Analysis of Bone Marrow Aspirate as an Osteoinductive Bone Graft Material

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INTRODUCTION: Bone grafts are the second most transplanted materials, surpassed only by blood transfusions[1]. Natural and synthetic substitutes have been developed to avoid the complications and pain associated with harvesting grafts from a patient's healthy bone, but these lack the osteoinductive properties desired by surgeons for proper repair. Mesenchymal stem cells (MSCs) derived from bone marrow have been successfully cultured *in vitro* but expansion protocols are needed to allow widespread clinical application. It is believed that the aspirate material obtained from femoral shaft reaming has the potential for use as a source of stem cells for expansion *in vitro*.

The objective of the present study was to evaluate the contents of aspirate material obtained from the femoral shaft using the Reamer/Irrigator/Aspirator (RIA) device (Synthes, USA. Paoli, PA). The aspirate material consists of three main components: bone fragments, liquid flow-through and a fat layer. The bone and fat layer were studied to determine the presence or absence of cells with osteogenic potential.

METHODS: Institutional Review Committee approval was obtained for this study. Reaming aspirate was collected from one male patient (age 33 years) and packed on ice for transport to a cell culture laboratory.

Bone & Fat Layer Cell Culture Bone fragments were rinsed in phosphate buffer saline (PBS) and samples of approximately 8 ml were transferred to culture flasks. Medium was added, but not removed, every two days through Day 8 to promote cellular attachment. Explants were then removed and the attached cells were fed every 2 days.

Samples from the fat layer were minced and placed into a type II collagenase mixture to digest the collagen tissue. Following digestion, samples were centrifuged to form a cell-rich pellet which was resuspended and expanded in culture medium.

Cell morphology was examined via light microscopy. Cells from each aspirate layer were frozen down at Day 12 for long-term storage. The frozen cells were thawed for use in the differentiation study.

Differentiation Study Cells isolated from the bone and fat layer were tested to determine their osteogenic potential. A human MSC line (Lonza Group Ltd, Switzerland) was used as a control. Cells from each group were seeded in triplicate onto tissue culture plates at a density of 1,000 cells/cm^2. Test wells (OB) were maintained in MSC Growth Medium (MSCGM, from Lonza) containing osteogenic supplements (dexamethasone, L-glutamine, ascorbate and B-glycerophosphate). A second set of control cells were maintained in plain MSCGM (Plain).

Days 8, 14 and 21 were selected to monitor the temporal changes in cell differentiation. At each time point, samples were collected for calcium testing and a plate was frozen at -80^{o}C. Alkaline Phosphatase (ALP, Sigma) and calcium deposition (Ca, Stanbio Laboratories) assays were conducted on all samples.

RESULTS & DISCUSSION: Viable cells were successfully isolated from both the bone and fat layer of the aspirate. Microscopic examination showed these cells had morphology comparable to those in a stromal stem cell line (Fig. 1).

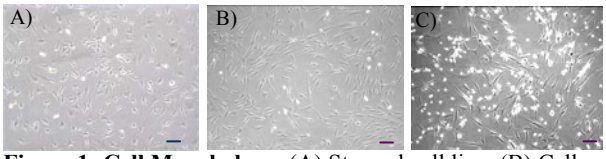

Figure 1: Cell Morphology. (A) Stromal cell line; (B) Cells isolated from bone chips; (C) Cells isolated from fat layer. Day 12 post-operation, images at 100x. (Scale bar = 100μm)

All OB test groups exhibited similar trends in ALP expression (Fig. 2). Test groups had significantly higher levels of ALP expression than the plain medium group at Day 21. This middle stage differentiation marker is expected to be highest between Days 15-28 in stromal cells differentiating into osteoblastic cells[2].

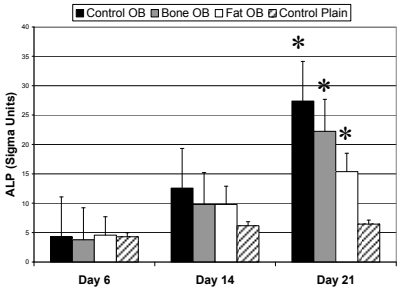

Figure 2: Alkaline Phosphatase Expression (n=3)

* Significantly higher values ($p<.05$) of ALP in OB medium groups.

OB groups displayed a slightly higher increase in Ca deposition between Days 14 and 21 compared to the control (Fig. 3). Additional time points will allow better characterization of differentiation using calcium which is considered a late-stage differentiation marker [2].

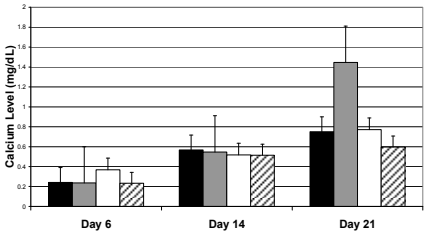

Figure 3: Calcium Deposition Levels (n=3, $p<.05$)

CONCLUSION: The results of this study suggest that cells isolated from both the bone and fat layer of RIA aspirate have the potential to differentiate along an osteogenic pathway. Previously considered waste, the lipid rich fat layer of aspirate may be a source of mesenchymal stem cells that could be used to stimulate new bone growth.

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2. Schecroun, N and Delloye, C. Bone. 2003; 32: 252-260