The effects of meniscal allograft transplantation on articular cartilage
Rijk, P.C.

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Chapter 5

Histological Changes of Articular Cartilage after Medial Meniscus Replacement in Rabbits

Paul C. Rijk, Wikky Tigchelaar-Gutter, Franz-Peter Bernoski and Cornelis J.F. van Noorden

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Abstract

**Purpose:** Meniscal allografts show capsular ingrowth, but it remains to be established whether meniscal transplantation can prevent long-term degeneration of articular cartilage. This study examines whether immediate or delayed transplantation of the medial meniscus can protect the knee from degenerative changes.

**Type of study:** Experimental study.

**Methods:** Thirty-five rabbits were divided into 5 groups. Three rabbits developed infective arthritis and were excluded from the study. Group A (6 animals) and Group C (6 animals) had meniscectomy only. Group B (7 animals) and Group D (6 animals) underwent meniscal transplantation immediately after meniscectomy. Group E (7 animals) had delayed transplantation 6 weeks after meniscectomy. Six nonoperated knees served as controls. Histological changes of the articular cartilage were examined 6 weeks (Groups A, B) and 1 year (Groups C, D, E, controls) after surgery.

**Results:** All operated groups showed more histological changes than the control group (p < 0.00001 for both medial tibial plateau and medial femoral condyle in all groups). At 6 weeks follow-up, no differences were found between the postmeniscectomy group and the transplanted group. At 1 year, immediately transplanted knees showed less degenerative changes than meniscectomized knees (p < 0.0001 for medial tibial plateau and p < 0.005 for medial femoral condyle). Delayed transplantation resulted in more degenerative changes than meniscectomy only and than immediate transplantation (for both comparisons, p < 0.00001 for both medial tibial plateau and medial femoral condyle).

**Conclusions:** Immediate meniscal transplantation in rabbits has a protecting effect on articular cartilage for the long term, whereas delayed transplantation leads to even more degenerative changes than meniscectomy only.
Introduction

Total meniscectomy leads to progressive degenerative arthritis of the knee on a long-term basis.\textsuperscript{1,4} Therefore, meniscal-retaining procedures are preferred over total or partial excision in the treatment of meniscal tears. Nevertheless, there remains a group of patients, in which meniscal preservation is not possible. In an attempt to restore the function of the native meniscus in these cases, allografts, autografts, and synthetic substitutes have been evaluated.\textsuperscript{5-8} Several experimental and clinical studies reported on implantation of meniscal allografts in meniscectomized knees and showed that ingrowth from the adjacent capsule into the transplant occurs and that allografts are not rejected.\textsuperscript{9-19} However, it has not yet been shown unequivocally that articular cartilage of the knee is protected and preserved after meniscectomy by meniscal allograft transplantation on a long-term basis. The purpose of the current study is to test the hypothesis that meniscal allograft transplantation provides protection of the articular cartilage by evaluating histological changes of articular cartilage in rabbit knees after meniscectomy and after immediate or delayed meniscal transplantation.

Materials and Methods

Thirty-five mature female New Zealand White rabbits weighing between 3.0 and 3.5 kg were divided into 5 groups (Table 1). All animals were purchased from the same supplier. Surgery was performed on the medial compartment of the right knee. Three animals (1 in Group A, 1 in Group C, and 1 in Group D) developed infective arthritis of the knee and were not included in the study. A sham procedure on the right knee was done in 4 rabbits. Six weeks after surgery, animals in Groups A and B and 2 sham-operated animals were euthanized by intravenous injection of sodium

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Follow-up (weeks)</th>
<th>Type of operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>6</td>
<td>Meniscectomy only</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>6</td>
<td>Meniscectomy followed by immediate transplantation</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>52</td>
<td>Meniscectomy only</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>52</td>
<td>Meniscectomy followed by immediate transplantation</td>
</tr>
<tr>
<td>E</td>
<td>7</td>
<td>52</td>
<td>Meniscectomy followed by delayed transplantation</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>52</td>
<td>None</td>
</tr>
<tr>
<td>Sham</td>
<td>2</td>
<td>6</td>
<td>Arthrotomy only</td>
</tr>
<tr>
<td>Sham</td>
<td>2</td>
<td>52</td>
<td>Arthrotomy only</td>
</tr>
</tbody>
</table>
pentothal. All other rabbits were euthanized 1 year after the first operation. The nonoperated left knees of 6 rabbits (3 animals in Group D and 3 animals in Group E) were selected at random before surgery to serve as the control group. The knees were removed for analysis at the time of death of the animals. Approval for this study was obtained from the local ethical committee for animal experiments.

