Meniscal transplantation has become a viable surgical option for patients with symptomatic meniscal deficiency. It is thought that the predictable long-term development of osteoarthritic degeneration in patients subjected to total meniscectomy can be decreased if the joint is protected by a meniscal substitute. Unfortunately, scientific evidence regarding the effectiveness of this surgical intervention is lacking. There has been limited long-term clinical investigation on the success of meniscal allografts.\textsuperscript{13-15,28,33} Furthermore, animal studies have demonstrated varying results, and to date, a reliable animal model has not been developed.\textsuperscript{7,10,16,31}

Although the optimal timing for meniscal allograft transplantation has not been well defined, it has become evident that surgical intervention must be performed before the development of advanced joint degeneration. The results of this procedure are suboptimal if transplantation is delayed.

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until advanced changes on radiographs are present.²⁸ The ability to better identify early signs of articular degeneration in a noninvasive manner would improve our ability to recommend reconstructive procedures in a timely fashion.

Our primary hypothesis was that a more anatomically based meniscal allograft transplantation procedure would be effective in reducing the degree of joint degeneration compared with meniscectomy as demonstrated by MRI, T2 mapping, biomechanical testing, and histologic analysis. Our secondary hypothesis was that measurements of T2 relaxation time could be used as a noninvasive measure of early cartilage degeneration in the postmeniscectomy model and that it could be used to monitor cartilage protection in the meniscal allograft transplantation model. Thus, the purposes of this study were (1) to assess the chondroprotective effects of meniscal allograft transplantation in the sheep postmeniscectomy model and (2) to evaluate the utility of T2 relaxation time mapping as a noninvasive measure of early articular cartilage degeneration.

MATERIALS AND METHODS

After Institutional Animal Care and Use Committee approval was obtained, 45 skeletally mature Columbian X Rambouillet ewes weighing approximately 70 to 80 kg were allocated for use. On arrival at the Colorado State University Veterinary Teaching Hospital, the sheep were placed in the large-animal research barn. They were dewormed and ear tagged for identification. Physical examination was performed, and only healthy animals with no clinically evident medical or orthopaedic problems were selected for the study.

Thirty nonstudy animals were allocated for meniscal allograft donation. These animals had been involved in unrelated research involving the spine or cranium and were scheduled for sacrifice on the day before meniscal allograft transplantation. During the harvest procedure, the entire lateral meniscus, including the full length of the anterior and posterior meniscal ligaments with a small amount of associated bone, was aseptically removed from the donor knees and stored in an antibiotic/nutrient medium according to the guidelines of the American Association of Tissue Banks.²² All donor knee menisci were size matched by the weight of the sheep as well as precise intraoperative measurements of length, width, and height of the meniscus. The allografts were stored in the antibiotic/nutrient medium for a maximum of 48 hours before transplantation. The animals had a high degree of genetic similarity, thus decreasing the risk for rejection of the tissue transplant.

STUDY GROUPS

The animals were randomly assigned to 1 of 3 groups. Four animals underwent a sham operation involving exposure of the lateral joint with a release and repair of the lateral collateral ligament and popliteus insertion. Twenty-four animals underwent a lateral meniscectomy after exposure of the lateral compartment. Seventeen animals underwent lateral meniscal allograft transplantation immediately after meniscectomy with size-matched donor menisci. For the sham and the meniscectomy groups, half of the animals were sacrificed at 2 months, and the other half were sacrificed at 4 months. For the meniscal allograft transplantation group, 8 were sacrificed at 2 months, 8 were sacrificed at 4 months, and the final animal was sacrificed at 1 year.

SURGICAL INTERVENTIONS

Meniscectomy

Before surgery, 10 sheep cadaveric knees were dissected to better define the surgical anatomy (Figure 1).²¹ A lateral incision was made over the lateral collateral ligament of the knee joint. The femoral attachment of the lateral collateral ligament and popliteus insertion site was elevated using an osteotome along with a small wedge of bone. The lateral collateral ligament and popliteus were then reflected distally to expose the entire lateral meniscus. Varus stress was applied to the knee joint to fully expose the meniscus. The peripheral rim of the meniscus was dissected sharply from the capsule, dividing the coronary ligament. The anterior and posterior horn attachments of the meniscus were then detached, and the meniscus was removed from the knee. Special care was taken to avoid any articular cartilage damage during the lateral meniscectomy. With detachment of the anterior meniscotibial ligament attachment, special care was taken to preserve the tibial insertion of the ACL, which lies in close proximity. With detachment of the posterior meniscofemoral ligament, the popliteal artery was carefully retracted to avoid vascular injury. The osseous femoral attachment of the lateral collateral ligament and popliteus was then reattached to the femur using a 4.0-mm cancellous bone screw. After thorough irrigation of the joint with saline solution, the joint capsule, overlying fascia of the tensor fascia lata, subcutaneous tissues, and skin were closed in layers.

Allograft Transplantation

Before the transplant procedure was performed in live animals, 10 sheep cadaveric knees were dissected. A surgical procedure was developed using anatomical placement of the anterior meniscotibial and posterior meniscofemoral attachment sites (Figure 1) with fixation through bone tunnels. At the start of the transplant procedure, lateral meniscectomies were performed using the technique described above. The previously harvested menisci for transplantation were then washed thoroughly with normal saline solution before transplantation. Accurate size matching was performed using 5 measurements: length from anterior horn to the posterior horn; width at the anterior horn, posterior horn, and central meniscus; and central height. The allograft meniscus was then prepared for transplantation by placing No. 2 Fiberwire (Arthrex, Naples, Fla) Krackow stitches through the anterior and posterior meniscal ligaments. The anterior meniscotibial attachment site was then exposed, and a drill tunnel was made entering at the anatomical insertion site and exiting the tibia on the medial side of the knee. The posterior meniscofemoral attachment site was then palpated, and a second
1466  Kelly et al  The American Journal of Sports Medicine

A drill tunnel was made entering the anatomical insertion site on the posterior aspect of the medial femoral condyle and exiting the femur on the medial side of the knee. The anterior and posterior meniscal allograft ligaments were then anchored to the anterior and posterior meniscal insertion sites by passing the Fiberwire sutures through the bone tunnels. The sutures were then fixed over buttons on the medial side of the knee. Tracking and appropriate sizing of the meniscus were confirmed after fixation. The outer rim of the meniscus was then reattached to the lateral capsule using 2 or 3 interrupted absorbable sutures. At the conclusion of the transplant fixation, the osseous femoral attachment of the lateral collateral ligament was reattached to the femur using a 4.0-mm cancellous bone screw, and the wound was closed in layers, as described above.

