Bone Production by Murine Osteoprogenitor Cells Treated With OP-1 and bFGF on Allograft Bone

Nelson ER; Huang Z; Ma Ting; Smith RL; Goodman SB
Stanford University School of Medicine, Stanford, CA

Statement of Purpose: Over 450,000 orthopaedic procedures are performed each year for a variety of conditions that require the use of bone grafts. Autograft bone is commonly used. Its benefits are that it provides an osteoconductive scaffold as well as osteogenic precursor cells and growth factors that facilitate its incorporation into native bone. However, autograft bone is limited in its supply and associated with morbidities because of the harvest procedure. Allograft bone is the preferred alternative because it provides an osteoconductive scaffold, the supply is less limited, and it does not require a harvest from the patient. Allograft bone, however, lacks both the osteogenic cells and the osteoinductive growth factors inherent in autogenous grafts. This in vitro study provides a model for the study and augmentation of corticocancellous allograft bone discs with bone marrow-derived mesenchymal stem cells treated with known osteogenic growth factors, OP-1 and bFGF.

Methods: Institutional guidelines for the care and use of laboratory animals were observed in all aspects of this project. The femora and tibiae of 10-week-old C57 black mice were excised and 1.5 mm slices were made from the metaphyses of the distal femora and proximal tibiae. Each slice was then characterized and quantitatively evaluated at a resolution of 10.5 µm using a Scanco Medical micro-CT (Southeastern, PA). The data collected included total volume of the graft (TV), bone volume (BV), total bone volume/graft volume (BV/GV), trabecular number (Tb.N.), trabecular thickness (Tb.Th.), and trabecular spacing (Tb.Sp.). Before seeding, the grafts were defatted and sterilized. Bone marrow was collected from the femora of C57 male mice. The cells were allowed to expand for 5 days, after which they were lifted and resuspended in augmented osteogenic medium [α-Eagle minimum essential medium supplemented with 10% fetal bovine serum, 100 µg/mL penicillin, 100 µg/mL streptomycin, 50 µg/mL L-ascorbic acid, and 0.01M ß-glycerophosphate, 10 nM dexamethasone]. $1x10^6$ cells in 300 µL of osteogenic medium was added onto each bone disc. A total of 35 grafts were used in this experiment, 7 for each treatment group. Treatment groups included allograft bone discs in osteogenic medium with: 1) no cells, 2) cells only, 3) cells + Osteogenetic Protein-1 (OP-1), 4) cells + bFGF, and 5) cells + OP-1 + bFGF. OP-1 and bFGF were added daily at concentrations of 100 ng/day and 50 ng/day, respectively. Each group was harvested after four weeks and scanned by high-resolution micro-CT. Data from each graft was then compared to the data from the original, pre-culture scan of that graft. A paired t-test was used to evaluate the statistical differences between the control (pre-culture scans) and the seeded bone discs.

Results/Discussion: Results from µCT scanning of the cultured grafts showed a statistically significant increase in the trabecular thickness (Tb.Th.) of grafts from both groups that were augmented with OP-1, which include treatment with OP-1 alone and OP-1+bFGF (Figure 1). In addition, significant increases in the BV/GV were seen in the cells only group, and in the groups treated OP-1 alone and bFGF alone (Figure 2).

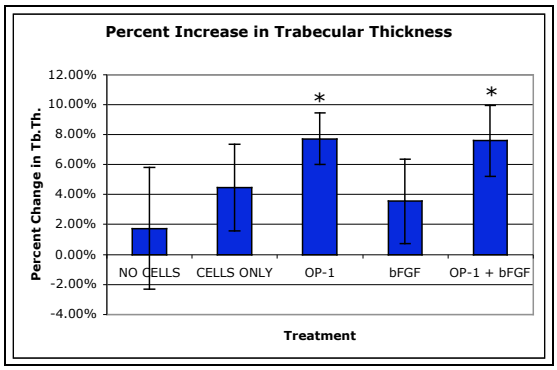

Figure 1. Shows the change in trabecular thickness over the control, pre-culture scan, given as the percent increase over controls. Groups treated with OP-1 or OP-1+bFGF showed a significant increase after 4 weeks. (* = p<.05)

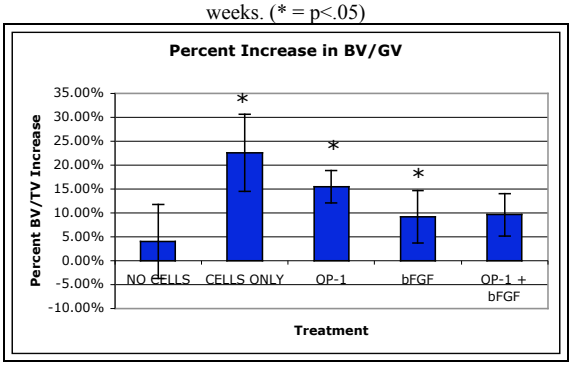

Figure 2. Shows the percent increase in bone volume/graft volume of each graft over the control. Significant increases were seen in the cells only group, the OP-1 treated, and the bFGF treated groups after 4 weeks. (* = p<.05)

Conclusions: These data show that the addition of bone marrow-derived mesenchymal stem cells (MSCs) plus exogenous growth factors can increase bone production onto allograft bone discs in vitro. This suggests that the combination of growth factors with a seeded allograft scaffold has the potential to provide the three key properties of autograft bone – osteoconductive scaffold, osteogenic MSCs, and osteoinductive growth factors - without the associated limitations and morbidities seen with the use of autografts. In addition, the model used in this project allows for the investigation of the effects of multiple pro-osteogenic growth factors and concentrations, as well as other interventions, on MSCs seeded onto allograft bone in vitro.