Therapeutic Strategy of Third Generation Autologous Chondrocyte Implantation for Osteoarthritis

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Introduction
Knee osteoarthritis (OA), a clinical syndrome with low-grade inflammation caused by abnormal wearing of the articular cartilage that covers joints and acts as a cushion, results in pain, destruction of the joints or decrease of synovial fluid that lubricates the joints. Because of the limited capacity for repair when damaged, abnormal wearing of the articular cartilage is a major clinical problem \(^1\). Various therapeutic strategies including bone marrow stimulation and transplantation of osteochondral autograft or allograft have been developed to restore articular cartilage and produce a permanent repair \(^2\). Among those, autologous chondrocyte implantation (ACI) is one of the promising choices for cartilage repair. The classical ACI was first described in 1994 and approved by the USA Food and Drug Association in 1997 \(^3\). The first generation ACI provided significant and long-term benefits for patients in terms of diminished pain and improved function. However, hypertrophy or ossification of patched periosteum and complex operative technique developed the second generation ACI, where bioengineered bi-layer collagen membranes were used without a periosteal flap. In addition, further technological advances have led to the third generation ACI, where biomaterials seeded with chondrocytes were used as carriers and scaffolds for cell growth. These “all-in-one” grafts do not need a periosteal cover or fixing stiches and can be trimmed to exactly fit the cartilage defect. Although the advantages of this new technique exists in its technical simplicity, shorter operating time, and the possibility to perform the surgery via a mini-arthrotomy or arthroscopy, the availability to OA is unknown and the effect is still controversial. Several clinical studies of the third generation ACI for partial cartilage injury have reported \(^4\)-\(^6\), but there is no study of the third generation ACI applying for OA-like change. Therefore, the purpose of this study is to evaluate the efficiency of the third generation ACI for the rat knee osteoarthritis produced by transaction of anterior cruciate ligament.

Materials and Methods

Animal model: The institutional animal care and committees of RIKEN Center for Developmental Biology approved all animal procedures. Athymic nude rats (F344/N Jcl rnu/rnu) aged 8 to 10 weeks were subjected to anterior cruciate ligament transaction \(^7\). Two weeks after the operation, we confirmed the degeneration of articular cartilage by macroscopic assessment and histology of toluidine blue staining.

ACI procedure: Following materials were transplanted at the part of cartilage injury with defect shape in patella groove after regulation by a periosteal flap. In addition, further technological advances have led to the third generation ACI, where biomaterials seeded with chondrocytes were used as carriers and scaffolds for cell growth. These “all-in-one” grafts do not need a periosteal cover or fixing stiches and can be trimmed to exactly fit the cartilage defect. Although the advantages of this new technique exists in its technical simplicity, shorter operating time, and the possibility to perform the surgery via a mini-arthrotomy or arthroscopy, the availability to OA is unknown and the effect is still controversial. Several clinical studies of the third generation ACI for partial cartilage injury have reported \(^4\)-\(^6\), but there is no study of the third generation ACI applying for OA-like change. Therefore, the purpose of this study is to evaluate the efficiency of the third generation ACI for the rat knee osteoarthritis produced by transaction of anterior cruciate ligament.

Contribution of transplanted chondrocytes: To examine human cell derived chondrogenesis, mRNA expression by RT-PCR analysis for chondrogenic markers (human specific collagen type 2 and SOX 9) and double immunofluorescence staining for human specific collagen type 2 and HLA-ABC were performed at week 4.

Morphological healing of OA: To confirm the recovery from OA-like arthritis, we performed macroscopic and histological evaluation at week 0, 4, 8, 20. We evaluated OA repair semi-quantitatively using a grading and staging system \(^8\). In this system, there are six histological grades and four histological stages. The total score (score= grade x stage) ranges from 1 point (normal articular cartilage) to 24 points (no repair).

Results

Contribution of transplanted chondrocytes: RT-PCR analysis using regenerated tissue samples from transplanted site demonstrated that the expressions of human specific collagen type 2 and SOX 9 were detected in the ACI group, suggesting that regenerated cartilage was derived from transplanted human chondrocytes. However, there were no expressions in collagen and sham groups. Double immunofluorescence staining for human specific collagen type 2 and HLA-ABC at week 4 demonstrated that double stained cells were seen in the ACI group, but not in the collagen and sham groups.

Morphological healing of OA: Macroscopic assessment showed better recovery with relative smooth surface of articular cartilage in the ACI group when compared to the other groups. Histological assessment with toluidine blue staining showed that the thickness of articular cartilage was significantly higher in the ACI group than the other groups at week 8 (ACI, 27.5±3.6; collagen group, 9.7±2.5; sham group, 8.3±1.5mm, respectively, p<0.05 for ACI vs. the other groups)(Fig.1). Semi-quantitative histological scoring at week 8 was also significantly better in the ACI group than the other groups (ACI, 1±0; collagen group, 7.8±1.6; sham group, 14±2.2, respectively, p<0.05 for ACI vs. the other groups). Although we could not detect the significant difference in the thickness of articular cartilage among the three groups at week 20, histological score was still better in the ACI group than the other groups (ACI, 8±2.8; collagen group, 12±2.8; sham group, 24±2.8, respectively, p<0.05 for ACI vs. the other groups).

Discussion

The present results indicated that the third generation ACI had a good capacity for repairing the articular cartilage defect of OA. And we also confirmed that the regenerated cartilage was controlled strongly by the transplanted human chondrocytes. At week 20, there had been found decreasing efficiency of ACI group for OA repair and healing, supposing that OA change had been lasted by the instability of ACL resected knee joint.

Conclusion

In the present study, using rat OA model, we showed the therapeutic potential of the third generation ACI for cartilage defect of knee OA. We believe that our findings provide a new insight to the field of OA treatment and widen the application of the third generation ACI for knee OA as a new strategy.

References