HIGH OSTEOPROGENITOR CELL RETENTION IN MINERALIZED BOVINE COLLAGEN BONE GRAFT SUBSTITUTES

*Seidel, J O; *Fang, C; *Holy, CE; *Geesin, J; *Bruder, SP
*Johnson & Johnson Regenerative Therapeutics, LLC, Raynham, MA
jseidel@rtxus.jnj.com

INTRODUCTION:

Bone graft substitutes offer an alternative to autograft, reducing the possibility of disease transmission and the morbidity associated with graft harvest. HEALOS®, a mineralized bovine collagen matrix (DePuy Spine, Raynham, MA), when combined with bone marrow aspirate (BMA) has been shown to be effective as an alternative bone grafting material in a rabbit model [1]. However, cell numbers in native bone marrow can vary and increasing cell numbers prior to implantation can offset this variability. In particular, increasing the number of osteoprogenitor cells on a graft material by selective retention prior to implantation has been shown to increase bone formation in a canine model [2].

Selective retention of osteoprogenitor cells onto a graft can be achieved by processing bone marrow with the CELLECT® Graft Preparation Device (DePuy Spine, Raynham, MA), a device that allows for controlled flow of bone marrow through the graft material. The system exposes the matrix to a higher volume of bone marrow than can be achieved via simple graft saturation. During this process a syringe pump controls flow parameters. Bone marrow is processed through the matrix in a unidirectional re-circulation flow pattern. Marrow can be passed through the system multiple times to optimize cell retention. Optimizing the flow conditions can maximize osteoprogenitor cell retention and thus, enrich the bone graft matrix.

This study reports the cell retention of bone marrow nucleated and osteoprogenitor cells processed through mineralized collagen materials in an effort to maximize osteoprogenitor cell retention: In a first set of experiments 5 mm thick mineralized collagen sponges were processed using either a flux of 0.6 cc/min/cm^2 or 2.4 cc/min/cm^2. In addition bone marrow was passed through the scaffold 1, 2 or 3 times. In a second set of experiments the cell retention was determined on 3 different matrices at a flux of 1.54 cc/min/cm^2: 1) 5 mm thick mineralized collagen sponge 2) 8 mm thick mineralized collagen sponge and 3) 8 mm thick layer of mineralized collagen fiber matrix. While the material composition of the three matrices is identical, the handling and consistency of the material varied. The sponge (Figure 1A) will maintain its rectangular shape after hydration with bone marrow and the fiber matrix (Figure 1B) will become moldable and injectable after hydration so that the matrix can thus be shaped for placement into a defect site.

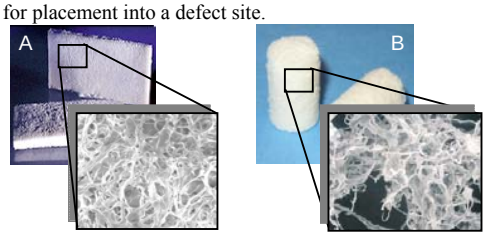

Figure 1: Figure 1A shows a 5 mm thick mineralized collagen sponge (HEALOS). A continuous fiber network can be seen. Figure 1B shows mineralized collagen fibers (HEALOS Fx) freeze-dried into a cylindrical form. Fibers are entangled but do not form a continuous network and the matrix can thus be shaped when hydrated with bone marrow.

METHODS:

All experiments were performed in a sterile manner in a biological safety cabinet. Human heparinized bone marrow (Cambrex, Walkersville, MD, 83U/cc heparin concentration) from healthy volunteers was processed on 5 mm and 8 mm thick mineralized collagen sponges or freeze-dried fiber matrix (DePuy Spine, Raynham, MA) using a selective cell retention prototype (CELLECT® Graft Preparation Device, DePuy Spine, Raynham, MA).

Marrow samples were prepared at a final heparin concentration of ~333U/ml. Aliquots of marrow solution were set aside for nucleated cell count and osteoprogenitor Colony-Forming Unit (CFU) assays. The cell retention efficiency was defined as the difference in nucleated cell and osteoprogenitor cell counts in the marrow solutions, before and after processing through the graft material.

