The effects of meniscal allograft transplantation on articular cartilage
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Chapter 7

Structural Analysis of Meniscal Allografts after Immediate and Delayed Transplantation in Rabbits

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Abstract

**Purpose:** To compare long-term performance of meniscal allografts transplanted immediately after meniscectomy and allografts transplanted at 6 weeks after meniscectomy.

**Type of Study:** Experimental study.

**Introduction:** Meniscus transplantation has been proposed as an alternative to total meniscectomy. A structural analysis was performed to compare long-term performance of meniscal allografts transplanted immediately after meniscectomy and allografts transplanted at 6 weeks after meniscectomy.

**Methods:** Twenty-one rabbits were subjected to meniscectomy and divided into 3 groups of 7 animals. Immediate meniscal transplantation was performed in group A (6 weeks follow-up) and group B (1 year follow-up). Group C underwent delayed transplantation at 6 weeks after meniscectomy. One animal in group B developed infective arthritis and was not included. Six nonoperated knees served as controls. Four other knees were subjected to a sham procedure. Menisci were examined macroscopically and histologically at 6 weeks (group A and 2 sham-operated animals) and 1 year (group B, C, controls, and 2 sham-operated animals).

**Results:** Capsular ingrowth was observed in all allografts. At 1 year, osteoarthritic changes in the delayed transplant group were more pronounced than in the immediate transplant group. Menisci in nonoperated controls and sham-operated knees appeared normal. No differences in shrinkage of allografts were observed between groups A and B. Group C showed significantly more shrinkage than allografts in both group A (p=0.004) and group B (p=0.005). Two allografts in group C were completely degenerated. Differences in architecture of the allografts were not found between groups A, B, and C. In both the peripheral and central areas of transplanted menisci, the number of cells was frequently increased due to repopulation even at 6 weeks follow-up.

**Conclusions:** Delayed meniscal allograft transplantation causes distinct structural damage to menisci in comparison with immediate transplantation.
Introduction

It has been demonstrated that menisci perform an important functional role in knee joints and that their removal results in degeneration of articular cartilage. Menisci are weight bearing, increase joint congruency, stabilize the knee, facilitate rotation of the opposing articular surfaces of the joint, and improve articular cartilage nutrition and lubrication. Recognition of the harmful effects of meniscectomy and the immunoprivileged character of meniscal tissue have led to efforts to replace menisci that have been irreversibly damaged. Transplantation of meniscal allografts has mainly been studied in animals, but its effects have been analysed in a number of clinical trials as well and the short-term and mid-term results are promising. However, experimental studies cannot be directly compared with clinical trials because transplantation in animals is performed mostly immediately after meniscectomy, whereas in the human, transplantation is delayed because the indication for meniscal transplantation is degenerative joint disease due to meniscectomy. To our knowledge, long-term studies have not yet been published, in which the performance of allografts after primary meniscal transplantation is compared with that of allografts after secondary transplantation. In the present histological study in rabbits, long-term performance of meniscal allografts transplanted immediately after removal of the original meniscus and allografts transplanted at 6 weeks after meniscectomy are compared at 1 year after (the first) operation.

Material and Methods

Twenty-one mature female New Zealand white rabbits weighing between 3.0 and 3.5 kg were divided into 3 groups of 7 animals each. In all animals, the right knee was the experimental knee. Group A and group B were subjected to meniscal allograft transplantation immediately after medial meniscectomy. Group C underwent delayed meniscus transplantation at 6 weeks after meniscectomy. One animal in group B developed infective arthritis of the knee joint and was not included in the study. A sham procedure on the right knee was performed in 4 other rabbits. Six weeks after operation, animals in group A and 2 sham-operated animals were killed by intravenous injection of sodium pentothal. All other rabbits were sacrificed at 1 year after (the first) operation. The nonoperated left knee joints of 6 rabbits (3 animals from group B and 3 animals from group C) were selected at random before operation to serve as control group. The knees were removed for analysis at the time of death of the animals. Approval of 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 carried out under general anesthesia with halothane, oxygen, and nitrous oxide inhalation via a mask. All operations were performed by the same surgeon. Using sterile operating procedures, the joint was entered following 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, the coronary ligament was divided, and detached from the anterior and posterior tibial bone at the junction of the ligamentous attachments 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 performed, an appropriately sized fresh allograft was selected from the removed menisci and sutured in the recipient bed using 3-6-0 polypropylene sutures. 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 performed by a 2-step procedure with an interval of 6 weeks between meniscectomy and transplantation using a fresh allograft.

