Introduction: Chondrocyte transplantation is one of the ideal methods for the treatment of large articular cartilage defect. However, we can observe a progressive thinning of regenerated cartilage in a time dependent manner post-transplant. One of the reasons for this phenomenon is that the repair cartilage was subsequently replaced with bone in a proximal to distal direction. However, there are several other explanations for this change. Isolated chondrocytes are susceptible to a myriad amount of damages during chondrocyte transplantation. The isolation procedure itself disrupts the integrin-related chondrocyte extracellular connection, leading to reduced chondrocyte function in perfusion, followed by apoptosis. Apoptosis, or programmed cell death, plays a key role in embryogenesis, immunological competence and tissue homeostasis for cell removal. Apoptotic chondrocytes have been described in cartilage at several levels: during endochondral ossification, during chondrogenesis, in the hypertrophic region of growth plates, and during osteoarthritis and rheumatoid arthritis. Since apoptosis might be responsible for cartilage breakdown in regenerated articular cartilage, we attempted to define apoptotic chondrocyte death in regenerated articular cartilage after chondrocyte transplantation in rabbit knee.

Methods: The research protocol was reviewed and approved by the Animal Research and Care Committee (ARCC) at the Children's Hospital of Pittsburgh and the University of Pittsburgh. Thirty 12-week-old New Zealand White rabbits were used in this study. Bovine type I collagen gels (Vitrogen 100®, Celtrix, Santa Clara, CA) containing allogenic chondrocytes were grafted to the experimental full-thickness osteochondral defects in rabbit knee. The repaired tissues were evaluated at 2 wk, 4 wk, 8 wk, 12 wk and 24 wk after operation by histology and histochemistry. The articular cartilage regeneration index (ACRI) was derived from Safranin-O stained sections. The ACRI is expressed as the ratio of the regenerated articular area to the imaginary normal articular area using histomorphometric analysis. Apoptotic cellular fractions were derived from in situ apoptosis analysis by TUNEL staining using the in situ cell death detection kit (Roche Molecular Biochemicals, Indianapolis, IN) along with the quantification of total cellularity. The average and standard deviation of all data were compared among the different groups using a Kruskal-Wallis H test following Student-Newman-Keuls test for statistical analysis. Statistical significance was defined as P < 0.05.

Results: Articular cartilage regeneration index: Histomorphometric analyses directed to the quantification of cartilage regeneration were performed using Meta Imaging Series 4.6 (Universal Imaging Corp. USA). As shown in figure 1 and Figure 3A, the ACRI decreased over time post-transplant. The ACRI after 24 weeks was 0.7 ± 0.10, which was significantly smaller than the other earlier time points (p < 0.05).

Cellularity: Before the determination of the apoptotic cell count, the total cellularity was evaluated from the center of the regenerated articular cartilage in a area measuring 400 um wide by 200 um deep. As shown in figure 3B, the total cellularity decreased with time.

Apoptotic Indices: Apoptotic cells were rare in normal articular cartilage but occasionally were seen along the superficial layer, where the apoptotic index was 8.6%. In situ study of DNA fragmentation in the regenerated articular cartilage showed that apoptotic cells were located in the superficial and middle zones. The percentage of apoptotic cells in the regenerated articular cartilage section was showed in figure 2 and Figure 3C. The highest percentage occurred 12 weeks after chondrocyte transplantation.

Discussion: Chondrocytes in the regenerated articular cartilage area showed morphologic changes that are characteristic features of apoptosis. Canine studies show that implanted chondrocytes undergo a sequential pattern of healing including proliferation (6weeks), transition (6-12weeks), and remodeling (after 12 weeks) with maturation. In our experiment, the beginning of the remodeling period coincides with the peak of apoptosis. This mechanism of cell death may play some role in the thinning change after chondrocyte transplantation. Since apoptosis plays a role in the thinning change of regenerated articular cartilage, anti-apoptotic gene therapy such as the Bcl-2 gene will protect transferred chondrocytes from apoptosis in regenerated articular cartilage.

Figure 1. Articular cartilage change after chondrocyte transplantation (A : Normal; B, C, D, E, F : 2, 4, 8, 12, and 24 weeks respectively.)

Figure 2. Results of TUNEL stains.

Figure 3.

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