The Effect of Addition of Unprocessed Bone Marrow clot in the Bone Healing Response in a Canine Multi Femoral Defect Animal Model

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Introduction:
Successful bone repair requires osteogenic connective tissue progenitors (CTP-Os). Many prior studies have demonstrated a positive effect of adding bone marrow-derived cells to an osteoconductive bone graft material. The majority of such studies have been conducted in small defects in rodent models1. Based on this literature, many surgeons now aspirate bone marrow and add marrow-derived cells to their bone graft materials with the expectation that the outcome is likely to be enhanced, and the premise that optimal performance from any osteoconductive or osteoinductive material may require augmentation with local CTP-Os2. As yet, no prospective clinical studies support this practice, however. This study was designed to provide quantitative means for examining the outcome both scaffold materials as well as the effect of modifying the local site of bone regeneration with cell therapy strategies or local bioactive agents. This study aims to test that the addition of bone marrow clot will increase the local bone formation on two different bone scaffold materials in the established Canine Femoral Defect Model.

Materials and Methods:
Study design: This study was conducted after approval from the Cleveland Clinic Institutional Animal Care and Use Committee. The addition with BMA cells were performed in two sets of experiments (10 animals in each) using two bone graft substitutes: 1) Poly(L-lactide:ε-caprolactone) and TCP granules (PLCL/TCP) scaffold fabricated by Integra Spine, Plainsboro, NJ; 2) Canine mineralized cancellous allograft (MCA) matrix fabricated by MTF (Edison, NJ). Graft Preparation: During surgery, 2 ml of non-heparinized bone marrow were added to prepared scaffolds and allowed to clot. Surgical procedure: Four identical 10mm diameter x 15mm deep unicortical cylindrical defects for grafting were created in the left femur. Scaffolds enriched with or without BMA cells were distributed randomly among the four defect locations. Due to a therapeutic focus on rapid bone formation, animals were euthanized at 4 weeks. The extent and distribution of new bone in-growth into each defect site was assessed using quantitative micro-CT analysis and qualitative undecalcified histology. The primary outcome parameter was Percent Bone volume (%BV), which was calculated throughout the defect volume as the % of pixels with intensity at above a bone threshold.

Results:
The 2D %BV contour plots in Figure 1 illustrate that new bone in-growth was found higher at the periphery of the defect volume and in the pericortical region where in-growth of cells from the periosteal and endosteal surfaces predominated. Figure 2 shows that overall in both experiments, there was no statistically significant increase of %BV with the addition of BMA clot (ANOVA). There was no evidence of inflammation response for all treatments. Figure 3 shows that bone in-growth was found extended in the PC region. Defects grafted with allograft were found to have advanced bone remodeling in the intramedullary canal region. Active resorption of the allograft bone by osteoclasts and deposition of new bone on the allograft surface by osteoblasts was present throughout all samples.

Discussion:
These results demonstrate that transplantation of unprocessed bone marrow including both marrow-derived cells and a large amount of peripheral blood-derived cells and red blood cells did not improve the performance of either the contemporary standard scaffold materials (MCA) or a less effective polymeric scaffold. The absence of improvement does not support the clinical use of unprocessed bone marrow as a cell source. However, one prior study conducted in a similar model has shown a positive effect on the performance of a bioactive scaffold delivering BMP-7 using the OP-1 Device3 (Stryker, Hopkinton, MA). The mechanism for failure in this study is not known. It is possible that the cell sources already available in these defects from periostium, endostium and vascularized bone marrow are optimized and that fractionation of marrow-derived cells to remove components that inhibit or compete with osteogenic and/or angiogenic cells may be needed. These questions are being addressed in further studies using the CFMD model and through the development of a more stringent segmental bone defect model, the Chronic Caprine Tibial Defect (CCTD) model.

Significance: The transplantation of unprocessed clotted bone marrow aspirates as a means of enhancing performance of bone grafts for large defect treatment is not supported by these data.

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References: