Effectiveness and Cell Fate of a Mesenchymal Stem Cell and Demineralized Bone Composite in Acute and Chronic Critical Sized Bone Defect Model

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Disclosures: 

Introduction: Autologous cortico-cancellous bone graft (autograft) is considered the ‘gold standard’ for bone regeneration and contains three components that are essential for bone grafting: an osteoconductive scaffold, osteoinductive signaling proteins and osteogenic cells. A recent publication demonstrated that a DBM scaffold containing human MSCs (hMSC/DBM) harvested from human adipose tissue contains the three components that are considered optimal for bone repair. Because the literature does not describe the contribution of adipose derived MSCs to bone repair in the presence of an osteoinductive scaffold, we completed a series of proof-of-concept in vivo studies, utilizing hMSCs obtained from human tissue donors, without culture expansion or pre-differentiation. The objective of these experiments was to compare and characterize the in vivo bone forming activity of hMSC/DBM to that of DBM alone, hMSCs alone, cortico-cancellous isograft derived from rat and human cortico-cancellous xenograft in a critical sized femur athymic rat defect model. An additional objective was to track the fate of the implanted cells after implantation in a critical sized defect.

Methods: Five in vivo experiments were performed to characterize the bone-forming properties of hMSC/DBM and its component parts and to assess cell fate. All in vivo experiments were performed in compliance with the USDA Animal Welfare Act and the NIH Guide for the Care and Use of Laboratory Animals and received IACUC approval.

Human Tissue: Human tissue donors with consent for research use were utilized. The hMSC control was prepared using the passage two cells derived from the adipose - derived stromal vascular fraction (SVF). The DBM control was processed using standard methods. hMSC/DBM combination was processed using previously described methods. For the cell fate study, SVF-derived mesenchymal stem cells (MSCs) from transgenic GFP-expressing rats were cultured and adhered to DBM. Rat tissue: Rat cortical and cancellous bone was morselized, rinsed in saline and stored at 80°C prior to implantation. Animal experiments: Male athymic rats were utilized for all studies. Intramuscular implantation: Four rats were randomly assigned to receive hMSC/DBM and 2 rats received hMSCs only. 0.2 cc of hMSC/DBM containing 50,000 hMSC/cc was implanted intramuscularly. In 4 limbs, 50,000 MSCs/cc in 0.2 cc of saline were implanted. Harvest occurred at 14 and at 28 days and the implant sites were examined histologically. Critical size defects: Two PEEK plates were fixed to the bone and a 5mm defect was surgically created in the femur. Three rats each received 0.5 cc of hMSC/DBM 50,000 hMSC/cc, 0.5 cc DBM, 50,000 hMSC/cc in 0.5cc saline and no treatment in the defect. At 42 days post-surgery, radiographic, μCT and histologic analysis was performed. Digital image analysis software was utilized to calculate new bone/mineralized area. For μCT, the threshold and the volume of interest (VOI) covering the entire length of the defect and 50 slices on each side of the defect were kept constant and new bone volume in the VOI was assessed. Cell Fate: Using the critical sized defect model, 28 rats received 0.5 cc of MSC-GFP+/DBM, 50,0000 MSC-GFP+/cc. After 3, 7, 14, 21, 56 and 84 days, five sections each of the bone, lung, liver, spleen, and kidney tissues were evaluated for GFP expression using immunohistochemistry.

Results: Acute Critical Sized Bone Defect

New bone area within the defect increased between day 21 and day 42 post-operative in all groups except the empty defect group. The rats treated with hMSC/DBM had the greatest mean new bone area by 42 days (Fig. 1&2). The empty defect group showed no new mineralized tissue within the defect throughout the study period.

Chronic Critical Sized Bone Defect

The empty defect treatment showed scant evidence of new radiographic bone formation and no unions were noted. The hMSC treatment group showed scant evidence of new bone formation, had a similar radiographic appearance to the empty defect group and no union was noted in this group. The DBM treatment group had more bone present on radiographs than empty defects or defects treated with hMSC with no unions noted by day 84. The hMSC/DBM treatment demonstrated moderate to robust new bone growth (Fig. 3). These animals demonstrated unions in 2 of 4 animals.

Comparison with Rat and Human Cortico-cancellous Bone Graft

Mineralized implanted material could be seen immediately after surgery on radiographs in the defects treated with rat and human corticocancellous bone, whereas no mineralized material was observed in defects treated with hMSC/DBM. However, by 42 days post-implantation, bony union across the critical-sized femur defect was radiographically present in 2/3 defects treated with hMSC/DBM, 0/3 defects treated with rat-derived cortico-cancellous graft and 0/3 defects with human-derived cortico-
cancellous bone. The mean bone volume within the defects as measured on microCT analysis was highest for hMSC/DBM (Fig. 4).

Histology was performed for all in vivo studies and was confirmatory to the radiological results.

Cell Fate

The greatest number of GFP+ cells was found in the bone defect after 7 days and cell numbers decreased thereafter. GFP+ cells were noted in mature callous and new bone 84 days after implantation and exhibited osteoblastic phenotypes. Few GFP+ cells were detected in the spleen, kidney and lung, but GFP+ cells were not detected in the liver.

Intramuscular Osteoinduction Assay

At both 14 and 28 days, histological analysis revealed the presence of osteoinduction in 3/3 DBM-treated, in 3/3 hMSC/DBM-treated rats and in 0/3 rats treated with hMSCs alone. Bone marrow elements were noted at the 28 day time points in the hMSC/DBM-treated groups.

All animals survived the in-life phases of the in vivo studies. No systemic illness or surgical site infection in any animal was noted.

**Discussion:** Optimized MSC-bone graft substitute combinations have enormous potential for augmentation of bone repair in severe trauma and other disease states where normal bone healing is compromised. To date, there is no single scaffold that is considered optimal for MSC osteogenesis. We explored the possible synergistic activity of the combination of non-culture expanded MSCs with demineralized bone. These pilot study results show clear evidence of potent osteogenic activity of the hMSC/DBM combination. The cell fate study confirms long term survival of MSCs following implantation and suggests differentiation to osteocytic phenotypes. These studies provide evidence that adipose derived MSCs on a human demineralized bone scaffold substantially contributes to bone formation and suggests that MSC differentiation to bone contributes to the mechanism of action.

**Significance:** This study provides unique research by showing that non-proliferated adipose derived mesenchymal stem cells substantially contributes to bone formation when a demineralized bone scaffold is used. This improved bone graft substitute may benefit patient outcomes in severe trauma and other disease states where bone healing is compromised.

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Fig 1

Mean New Bone Area

Day 21 cm²
Day 42 cm²
Fig. 2

Micro CT-Mean New Bone Volume

New Bone Volume mm³

No Treatment  
hMSC  
DBM  
DBM/hMSC
Fig 3

Mean 12 Week New Bone Volume (mm³)

Mean New Bone Volume (mm³)

No Treatment  hMSC  DBM  DBM/hMSC
Fig 4

Micro CT New Bone Volume

New Bone Volume mm$^3$

Rat C-C  hMSC/DBM  Human C-C

Treatment Group