**Surgical Procedures**

The rabbits were premedicated with an intramuscular dose of ketamine (50 mg/kg) and xylazine hydrochloride (8 mg/kg). Surgery was performed with the animals under general anesthesia with halothane, oxygen, and nitrous oxide inhalation via a mask. All operations were done by the same surgeon. Using aseptic operating procedures, the joint was entered after an anterior midline incision, a medial parapatellar capsulotomy through the patellar fat pad, and gentle lateral displacement of the extensor mechanism. Medial menisci were resected sharply along the periphery, dividing the coronary ligament, and detached from their anterior and posterior tibial bone attachments at the junction of the ligamentous attachment and the meniscal fibrocartilage. Care was taken not to injure the medial collateral ligament, cruciate ligaments, or articular cartilage. The meniscal grafts were immersed in sterile saline after harvesting. When an immediate transplantation was done, an appropriately sized fresh allograft was selected from the removed menisci and sutured in the recipient bed using 6-0 polypropylene sutures. Allografts were selected from removed menisci by the eye on the basis of its match in size and shape with the original meniscus. Immunology mismatching was not performed before implantation. All allografts were obtained from animals used in this study and were reimplanted within 2 hours after harvesting. The anterior and posterior horns of the graft were reattached to the appropriate ligamentous structures; the midportion was sutured to the medial collateral ligament. The allograft position and mobility were controlled in knee flexion and extension and under valgus and varus stress. The capsule, periarticular tissues, and skin were closed with interrupted 3-0 polyglactin sutures. Delayed transplantation was done by a 2-step procedure with an interval of 6 weeks between meniscectomy and transplantation using a fresh allograft. In the sham operations, the joint capsule was opened but the meniscus was not removed. After surgery, animals received a subcutaneous injection of buprenorphine (0.05 mg/kg) for pain relief and could move and exercise freely. Five milligrams enrofloxacin 5% (weight per volume) was given as an antibiotic prophylaxis for 72 hours.

**Preparation of Sections**

After dissection of the knees, skin and superficial muscle layers were removed and the joints were immediately embedded in an aqueous solution of 8% (weight per volume) gelatin white (Sigma, St. Louis, MO, USA) and frozen slowly in liquid nitrogen as previously described.\textsuperscript{20,21} Sections were cut on a motor-driven cryostat fitted with a retraction microtome (Bright, Huntingdon, UK) and a tungsten carbide-
tipped knife (Spikker, Zevenaar, The Netherlands) at a cabinet temperature of \(-25^\circ\) C.\(^{21,22}\) The angle between the knife and surface of the tissue block was \(8^\circ\). After the block was trimmed to the desired level, transparent tape (Scotch Tape 800; 3M, St. Paul, MN, USA) was fastened with a stiff brush onto the section surface of the block. The microtome knife then cut underneath the tape at an extremely low but constant speed and 10 \(\mu\)m-thick sections attached to tape were obtained without loss of tissue integrity.\(^{21,22}\) Pieces of tape with adherent sections were fixed on glass slides with ordinary tape. Coronal sections of the intact knees were prepared including menisci, femur, and tibia. Sections made in this plane allow for comparative observations in medial and lateral compartments in each section. Three to 6 sections from the anterior and posterior region of each knee were prepared and stained with Giemsa (BDH, Poole, UK).

