Effect of Hydroxyapatite Content in Biomimetic Composite Scaffolds on Bone Marrow Stromal Cell Differentiation

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Statement of Purpose: Tissue engineering utilizes the life sciences, medicine and engineering to develop techniques to restore, maintain or improve tissue function.[1] Tissue engineers accomplish this through scaffold design, combined with cell and factor-based therapies. One important approach in scaffold design is *biomimetics*, or the application of and improvement upon naturally occuring phenomena to bring about desired biologic effects. One biomimetic approach is to mineralize a polymer scaffold using simulated body fluid (SBF). This process results in the inclusion of apatite crystals, chemically similar to those found in the mineralized extracellular matrix (ECM). While the use of hydroxyapatite (HA) as a scaffold material has been deemed beneficial[2], work has not been done to determine the optimal volume fraction of HA in composite scaffolds. If the amount of HA can be limited, while maintaining its beneficial properties and reducing the negative effects of SBF treatment, scaffold properties potentially be improved upon.

Methods: Poly (L-lactic) acid (PLLA, Resomer L207, Boehringer Ingelheim, GmbH)) films were fabricated by spin coating, where a 5% solution of PLLA/1,4 dioxane was coated onto 15mm glass coverslips. Films were then subjected to treatment with 1.5X SBF for times of 3, 7 and 14 days. These treatment times yielded films with HA coverage of >10%, 50% and 100%, respectively. An untreated control group was also included. Rat bone marrow stromal cells (MSC) were harvested from 6-week-old male Fisher rats, by flushing the femurs with Eagle's minimum essential medium (α-MEM, Invitrogen; Carlsbad, CA). Adherent cells were cultured and expanded and osteoblastic differentiation induced using osteogenic medium (α-MEM supplemented with 10% FBS, 50 μg/ml L-ascorbic acid, 10mm glycerophosphate and 100 nM dexamethasone) for 10 days. Cell response was assessed as a function of HA content through observation of proliferation at 3 days, Alkaline Phosphatase activity at 7 days and gene expression (RT-PCR) at 14 days. MSCs were seeded onto films at 5,000/cm^2 for proliferation and 20,000/cm^2 for ALP and RT-PCR. The culture medium was changed twice a week.

Results: Figure 1 shows relative MSC population as a function of HA content. An initial increase occurs with 10% inclusion, but 50 and 100% inclusion do not lead to a significant increase over the control films. Figure 2 is a plot of ALP activity versus HA content and does not indicate any significant increase for any amount of HA compared to PLLA control films. HA content of 50% leads to a decrease in ALP activity compared to PLLA control, while ALP activity at 100% HA returns to control levels.

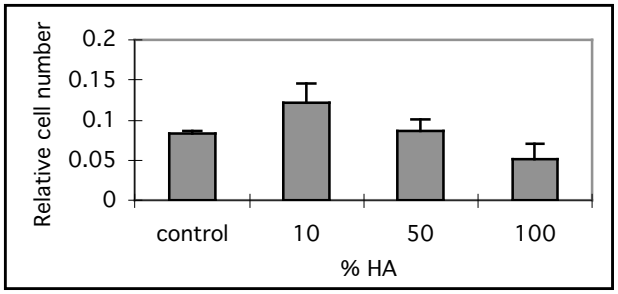

Figure 1 – BMSC population on PLLA/HA at 3 days

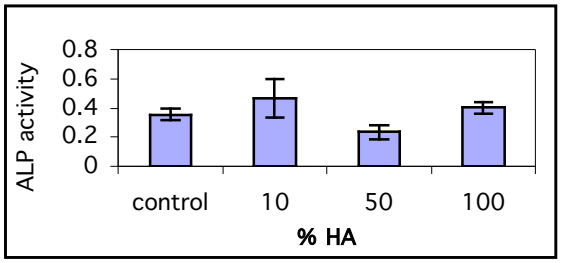

Figure 2 – ALP activity at 7 days

Figure 3 is a plot of the expression of Collagen 1 (col1), Bone Sialoprotein (BSP) and Osteocalcin (OCN) at 14 days as a function of HA content. Collagen 1 expression did not change significantly until 100% HA. BSP and OCN expression both reached their plateau at 50% HA.

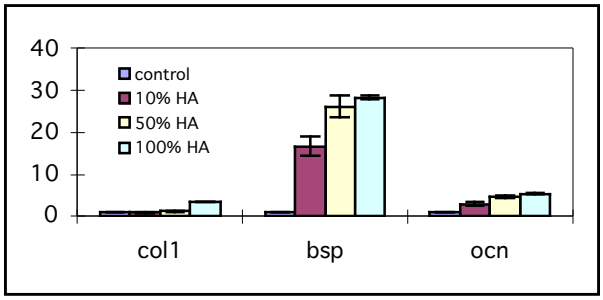

Figure 3 – Gene expression at 14 days

Conclusions: These results indicate that the addition of HA to a 2D polymer substrate, specifically an incorporation of >10% HA to PLLA improves BMSC proliferation, as well as collagen 1, BSP and OCN expression. However, further increases in HA led to a decrease in proliferation at 3 days. BSP and OCN expression reach a plateau at 50% HA. These results indicate that there exists a potential cut-off point for HA inclusion in a 2D HA/PLLA composite system for cell culture. By extension, a similar point may exist for three-dimensional scaffolds as well. Limiting the amount of HA in a 3D HA/PLLA scaffold system can reduce treatment time and preserve scaffold integrity. Therefore, further work is being conducted to extend this study into 3D scaffold systems.

References: [1]Langer R, Vacanti JP. Science 1993;260:920-926. [2]Hench LL. J Am Ceram Soc 1991;74:1487-1510.