Sham Operation

The sham operation was performed using the same approach to the lateral compartment required for the meniscectomy procedure and transplant procedure. In this group, no further surgery was performed after exposure of the joint.

POSTOPERATIVE CARE

After surgery, postoperative activity was restricted for the first 2 weeks by housing the animals in closed confinement. The limbs were not immobilized. At 2 months, 2 animals from the sham group, 12 animals from the meniscectomy group, and 8 animals from the allograft group were sacrificed. Both the operated and nonoperated limbs were harvested for analysis. At 4 months, the remaining animals in the sham and meniscectomy groups and all but 1 of the remaining transplant animals were sacrificed, and both limbs were sent for analysis (2 sham, 12 meniscectomy, 8 allograft). The final allograft animal was sacrificed at 1 year.

POSTSACRIFICE ANALYSIS

After sacrifice, both the operative and nonoperative knees were detached at the level of the midfemur and midtibia. All but 7 of the limbs were immediately frozen and stored for analysis. The remaining 7 limbs were kept fresh for cell viability analysis of the allograft menisci using a paravital staining technique. The time between euthanasia and analysis of the frozen limbs was between 5 and 7 days. The fresh limbs were evaluated within 24 hours after sacrifice. The frozen knees were thawed before analysis and then assessed sequentially with cartilage-sensitive MRI, MRI with T2 mapping, gross inspection with india ink staining, biomechanical testing of cartilage stiffness, and semiquantitative histology. Analysis of the vascularity of the transplanted menisci was performed using the Spälteholz technique on 3 of the specimens from the 4-month transplant group. Complete analysis of each limb was performed within a 24-hour period to avoid any potential degenerative effects from exposure to air. The specimens were kept refrigerated between tests during that 24-hour period, and all tests were performed in a temperature-controlled laboratory set at 65°F. None of the tests resulted in heating of the tissue. All limbs were treated with the same protocol, eliminating the potential for any significant differences due to prolonged exposure. Seven of the limbs (5 at 2 months and 2 at 4 months) were delivered fresh and analyzed within 24 hours of sacrifice. In addition to the routine analyses described above, the fresh menisci from these animals were evaluated for cell viability using paravital staining techniques.

Figure 1. Normal anterior tibial (A) and posterior femoral (B) meniscal attachment sites.
MAGNETIC RESONANCE IMAGING

All animals were imaged in a clinical 1.5-T superconducting magnet (Signa Horizon LX, General Electric Medical Systems, Milwaukee, Wis) with a 5-inch, curved, receive-only linear shoulder coil (IGC-Medical Advances, Milwaukee, Wis). Animals were imaged using a fast spin echo sequence in the coronal and sagittal planes with a repetition time of 4200 to 5000 milliseconds, effective echo time of 17 milliseconds, and field of view of 8 × 420 cm with a matrix of 512 × 384, yielding an in-plane resolution of 156 µm × 156 µm × 1 mm slice resolution. Images were obtained with 3 excitations. Tailored radiofrequency (GE Health Care, Milwaukee, Wis) was used to reduce interecho spacing, with echo train lengths ranging between 8 and 12.

Subsequent spin echo T2 relaxation maps were obtained using repetition time of 500 to 650 milliseconds; echo times of 11, 40, and 80 milliseconds; at one excitation. Field of view was 8 × 6 cm with a matrix of 256 × 160, yielding an in-plane resolution of 312 µm × 375 µm with 2-mm slices. Receiver bandwidth was 15.6 kHz (over the entire frequency range). T2 relaxation maps were then reconstructed using a monoexponential fit model to calculate T2 relaxation pixel by pixel (Functool 3.1.10, Advantage Windows work station, GE Health Care).

The fast spin echo images were analyzed to assess morphologic changes in cartilage, bone, and bone marrow edema, as well as the presence or absence of osteophytes. Maximum cartilage thickness in the nonosteoarthritic sheep model was 0.8 mm based on MRI measurements. An MRI score was calculated based on the observed changes in cartilage, subchondral bone, and bone marrow edema, as well as the presence or absence of osteophytes. A similar system for cartilage degeneration has been previously validated in a clinical knee study using arthroscopy as the standard.27 Cartilage was scored at the anterior, central, posterior, and peripheral margin over the lateral condyle and lateral plateau using a 1 to 4 grading system: 1, increased signal intensity; 2, <50% cartilage loss; 3, >50% cartilage loss; 4, exposed subchondral bone. The scoring was performed by a single attending MRI radiologist who was blinded to the surgical procedure. A sample of the images were scored a second time to confirm intraobserver reliability; no significant differences were found between scoring sessions. The subchondral bone was also assessed in each of these locations for the presence of sclerosis, scoring 1 (none apparent), 2 (mild), 3 (moderate), and 4 (severe). Bone marrow edema and osteophyte formation were both scored as absent (0) or present (1). A total MRI score for the lateral compartment was calculated by summing the mean values for each of the 4 categories. With this system, the minimum value of 2 corresponded to normal joint characteristics with no evidence of degeneration, whereas the maximum score of 10 corresponded to advanced degenerative changes.