All allografts were obtained from animals used in this study and were reimplanted within 2 hours after harvesting. In the sham operations, similar skin and capsular incisions were made but the meniscus was not removed. After surgery, animals received subcutaneous analgesic (buprenorphine, 0.05 mg/kg) during 24 hours and were allowed to bear weight immediately and to move freely. Antibiotic prophylaxis was given for 72 hours (enrofloxacin 5%, 5 mg/kg).

**Preparation of Sections**

After dissection of the knee joints, skin and superficial muscle layers were removed and the joints were immediately embedded in an aqueous solution of 8% gelatin white (Sigma, St. Louis, MO, USA) and frozen slowly in liquid nitrogen as described previously.\(^ {16,17}\) 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.\(^ {17,18}\) The angle between 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.\(^ {17,19}\) Pieces of tape with adherent sections were fixed on glass slides with ordinary tape. Coronal sections of the intact knee joints 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 joint were prepared and stained with Giemsa (BDH, Poole, UK).

**Histological Evaluation**
Light microscopical assessment of the menisci was performed by 1 observer who was blinded to the experimental groups. Capsular ingrowth, coverage with synovial tissue, surface irregularities, and calcifications were described and scored as 0 (absent) or 1 (present). Features were presumed to be present if they were observed in more than 1 section. The number of viable cells was scored separately for peripheral and central areas as -1 (decreased), 0 (normal), or 1 (increased). Shrinkage was examined separately for the anterior and posterior horn of the meniscus and scored as 0 (absent), 1 (only present in the anterior or posterior horn), 2 (present in both the anterior and posterior horn), or 3 (meniscus completely degenerated). In order to limit subjectivity of the assessment technique, all sections were randomized before they were described.

A mean histological score for the articular cartilage was obtained per animal for both the medial tibial plateau (MTP) and medial femoral condyle (MFC) using a slight modification of the Mankin scoring system as described previously. The mean scores for all animals per group were registered but not evaluated in the present study.

**Statistical Analysis**
The Fisher exact probability test was used to compare differences in frequencies of coverage with synovial tissue, surface irregularities, and calcifications. The Mann-Whitney rank-sum test was used to compare scores for cellularity and shrinkage between different groups. Statistical significance was set at $p \leq 0.05$.

**Results**

**Macroscopical Evaluation**
Two allograft menisci in the group that underwent delayed transplantation were completely degenerated. All other allografts showed integration into the host capsular tissues. None of the menisci showed extrusion.

**Histological Evaluation of the Articular Cartilage**
In all transplanted knees some degeneration of the medial tibial and femoral surface was observed in comparison with the nonoperated knees (Figure 1). At 1 year follow-up, osteoarthritic changes were more pronounced in the delayed transplant group (histological score, $9.3 \pm 3.6$ and $9.8 \pm 3.9$ for the MTP and MFC, respectively) than in the immediate transplant group (histological score, $2.7 \pm 1.7$ and $2.7 \pm 2.0$ for the MTP and MFC, respectively)(Figures 1C and D). Degenerative changes of the
cartilage were similar at 6 weeks (histological score, 3.1 ± 2.0 and 2.6 ± 2.4 for the MTP and MFC, respectively) and at 1 year after immediate meniscal transplantation (Figures 1B and 1C). Degenerative changes were not found in knees of animals subjected to a sham procedure.

**Histological Evaluation of Menisci**

Histological evidence of rejection was never observed although mild proliferation of synovial tissue surrounding the meniscus was observed in all transplanted animals. In most animals, blood vessels were present in areas adjacent to the peripheral meniscal attachment. Histological changes in groups A, B, and C are summarized in Table 1. No scores for synovial coverage, surface irregularities, calcifications, and number of cells could be obtained for the 2 completely degenerated allografts.

