Multifunctional Biodegradable Porous Microspheres to Act as Stem Cell Delivery Vehicles and Local Drug Delivery Platform for Bone Tissue Regeneration

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Statement of Purpose: Current challenges to bone tissue engineering are the difficult expansion of a cell population while maintaining their undifferentiated state, and directing them toward the desired cell type. The wound environment is complex and the path toward tissue regeneration and healing involves many well-timed and overlapping steps. Our hypothesis focuses on the development of injectable biomaterials to facilitate effective would healing by addressing the challenges currently facing tissue engineers. Our approach seeks to provide a 3D matrix that mimics the microenvironment of bone tissue by using physical cues to maintain their stemness until cross-talk with the natural tissue can direct the cells at the appropriate time. The microsphere carrier seeks to provide a local, controlled release of drugs, deliver growth factors and facilitate stem cell-based wound healing. In this study we prepared 3D porous composite microspheres to act as stem cell carriers with a mineralized surface for binding the bisphosphonate drug, alendronate and facilitate a controlled, local delivery. This unique polymer/bioceramic design was seeded with mammalian Mesenchymal Stem Cells (MSCs) and murine preosteoblasts and various cell-microsphere interactions were examined. These preliminary results indicate we have produced a promising biomaterial strategy for local drug delivery and stem cell-based tissue engineering and regenerative medicine.

Materials and Methods: Using a double emulsion and porogen leaching techniques, various amounts of poly (lactic-*co*-glycolic acid) (PLGA), and gelatin were combined to yield microspheres of varying porosity and microstructure. In this experiment the gelatin in the aqueous phase was leached from the PLGA matrix to yield 3D porous microspheres. In addition to a cell delivery vehicle, we functionalized the surface with hydroxyapatite (HA) minerals by immersion in a 10x simulated body fluid (SBF) for various time periods. Following crystal precipitation, the polymer/bioceramic microspheres were immersed in a 1000 nM solution of alendronate. The release kinetics of alendronate delivery were studied by subjecting the drug-loaded microspheres to dynamic conditions and measuring the UV/Vis of the supernatant at 1, 2, 6 and 12 hours and 1, 3, 5, 7, 14 and 21 days. Cell-microsphere interactions were examined by fixing and staining various cell types and imaging their morphology with confocal microscopy. ALP activity was quantified at 7 days and calcium mineralization was analyzed at 21 days.

Results and Discussion: The porous composite microspheres were created with uniform size distribution of 100-300µm in diameter and average pore size of 15µm and a porosity >90%. Our previous studies suggest that scaffolds of similar micro and macro structure to these microspheres are suitable for cell growth *in vitro* and further encourage tissue formation *in vivo*. We seeded various cell types onto spheres and saw porous neat and porous HA microspheres had higher viability over 24 hours compared to solid PLGA control microspheres. Cells exhibited excellent attachment and proliferation at early time points and demonstrated the ability to differentiate down different lineages. ALP activity and calcium mineralization of porous microsphere groups increased significantly over solid controls. Drug release studies showed a sustained and controlled release over the duration of the time points.

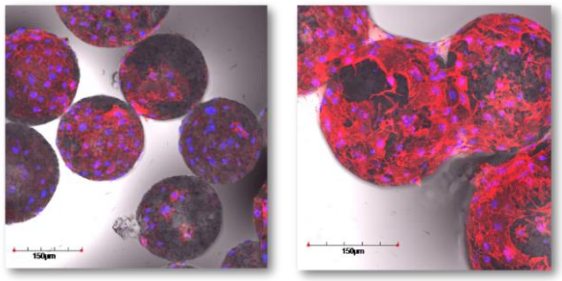

Figure 1. Confocal microscopy maximum projection of mMSCs as stained by Phalloidin-Texas Red (red-cytoskeleton) and DAPI (blue - nucleus) (Scale bar=150) µm. (Left) 1.1x zoom, after 1 Day in culture, (Right) 1.5x zoom, after 3 Days in culture.

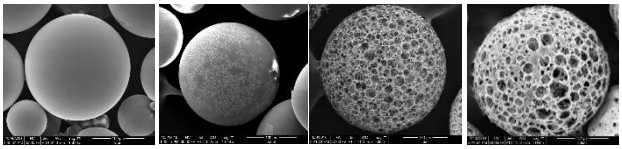

Figure 2. SEM images of microspheres from left to right: solid neat, solid with HA crystals precipitated on the surface, porous neat, and porous with HA crystals precipitated on the surface. (Scale bars=100 µm).

Conclusions: This unique cell carrier and drug delivery platform composed of a polymer/bioceramic composite design incorporates PLGA and HA, as well as chemically conjugated alendronate. The data from this *in vitro* model demonstrated that our multifunctional biomaterial supports cellular attachment and proliferation via engineered micro- and macrostructure, HA crystal precipitation and local controlled drug delivery. The results indicate we have produced a promising biomaterial strategy for both local drug delivery and stem cell-based tissue engineering and regenerative medicine.