Spin echo T2 maps of the lateral tibial plateau were created in this study to reduce the potential error introduced by stimulated echo formation generated in most multiecho fast spin echo techniques, yielding additive T1 contrast.20 Quantitative T2 values were then obtained from the anterior, central, and posterior regions of the central weightbearing zone of the lateral tibial plateau using a standardized region of interest analysis. These areas of interest corresponded to the areas in which maximal cartilage degeneration was seen in all animals due to the loading characteristics of the postmeniscectomy ovine knee. They also corresponded to the 3 areas of interest identified for mechanical testing using indentation probe testing. Subsequent color maps were generated using the same Functool program in which T2 value stratification was depicted as prolonged values in green to blue and shorter values in orange to red, using an expanded color scale stratified from a minimum echo time of 0 milliseconds to a maximum echo time of 150 milliseconds.

GROSS INSPECTION

After MRI, all limbs were dissected, and the tibial plateaus were cleared of all surrounding soft tissue. The cartilage surfaces were stained with a dilute (1%) india ink solution (Higgins waterproof black india ink), and all specimens were photographed for determination of gross evidence of cartilage degeneration as demonstrated by uptake of india ink stain. Average stain areas overlying the tibial plateaus were measured using a Metamorph computer analysis (square millimeters; Universal Imaging Corporation, Downington, Pa).

BIOMECHANICAL TESTING

Cartilage stiffness was measured over the central weightbearing zone of the lateral tibial plateau. The area tested represented the area of greatest wear in the postmeniscectomy knees. Three measurements were collected from 3 different locations along the central contact area of the tibia: anterior, central, and posterior. The location of these data points was the same in all specimens and was verified by dividing the central weightbearing zone into thirds and finding the center point of each section. The location of these data points also corresponded to the areas sampled for the T2 analysis. The 3 data points from each location were averaged. Stiffness was measured using a cartilage indentation probe specifically designed for thin cartilage (Artscan Inc, Helsinki, Finland). The probe consisted of a measurement rod with a 100-µm-diameter indenter located in the center of a reference plate at the end of the probe. The value of the indenter force (newtons) reflects the force that the cartilage exerts against the indenter and can reliably be used as an index of cartilage stiffness. Several studies have demonstrated the association between cartilage degeneration and decreased cartilage stiffness.

HISTOLOGIC ANALYSIS

Histologic sections were subsequently prepared and scored by an attending pathologist blinded to the procedure performed. The specimens were sectioned in the coronal plane at the midpoint of the weightbearing zone of the tibial plateau. The osteochondral specimens were then fixed in 10% neutral buffered formalin (Sigma Diagnostics, St Louis, Mo) and then
decalcified in 5% nitric acid (50 mL concentrated nitric acid to 950 mL dH2O). The tissues were checked daily; as soon as the decalcification was complete, the tissues were removed, so as to avoid overdecalcification. Tissue processing and paraffin embedding were then performed using the Tissue Tek VIP1000 tissue processor. Five-micrometer-thick sections were cut and then stained with H&E and safranin O. The histologic sections were taken from the middle third of the tibia and were directed from the lateral edge (peripheral zone) to the intercondylar notch (central zone). The central zone corresponded to the area of greatest wear that was evaluated with the biomechanical testing.

The histologic sections were graded using the modified Mankin grading scale for hyaline cartilage degeneration.21,32 This semiquantitative analysis assessed cartilage structure (0-6), cellular abnormalities (0-3), matrix staining (0-4), and tidemark integrity (0-1). A minimum score of 0 denotes no cartilage degeneration, and a maximum score of 14 indicates severe cartilage destruction. Regional differences between the central zone and peripheral zone were evaluated.

Polarized light microscopy was performed to further assess collagen organization within 4 layers of the matrix: superficial layer, transitional zone, radial zone, and calcified zone. The collagen was scored as organized (0) or disorganized (1) in each of these 4 zones, resulting in a score range between 0 and 4.

Paravital staining to assess cell viability of the transplanted menisci was performed on 7 fresh specimens (5 from the 2-month allograft transplant group and 2 from the 4-month allograft transplant group).24 A full detail of the technique for cell viability paravital staining has been previously reported.16 The paravital technique was made up of a combination of 2 indicators. Propidium iodide is a cell impermeable dye that labels nucleic acids only in dead cells and is made up of 3 parts benzyl benzoate and 5 parts methyl salicylate. The specimens were then visualized under direct magnification and photographed using high-resolution film for further review.

STATISTICAL METHODS

Means and standard deviations were calculated for all measurements. A paired Wilcoxon nonparametric test was used to make direct comparisons between sham operation animals and nonoperated control animals, between the central tibial plateaus and peripheral tibial plateaus of the allograft transplant animals, between the 2-month meniscectomy and 4-month meniscectomy groups, and between the 2-month allograft transplant and the 4-month allograft transplant groups. Significant differences (P < .05) between allograft animals, meniscectomy animals, and nonoperated control animals were calculated with a 1-way analysis of variance followed by t tests with a Bonferroni correction or a Kruskal-Wallis test followed by Mann-Whitney tests. Correlations between T2 data and all other variables were calculated with Spearman rank correlation (r values) tests for nonparametric data. The collected data were analyzed and interpreted by each of the investigators to ensure accuracy, and all data were analyzed in a blinded fashion.

RESULTS

Operative Observations

At the time of the initial surgery, there was no evidence of gross cartilage degeneration within the lateral compartment of the joint. Complete visualization of the lateral tibial plateau was feasible in all operative specimens using the approach described.

