The Effect of Crosslinking Density and Pore Geometry of Poly(propylene fumarate)/Diethyl Fumarate Composite Scaffolds on Osteogenic Signal Expression of Rat Bone Marrow Stromal Cells

Kyobum Kim[1], Anqi Lu[1], Rob Breithaupt[2], David Dean[3], Antonios G. Mikos[4], and John. P. Fisher[2]
[1]Department of Chemical and Biomolecular Engineering, University of Maryland, College Park, MD
[2]Fischell Department of Bioengineering, University of Maryland, College Park, MD
[3]Department of Neurological Surgery, Case Western Reserve University, Cleveland, OH
[4]Department of Bioengineering, Rice University, Houston, TX

Statement of Purpose: Diethyl fumarate (DEF) can be incorporated within poly(propylene fumarate) (PPF) to increase crosslinking density and stiffness of composite scaffolds due to the creation of additional bridges between PPF polymer chains. Using these composites, we hypothesize that increasing DEF content and increasing pore size of 3D macroporous scaffolds would promote osteogenic signal gene expression by augmenting substrate rigidity[1] and facilitating nutrient transport, therefore enhancing the osteogenic differentiation of progenitor cells. Therefore, we investigated the effect of DEF content and scaffold pore sizes on osteogenic signal expressions of rat bone marrow stromal cells (BMSCs) on PPF/DEF macroporous composite scaffolds.

Methods: PPF was synthesized according to previous methods[2]. 3D macroporous composite scaffolds were fabricated with 100:0, 90:10, 75:25, and 66:33 ratio of PPF:DEF DEF as well as 180-300 and >500 μm pore sizes by simple porogen (NaCl) leaching method with the aid of photoinitiator, bis(2,4,6-trimethylbenzoyl) phenylphosphine oxide (Ciba Specialty Chemicals, Tarrytown, NY). Scaffolds were first characterized with (1) sol fraction to determine crosslinking density of samples, and (2) compressive modulus and offset yield strength were tested to measure mechanical strength. To determine the initial metabolic activity of rat BMSCs on these 3D scaffolds, a MTT assay was performed with samples of the four different PPF:DEF ratios. Along with this test, the effect of dissolved DEF in culture media for a monolayer cell was also investigated for up to 4 hrs of incubation. To see the effect of both fabrication parameters on osteogenic signal expression of BMSCs, reverse-transcription polymerase chain reaction (RT-PCR) was performed with 100:0 and 66:33 ratio of PPF:DEF as well as two pore sizes. Half million cells per scaffold were cultured for 8 days, and isolated total RNA was reverse transcribed to cDNA. Using Taqman gene expression assays (Applied Biosystems, Foster City, CA) including fibroblast growth factor-2 (FGF-2), transforming growth factor beta-1 (TGF- β1), vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMP)-13 as well as two osteogenic differentiation markers including alkaline phophatase (ALP) and osteocalcin (OC), relative gene expression levels were assessed.

Results: Results demonstrated that incorporation of DEF decreased sol fraction in composite scaffolds (Figure 1), and therefore likely increased level of crosslinking density. In addition, the 75:25 of PPF:DEF ratio exhibited the highest compressive modulus and offset yield strength (Figure 2A and 2B). Initial metabolic activity of cells in DEF incorporated scaffolds was similar to those in the PPF control (Figure 3A). However, DEF dissolved in culture media showed a negative effect on monolayer cell metabolic activity for 4 hrs (Figure 3B). RT-PCR data on day 8 demonstrated that osteogenic signal gene expression including FGF-2, TGF- β1, VEGF, and MMP-13 could be controlled by both DEF content and pore size (Figure 4A). These signals altered early osteogenic differentiation, as demonstrated by ALP, but did not significantly alter late osteogenic differentiation, as demonstrated by OC expression (Figure 4B).

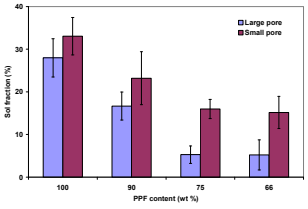

Figure 1

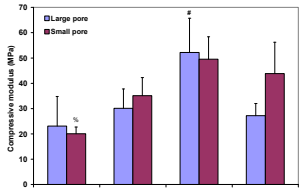

Figure 2A

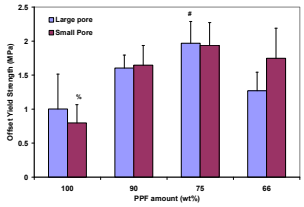

Figure 2B

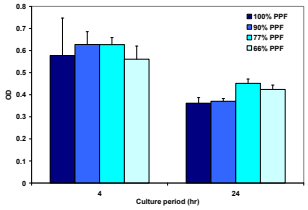

Figure 3A

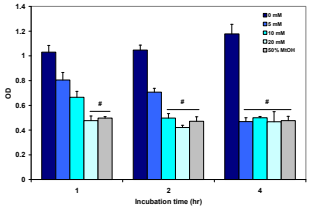

Figure 3B

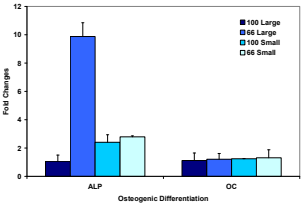

Figure 4A

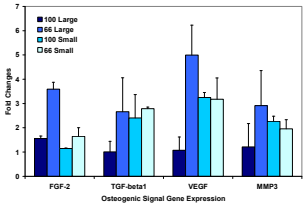

Figure 4B

Conclusions: We concluded that the DEF content and pore size of macroporous composite scaffolds are parameters that are critical to control osteogenic signal gene expression during early osteogenic differentiation of cultured progenitor cell populations.

References:

1. Fisher et al., Biomaterials, 2002;23: 4333–4343
2. Kim et al., Biomacromolecules, 2009;10: 1810-1817