An Experimental Study on Allograft of Cryopreserved Articular Cartilage

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Abstract: Allografting is a very useful means of reconstructing cartilage. An attempt was made to transplant a very thin cryopreserved articular cartilage film with no subchondral bone into the recipient cartilage defect, with the aim of reducing the rejection process.

1. Articular cartilage preservation
The knee joints of adult rabbits were pretreated with various kinds of cryopreservatives and saline, then rapidly cooled to -80°C and stored for 2 months. Pretreatment with 5% DMSO was useful because the columnar arrangement of the cartilage cells was maintained and a great number of typical cartilage cells were observed.

2. Transplantation of articular cartilage
A thin circular shaped cartilage film (3mm in diameter) was removed from the condylar surface of the patello-femoral joint and the weight bearing surface of the tibio-femoral joint which was preserved for 2 months with 5% DMSO. The thin film was transplanted into the cartilage defect. No apparent immunological rejection was revealed histologically. The success rate was 80% (32 out of 40 sites). Successful cartilage film allografting with no apparent rejection was obtained in the specimen fitted with no gap into the recipient cartilage bed and most frequently at the non-weight bearing cartilage surface.

Key words: Chondral allograft, Cryopreservation, Articular cartilage

INTRODUCTION
The repair of defects in articular cartilage, such as those occurring in osteochondritis dissecans, depressed fractures of the tibial plateau and other injuries to the articular surface, have presented a difficult problem in cartilage reconstruction. Allografting is a very useful means of reconstructing cartilage, depending on the volume and the site of the recipient cartilage. The procedure of cartilagenous preservation and/or transplantation, however, is not yet established. Many investigators reported that it was not easy to fit and incorporate a thin slice of articular cartilage into the chondral defect of the recipient cartilage. In 1961, Pap and Krompecher used cartilage grafts with adjoining cancellous bone, not exceeding five mm in thickness and suggested the long survival of the cartilage, but others have been unable to confirm these findings using the same method, and McKibbin reported the problem of immunological rejection. It is believed that subchondral bone itself has antigenicity, and further rejection process may occur. An attempt was made to transplant a very thin cryopreserved articular cartilage film with no subchondral bone into the recipient cartilage defect, with the aim of reducing the rejection process.
MATERIALS AND METHODS

I. Articular cartilage preservation

Knee joints taken from adult rabbits were pretreated with 5% and 10% dimethyl sulfoxide (DMSO), 5% glycerol, 0.05% hyaluronic acid and saline, as control, for 30 minutes and then rapidly cooled to -80°C. They were stored for 2 months, rapidly thawed and studied histologically (H & E, Safranin-O staining).

II. Transplantation of articular cartilage

Ten adult rabbits weighing 3kg were used for this study. The patello-femoral articular region and the medial femoral condyle were chosen as the site for allografting. Circular-shaped cartilage film (3mm in diameter) was removed from the condylar surface of patello-femoral joint (P-F joint) and the weight-bearing surface of tibio-femoral joint (T-F joint) of the knee joint cryopreserved for 2 months with 5% DMSO. The cartilage grafts were transplanted into the defect previously made at the distal end of the femur as shown in Fig. 1 and fixed without biobonding. The hind legs were not immobilized and free movement was allowed soon after surgery. The cartilage grafts in the condyles were removed en bloc at 4 and 8 weeks after transplantation and evaluated histologically.

Control autograft were performed by swapping the cartilage films obtained from the knee with those of the opposite side, using an additional 5 rabbits. These rabbits were also killed at 4 and 8 weeks after the sham surgery and evaluated histologically.

