The Effect of Osteoclast Conditioned Medium on Mesenchymal Stem Cells

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Statement of Purpose: Greater than 5.5 million fractures are sustained by Americans each year, accounting for more than 500,000 bone graft surgeries[1]. The current "gold standard" for bone grafting involves harvesting bone material from the patient's iliac crest; due to its osteoinductive properties[2]. Unfortunately, the surgery required to harvest material from the iliac crest, yields a limited amount of tissue and a 30% rate of donor site morbidity [1,3]. Both natural and synthetic substitutes have been developed to avoid the complications associated with harvesting grafts from a patient's healthy bone, but the substitutes lack the osteoinductive properties desired by surgeons for proper repair. The incorporation of osteogenic cells and growth factors within a bone graft can improve the substitute viability, but the optimal mix of cells has yet to be determined.

The objective of this study was to evaluate the effects of osteoclast conditioned medium on mesenchymal stem cell (MSC) maturation to osteoblasts.

Methods: Mouse monocytes from the RAW 264.7 cell line (ATCC, Manassas, VA) were seeded onto tissue culture plates at a density of $3.8x10^4$ cells per well. Minimum Essential Media Alpha (a-MEM) supplemented with 10% FBS, 1% antibiotics and 10ng/ml soluble murine RANKL (Peprotech, Rocky Hill, NJ) was used to induce osteoclast differentiation. Medium from RAW cells at 2-4 days of culture was used as the "conditioned medium" described below.

Multipotent mouse marrow stromal cells from the D1 cell line (ATCC) were seeded onto tissue culture plates at a density of 3.4 x 10^5 cells per well. Three wells on each plate (OB) were fed α-MEM with osteogenic supplements (10mM B-glycerophosphate and 50ug/ml ascorbic acid (both from Sigma)). Three additional wells (CM) were fed a 50/50 % mixture of "conditioned medium" and α-MEM with osteogenic supplements.

All plates were cultured under standard conditions. Days 8, 14, 21 and 26 were selected as the four time points to monitor the temporal changes in cell differentiation. One plate was seeded for each time point. Microscopic images were taken at each designated day, and cells were frozen at -80°C. An Alkaline Phosphatase (ALP, Sigma) assay was conducted on all samples at the conclusion of the study.

Results/Discussion: The cells were observed over the course of the culture period. Both cell groups appeared similar to fibroblasts at Day 2 (Fig 1: A.1, B.1). By Day 8, the morphology of the CM cells began to change to a more rounded shape, while the OB cells acquired a tightly packed, polygonal shape more consistent with differentiating bone cells (Fig 1: A.2, B.2). There were marked differences in morphology at Day 26; the cells of the CM group were less dense and included cells with both spindle and rounded morphology, while the OB group had a more uniform appearance (Fig 1: A.3, B.3). There were significant differences in ALP expression between the two groups at each time point, with a peak value at day 21 in the OB group (Fig 2). This marker for middle stage osteoblast differentiation has been shown to be highest between days 14-28 in stromal cells differentiating into osteoblastic cells.

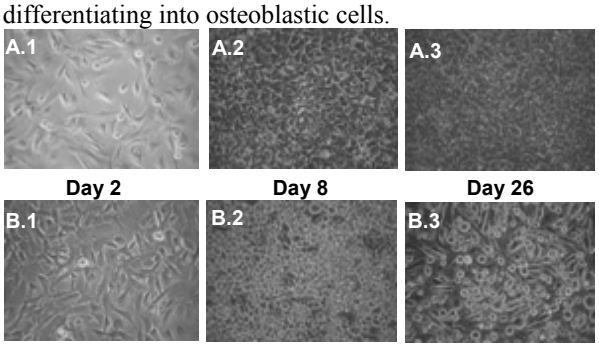

Figure 1: Cell Morphology
Cells grown in osteogenic supplements (Top, A.1-3). Cells grown in osteogenic supplements with osteoclast conditioned media (Bottom, B.1-3). All images 320x total magnification.

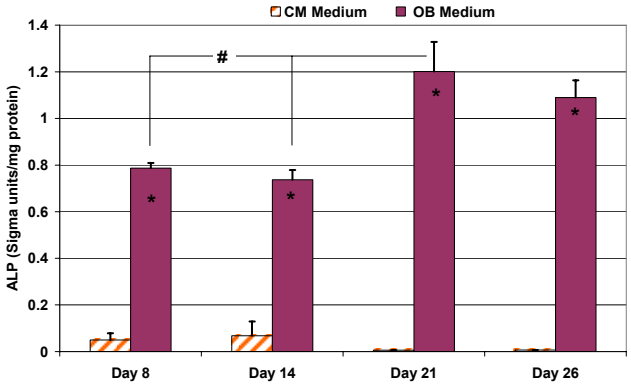

Figure 2. ALP Expression Assay
* Significantly higher values of ALP for OB group versus CM group ($p<.05$). # Significant increase between OB Day 14 and Day 21 ($p<.05$).

Conclusions: These results suggest that the differentiation of MSCs into osteoblasts is inhibited by the presence of osteoclast conditioned medium. Future work will focus on the physiological significance of the levels of protein expression and on optimizing MSC culturing conditions for use in bone grafts.

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References:

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