Nucleated cell counts were determined in quadruplicate after lysing red blood cells from the bone marrow using 3% acetic acid. Osteoprogenitor cell counts were determined in triplicate by plating five million nucleated cells from each marrow sample in a T-75 culture flask containing 20 ml of osteogenic differentiation media (DMEM + 10% fetal calf serum, 10mM β-glycerophosphate, 100nM dexamethasone, 50μM ascorbic phosphate and 1X antibiotic-antimycotic: 100 units of penicillin, 100 units streptomycin and 250 ng/ml amphotericin B). The flasks were incubated at 37°C, 5% CO_2, for 12-14 days until colonies were visible by naked eye. The flasks were then fixed with 10% formalin and stained for alkaline phosphatase using an alkaline phosphatase (AP) kit (Sigma, St. Louis, MO). AP-stained colonies were then manually counted.

An overall statistical analysis of the nucleated and osteoprogenitor cell retention efficiency was done using a one-way analysis of variance (ANOVA) for all groups (SigmaStat software, SPSS, Chicago, IL). If differences in the mean values among the groups were greater than 0.05, an all-pairwise multiple comparison procedure was performed (Tukey Test). A paired student T-test was also performed using Excel software.

RESULTS:

Bone marrow and osteoprogenitor cell retention on CELLECT-processed mineralized collagen matrices was investigated using a flux of 0.6 cc/min/cm^2 or 2.4 cc/min/cm^2, and 1, 2 or 3 passes. Nucleated cell retention ranged from 10.3±4.9% to 56.5±11.0% for all tested configurations, while osteoprogenitor cell retention ranged from 52.7±15.4% to 93.6±4%. Cell retention was higher at low flux compared to high flux. At the low flux, 1 pass showed a significantly lower cell retention compared to 2 and 3 passes (73.1±2.7% compared to 77.9±2.7% and 81.3±2.9%, p= 0.025 and p= 0.002 respectively).

Results of cell retention on the different matrices (i.e. 5 mm thick mineralized collagen sponge, 8 mm thick mineralized collagen sponge and 8 mm thick layer of mineralized collagen fibers) at a flux of 1.54 cc/min/cm^2 and 2 passes are shown in Figure 2. Nucleated cell retention was 47%±14% and 39%±10% for 5mm and 8mm thick sponges respectively and 38%±14% for the injectable mineralized collagen formulation. Osteoprogenitor cell retention was 87%±8% and 94%±2% for 5 mm and 8 mm thick sponges respectively and 88%±6% for the injectable mineralized collagen formulation. No statistical differences were observed between these three groups.

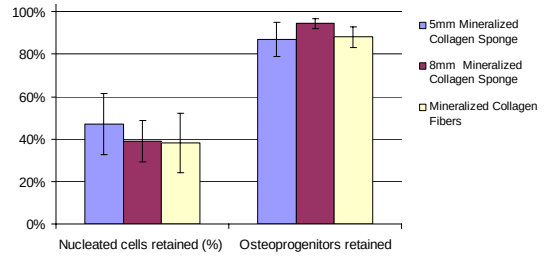

Figure 2: Percent of nucleated and osteoprogenitor cells retained in: 1) 5 mm thick mineralized collagen sponge, 2) 8 mm thick mineralized collagen sponge, and 3) 8 mm thick layer of mineralized collagen fibers.

DISCUSSION:

This research further highlights differences in cell attachment properties between bone marrow-derived osteoprogenitor cells and all other nucleated cells contained in human bone marrow. Osteoprogenitor cells were selectively retained to the graft matrices tested in this research (collagen and hydroxyapatite), as shown by retention scores of ~>80% while most other nucleated cells simply passed through the matrices. Kinetics of cell attachment were not investigated in this study, however, even using a higher flux, selective osteoprogenitor attachment could be achieved, indicating a fairly rapid cell attachment mechanism. A key parameter to increasing cell retention was the number of bone marrow passes through the matrix. After 2 passes, most cells were found to be retained on the graft, and osteoprogenitor cell retention did not significantly increase between 2 and 3 passes. Interestingly, all tested matrices showed similar cell retention. These matrices were all composed of collagen and hydroxyapatite, with slightly different morphological configurations. These results thus demonstrate that the differences in morphology did not affect cell attachment using the selective cell retention methodology.

REFERENCES:

1) Tay, R et al., Spine (23)21; 2276-81, 1998
2) Brodke, D et al., J Orthop Res (24)5; 857-66, 2006