PERFORMANCE OF MOLDED VASCULARIZED BONE GRAFTS INDUCED BY BMP-2 AND MARROW IN A RAT SEGMENTAL DEFECT MODEL

INTRODUCTION: Previous experiments demonstrated that desired shape and size bone could be generated using silicone molds containing the inferior epigastric bundle with osteoinductive rhBMP-2 and osteogenic bone marrow in vivo. This experiment tests the hypothesis that molded vascularized bone graft is comparable to “gold standard” autogenous bone graft in healing critically sized rat femoral defects.

METHODS: 112 male syngeneic Lewis rats (300-325 gr.) were divided into 4 groups (28 rats/group). Animals in Group 1 (Autogenous Bone Graft = ABG) received syngeneic cancellous bone graft obtained from distal femur and proximal tibia of a donor rat. Group 2 (molded vascularized ABG = vmABG) received the same amount of syngeneic cancellous bone after forming neo-ossicle in the chamber for 4 weeks. Group 3 (recombinant human Bone Morphogenetic Protein-2 + Bone Marrow vascularized for 2 weeks = vmBMP+BM 2W) received 20 μg of rhBMP-2 and bone marrow obtained from medullary canal of one donor femur and tibia (1x 10⁶ cells) and molded for 2 weeks in the chamber. Group 4 (vmBMP+BM 4W) received the same amount of rhBMP-2 and marrow for 4 weeks in the chamber. Previous studies histologically demonstrated that at 2 and 4 weeks chambers were filled with woven and lamellar bone respectively. Cancellous graft chips in group 1 and the vascularized grafts formed within the chambers in groups 2, 3 and 4 were transferred into a 5 mm osteoproteostal segmental defects. Serial lateral radiographs were performed at 3, 6, 9 and 12 weeks postoperatively. The area of the defect occupied by bone was estimated by 3 blinded observer using 5 scale scoring system. Union rates were assessed radiographically and confirmed by torsion testing using the Lane modified White scoring system. Statistical analysis utilized the ANOVA and Fisher exact tests.

RESULTS: Eighty –two percent (23/28) of the BMP+Bone Marrow molded chambers in both groups produced vascularized neo-bones for transfer while all of the autografted chambers produced bone ossicles. Only the neo-formed bones in groups 2, 3 and 4 were transferred in to the defects.

Bone Formation: Using ANOVA, between 3 and 6 weeks bone formation increased significantly in group (vmBMP+BM 2W) (p<0.001) and reached the maximum level at 6 week post operatively. Groups ABG chips, (vmBMP+BM 2W) and (vmBMP+BM 4W) were statistically better than group 2 (vascularized cancellous bone ossicle) at 12 weeks postoperatively (p<0.001). All of the other groups, except group (vmABG), improved in terms of bone formation for each time point. At 12 weeks, bone formation in the defects of (ABG) and (vmBMP+BM 2W) groups were significantly better than groups (vmABG) and (vmBMP+BM 4W). Group (vmBMP+BM 2W) had the best overall performance at this point, but it was not significantly better than group ABG. Group (vmBMP+BM 2W) was significantly superior to group (vmBMP+BM 4W) at this time point (p<0.001).

Union: There was no united defect in (vmABG) animals 0/23 (0%). (ABG) group presented 39% (9/23), (vmBMP+BM 2W) group presented 46% (6/13) and (vmBMP+BM 4W) group presented 10% (2/20) union at the time of sacrifice (12 week post operatively). There was no statistical difference between woven bone (vmBMP+BM 2W) and autogenous cancellous bone (ABG) at this time in terms of union. The Lane modified White scoring system was applied to biomechanically tested femurs. There was no statistical difference between ABG and (vmBMP+BM 2W) neo-bone groups in terms of Lane-White biomechanical evaluation.

DISCUSSION: This study demonstrated that a vascularized neo-bone can be molded and transferred to heal a critically sized femoral defect. The BMP and bone marrow in combination give rise to a viable bone ossicle that is comparable to autogenous cancellous bone graft. The earlier less mature bone (2 weeks woven) proved to be significantly superior to the more mature (4 weeks lamellar) ossicle in terms of bone formation, union and mechanical properties. Autogenous bone graft does not fare well in a molded vascularized application. The project provides an opportunity to shape bone grafts to order from a combination of that vessels, bone marrow osteoprogenitor cells and the osteoinductive growth factor rhBMP-2.