Sham Operation

No statistical differences were found between any of the sham operation animals and the nonoperated control limbs with regard to MRI, T2 mapping, gross inspection with india ink, biomechanical testing, and histologic analysis. Thus, the nonoperated limbs were subsequently used as the control group in all comparisons between the meniscectomy and allograft groups.

STUDY GROUPS

Postmeniscectomy Animals

There were statistically significant differences (P < .05) between the nonoperative control limbs and the meniscectomy limbs in all outcome measures at both 2 months and 4 months after meniscectomy (MRI, T2 mapping, gross inspection with india ink, biomechanical testing, and histologic analysis) (Tables 1 and 2).

Magnetic Resonance Imaging. The MRI appearance of the nonoperated control limbs demonstrated good preservation...
of the lateral compartment joint space, no cartilage wear (mean cartilage thickness was approximately 0.8 mm), no subchondral sclerosis or edema, and no peripheral osteophyte formation (mean score, 2.0) (Figure 2A). By 2 months after meniscectomy, MRI demonstrated moderate cartilage degeneration, with significant focal cartilage wear over the central weightbearing zone of the tibial plateau, mild to moderate subchondral sclerosis, and the presence of osteophytes and subchondral edema in the majority of the specimens (mean score, 7.1 ± 0.6). By 4 months, advanced degeneration was clearly seen with large areas of full-thickness cartilage wear, significant sclerosis, and the presence of osteophytes and edema in all specimens (mean score, 8.4 ± 0.7) (Figure 2B).

**T2 Mapping.** T2 maps demonstrated significant prolongation ($P < .05$) of T2 relaxation times in the lateral tibial plateau after meniscectomy compared with nonoperated controls at both 2 months (Figure 3) and 4 months (Figure 4; Tables 1 and 2). The mean T2 relaxation time for the nonoperated controls was 38.7 ± 10.0 milliseconds. There was no significant difference between the increased T2 relaxation time seen at 2 months and 4 months, suggesting that a threshold of cartilage degeneration was reached by 2 months that was beyond the limits of resolution of the T2 mapping to detect further cartilage deterioration. Little to no stratification of T2 relaxation times was noted over the plateau, likely because of the advanced degree of degeneration and the limitations of the in-plane resolution of the T2 mapping sequence, given the time requirements for obtaining 3 echo samples.

In an attempt to identify correlations between the T2 mapping and the other outcome measures, Spearman rank correlations were calculated and subjected to post hoc analysis. There was no assumption of a linear relationship between variables at 2 months and 4 months (Table 3). Significant correlations were identified between prolongation of T2 relaxation times and all other outcome measurements except the morphologic MRI score and the India ink staining area ($P < .05$).

**Gross Inspection.** Representative gross specimens at 4 months stained with 1% dilute India ink are seen in Figures 5A and B. The nonoperated control limbs (Figure 5A) demonstrated minimal staining over the lateral plateau at 4 months (mean stain area, 3.0 ± 6.2 mm$^2$). This signal intensity seen at both 2 months and 4 months compared with control limbs. There was no significant difference between the increased T2 relaxation time seen at 2 months and 4 months, suggesting that a threshold of cartilage degeneration was reached by 2 months that was beyond the limits of resolution of the T2 mapping to detect further cartilage deterioration. Little to no stratification of T2 relaxation times was noted over the plateau, likely because of the advanced degree of degeneration and the limitations of the in-plane resolution of the T2 mapping sequence, given the time requirements for obtaining 3 echo samples. In an attempt to identify correlations between the T2 mapping and the other outcome measures, Spearman rank correlations were calculated and subjected to post hoc analysis. There was no assumption of a linear relationship between variables at 2 months and 4 months (Table 3). Significant correlations were identified between prolongation of T2 relaxation times and all other outcome measures at 4 months ($P < .05$). At 2 months, significant correlations were identified between prolongation of T2 relaxation times and all other outcome measurements except the morphologic MRI score and the India ink staining area ($P < .05$).

### TABLE 1
Evaluation of Tibial Plateaus in Postmeniscectomy Animals and Nonoperated Controls at 2 Months After Surgery $^a$

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>P</th>
</tr>
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<tbody>
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<td>MRI score</td>
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<td>7.1</td>
<td>0.6</td>
<td>12</td>
<td>2.0</td>
<td>0.0</td>
<td>&lt;0.001</td>
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<td>T2 map, ms</td>
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<td>57.2</td>
<td>11.1</td>
<td>6</td>
<td>41.6</td>
<td>7.0</td>
<td>0.01</td>
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<tr>
<td>India ink, mm$^2$</td>
<td>12</td>
<td>36.1</td>
<td>7.9</td>
<td>12</td>
<td>3.0</td>
<td>6.2</td>
<td>&lt;0.001</td>
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<tr>
<td>Biomechanics, N</td>
<td>12</td>
<td>0.37</td>
<td>0.13</td>
<td>12</td>
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<td>0.08</td>
<td>&lt;0.001</td>
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<td>Mankin</td>
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<td>1.5</td>
<td>12</td>
<td>0.25</td>
<td>0.45</td>
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<tr>
<td>Polarized</td>
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<td>2.2</td>
<td>0.8</td>
<td>12</td>
<td>0.0</td>
<td>0.0</td>
<td>&lt;0.001</td>
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</tbody>
</table>

$^a$Modified Mankin scores are between 0 and 14; polarized light scores are between 0 and 4; MRI scores are between 2 and 10. Highly significant differences were seen in all outcome measures ($P < .05$).

