• Novel Technology to Provide an Enriched Therapeutic Cell Concentrate from Bone Marrow Aspirate

INTRODUCTION:

Bone marrow is a rich source of osteogenic progenitor cells that have the potential to enhance bone healing and regeneration. However, clinical use of BMA has been limited due to highly variable outcomes in clinical practice. One potential cause of these variable results is that standard aspiration techniques yield bone marrow aspirates (BMA) that are diluted with peripheral blood. To address this issue Hernigou et al has performed clinical studies using concentrated BMA and has been able to demonstrate that reliable healing can be achieved when the osteoprogenitor cell population is in excess of 1000 colony forming units per ml. Current devices used to concentrate BMA are dependent on centrifugation or capture the cells directly onto the graft material requiring either capital equipment or limiting application to open grafting procedures. To address these issues we have developed a novel, compact, automated filtration device that allows rapid concentration and recovery of nucleated cells from BMA in an aqueous suspension. The aims of the investigation presented were to determine:

i. the efficiency of nucleated cell recovery,

ii. cell viability

iii. osteogenic capacity of the recovered nucleated cells.

METHODS:

The novel BMA concentrator (Fig.1) utilizes controlled vacuum pressure and acoustics to maintain steady state filtration. Nine Human BMA were purchased from Lonza (Rockville, MD). Volumes of BMA ranging from 5ml to 40ml were processed through the device. Unprocessed BMA for each aspirate was used as control. Nucleated cell number was determined using a Coulter Counter, cell viability using a Guava EasyCyte and osteoprogenitor and stem cell number by colony forming unit assays (CFU-f, stem cells; CFU-ob, osteoprogenitors). CFU assays were performed by culturing cells in basal (CFU-f) and osteogenic (CFU-ob) media for 14 days and visualised by total cell staining (CFU-f) and alkaline phosphatase activity (CFU-ob).

RESULTS:

The time for each run, irrespective of starting volume (5-40ml), was under 15 minutes.

Nucleated cell recovery. There was a linear relationship between BMA volume reduction and increased concentration of total nucleated cells (TNC) up to 8 fold volume reduction (Fig. 2). Closer analysis of samples where the volume reduction was >4 fold (our target clinical minimum) revealed that the mean recovery of TNC was 89.1% (range 82.6-96.9%) irrespective of the starting total nucleated cell (TNC) concentration. (Table 1). When the volume reduction was increased above 8 fold the linear relationship between volume reduction and TNC concentration was lost with efficient of recovery of TNC falling to 69% when the volume reduction was above 9 fold.

Cell viability. Cell viability in the unprocessed and processed aspirates were comparable irrespective of volume reduction and were in excess of 95% for all samples.

Stem cell and osteoprogenitor cell number. The number of CFU-f and CFU-ob ml of aspirate increased linearly with increasing volume reduction. Figure 3 shows representative cultures of unprocessed and processed BMA stained for CFU-ob. An interesting and unexpected observation is that compared to TNC recovery CFU-f and CFU-ob recovery was consistently greater than 90% irrespective of concentration factor (CFU recovery > TNC recovery). This preferential retention of CFU-F/CFU-ob was most marked when the volume reduction was greater than 9 fold when there was a significant fall off in TNC recovery.

DISCUSSION:

The data presented here support the concept that our novel filtration device rapidly, and consistently concentrates the nucleated cell fraction of BMA. The data further suggest that the device preferentially selects the CFU fractions and thus enriches the therapeutic cell fraction in the concentrated BMA. The rapid processing times (less than 15min) and automated functioning of the device are key features required for the intra-operative application of this device to enhance bone healing. Based on these observations we propose to advance our development of the technology and test the efficacy of the cell fraction generated by the device in clinically relevant in vivo models.

REFERENCES: