Characterization of Human Bone Marrow Aspirate Concentrate Derived Mesenchymal Stem Cells and the Effect of Commonly Used Clinical Drugs on Their Viability and Proliferation

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INTRODUCTION: Bone marrow aspirate concentrate (BMAC) is an expanding orthobiologic treatment for cartilage defects that contains mesenchymal stem cells (MSCs) with chondrogenic differentiation potential. Many common pharmaceutical agents, including anticoagulants, local anesthetics, and corticosteroids, are routinely used during BMAC procedures. However, the effect of these agents on MSC viability, proliferation, and functionality remain unclear. This study aims to characterize patient-derived BMAC MSCs by assessing their colony-forming potential, expression of specific cell surface markers, and capacity for multilineage differentiation, as well as to quantify the effects of commonly used clinical drugs on their viability and proliferation.

METHODS: BMAC was obtained using published methods from five patients. Initial cell counts were performed to determine colony-forming potential. Cells were then expanded and subjected to various assays. Flow cytometry confirmed MSC surface marker expression (CD73, CD90, CD105) and absence of hematopoietic (CD45) and endothelial (CD31) markers. Trilineage differentiation capacity was verified through staining for chondrogenic, adipogenic, and osteogenic induction *in vitro*. Finally, the cells were exposed to nine drug treatments, and viability and proliferation assays were conducted at three time points following treatment to determine the effect of each drug. This study was approved by the institutional review board, and consent was obtained from all patients providing samples.

RESULTS: Cells from all five patients expressed MSC markers and were confirmed to be capable of differentiating into chondrocytes, adipose tissue, and bone *in vitro*. MSCs treated with bupivacaine, lidocaine, methylprednisolone, epinephrine, ACD-A alone, and ACD-A + heparin combination showed significantly reduced viability and proliferation at multiple different time points (P<0.05). Ropivacaine and heparin alone showed no detrimental effects. Thrombin treatment showed significantly enhanced viability and proliferation (P<0.05).

DISCUSSION: These findings suggest that while BMAC-derived MSCs consistently exhibit colony-forming ability, express standard MSCs markers, and retain multipotent differentiation capacity, their viability and proliferation can be significantly affected by certain intraoperative medications. Specifically, bupivacaine, lidocaine, methylprednisolone, epinephrine, ACD-A alone, and the combination of ACD-A with heparin negatively impacted MSCs function. In contrast, ropivacaine and heparin alone had no detrimental effects, while thrombin enhanced MSCs viability and proliferation. These results highlight the importance of carefully selecting intraoperative agents when using BMAC-derived MSCs in regenerative procedures.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings demonstrate that certain local anesthetics, corticosteroids, and anticoagulants should be avoided when possible in order to preserve MSC cell viability for BMAC procedures.

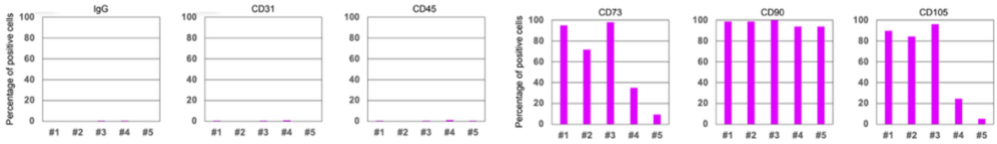

Figure 1. Flow cytometry analysis of MSC surface marker expression.
MSC populations from five patients were analyzed for expression of CD73, CD90, and CD105 (positive markers), and CD31 and CD45 (negative markers). IgG was used as an isotype control. The Y-axis represents the percentage of marker-positive cells; the X-axis shows patient ID numbers.

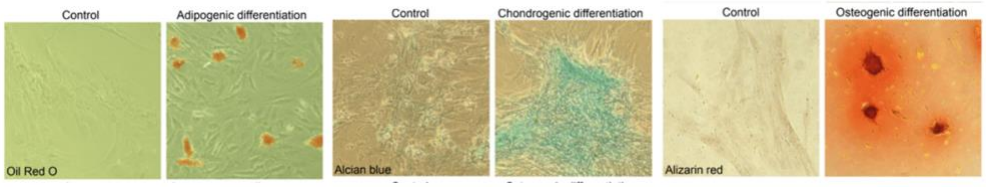

Figure 2. Multilineage differentiation potential of MSCs *in vitro*.
MSCs derived from five patients were cultured in either control basic medium (negative controls, left panels) or lineage-specific induction medium (right panels). Adipogenic differentiation was confirmed by Oil Red O staining of lipid droplets; chondrogenic differentiation by Alcian Blue staining of sulfated proteoglycans; and osteogenic differentiation by Alizarin Red staining of calcium deposits.

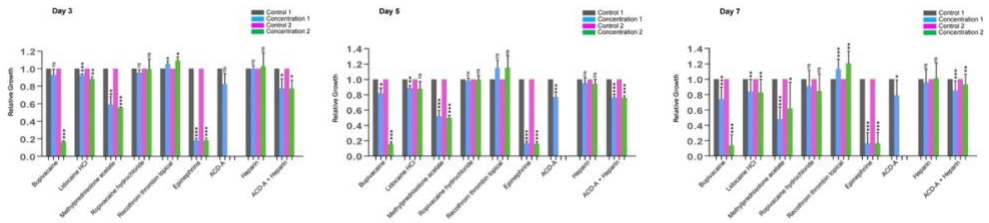

Figure 3. Colorimetric assay of MSC viability and proliferation following drug treatment. Quantification of MSC viability and proliferation at days 3, 5, and 7 following treatments with the indicated clinical drugs. Data is presented as mean ± SD. *P<0.05; **P<0.01; ***P<0.001.