### TABLE 2
Evaluation of Tibial Plateaus in Postmeniscectomy Animals and Nonoperated Controls at 4 Months After Surgery $^a$

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Mean</th>
<th>SD</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>P</th>
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<td>T2 map, ms</td>
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<td>13.0</td>
<td>6</td>
<td>35.7</td>
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<td>India ink, mm$^2$</td>
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<td>2.8</td>
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<tr>
<td>Biomechanics, N</td>
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<td>0.25</td>
<td>0.08</td>
<td>12</td>
<td>0.63</td>
<td>0.08</td>
<td>&lt;0.001</td>
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<td>Polarized</td>
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<td>3.5</td>
<td>0.8</td>
<td>12</td>
<td>0.0</td>
<td>0.0</td>
<td>&lt;0.001</td>
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</table>

$^a$Modified Mankin scores are between 0 and 14; polarized light scores are between 0 and 4; MRI scores are between 2 and 10. Highly significant differences were seen in all outcome measures ($P < .05$).
finding was consistent across all animals, with virtually no evidence of baseline cartilage degeneration in the lateral plateau. At 2 months after meniscectomy, there was a significantly increased stain consistent with moderate cartilage degeneration (mean stain area, 36.1 ± 7.9 mm²). By 4 months, advanced cartilage wear was evident by gross inspection (mean stain area, 46.7 ± 13.0 mm²) (Figure 5B).

**Biomechanical Testing.** The central weightbearing zone of the tibial plateau represented the area of most rapid degeneration and was the area that was focused on for biomechanical and histologic testing. The biomechanical testing was performed in the anterior, central, and posterior regions of the weightbearing zone depicted by the areas of

**Figure 2.** A, magnetic resonance imaging of the nonoperated control limbs demonstrated no evidence of cartilage wear or subchondral bony changes. B, by 4 months after meniscectomy, advanced changes were seen in all specimens, including subchondral depression, edema pattern, and marked thinning of the cartilage.

**Figure 3.** Quantitative T2 relaxation time maps comparing the nonoperated control (A) and the 2-month postmeniscectomy (B) tibial plateau. The color maps are coded to capture $T_2$ values ranging from 0 to 150 milliseconds, with green and blue reflecting longer $T_2$ values, yellow intermediate values, and orange/red the shorter values. The apparent prolongation in $T_2$ values to the posterior margin (green regions) is owing to the magic angle effect, yielding the expected prolongation of $T_2$ when the collagen is oriented close to 55° relative to the magnetic field. Note diffuse prolongation of $T_2$ values over the anterior margin (arrow; blue region in B) after meniscectomy (B) compared with the nonoperated control (A).
There was a significant decrease in cartilage stiffness at both 2 months ($0.37 \pm 0.13 \text{ N}$) and 4 months ($0.25 \pm 0.08 \text{ N}$) compared with controls ($0.64 \pm 0.9 \text{ N}$). Significant differences between the 2- and 4-month animals were also detected ($P < .05$). Biomechanical testing of 4-month meniscectomy knees demonstrated significantly decreased articular cartilage stiffness over the entire lateral tibial plateau (anterior, central, and posterior data points) compared with controls ($P = .006$), whereas at 2 months, stiffness was significantly decreased over the anterior lateral tibial plateau only ($P = .01$).

**Histologic Analysis: H&E.** Histology specimens were cut in the coronal plane and extended from the periphery of the tibial plateau to the central (intercondylar notch) region at the midpoint of the tibial plateau. Normal articular cartilage was seen in all nonoperated controls and sham animals and was consistent with the absence of any baseline cartilage wear over the lateral plateau (mean modified Mankin score was $0.46 \pm 0.67$). At 2 months, histologic evidence of moderate cartilage degeneration was seen (modified Mankin score was $7.3 \pm 1.5$), and by 4 months, advanced cartilage degeneration was confirmed.
in all specimens (mean modified Mankin score was 11.2 ± 1.6). Characteristic histologic findings included disorganization of cartilage structure with fissure formation, hypocellularity, reduced matrix staining, and loss of tidemark integrity.

**Histologic Analysis: Polarized Light Microscopy.**

Polarized light microscopy was used to further evaluate the collagen organization. The control animals demonstrated normal cartilage with polarized light analysis, which was reflected by maintenance of collagen organization within the 4 layers of the matrix: superficial layer, transitional zone, radial zone, and calcified zone (polarized light score of 0). Significant loss of collagen organization was seen at both 2 months (polarized light score of 2.2 ± 0.8) and 4 months after meniscectomy (polarized light score of 3.5 ± 0.8). Polarized light scores demonstrated significantly decreased collagen organization at 4 months compared with 2 months ($P = .002$). Collagen disorganization was evident in the superficial and transitional zones by 2 months, with extension into the deeper layers (radial zone and calcified zone) at 4 months.

**Allograft**

There were statistically significant differences between the allograft limbs and the meniscectomy limbs in all objective categories at 2 months (MRI, T2 mapping, gross inspection with india ink, biomechanical testing, and histologic analysis). At 4 months, allograft limbs demonstrated significant differences in all objective categories except the T2 mapping (Table 4). In comparing the allograft limbs to the nonoperated control limbs, no significant differences were seen at 2 months in any of the categories except the MRI data. At 4 months, the nonoperated control limbs demonstrated significantly less wear compared to the allograft limbs in all categories except the modified Mankin scores (Table 5). One animal was sacrificed at 12 months after surgery. The scores in all categories for this animal remained better than the mean scores at 2 months in the postmeniscectomy animals.

**Magnetic Resonance Imaging.** The MRI appearance of the allograft limbs demonstrated significant improvements in all morphologic criteria compared with the meniscectomy limbs at both 2 and 4 months, although there was a statistically significant increase in the MRI score between 2 and 4 months (2.7-4.2, $P < .05$). Compared with the nonoperated control limbs, however, MRI scores were significantly worse at both 2 and 4 months after allograft transplantation (Table 5). Magnetic resonance imaging further evaluated the transplanted meniscus with regard to intrameniscal signal, extrusion in the sagittal and coronal planes, and meniscal morphologic characteristics. At 2, 4, and 12 months, the transplanted allografts demonstrated minimal intrameniscal signal, minimal extrusion in either plane, and preservation of normal meniscal shape. The allografts were healed at the capsular attachment site. Several specimens demonstrated evidence of fibrous tissue ingrowth into the anterior and posterior meniscal-ligament attachment sites. No meniscal tears were seen on MRI.