*Group A (6 weeks after immediate transplantation, Figure 1B).* All allografts showed a synovial membrane covering the peripheral surface. The histological appearance of the transplanted menisci was completely normal in 2 of 7 animals. An increased number of cells was found in peripheral and central areas in 1 allograft. Four other allografts showed an increase in cells in the peripheral edge only. The majority of these cells were found in the superficial layers of the transplants. Some
Table 1. Histological changes in medial meniscal allografts at 6 weeks after meniscectomy followed by immediate transplantation (group A), 1 year after meniscectomy followed by immediate transplantation (group B), and 1 year after meniscectomy followed by 6 weeks delayed transplantation (group C).

<table>
<thead>
<tr>
<th>Animal</th>
<th>Peripheral coverage with synovial tissue</th>
<th>Surface irregularities</th>
<th>Increased number of cells (peripheral/central area)</th>
<th>Calcification$^1$</th>
<th>Shrinkage$^3$</th>
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<tr>
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</tr>
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<td>1</td>
<td>1/0</td>
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</table>

$^1$ Score for peripheral coverage with synovial tissue, surface irregularities and calcification: 0 = absent; 1 = present

$^2$ Score for number of cells: -1 = decreased; 0 = normal; 1 = increased

$^3$ Score for shrinkage: 0 = no shrinkage; 1 = shrinkage of only the anterior or posterior horn; 2 = shrinkage of both the anterior and posterior horn; 3 = meniscus completely degenerated

* Calcification observed in the central area of the lateral meniscus

shrinkage of the anterior horn was observed in 3 specimens. No signs of shrinkage were observed in the posterior horns of the transplanted allografts. None of the allografts showed calcifications. Mild surface irregularities were found in 1 allograft.

Group B (1 year after immediate transplantation, Figure 1C). In 3 out of 6 transplants, the peripheral surface was covered with synovial tissue. The histological appearance was completely normal in 1 transplanted specimen. Shrinkage of the anterior horn was observed in 2 allografts, whereas shrinkage of the posterior horn was never found. Increased number of cells in only the peripheral areas of the allograft was found in 2 animals. These cells were located both in the deep and superficial
layers. One other transplant showed an increased number of cells in the deep and superficial peripheral areas as well as in the superficial central areas. Calcifications in the central area of the allografts were observed in 2 rabbits. One of these animals also showed calcification of the central area of the lateral meniscus. Mild surface irregularities were observed in 2 allografts.

Group C (1 year after delayed transplantation, Figure 1D). Two of 7 allografts were completely degenerated. In the other animals, synovial lining of the peripheral surface of the meniscal transplants was observed in 3 allografts. Increased numbers of cells in both deep and superficial layers of peripheral areas were found in 2 transplants. One of these allografts also showed an increase in cells in the central area predominantly located in the superficial layers. One allograft showed shrinkage of the anterior horn only and 4 other allografts showed shrinkage of both the anterior and posterior horn. Calcifications in the central part of the transplant were observed in 2 allografts. Another animal showed a calcification in the central part of the lateral meniscus. Four allografts showed surface irregularities.

Sham procedure and controls (Figure 1A). All menisci in control and sham-operated knees appeared to be normal. Compared with the control group, a mild hyperplasia of synovial cells was found in all knees subjected to sham procedures.

Statistical Analysis
When compared to the nonoperated controls, increased cellularity in the peripheral areas of the allografts and peripheral synovial coverage were significantly more often observed in allografts at 6 weeks after transplantation (p = 0.04 and p = 0.0006, respectively). No other significant differences were observed between these groups. In the immediate transplant group at 1 year follow-up, significant differences in examined features were not found when compared to both the control group and the immediate transplant group at 6 weeks follow-up. For the completely degenerated allografts, grading scores could not be obtained for peripheral coverage with synovial tissue, surface irregularities, number of cells, and calcifications. Therefore, it was not possible to analyse statistically differences in these features between the delayed transplant group and the immediate transplant groups. However, allografts showed significantly more shrinkage after delayed transplantation than controls (p = 0.003) and immediately transplanted allografts at 6 weeks and at 1 year follow-up (p = 0.004 and p = 0.005, respectively).