**Histological Evaluation**

The transplanted menisci were assessed macroscopically for capsular ingrowth and degenerative changes. The quality of cartilage of tibial and femoral surfaces was examined separately for the medial and lateral compartments using a scoring system that was slightly modified on the basis of histological criteria for quality assessment of cartilage structure and chondrocytes (Table 2).\(^{24}\) To obtain a score that corresponds to the severity of degenerative changes in the cartilage, the scores for each of the criteria were added, resulting in a semiquantitative value ranging from 0 to 21. A

<table>
<thead>
<tr>
<th>Changes in articular cartilage</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Advanced</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cartilage structure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface irregularities</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Clefts into transitional zone</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Clefts into radial zone</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Transverse clefts</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Tissue loss</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><strong>Chondrocytes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cloning</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Hypocellularity</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

*Total score (range, 0-21) is the sum of the grades for each parameter.*
value for each tibial and femoral surface of medial and lateral compartments was obtained in each section. To limit subjectivity of the assessment technique, all sections were randomized before scoring and the grading was done separately by 2 observers in a training session. After these individual assessments, the observers assessed the sections together to reach agreement on standards for grading. Four months later, both assessors separately graded all sections again to obtain definitive scores and to assess interobserver reliability. Eight months later, 1 observer scored the images again to establish intraobserver differences. All assessments were performed in a blinded manner.

Statistical Analysis
Data were analyzed statistically using the Mann-Whitney rank-sum test for nonparametric variables. The Spearman correlation coefficient was used for determining intraobserver and interobserver reliability. Statistical significance was set at \( p \leq 0.05 \).

Results

Macroscopical Assessment of Menisci
None of the menisci showed extrusion. Menisci in sham-operated knees appeared to be normal. Capsular ingrowth was observed in all menisci that were transplanted immediately after meniscectomy. Six weeks after transplantation, 2 of 7 meniscal allografts in Group B showed degenerative changes. One year after immediate transplantation (Group D), degenerative changes were found in 3 of 6 transplants. After delayed transplantation (Group E), 2 of 7 menisci were degenerated completely after 1 year follow-up. All other transplants in Group E healed to the periphery, but all showed degenerative changes and shrinkage. One of these transplants had a calcification developed in the anterior horn. Evidence of meniscal regeneration was observed in 5 of 6 rabbits at 6 weeks after meniscectomy without transplantation (Group A) and in 3 of 6 rabbits at 1 year after meniscectomy only (Group C). These observations were not correlated with the subsequent analysis of the articular cartilage.

Histological Changes of Articular Cartilage
The mean grading scores of both observers for each section in each group are summarized in Figure 1. Abnormalities were not observed in the lateral compartment except for 1 animal in the delayed transplant group (Group E) showing minimal degenerative changes. None of the assessed knees showed advanced loss of tissue in the medial compartment. In the sham-operated animals, minimal or no histological degenerative changes were found in the lateral and medial compartments. Because of their small numbers, these sham-operated animals were not included in the statistical analysis.
CARTILAGE CHANGES AFTER MENISCUS REPLACEMENT

Figure 1. Box and whisker diagram comparing semiquantitative values of degenerative changes of the medial tibial plateau (MTP) and the medial femoral condyle (MFC) in the nonoperated group (control), Group A (6 weeks after meniscectomy), Group B (6 weeks after meniscal transplantation), Group C (1 year after meniscectomy), Group D (1 year after immediate meniscal transplantation), and Group E (1 year after meniscectomy followed by delayed meniscal transplantation 6 weeks later) by showing the median (bar), 25% and 75% percentiles (box), and range (whisker) of the mean scores of both observers for each section.

Statistical Analysis

The scores of both observers for each section were used for statistical analysis. Compared to the nonoperated control group (Figure 2A), all groups of animals that had been operated on (Figures 2B-F) showed significantly more degenerative changes (p < 0.00001 for medial tibial plateaus and medial femoral condyles in all groups). In the knees examined 1 year after meniscectomy (Figure 2D), significantly more osteoarthritic changes were found than in those examined 6 weeks after meniscectomy (Figure 2B) (p < 0.0001 for medial tibial plateaus and medial femoral condyles).

Comparing the immediate transplant groups 6 weeks after transplantation (Figure 2C) and 1 year after transplantation (Figure 2E), no significant differences were observed for the medial tibial plateau (p = 0.99), but the medial femoral condyle showed significantly more degenerative changes at 1 year follow-up (p = 0.022).

Six weeks after immediate transplantation, less degenerative changes of the medial tibial plateau and more degenerative changes of the medial femoral condyle were found than at 6 weeks after meniscectomy, but these differences did not reach statistical significance (p = 0.37 and p = 0.45 for medial tibial plateau and medial femoral condyle, respectively). At 1 year follow-up, there were significantly more degenerative changes after meniscectomy than after immediate transplantation (p < 0.0001 for the medial tibial plateau and p < 0.005 for the medial femoral condyle).