**Figure 5.** Gross inspection with 1% dilute india ink comparing the nonoperated control tibial plateau (A), the 4-month postmeniscectomy tibial plateau (B), and the 4-month meniscal allograft transplant tibial plateau (C).
T2 Mapping. T2 maps demonstrated improved maintenance of T2 relaxation times at 2 months and 4 months compared with meniscectomy (Table 4 and Figure 4). This improvement was statistically significant at 2 months but not at 4 months. This finding suggests improved preservation of collagen organization resulting from the meniscus transplant and is correlated with the findings observed under polarized light microscopy. There were no significant differences in the T2 maps between the nonoperated control limbs and the allograft limbs at 2 months. However, by 4 months, the allograft limbs demonstrated a significant prolongation in T2 relaxation times compared with the nonoperated controls (Table 5).

Gross Inspection. A representative gross specimen 4 months after allograft transplantation is depicted in Figure 5C. Compared with the meniscectomy limbs, there were significantly decreased India ink stain areas over the lateral plateau at both 2 and 4 months. However, there was a significant increase in stain area between 2 and 4 months (6-18 mm², P < .05). The wear pattern, as evidenced by the India ink staining, demonstrated additional wear along the periphery of the tibial plateau in some specimens. This wear pattern differed from the meniscectomy animals, in which the wear was isolated to the central weightbearing zone. At 2 months, there was no difference in the gross appearance between the allograft animals and control animals. However, by 4 months, the allograft animals demonstrated significantly increased stain area compared with the control animals.

Biomechanical Testing. Biomechanical testing was performed in the same locations along the central weightbearing zone of the lateral plateau as was done for the meniscectomy animals. There was significantly improved maintenance of cartilage stiffness at both 2 and 4 months compared with the meniscectomy animals (Table 4); however, stiffness values were significantly lower at 4 months compared with 2 months (0.4 N vs 0.6 N, P < .05). Similar to the other outcome measures, 2-month allograft limbs demonstrated no differences compared with control limbs, while 4-month allograft limbs showed significantly decreased stiffness compared with control limbs.

### Table 4

<table>
<thead>
<tr>
<th>Variable</th>
<th>Meniscectomy 2 Months</th>
<th>Allograft 2 Months</th>
<th>P</th>
<th>Meniscectomy 4 Months</th>
<th>Allograft 4 Months</th>
<th>P</th>
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</thead>
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<tr>
<td>MRI</td>
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<td>n=12 M=8.4 SD=0.7</td>
<td>n=5 M=4.2 SD=0.9</td>
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<td>n=11 M=55.1 SD=13.0</td>
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<td>India ink, mm²</td>
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<td>&lt;.001</td>
<td>n=12 M=46.7 SD=13.0</td>
<td>n=8 M=18 SD=6</td>
<td>&lt;.001</td>
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<td>n=8 M=0.6 SD=0.1</td>
<td>&lt;.001</td>
<td>n=12 M=0.25 SD=0.08</td>
<td>n=8 M=0.4 SD=0.06</td>
<td>.001</td>
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<td>Mankin</td>
<td>n=12 M=7.3 SD=1.5</td>
<td>n=8 M=0.3 SD=1.0</td>
<td>&lt;.001</td>
<td>n=12 M=11.3 SD=1.6</td>
<td>n=8 M=0.7 SD=1.0</td>
<td>&lt;.001</td>
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<tr>
<td>Polarized</td>
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<td>n=8 M=0 SD</td>
<td>&lt;.001</td>
<td>n=12 M=3.5 SD=0.8</td>
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<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Modified Mankin scores are between 0 and 14; polarized light scores are between 0 and 4; MRI scores are between 2 and 10. All outcome measures demonstrated significant differences between the 2 groups except the T2 mapping at 4 months (P < .05).

### Table 5

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control 2 Months</th>
<th>Allograft 2 Months</th>
<th>P</th>
<th>Control 4 Months</th>
<th>Allograft 4 Months</th>
<th>P</th>
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<td>.3</td>
<td>n=6 M=29 SD=3</td>
<td>n=5 M=50 SD=5</td>
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<td>n=8 M=6 SD=7</td>
<td>.4</td>
<td>n=12 M=3 SD=7</td>
<td>n=8 M=18 SD=6</td>
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<td>Biomechanics, N</td>
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<td>Polarized</td>
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<td>n=8 M=0 SD</td>
<td>.9</td>
<td>n=12 M=0 SD=0.0</td>
<td>n=8 M=0.9 SD=0.7</td>
<td>.01</td>
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</tbody>
</table>

*Modified Mankin scores are between 0 and 14; polarized light scores are between 0 and 4; MRI scores are between 2 and 10. At 2 months, the MRI score was the only outcome measure that was statistically different from the control animals. At 4 months, all values except the modified Mankin scores were significantly worse compared with the control animals (P < .05).
but by 4 months, the allografts were significantly worse compared with controls (Table 5).

**Histologic Analysis: H&E.** Compared with the meniscectomy animals, there were statistically significant improvements in the modified Mankin scores over the central weightbearing zone at both 2 months and 4 months. Excellent preservation of the cartilage structure, cellularity, matrix staining, and tidemark integrity was observed in this central zone. However, there was significantly more cartilage wear in the peripheral zone beneath the allograft meniscus compared with the central weightbearing zone of the tibial plateau (Table 6). This finding was true for the modified Mankin scores at both 2 and 4 months (\(P = .007\) and \(P = .02\), respectively). Scores along the periphery of the tibial plateau for the allograft animals were still lower than were the Mankin scores for the meniscectomy animals in the central weightbearing zone. No significant differences were noted between the Mankin scores at 2 months and 4 months in the allograft animals. In comparing the central zone histologic findings of the meniscal allograft animals and the nonoperated control animals, no significant differences were noted at 2 or 4 months (Table 5).

