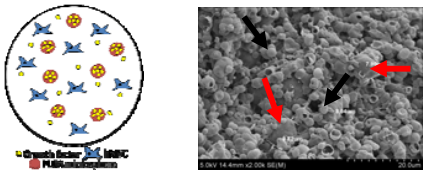

Figure 1. A) Schematic of growth factor-loaded PLGA microspheres and hMSCs encapsulated in alginate scaffold. B) SEM image of bead showing homogenous incorporation of microspheres (black arrows) and cells (red arrows).

Results: Histology results showed dynamic cell culturing significantly impacted osteogenic hMSC differentiation compared to those cultured in static conditions. In addition, those cells supplemented with growth factor-loaded PLGA microspheres had increased expression of osteogenic markers as compared to those cultured with empty microspheres. Specifically, cells exposed to BMP-2 produced greater amounts of ALP, OCN (Figure 2), and OPN for all three time points as evident in the darker and more intense staining, compared to the unloaded microsphere control group. VEGF had the least visible effect on osteogenic marker expression, although still greater than the control. In addition, histology indicated that mixed growth factor supplement had comparable osteogenic effects on the hMSCs as the BMP-2 growth factor alone, further confirming VEGF's limited impact. Lastly, ELISAs showed sustained growth factors released from microspheres over a 21-day period confirming continuous diffusion to cells.

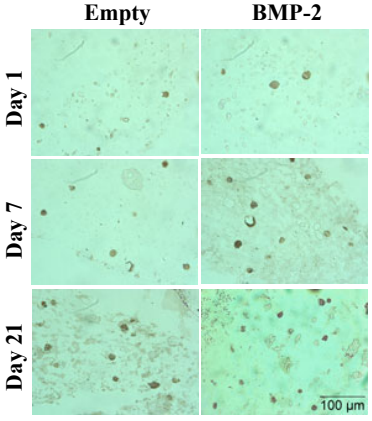

Figure 2.Osteocalcin immunostaining of alginate beads with empty or BMP-2-loaded microspheres cultured in TPS bioreactor. Greater intensity of osteocalcin staining observed for all time points cultured with BMP-2 compared to control. All images taken at 40x.

Conclusions: The release study showed that SEE is a suitable fabrication method of PLGA microspheres to provide sustained release of growth factors. Also, BMP-2 had a dominant effect on osteogenic marker production compared to VEGF in both static and dynamic conditions, indicating that it is an important growth factor in the differentiation pathway. Hence, dynamic flow culturing environment in the TPS bioreactor and sustained-release growth factors from PLGA microspheres show to have a synergistic effect on osteogenic differentiation of hMSCs. Future studies will include optimization of the BMP-2 dose delivered to hMSCs.