RESULTS

I. Articular cartilage preservation

Under the light microscope, the irregular surface and the cleft in the surface were more or less visible in the specimens taken at 2 months after preservation regardless of the kind of pretreatment. There was histological evidence of cell degeneration in the cartilage pretreated with 10% DMSO and saline. With the 5% and 10% DMSO pretreatments, the normal columnar arrangement of cartilage cells was maintained and a great number of typical cartilage cells were observed in the specimen pretreated with 5% DMSO. Metachromasia in the cartilage matrix with

![Fig. 1. Transplantation technique for articular cartilage: The cartilage film was transplanted onto the defect previously made by the same trocher (arrow) at the condylar of the patello-femoral joint.](image-url)
Fig. 2. Cartilage-tissue cryopreserved with 5% DMSO: Columnar arrangement of the cartilage cells and a great number of typical cartilage cells were observed.
II. Transplantation of articular cartilage

Forty sites were allografted by the cartilages pretreated with 5% DMSO, using 10 rabbits in total. No immunological rejection appeared in any of the specimens histologically. The grafts were assessed histologically in term of success in filling the defects: successful—where there was complete or almost complete filling of the defect with graft tissue; failed—where there was only minimal cartilage present with fibrous tissue, or fibrous tissue alone in the defect. Success rate in our study was 80% (32 out of 40 sites).

A. Gross findings

Ten sites received autografts, using 5 rabbits as a control group. These grafts were well incorporated into the surrounding cartilage. The grafted cartilage appeared to be healthy with an even surface.

All of the successful allografts were found to be firmly attached to the host cartilage at 4 or 8 weeks after transplantation. In particular, at the femoral articular surface of the P-F joint, the interface between donor and recipient cartilage was very smooth with no fibrous tissue (Fig. 3. a.). On the contrary, the surface of the graft from the medial femoral condyle was more likely to be irregular and deformed and some destruction of articular cartilage was found. In a few areas there was some proliferation of fibrous tissue at the margin of the graft (Fig. 3. b.).

B. Microscopic findings

An autograft study showed no evidence of abnormal reaction, and normal columnar arrangement of the cartilage cells was seen. As to the allograft study, histological ex-
Fig. 4. Allograft at eight weeks after transplantation: the superficial layer was maintained and no evidence of cell atrophy or degeneration was found in the middle layer.
Fig. 5. Allograft at eight weeks after transplantation

a: The grafted cartilage is located on the right side. Neither inflammatory cell nor fibrous tissue infiltration were observed at the junction.

b: No fibrous tissue infiltration was observed at the junction between the host cartilage and the base of the grafted cartilage.
amination revealed that the thickness of the cartilage layer and the normal columnar arrangement of the cartilage cells were maintained in the superficial and middle layers of the specimens from the P-F joint and T-F joint at 4 weeks after surgery. Surviving chondrocytes were demonstrated clearly by Safranin-O staining. However, deeper layer near the junction of the graft and host cartilage, the cells were decreased in number and open lacunae of chondrocytes were found.

The specimen from the P-F joint at 8 weeks after surgery showed that the superficial layer was maintained and no evidence of cell atrophy or degeneration was found in the middle layer with H.E staining (Fig. 4. a.). The middle layer of the matrix in the P-F joint specimen 8 weeks after surgery was also clearly demonstrated with safranin-O staining (Fig. 4. b.). Neither inflammatory cells nor fibrous tissue infiltration were seen at the junction between the host cartilage and the grafts (Fig. 5. a.). The arrowed V-shaped defect was not due to a tissue atrophy, but mechanical defect caused by the trocher and/or sheath wall. No fibrous tissue infiltration was observed at the junction between the host cartilage and the bottom of the grafted cartilage (Fig. 5. a.). There was no difference seen in staining by safranin-O between the host matrix and the graft. Almost all of the failed cases were specimens from the medial femoral condyle and slight infiltration of fibrous tissue and degeneration of the cartilage were observed. In such cases, the gap existing between the allograft and the recipient site was partly covered with a thin fibrous tissue layer. There was also moderate inflammatory cell infiltration in the deep layer of the graft.

**Discussion**

I. Articular cartilage preservation

Studies on isolated chondrocytes have suggested that viability and function may be retained following freeze-preservation. The successful cryopreservation of these cells is dependent on several factors. Smith(1) found that by pretreatment with DMSO, chondrocytes thawed after one week of storage at -79°C appeared normal under phase-contrast microscopy. Tomford(3) reported that chondrocytes frozen with DMSO produced cartilage similar to that of fresh cells, whereas frozen cells not treated with DMSO did not survive. However, cryopreservatives are known to be toxic to cells. Tomford reported that the upper limit of concentration of glycerol and DMSO was found to be approximately 12%, with exposure for one and one half hours before toxic effects began to appear. We, therefore, chose 5 to 10% as the concentrations of cryopreservatives.