Discussion
Several animal studies evaluated meniscal allograft transplantation immediately after meniscectomy in normal knee joints. Arnoczky et al.\textsuperscript{22} reported that allografts had healed to the capsule at 6 months after transplantation showing normal cell populations in a dog model. In another study, Jackson et al.\textsuperscript{11} performed immediate
transplantation of meniscal allografts after meniscectomy in goats and demonstrated that at 6 months after operation, the allografts did not show gross signs of degeneration although a decreased uronic acid and an increased water content was observed. However, the indication for meniscal transplantation in humans is usually symptomatic degenerative joint disease secondary to meniscectomy and therefore, immediate transplantation in animal models cannot be transposed to the human situation. Long-term histological studies have not been published yet, in which functional aspects of secondarily transplanted meniscal allografts are compared with primarily transplanted specimens.

In our study, secondarily transplanted allografts showed significantly more shrinkage than immediately transplanted menisci at 1 year after transplantation (Table 1). Two allografts in the delayed transplant group were even completely degenerated. These findings are in agreement with Aagaard et al., who reported that the mean macroscopical shrinkage of allografts in sheep was 2.5% after immediate transplantation and 23.8% after delayed transplantation at 6 months follow-up. The more pronounced shrinkage in secondarily transplanted menisci could be due to ridge formation following meniscectomy as described by Fairbanks, which may lead to a mechanical conflict between allograft and condyle. Furthermore, the twofold insult to knee joints subjected to delayed meniscal transplantation could at least be partly responsible for the pronounced graft shrinkage in this group. The finding that knee joints subjected to a sham operation did not show shrinkage of the meniscus, suggests that a first arthrotomy by itself does not lead to shrinkage. Histologically, obvious differences were not found between immediately and secondarily transplanted allografts at 1 year follow-up. In comparison with menisci in sham-operated knees and menisci in control knees, the number of cells was frequently increased in transplanted menisci both at 6 weeks and 1 year follow-up. None of the allografts showed a decrease in cellularity. These findings are in contrast to those of Mikic et al., who demonstrated an obvious cellular depopulation in meniscal allografts during the first 4 - 5 months after transplantation in dogs. On the other hand, Arnoczky et al. observed a decreased cellularity at 2 weeks after transplantation, but an increase in cellularity at 1 month follow-up in dogs. Jackson et al. stated that this increased cellularity is caused by a repopulation of the allograft with host cells replacing donor cells. The increased peripheral coverage of the meniscal allografts with synovial tissue at 6 weeks follow-up is in agreement with animal studies that demonstrated synovial-cell repopulation of frozen meniscal transplants. Apparently, repopulation has already taken place at 6 weeks follow-up in the present study in rabbits, probably after a temporary decrease in cellularity during the first weeks.

Several studies reported that articular cartilage that is not covered by a meniscus shows more degenerative changes than cartilage directly beneath a transplant. This phenomenon suggests that pronounced shrinkage of secondarily transplanted allografts is responsible for the increased degenerative changes of articular cartilage as observed in the delayed transplant group.
It is recognized that the semiquantitative method of interpretation in the present study may be subjective. However, no quantitative or semiquantitative scoring system with an acceptable reproducibility for grading histological changes in meniscal tissue has been described thus far.

A clinical consequence of our study is that when meniscal allograft transplantation is performed to protect articular cartilage, it has to be performed as soon as possible after meniscectomy. However, our data obtained in rabbits cannot simply be extrapolated to the situation in man. Rabbit models have proven to be one of the better models for the human situation because of similarities in histological and biochemical aspects of rabbit and human cartilage. On the other hand, 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 rather difficult and may introduce artefacts that are hard to interpret when comparing data obtained in small animals with those obtained in larger animals. Due to the small size of rabbit knee joints, the horn ligamentous attachments of the transplanted allografts were not retained via transplantation of bone plugs, but the anterior and posterior horn were reattached to the ligamentous tibial bone attachments. 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. On the other hand, bone plugs increase the antigenic load since bone allografts elicit an immune response. The effect of the anchoring technique on immune response, load distribution, and shock absorption could not be determined in our study. More future experimental and clinical studies are needed to evaluate these effects and to investigate the biologic and biomechanical properties of meniscal transplants on a long-term basis.

In conclusion, our findings suggest that delayed meniscal allograft transplantation leads to more graft shrinkage than immediate allograft transplantation, whereas no clear differences in histological architecture were observed between both groups.

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References