**Histologic Analysis: Polarized Light.** Polarized light microscopy demonstrated maintenance of collagen organization after allograft transplantation at both 2 and 4 months compared with the meniscectomy animals (Table 4); however, there was loss of collagen organization at 4 months compared with 2 months (\(P < .05\)), thus correlating with the prolonged T2 relaxation times noted in the 4-month allograft group. Compared with the control limbs, polarized light scores were not significantly different at 2 months, but by 4 months, allograft limbs demonstrated significantly decreased collagen organization. In comparing the peripheral zone to the central weightbearing zone of the tibial plateau, the polarized light scores were significantly different at 2 months only (\(P = .006\)) (Table 6).

**Allograft Explant Analysis**

All allograft explants were evaluated by gross examination (Figure 6). There was tissue ingrowth into the allograft at the anterior and posterior attachment sites, as well as along the capsular periphery. The allografts appeared to be firmly fixed within the joint, with no evidence of lateral extrusion or any evidence of rupture of the attachment sites. No meniscal tears were seen at any location along the length of the allograft at any of the sacrifice time points (2, 4, and 12 months).

Routine histologic examination of the meniscus was performed on 3 of the specimens and demonstrated normal-appearing fibrocartilage that was indistinguishable from the native medial meniscus. Histologic evaluation of the anterior and posterior attachment sites demonstrated fibrous tissue loosely integrated into the recipient bone. Cell viability of the allograft menisci was evaluated using paravital staining techniques in 7 allograft specimens (5 at 2 months and 2 at 4 months). These specimens were

<table>
<thead>
<tr>
<th>Variable</th>
<th>Central Zone</th>
<th>Peripheral Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n  Mean SD</td>
<td>n  Mean SD</td>
</tr>
</tbody>
</table>
| 2 mo        | 9  0.3 1.0   | 9  3.78 1.79    | \(P = .007\)  
| Mankin      | 9  0 0     | 9  1.33 0.5     | \(P = .006\)  
| Polarized   | 4 mo        |                 |  
| Mankin      | 7  0.7 1.0  | 7  3.86 1.52    | \(P = .02\)   
| Polarized   | 7  0.9 0.7  | 7  1.29 0.49    | \(P = .08\)   |

| Modified Mankin scores are between 0 and 14; polarized light scores are between 0 and 4. Significantly greater wear was noted at the periphery compared with the central zone by modified Mankin scores at both time points. The polarized light scores were significantly worse at the periphery for the 2-month animals only (\(P < .05\)).

**Figure 6.** Gross examination of the allograft transplants demonstrated good fixation at the anterior and posterior ligament insertion sites, as well as along the capsular periphery. Good preservation of the central weightbearing cartilage on the tibial plateau was also seen by gross examination.
evaluated fresh and not subjected to a freeze-thaw cycle as were the other specimens. At both 2 and 4 months, there was greater than 90% cell viability in all allograft menisci examined. Again, the allograft tissue was indistinguishable from the native medial meniscus. Evaluation of the vascularity of the allograft meniscus was assessed using the Spälteholz technique in 3 of the 4-month specimens. The nonoperated limbs of 2 animals were also assessed with the vascular injection study to determine the vascularity of the native lateral meniscus. Extensive vascular ingrowth was observed at both the anterior and posterior attachment sites as well as along the periphery. The allografts were hypervascular compared with the native meniscus (Figure 7).

DISCUSSION

Currently, there is no consistently reproducible animal model for meniscal allograft transplantation in the literature. Previous animal models have demonstrated variable results; however, these earlier studies have not emphasized anatomical reconstruction of the native meniscal insertion sites, nor have they used bone tunnel fixation as is typically performed in humans. Arnoczky et al\(^5\) and Mikic et al\(^22\) reported some degree of protection of the cartilage after meniscal allograft transplantation in dogs; however, these findings were not compared with meniscectomy. Edwards et al\(^18\) found no radiographic differences between meniscal transplants and those of meniscectomy at 21 months and concluded that meniscal autogenous grafts and allografts did not protect cartilage. Cummins et al\(^1\) found that meniscal allografts offered some histologic evidence of articular cartilage protection in a rabbit model. Szomor et al\(^31\) reported perhaps the best published large-animal model for the protective effects of autograft and allograft meniscal transplantation. They demonstrated a 50% decrease in cartilage wear as determined by macroscopic grading of the cartilage; however, they found no differences between the meniscectomy and transplant groups when histologic criteria were examined. No further outcome measures were used in any of these studies.

The surgical technique used in this study attempted to restore the anatomical anterior tibial and posterior femoral meniscal attachments. In our dissections and cadaveric transplant trials, re-creation of the normal, anatomical anterior and posterior lateral meniscal attachments grossly reproduced normal meniscal kinematics. Adhering to these anatomical considerations resulted in significantly decreased cartilage wear compared with meniscectomy and may provide a more accurate evaluation of the chondroprotective effect of a meniscus transplant. We used a wide array of outcome measures to provide a comprehensive evaluation of the chondroprotective effects of this surgical procedure. We were able to demonstrate the protective effects of allograft transplantation by gross inspection, histologic analysis, biomechanical testing, MRI, and T2 mapping of the tibial plateau to 4 months. A single animal was sacrificed at 1 year with promising results; however, further studies with larger numbers of long-term survival animals are necessary. Each of these outcome measures provides useful information regarding the status of the underlying cartilage and subchondral bone. Although this model demonstrated significant improvements compared with meniscectomy, progressive cartilage degeneration still occurred. As early as 4 months after allograft transplantation, we noted significantly more cartilage degeneration compared with control animals in all outcomes except the modified Mankin scores, even in this “best-case scenario” of transplantation immediately after meniscectomy.

