EVALUATION OF VIABILITY OF OSTEOPROGENITOR CELLS RETAINED ON HEALOS PROCESSED WITH CELLECT

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INTRODUCTION

Bone marrow contains two primary cell types: hematopoietic lineage cells and mesenchymal lineage cells (MSC). MSCs are responsible for the osteogenicity of bone marrow. A new method called selective cell retention (SCR) was developed to concentrate the MSC fraction of whole bone marrow; grafts enriched with osteoprogenitor cells using the SCR method were shown to have improved osteogenicity as compared to a graft soaked in whole bone marrow[1].

Cellect (CELLECT™, DePuy Spine) is a commercialized device that allows surgeons to apply this technology for preparation of grafts in various fusion procedures. The Cellect process involves circulation of bone marrow over a graft matrix under a controlled flow rate and after two cycles, the grafts enriched with osteoprogenitors are used for implantation.

Type I collagen and hydroxyapatite (HA) represent the predominant organic and inorganic components of natural bone. These components were shaped into a three-dimensional matrix comparable to natural bone (HEALOS™, DePuy Spine). When combined with BM aspirate, HEALOS was shown to perform similar to autograft in a spinal fusion model.[2]

While in vitro studies show significant osteoprogenitor retention efficiency on various scaffolds including HEALOS through the Cellect device by measuring the difference in viable cell counts between input and output samples, there is no study directly showing if sheer force during the Cellect process has any effect on viability of the cells retained in a scaffold. Before the osteogenicity of a stem cell enriched graft is evaluated in preclinical or clinical studies, questions about viability and attachment of bone marrow stem cells in graft materials after the Cellect process must be addressed.

In this study, viability of MSCs in HEALOS loaded through a Cellect prototype was assessed and compared with viability of MSCs in Healos loaded in the absence of sheer force by hydration with whole marrow.

MATERIALS AND METHODS

Materials:

Human heparinized BM (Cambrex) was withdrawn from healthy volunteers and human mesenchymal stem cells (MSC s) were purchased through Cambrex. Live/Dead staining kit was obtained from Invitrogen-Molecular Probes.

MSC Viability assessment:

The expanded MSCs were diluted to $1X10^6$ cells/ml in PBS and stained with 2µM Calcein AM for 30min at room temperature. Cells were then washed 3 times with PBS and resuspended in human bone marrow at a concentration of $0.5X10^6$ cells/ml. Aliquots of this bone marrow suspension were processed on one HEALOS strip either through a Cellect prototype or by hydration with whole marrow. At 0 and 2h incubation at RT, 5mm punches from BM loaded HEALOS were taken and stained with 4µM Ethidium Bromide at room temperature for 5min. The punches were then manually sliced into pieces with around 0.5mm in thickness for examination of live and dead cells under fluorescent microscope. The viability of MSCs in Cellect processed Healos was then compared with those in marrow hydrated Healos.

RESULTS AND DISCUSSIONS

Like cells in HEALOS loaded in the absence of sheer force by hydration with whole bone marrow, cells in HEALOS loaded through the Cellect process with a flux of 1.35 cc/min/cm^2 are viable and there were no detectable dead cells found in the scaffold, even after 2hrs incubation at RT (Fig. 1). These results demonstrate that cells enriched in HEALOS through Cellect well tolerate the sheer force during the Cellect process, consistent with our previous studies that the Cellect retained osteoprogenitors could populate within HEALOS as well as cells retained by hydration (data not shown). Further, the cells in Cellect processed Healos remain viable and there is no increase in dead cells after 2hr incubation at RT, indicating the cell loaded construct is stable for surgical implantation.

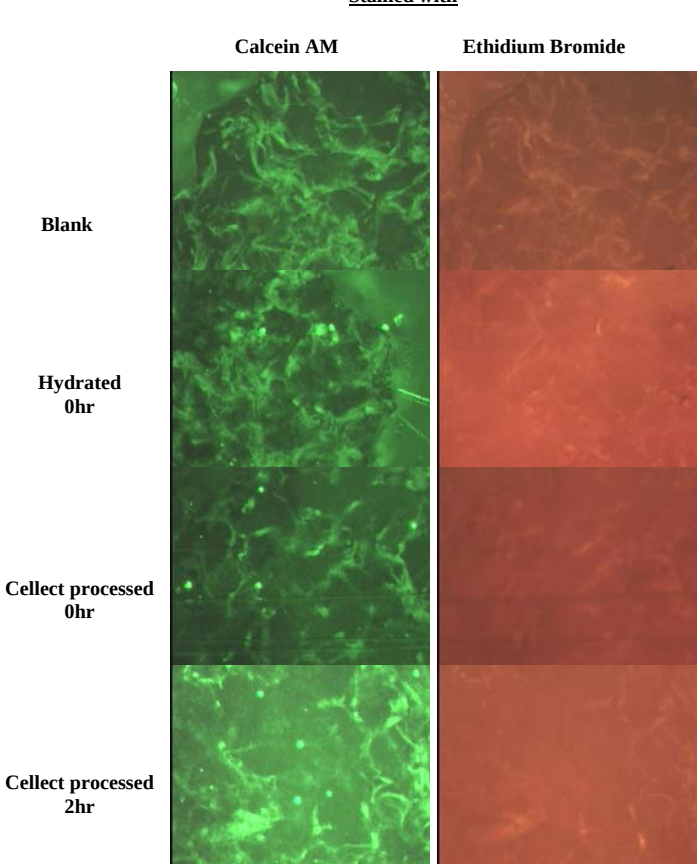

Fig. 1. Purified MSCs were prestained with 2µM Calcein AM and mixed with human bone marrow at $0.5X10^6$ cells/ml. One strip of HEALOS was either untreated (Blank) or hydrated with 1X graft volume of MSC containing marrow or Cellect processed using 3X graft volumes of the same marrow. 5mm punches of each HEALOS were stained with 4µM Ethidium Bromide before visualized under fluorescent microscope for distribution of live or dead cells.

REFERENCES

1. Muschler GF *et al*. Clin Orthop Rel Res 407:102-118. 2003
2. Tay BK-B *et al*. Spine 23:2276-2281. 1998