The delayed transplant group (Figure 2F) showed significantly more degenerative
Figures 2A-F. Photomicrographs of Giemsa-stained rabbit knees showing the effects of meniscal transplantation on articular cartilage after meniscectomy: (A) control; (B) 6 weeks after meniscectomy; (C) 6 weeks after meniscectomy immediately followed by transplantation; (D) 1 year after meniscectomy; (E) 1 year after meniscectomy immediately followed by transplantation; and (F) 1 year after meniscectomy followed by delayed transplantation. (M, meniscus; R, regenerating meniscus; T, tibia; F, femur; J, joint space; C, cartilage; Hc, hypocellularity of cartilage; Th, suture; *, surface irregularities of cartilage; arrow, vertical clefts into the transitional zone of cartilage; double arrow, vertical clefts into the radial zone of cartilage. Original magnification x10).
changes after 1 year than the postmeniscectomy group and the immediate transplant group (for both comparisons, \(p < 0.00001\) for medial tibial plateaus and medial femoral condyles). The scoring system used in this study showed an intraobserver and interobserver reliability of 0.84 and 0.91, respectively (\(p < 0.00001\) for both).

**Discussion**

The prospect of restoring the function of the meniscus by meniscal allograft transplantation is appealing to prevent degenerative osteoarthritic changes that occur after total meniscectomy. The use of meniscal allografts has progressed to a point where relief of pain may be expected for the short term.\(^{11,12}\) Arnoczky et al.\(^{13}\) reported that transplanted medial meniscal allografts in dogs were able to minimize degenerative changes of tibial cartilage at a 6-month follow-up. However, Jackson et al.\(^{9}\) found an increase in water content and a decrease in uronic acid in meniscal allografts in goats 6 months after transplantation, suggesting degeneration. These biochemical data cast doubt on the protective effects of meniscal allografts on articular cartilage on a long-term basis. However, follow-up studies for longer than 6 months are not available with respect to protective effects of meniscal allografts on articular cartilage. In the current study, meniscal transplantation in rabbits matched for size and weight showed good healing of the grafts within 6 weeks after meniscus transplantation, which is consistent with the results of Shibuya.\(^{14}\) After 6 weeks, no significant differences were observed between the postmeniscectomy group and the transplant group. These findings suggest that meniscal transplantation does not protect the articular surface during the initial period of capsular ingrowth. The results at 1 year after surgery, which can be considered long-term follow-up, showed clearly that knees that received a meniscal transplant immediately after meniscectomy developed fewer degenerative changes than knees that were subjected to meniscectomy only. On the other hand, knees that underwent immediate transplantation showed more degenerative changes in the medial femoral condyle at 1 year than at 6 weeks after surgery. These findings suggest that immediate transplantation can delay but not prevent progression of degenerative changes. In contrast, rabbits that received a transplant 6 weeks after meniscectomy showed significantly more degenerative changes than rabbits in the postmeniscectomy group and the immediate transplant group. Cummins et al.\(^{15}\) reported that immediate and delayed meniscal allograft transplantation in rabbits at 3 months follow-up offer protection to articular cartilage of knees after meniscectomy and that delayed transplantation may reverse initial degenerative changes. The current results suggest that if initial degenerative changes attributable to meniscectomy are reversed, it is only temporary. Aagaard et al.\(^{16}\) stated that the altered shape of the condyles attributable to ridge formation after meniscectomy may cause a mechanical conflict between the transplant and the condyles, which may be responsible for the poor outcome in the delayed transplant group.
In the current study, 2 of 7 delayed transplanted allografts were totally degenerated 1 year after implantation, whereas the other delayed transplants all showed degenerative changes. In the group that had immediate transplantation, degenerative changes were observed in only 3 of 6 allografts at 1 year follow-up. A clinical implication of these findings may be that when the aim of meniscal transplantation is to reduce degenerative changes of articular cartilage, it has to be performed as soon as possible after meniscectomy. However, in the human situation the question how early to consider meniscal allograft transplantation is difficult to answer. In the first years after meniscectomy, patients have no symptoms and meniscal transplantation cannot be recommended at present for a patient without complaints.