Several investigators(4) have reported the effectiveness of the use of a two-stage cooling technique by which to achieve maximum viability of isolated cells. In our experiments, however, the articular cartilages were directly cooled to -80°C after pretreatment with the cryopreservative (5% DMSO). This procedure is simple in practice and applicable for storing bone in banks.

II. Transplantation of articular cartilage

It is reported that transplanted of articular cartilage without subchondral bone is unlikely to be incorporated into the host cartilage as a viable member of cartilage. It is believed that the articular cartilage graft with subchondral bone was quickly revascularized and incorporated into the recipient bone surface(5). The nutritional support of the avascular articular cartilage was not apparent. Some authors believed that it is fed exclusively by the synovial fluid and others believe that subchondral circulation is necessary for the cartilage to survive. Hishikawa(4) reported that the autografted articular cartilage, without access to the subchondral circulation, would survive for one year after transplantation. Ninomiya(8) noted that the subchondral circulation could not be taken into account. Honner(5) described that the nutrition for adult articular cartilage
comes through the synovial fluid. In our experiment, allografted cartilage film fed only by synovial fluid survived. This result leads us to believe that subchondral blood circulation is not necessary for allografted cartilage film.

All of the failed allografts were specimens of the weight bearing surface of the medial condyle of T-F joint. This suggests that one of the factors contributing to this failure was allowing weight bearing soon after surgery. We conclude that weight bearing should not be permitted for a certain period of time until the cryopreserved chondrocytes would recover from the shock of being thawed.

III. The junction between the graft and the host cartilage
In the case of the wide gap between the host and graft cartilage, Tanaka\(^{12}\) reported that the gap was filled with connective tissue and that the chondrocytes were decreased in number and the immunological reaction was increased. Our findings indicate that once the homogeneous cartilage film is exactly fitted into the recipient cartilage bed with no gap, the graft is successfully incorporated into the host cartilage with reduced immunological reaction. Maintaining the natural contour of the cartilage surface is also important for the incorporation of the graft.

IV. Antigenicity of chondrograft
It has been shown that homogenous transplants of cartilage survive longer than those of any other tissue except the cornea. Not only is cartilage avascular but its intracellular matrix seems to be resistant to destruction by granulation tissue, fibrous tissue and tumors\(^{17\text{-}10}\). Cartilage graft causes very little immunological reaction, as manifested by the lack of lymphocytic or fibroblastic reaction along the margins. Enclosed within a protective avascular intracellular matrix, chondrocytes survive for long periods of time if adequate nutrition is present. Autogenous cartilage grafts survive and maintain their size and shape, whereas allografts gradually diminish in size due to absorption at the areas of contact with the vascular tissue of the host, resulting in a slow process of replacement by fibrous tissue. This would suggest that the allograft response to cartilage differs from that of the other tissues, such as skin or bone. Our hypothesis is that the subchondral blood circulation must be directly responsible for the allograft rejection and the gap between the allograft and the host cartilage may allow the further infiltration of a various kind of inflammatory cells. We used the cartilage allograft as a thin film with no subchondral bone in order to omit the subchondral circulation. Furthermore, we tried to transplant the allograft into the recipient cartilage bed with no gap. Our results showed that no obvious rejection was successfully demonstrated in the specimens, 8 weeks after surgery.

CONCLUSION

I. As a preservation procedure for chondral allograft, pretreatment with 5% DMSO was relatively useful.

II. Homogeneous cartilage film preserved for 2 months with 5% DMSO at \(-80^\circ\text{C}\) mostly maintained the size and shape of chondrocytes after transplantation.

III. Successful cartilage film allografting with no apparent rejection was obtained in the specimen exactly fitted with no gap into the recipient cartilage bed and more frequently at the non-weight bearing cartilage surface.

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