TISSUE-ENGINEERED BONE REPAIR OF GOAT FEMUR DEFECTS WITH OSTEOSTIC INDUCED AND BMP7-GENETIC MODIFIED BONE MARROW STROMAL CELLS

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**Introduction:** Segmental defect of bone remains a major challenge in orthopaedic surgery. Development of tissue engineering technique offers a tool for bone regeneration and bone defect repair. Bone marrow stromal cells (BMSCs) have the potential for multi-lineage (including osteogenic) differentiation and thus are the ideal seed cells for bone tissue engineering. Bone morphogenetic protein 7 (BMP-7) gene transfection has also been shown to promote bone formation *in vivo*. This study investigated the possibility of tissue-engineered bone repair of sheep femoral defects with induced and BMP-7 genetic modified BMSCs.

**Methods:** Recombinant BMP-7 adenovirus was constructed by direct gene cloning technique using a kit from Clontech. Briefly, BMP-7 cDNA was first cloned into pShuttle vector and then excised with two unique restriction sites following the direct cloning of BMP-7 cDNA into adenoviral genome. BMP-7 recombinant virus was amplified in 293 cells and purified with cesium chloride gradient centrifugation. Isolated sheep BMSCs were *in vitro* expanded and either induced with DMEM containing 10%FBS, 10⁻⁷M dexamethasone and 10mM β-phospherglycerol to become osteogenic, or infected with BMP-7 adenovirus (100 pfu/cell). RT-PCR, immunocytochemistry and Western-Blot were used to analyze BMP-7 expression. Osteogenic phenotype of modified cells was evaluated with transmission electron microscope (TEM), and cell cycle was monitored by FACS analysis. Either induced or modified BMSCs (4×10⁵) were seeded onto a cylinder coral construct (2.5x1.6cm) and co-cultured for 1 week. A 2.5cm long defect was created in right femur of each sheep and fixed internally. The defects were either repaired with coral construct alone (G1, n=10) or with coral construct plus induced cell (G2, n=10) or construct plus modified cells (G3, n=7). Bone healing was monitored by radiograph and histological examination at various time points. Radiodensity and three-point bending strength were quantitatively analyzed with F-test and T-test respectively.

**Results:** *In vitro*, strong expression BMP-7 at both mRNA and protein levels were observed in Adeno-BMP7 infected BMSCs but not in induced BMSCs. In BMP-7 modified cells, enhanced cellular metabolism was observed with TEM and more calcium was deposited in the culture dishes compared to non-modified cells. FACS did not detect abnormal change of cell cycle in the modified cells. *In vivo*, radiography demonstrated that coral construct was degraded partially at 1 month and completely at 2 month in G1 along with limited bone formation between 3 and 8 months post-repair. In G2, new bone was formed during months 1 to 8 with increased radiodensity, significantly different from G1 (p<0.05). At 8 months, engineered bone of G2 was remodeled into cortex bone at outer layer, and no significant difference in bending load strength and bending rigidity was found between G2 and normal control femur (p>0.05). Histologically, Non-union healing by fibrous tissue was observed in G1, while matured tissue engineered bone with irregular osteon structure was observed in G2. In G3, X-ray demonstrated accelerated bone formation. Abundant callus was formed in G3 as early as 1 month, which is similar to that of G2 at 3 months. Histology showed wider trabecular and more woven bone tissue formed at 4 months compared with G2 specimen at the same time point. In addition, irregular osteons formed two month earlier than G2. Furthermore, biomechanical test demonstrated that repaired segments of G3 were stronger than G2 segment at 4 months post-repair (p<0.05).

**Discussion:** This study demonstrated that weight-bearing bone defect could be successfully repaired by bone tissue engineered with induced BMSCs and coral. In addition, the engineered bone reveals a normal histological structure and biomechanical properties similar to normal bone. Furthermore, we also demonstrated that genetic modification of BMP-7 recombinant adenovirus not only enhanced osteogenic phenotype of BMSCs *in vitro*, but also promoted the bone formation and repair *in vivo*, indicating that combination of gene therapy and tissue engineering is better than either single method for bone repair.

**Figures:**

Tissue engineered bone repair of sheep femoral bone defect at 8 months. Left picture: group 1; Right picture: group 2.

BMP-7 modification promotes bone formation *in vivo*. More calluses were formed in group 3 at 2 month (right picture) than in group 2 at 4 months (left picture).