Our outcome analyses focused on the central tibial plateau. This was the area of greatest cartilage degeneration noted after meniscectomy. In the absence of the meniscus, the lateral femoral condyle focally loaded the central tibial plateau, resulting in rapid cartilage destruction. The anatomical allograft transplantation resulted in a redistribution of the load away from the central tibia, so that the focal wear was eliminated. Histologic analysis of the central tibia as well as biomechanical testing of the central cartilage stiffness demonstrated significant “chondroprotection” after transplantation of the meniscus. However, we did observe increased wear along the periphery, where the allograft meniscus had direct contact with the peripheral tibial plateau. We believe that this increased peripheral wear was
in part a result of the surgical approach (release of the lateral collateral ligament and popliteus with open exposure to the joint); however, it may have also been a result of allograft size mismatch and subsequent increased cartilage loading along the periphery. Despite the increased wear along the periphery, this area of cartilage wear in the allograft animals was still less than the cartilage wear seen in the central weightbearing zone of the meniscectomy animals.

The second main purpose of this study was to evaluate the utility of T2 relaxation time mapping as a noninvasive measure of early articular cartilage degeneration and to determine if it could be used to monitor cartilage protection in the meniscal allograft transplantation model using clinically relevant MRI field strengths of 1.5 T. Although cartilage-sensitive techniques are helpful in assessing morphologic changes, a study of healthy bovine and degenerative human cartilage samples indicated that routine clinical images do not reveal early degenerative changes, thus emphasizing the need for more sensitive MRI techniques. T2 mapping techniques have been shown in very high-field magnetic resonance microscopy systems to reflect the structure and orientation of the collagen component of the extracellular matrix, exploiting the depth-sensitive internuclear dipolar interaction of the hydrogen nuclei.

Clinically, T2 relaxation time is a quantifiable MRI parameter that at high-field strengths of 3 T has demonstrated a relationship between the water content of hyaline cartilage, as well as the relative restricted mobility of water in the cartilage within an anisotropic solid matrix. Further work at 3 T has demonstrated that aging is associated with an asymptomatic increase in T2 relaxation times in the transitional zone of articular cartilage, compatible with a relative increase in water mobility. In this study, we were able to demonstrate maintenance of T2 mapping times 2 months after allograft transplantation compared with 2 months after meniscectomy. This difference was no longer significant after 4 months (Table 5), whereas all other outcome measures were significantly different between the transplant and meniscectomy groups. This finding suggests that T2 mapping may be able to detect more minor changes in collagen organization that are not detectable with the other traditional outcome modalities and, therefore, has important clinical applications. We are currently using T2 mapping in select patients in an attempt to identify early cartilage degeneration that is not appreciated with traditional MRI techniques.

We found significant correlations between T2 mapping values and our other more traditional outcome measures (routine MRI, gross inspection, biomechanical testing, and histology). Perhaps the most important correlation seen in this study was between the T2 mapping and the polarized light scores. Polarized light microscopy evaluates collagen organization and provides a histologic corollary to the structure and orientation of the collagen component of the extracellular matrix reflected by the T2 mapping.

Of interest, there was a significant 30% increase in surface area of cartilage degeneration from 2 to 4 months detected by India ink staining that was not detected by T2 relaxation times. This finding suggests that a threshold of detectable degeneration reflected in the T2 relaxation times was met and exceeded by 2 months after meniscectomy. This threshold is likely owing to the limits of the resolution of T2 mapping in the context of the moderate to severe degree of cartilage degeneration seen in these animals at the sacrifice time points analyzed. Further cartilage degeneration beyond the 2-month time point was not reflected in additional changes in the T2 mapping score. This is a fair limitation of this technique for looking at differences between moderate to severe cartilage degeneration as seen in the postmeniscectomy animals but does not reflect an inability of T2 mapping to detect more subtle changes in early cartilage degeneration.

In summary, this meniscal allograft transplantation model demonstrated significantly decreased cartilage degeneration compared with meniscectomy, as well as a high degree of allograft cell viability and vascular ingrowth. We developed a more anatomical surgical technique that accurately reproduces normal meniscus anatomy and more securely fixes the transplanted meniscus compared with previously described techniques. We used a wide array of outcome measures to more fully and accurately characterize the cartilage surfaces after meniscal transplantation, as well as the allograft tissue after transplantation, and have provided a comprehensive baseline against which future animal studies investigating this procedure may be compared. We recognize the importance of early detection of cartilage wear to optimize the results of meniscal allograft transplantation, and our findings help to validate, by standard MRI, histology, and biomechanical testing, the use of T2 mapping of cartilage to assess collagen in a noninvasive manner. In this study, MRI with T2 mapping proved to be very sensitive for the early detection of hyaline cartilage matrix changes and promises to be a valuable clinical tool for the early detection of degenerative joint disease. By increasing the sensitivity and sophistication of our testing parameters through noninvasive imaging of early cartilage breakdown, and integrating these tools with surgical planning and timing, the outcome of these surgical interventions should be improved. Future studies will be aimed at evaluating the efficacy of delayed allograft transplantation as well as the use of novel biomaterials to provide cartilage protection after meniscectomy.

ACKNOWLEDGMENT

Research assistance was provided by Peter Torzilli, Manjula Bansal, Stephen Lyman, Li Foong Foo, and Chris Chen. This research was supported by grants from the American Orthopaedic Society for Sports Medicine Young Investigator’s Award, National Football League Charities, and Aircast Foundation.

REFERENCES