Osteogenic Potential of Cells Isolated from Lipid-Rich Layer of Reamer Aspirate

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INTRODUCTION: Bone grafts are the second most transplanted materials, surpassed only by blood transfusions[1]. 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 optimal fracture repair. It is believed that the aspirate material obtained from femoral shaft reaming has the potential for use at the surgery site as a source of mesenchymal stem cells (MSCs) with osteogenic potential and as a source for *in vitro* MSC expansion. The aspirate material consists of three main components: bone fragments, liquid flow-through, and a fat layer. All three layers are being evaluated by researchers for their osteogenic capabilities[2, 3].

The objective of the present study was to evaluate the fat layer of aspirate obtained from the femoral shaft using the Reamer/Irrigator/Aspirator (RIA) device (Synthes, USA. Paoli, PA). Cells isolated from this lipid-rich layer were studied to determine osteogenic potential. Cellular differentiation was examined on clinically available ceramic granules.

METHODS: Institutional Review Committee approval was obtained for this study. Reaming aspirate was collected from one female patient (age 23 years) and packed on ice for transport to a cell culture laboratory.

Cell Culture: Samples from the fat layer were minced and placed into a type II collagenase mixture to digest the tissue. Following digestion, samples were centrifuged to form a cell-rich pellet which was resuspended and expanded in MSC Growth Medium (MSCGM, from Lonza).

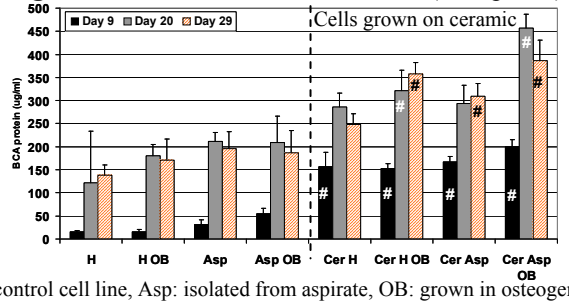
Differentiation Study: Primary cells isolated from the fat layer were tested to determine their osteogenic potential (Asp). A human MSC line (Lonza Group Ltd, Switzerland) was used as a control (H). Cells from each group were seeded, at a density of 1,000cells/cm^2, in triplicate directly onto tissue culture polystyrene well-plates or onto 0.13 g of ceramic granules (Fig 1) covering the bottom of the wells. Test wells (OB) were maintained in MSC Growth Medium (MSCGM, Lonza) containing osteogenic supplements (dexamethasone, L-glutamine, ascorbate and B-glycerophosphate). Control groups were maintained in plain MSCGM.

Days 9, 20 and 29 were selected to monitor the temporal changes in cell differentiation. At each time point a plate was frozen at -80^{o}C. Alkaline phosphatase (ALP, Sigma) and intracellular protein assays (Pierce) were conducted on all samples.

Figure 1: Ceramic Granules. β-tricalcium phosphate ceramic granules of bone void filler. 0.5-0.7 mm in size. (Synthes, USA. Paoli, PA)

RESULTS & DISCUSSION: Viable cells were successfully isolated from the aspirate layer and seeded onto the ceramic granules (Cer) after two passages. Cells grown on the granules had significantly (#, Fig 2) higher levels of intracellular protein compared to cells grown directly on tissue culture plastic. This result indicates that an increased number of cells were present on the ceramic granules at the initial and final time points, in particular.

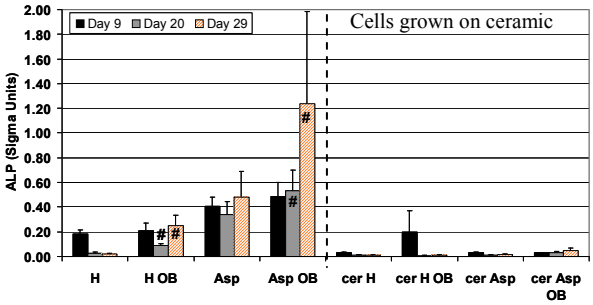
Figure 2: Total Intracellular Protein (n=3, p<.05)



H: control cell line, Asp: isolated from aspirate, OB: grown in osteogenic medium

Figure 3: Alkaline Phosphatase Expression (n=3)

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



OB polystyrene test groups, at Days 20, 29, had significantly higher levels of ALP expression than the plain medium, polystyrene groups (Fig 3). This middle stage osteoblast marker indicates that the cells have osteogenic potential. Cells grown on the ceramic granules expressed significantly lower levels of ALP which may be attributed to the fact that the cells were proliferating at higher levels and, therefore, not yet differentiating.

CONCLUSIONS: The results of this study suggest that cells isolated from the fat layer of RIA aspirate have high levels of cellular growth on ceramic bone void filler and high 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 alone or in conjunction with currently available synthetic bone graft material.

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