REPAIR OF PORCINE FULL-THICKNESS ARTICULAR CARTILAGE DEFECT WITH AUTOLOGOUS BONE MARROW STROMAL CELLS

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Introduction: In a previous study, we have shown that full-thickness articular cartilage defect could be satisfactorily repaired in a porcine model with autologous chondrocyte engineered cartilage (1). Nevertheless, it needs to harvest autologus cartilage for isolating chondrocytes. Additionally, the underlying cancellous bone was repaired by the engineered cartilage rather than bone tissue. Bone marrow stromal cells (BMSCs) have the potential for multi-lineage differentiation, which can be induced into osteogenic or chondrogenic cells. The purpose of this study was to test the possibility of using BMSCs and biodegradable polymers to repair full-thickness articular cartilage defect and their underlying bone defect respectively, taking the advantage that BMSCs could be induced into osteoblast and chondrocyte given a proper local environment.

Methods: Bone marrows were harvested from 16 Changfeng hybrid pigs respectively and BMSCs were isolated by their adhesion to culture dish and allowed for in vitro expansion in DMEM plus 10%FBS. For general induction, BMSCs were treated with dexamethasone (40ng/ml). For chondrogenic induction, cells were treated with dexamethasone (40ng/ml) and transforming growth factor-ß1 (TGF-ß1, 10ng/ml). Expression of collagen II and aggrecan were examined with immunohistochemistry, in situ hybridization and RT-PCR with dermal fibroblasts as negative control cells and chondrocytes as positive cells. After induction and expansion, BMSCs (1.5×10^6) were seeded on a construct of polyglycolic acid (PGA) and polyactic acid (PLA) and co-cultured for 1 week before in vivo implantation. Cell attachment to polymer and their matrix production on polymer were observed with light microscope and electronmicroscope. For surgical procedure, in each animal, two full-thickness articular cartilage defects (8mm in diameter) as well as their underlying cancellous bone defects were created at the non-weight bearing areas of one knee joint. The other two were similarly created on the other side. Among these 4 defects, the defect was repaired either with PGA/PLA construct and generally induced BMSCs in group A or with the construct and chondrogenically induced BMSCs in group B, or with the construct alone in group C or left unrepaired in group D. To trace the implanted BMSCs, cells retrovirally-labeled with Green Fluorescent Protein (GFP) were implanted in the group B of two animals. Total 16 pigs were included and 6 of them were sacrificed at 3 months post-repair and the rest were sacrificed at 6 months. Gross observation, histology, glycosaminoglycan (GAG) quantification and biomechanical test were applied to analyze the results. Paired t-test (for in vitro data) and F-test (for in vivo data) were used for statistical study and a p-value less than 0.05 was considered as statistically significant.

Essential result: Stronger expressions of type II collagen and aggrecan at both mRNA and protein levels were observed in BMSCs induced with both dexamethasone and TGF-ß1 than in cells induced with dexamethasone alone (p<0.05). However, these cells had good biocompatibility to polymer regardless of the induction methods, including good adherence between cells and polymer and the matrix production as evaluated by light microscope and electron microscope. Gross observation showed that defects were completely repaired by tissue engineered cartilage in group B at 3 months post-repair, which exhibited a smooth surface closely resembling adjacent articular cartilage at 6 months. In group A, the defects were partially repaired at 3 months and mostly repaired at 6 months. No repair was observed in groups C and D at both time points except for partial repair in group C of two animals. Histology at 3 months demonstrated that the defects were repaired in group A with fibrous tissues (2/6) or fibrocartilage (4/6). In group B, the defects were repaired with hyaline cartilage in 4 of 6 cases and with fibrocartilage (2/6) in the rest. In contrast, only fibrous tissue was observed in both groups C and D. At 6 months, 4 of 10 defects in group A were repaired with hyaline cartilage and 6 with fibrocartilage. In group B, 7 of 10 defects were repaired with hyaline cartilage and the rest (3/10) with fibrocartilage. Furthermore, the underlying cancellous bone defects were completely repaired or mostly repaired in both group A and group B at 6 months post-repair. Contrarily, only 2 of 10 defects in group C were repaired with fibrocartilage, the other cartilage defects in group C and group D and their underlying bone defects of both groups were not repaired. Besides, the compressive moduli of groups A and B have reached 60% and 80% of normal cartilage amount respectively at 6 months, which were further supported by the high levels of GAG contents in engineered cartilage of group A (75% of normal contents) and group B (no statistical difference from normal contents). More importantly, confocal microscope revealed the presence of GFP-labeled cells in both engineered articular cartilage and engineered cancellous bone underlying the repaired cartilage, providing the direct evidence that BMSCs were transformed into chondrocytes and osteoblasts respectively in vivo.

Discussion: The results of this study demonstrated that chondrogenic phenotype can be better induced by both dexamethasone and TGF-ß1. In addition, induced cells have good biocompatibility to polymer construct. Implanted BMSCs can differentiate into either chondrocytes or osteoblasts at different local environments and repair a complex articular defect with both engineered cartilage and bone. Therefore, BMSCs can serve as seed cells better than chondrocytes for repairing articular defect. Moreover, using BMSCs can avoid the harvest of autologous cartilage and thus preventing secondary tissue defect.

This study also showed that engineered cartilage not only had a normal structure but also was fully functional as demonstrated by their high levels of GAG contents and the compressive moduli similar to normal counterpart. Next step of study will focus on the use of BMSCs to repair articular cartilage defect at weight-bearing area and on investigating the long-term result (up to 1 year) of tissue engineered repair for articular cartilage defect.

Reference