Calcium Phosphate-Containing Scaffolds Stimulate Early Stage Osteogenic Differentiation

Jenni R. Popp[1], Brian J. Love[2], Kate E. Laflin[3], Aaron S. Goldstein[1,3]

1. Virginia Tech - Wake Forest School of Biomedical Engineering and Sciences
2. Departments of Materials Science and Biomedical Engineering, University of Michigan, Ann Arbor
3. Department of Chemical Engineering

Statement of Purpose: Tissue grafts engineered to facilitate healing of bone defects require scaffolds capable of bearing load and supporting the growth of osteoprogenitor cells. Hydroxyapatite (HAP) has been widely used as a scaffold for bone grafts due to its osteoconductivity and biocompatibility [1]. However, HAP lacks osteoinductive properties to stimulate osteogenesis and is resistant to biodegradation.

In contrast, amorphous calcium phosphate (ACP) is a mineral that solubilizes under aqueous conditions, releasing calcium and phosphate ions [2]. We propose that the immobilization of ACP particles within a biodegradable PLGA scaffold will enhance the osteoconductivity of the scaffold while providing calcium and phosphate ions to stimulate osteogenic differentiation. The goal of this study was to fabricate composite scaffolds of PLGA microspheres and ACP and determine their effect on in vitro osteogenic differentiation of MC3T3-E1 cells.

Methods: Microspheres were fabricated using an oil-in-water emulsion technique. Poly(lactic-co-glycolic acid)(PLGA) [75:25] (M_w = 97,100, [η] = 0.55-0.75 dL/g, Lactel Biodegradable Polymers, Birmingham, AL) was dissolved in methylene chloride, added to a stirred solution of 1% poly(vinyl alcohol) (PVA; M_w = 25,000, 88% mole hydrolyzed; Sigma-Aldrich, St. Louis, MO) and stirred at 200 rpm for 24 h to allow for complete evaporation of the solvent [3]. The microspheres were isolated from the PVA solution by vacuum filtration, washed with deionized water, and then vacuum-dried for an additional 24 h.

For composite scaffolds containing 0.5% (w/v) ACP, mineral particles with diameter less than 106 μm were mixed with PLGA microspheres (300-500 μm diameter), added to a 24-well plate and heated at 70°C for 24 h.

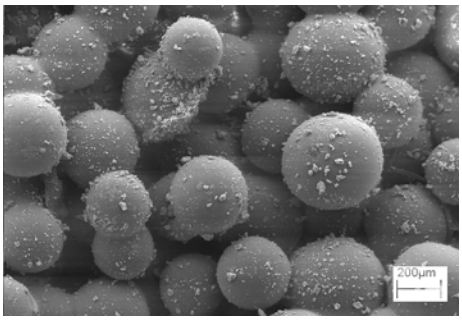

Figure 1: SEM image of PLGA microsphere scaffold with 0.5% ACP.

Scanning electron microscopy (SEM) was used to evaluate scaffold morphology. Scaffolds were sputter-coated with palladium (Model 208HR, Cressington Instruments, Cranberry Township, PA) and images were acquired using a LEO 1550 Field Emission SEM (Carl Zeiss SMT, Thornwood, NY).

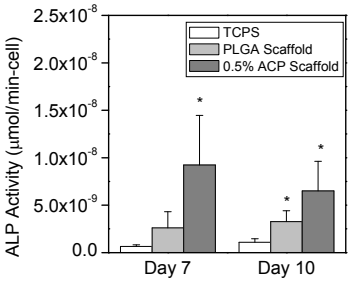

Figure 2: ALP activity of pre-osteoblasts on composite scaffolds.

Cell growth and osteogenic differentiation on the scaffolds were assessed by culturing MC3T3-E1 pre-osteoblasts on the surface of composite scaffolds. Cell number and alkaline phosphatase activity were measured at days 7 and 10 using Hoechst dye (Sigma, St. Louis, MO) and a commercially available kit (Biotron Diagnostics, Hemet, CA), respectively.

Results: SEM images of the scaffolds demonstrate the distribution of calcium phosphate particles on the surface of the microspheres (Figure 1). Cells proliferated on all surfaces (data not shown). Cells cultured on composite scaffolds had elevated ALP activity at both time points when compared to tissue culture polystyrene and scaffolds containing no ACP (Figure 2).

Conclusions: This study investigated the effect of scaffolds containing ACP on the early-stage differentiation of a pre-osteoblast cell line. Scaffolds containing 0.5% ACP supported cell growth and stimulated ALP activity 7 and 10 days post-seeding.

Further studies will characterize the morphology and mechanical properties of composite scaffolds. In addition, we aim to analyze late-stage osteoblast differentiation on the composite scaffolds using PCR.

References:

1. Whited, B.M., et al., J Biomed Mater Res A, 2005.
2. Skrtic, D., et al., J Biomed Mater Res, 2002. **59**(4): p. 597-604.
3. Borden, M., et al., Biomaterials, 2002. **23**(2): p. 551-9.