The knees that underwent a sham operation showed only minimal or no histological changes in cartilage after 6 weeks and 1 year. These findings are in agreement with those of Shapiro and Glimcher, who observed no significant degenerative changes in sham-operated rabbit knees at 6 months after surgery. On the basis of these considerations, it can be concluded that a first arthrotomy does not lead to degenerative changes by itself. However, the twofold insult to knees subjected to delayed meniscal transplantation where the cartilage has been exposed to the effects of drying for a longer period, may be at least partly responsible for the poor outcome in this group.

Degenerative changes of the cartilage were graded by 2 observers in sections of knees using a modified Mankin scoring system. The overall interpretation of assessments by the first and second observer in the current study showed a more than acceptable interobserver and intraobserver reliability. However, it is recognized that this semiquantitative method of interpretation is subjective to a considerable extent. Moreover, despite the fact that some criteria that were assessed are likely more important than others in the grading of degenerative changes, each of the criteria was given the same numerical rating.

The current data obtained in rabbits cannot be extrapolated to the situation in man. Fresh allografts were reimplanted within 2 hours after harvesting. Several studies have suggested that the presence of viable cells in meniscal allografts may have a beneficial effect in maintaining the extracellular matrix and the mechanical integrity of the transplant. Allografts can be kept at 4°C in sterile tissue culture medium for 7 days without loss of viability. However, the availability of fresh transplants in clinical practice is limited and the impossibility of matching the meniscal size of donor and receiver may limit further the applicability of this type of graft in humans.

Furthermore, all rabbits were purchased from the same supplier and immunological mismatching was not performed. Therefore, it is possible that these animals have genetic similarities in their major histocompatibility complex haplotypes. Rodeo et al. found a small number of immunologically active cells (B lymphocytes and/or T-cytotoxic cells) in the majority of transplanted allografts in humans. The presence of these cells suggests a subtle immune reaction which may modulate graft healing, incorporation, and graft revascularization. The clinical importance of these
immune responses to meniscal allografts is not exactly known yet, but in general, there is no evidence for graft failure or rejection.

In 5 of 6 rabbits meniscal regeneration was observed after 6 weeks, which could be responsible for some protection of the articular cartilage in meniscectomized knees. The role of meniscal regeneration in the clinical situation is uncertain. Whether human meniscal allograft transplantation can prevent or postpone the onset of osteoarthritis has not been established. Nevertheless, rabbit models have proven to be one of the better models because of similarities in histological and biochemical aspects of rabbit and human articular cartilage. However, the weight-bearing profile in rabbit knees is different from that in human knees. Furthermore, surgical procedures in small animals such as the rabbit are difficult and may introduce artefacts that are hard to interpret when comparing data obtained in small animals with those obtained in larger animals. Because of the small size of rabbit knees, the horn ligamentous attachments of the transplanted allografts were not retained via transplantation of bone plugs, but the anterior and posterior horns were reattached to the ligamentous tibial bone attachments. In the current study, the effect of the anchoring technique on immune response, load distribution, and shock absorption could not be determined. However, Szomor et al., who reported comparable results at 4 months after immediate meniscal transplantation in sheep, hypothesized that the damage to articular cartilage observed in the group that underwent immediate transplantation was secondary to nonisometric positioning and tensioning and to rigidity of fixation of the grafts. Alhalki et al. reported that implantation of meniscal autografts in human cadaver knees with bone plugs resulted in contact mechanics that were significantly closer to normal as compared with fixation with sutures. However, bone plugs increase the antigenic load since bone allografts elicit an immune response. More future experimental and clinical studies are needed to evaluate these effects and to investigate the biological and biomechanical properties of different anchoring techniques on a long-term basis. The current findings suggest that meniscal allograft transplantation immediately after meniscectomy has a protecting effect on articular cartilage on a long-term basis, whereas delayed transplantation results in more degenerative changes than meniscectomy only. If the results of the current study are applicable to humans, they would support immediate but not delayed transplantation after removal of a meniscus. As a consequence, the question remains to be answered whether meniscal allograft transplantation after meniscectomy is indicated in asymptomatic patients.

**Acknowledgments**

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References


