Solution-Mediated Effect of Biomimetically-Mineralized Poly*(lactide-co-glycolide)* Scaffold on Osteogenic Differentiation of Mouse Bone Marrow Stromal Cell

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Statement of Purpose: Biomimetic apatite coating on polymer scaffolds promotes osteoblastic differentiation *in-vitro* [1]. Not only does the Ca-P material provide a surface for cells to grow on, but its dissolution products may also evoke a cellular response and/or reprecipitate back onto the surface. However, it is not understood how soluble products from biomimetic apatite change the local composition of the medium, and lead to a solution-mediated effect on the osteoblastic differentiation. To address this question, changes in osteogenic differentiation of mouse bone marrow stromal cells (BMSCs) were investigated under solution-mediated influence of a biomimetically-mineralized poly(*lactide-co-glycolide*) (PLGA) scaffold.

Methods: Three dimensional porous PLGA scaffolds and mineralized PLGA scaffolds were fabricated as previously described [2]. PLGA scaffolds were prepared by a solvent casting–particulate leaching process to a thickness of 5mm, diameter of 1mm and pore size of 250-425μm. Mineralized scaffolds were prepared by incubating PLGA scaffolds at 37°C for 12 days in 2x simulated body fluid (2xSBF), an ionic solution that includes 2X the ionic concentrations of plasma.

BMSCs were isolated from the femora and tibiae of 5-week old mice and plated at a density of 5 x 10^4 cells/well of 24-well tissue culture plate. A PLGA scaffold or a mineralized PLGA scaffold was placed on an insert membrane with 8μm pores so that the scaffold was separated from the cells and only solution could penetrate through the insert. Empty inserts were placed as a control group. Cells in each well were grown in either 1ml of cell culture medium or osteogenic medium which contained 50mg/L ascorbic acid, 10mM β-glycerophosphatase and 10^{-8} M dexamethasone. Each medium was changed every 3 days. Cell growth was determined by an MTS assay. At days 3, 6, 9, 12, 15 and 18, osteocalcin (OCN) released into the medium was measured by mouse OCN EIA (Biomedical Tech Inc). Ionic concentrations of Ca in the media were measured by optical emission spectrometry. The sample size of each group was 4 and 1-way ANOVA was used to test for effects of Ca content on cellular propagation and differentiation.

Results/Discussion: Mineralized PLGA lowered the Ca concentration of the medium to less than 40% of the normal Ca level in a medium (84.30 ±0.96 → 32.53 ±1.90 mg/L), whereas PLGA did not change the Ca level of the medium. OCN production significantly increased from day 12 to 18 for every group (control, PLGA and mineralized PLGA) cultured in the osteogenic differentiation medium ($p<0.05$). On each day, OCN production was significantly less ($p<0.001$) at lower concentrations of extracellular Ca in the media (Fig.1). These outcomes may have resulted from Ca adsorption from the medium to the surface of the mineralized PLGA scaffold, which enriches the scaffold with Ca and lowers Ca concentration in the medium. Cell growth was significantly inhibited in the medium with low Ca level (32.53 ±1.90 mg/L) at day 6 and 12 compared to the control group ($p<0.001$) (Fig.3)

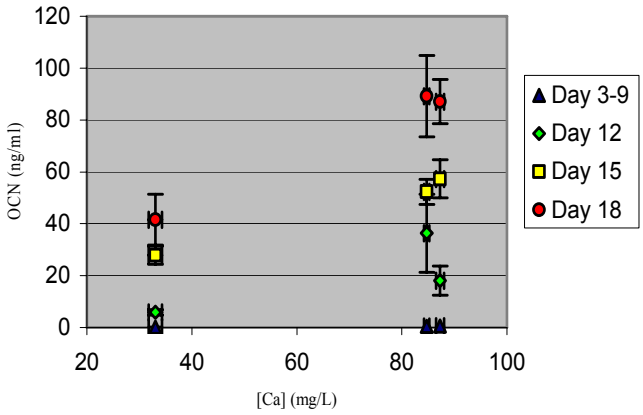

Figure 1: OCN production in different [Ca] of the media (Mineralized PLGA, control and PLGA from the left colume)

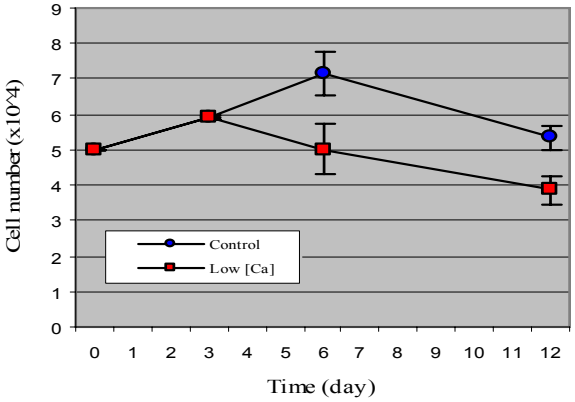

Figure 2: Inhibited cell growth in media with low Ca concentration

Conclusions: Osteogenic differentiation of BMSCs was inhibited by low Ca concentration in the media, and the cellular response to a Ca-P biomaterial is modulated by solution as well as surface mediated effects.

References:

[1] Chou YF. J Biomed Mat Res. Part B 2005:81-90
[2] Shin K. 7Th World Biomaterials Congress 2005