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Evaluation of Small Intestinal Submucosa Grafts for Meniscal Regeneration in a Clinically Relevant Posterior Meniscectomy Model in Dogs

James L. Cook, DVM, PhD
Derek B. Fox, DVM, PhD
Prasanna Malaviya, PhD
James L. Tomlinson, DVM, MVSc
Jack Farr, MD
Keiichi Kuroki, DVM, PhD
Cristii Reeves Cook, DVM, MS

ABSTRACT: Large meniscal defects are a common problem for which treatment options are limited. Successful meniscal regeneration has been achieved by using grafts of small intestinal submucosa in posterior, vascular meniscal defects in a dog model. This study investigates the long-term effects of a tibial tunnel fixation technique and a clinically based meniscectomy defect on meniscal regeneration using this model. Eight mongrel dogs underwent medial arthrotomy and partial meniscectomy. The dogs were divided into groups based on defect treatment: small intestinal submucosa (n=4) or meniscectomy (n=4). Dogs were scored for lameness by subjective scoring postoperatively, sacrificed at 6 months, and assessed for articular cartilage damage, gross and histologic appearance of the operated meniscus, amount of new tissue in the defect, and relative compressive stiffness of articular cartilage.

Dogs in the meniscectomy group were significantly (P=0.002) more lame than dogs treated with small intestinal submucosa. Small intestinal submucosa-treated joints had significantly (P=0.01) less articular cartilage damage than meniscectomy joints. Small intestinal submucosa meniscal implants resulted in production of meniscal-like replacement tissue, which was consistently superior to meniscectomy in amount, type, and integration of new tissue, chondroprotection, and limb function during the study period. Small intestinal submucosa implants may be useful for treatment of large posterior vascular meniscal defects in humans. The tibial tunnel technique used for fixation may have clinical advantages and therefore warrants further investigation.

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INTRODUCTION

The meniscus is recognized as an integral component of knee function as it provides stability, congruency, and biomechanical functions that protect the articular cartilage of the joint. Meniscal tears are the leading cause for orthopedic surgery, contributing to the need for approxi-

mately 1 million meniscal surgeries each year in the United States.11 The majority of meniscal tears are considered non-repairable, making partial meniscectomy the most common form of treatment for meniscal injury. Although partial meniscectomy provides pain relief and return to function, the loss of meniscal tissue results in long-term dysfunction and secondary osteoarthritis.1,2,5,6,12,26-28 Two consistent findings are present in published studies evaluating the long-term results of meniscectomy: 1) meniscectomy causes or exacerbates long-term articular cartilage pathology based on gross, histologic, imaging (radiographic and magnetic resonance imaging), and functional analyses, and 2) the amount of remaining meniscus influences the rate and severity of the pathology.1,2,5,6,12,26-28 These findings have stimulated clinicians to preserve


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Drs. Cook, Fox, Tomlinson, Kuroki, and Cook are from the Comparative Orthopaedic Laboratory, University of Missouri, Columbia, Mo. Dr Malaviya is from DePuy Orthopaedics, Warsaw, Ind; and Dr Farr is from OrthoIndy, Indianapolis, Ind.

Reprint requests: James L. Cook, DVM, PhD, Comparative Orthopaedic Laboratory, University of Missouri, 379 E Campus Dr, Columbia, MO 65211.

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as much healthy, functional meniscal tissue as possible, and researchers to attempt to find ways to replace menis-
cal tissue that must be removed. Meniscal allografts and xenografts can be used to replace damaged meniscal tis-
uce. However, currently available materials and techniques do not always allow for complete re-
generation of functional meniscal tissue in a reproducible manner, and sizing problems and concerns about disease transmission limit their usefulness. In addition, availability of appropriate allograft tissues does not address the current need.

Porcine small intestinal submucosa has been used with success to enhance meniscal regeneration. From our previous work using small intestinal submu-
cosa, it appears that this biomaterial works to augment the amount and type of meniscal replacement tissue primarily by providing a three-dimensional scaffold for clot forma-
tion and cell conduction allowing for new tissue produc-
tion. Small intestinal submucosa may also have miogenic properties for some cell types involved in the tissue regeneration that occurs in this application. Our data suggest that small intestinal submucosa-induced meniscal tissue replacement can be optimized by ensuring the following criteria are addressed:

- The meniscal defect should extend to the vascular zone.
- A source for initial clot formation should be present/ created.
- The small intestinal submucosa implant should be ade-
quately stabilized in the meniscal defect.
- The small intestinal submucosa implant meniscus should be protected during the initial phases of healing.

When these criteria have been addressed, we have reached excellent outcomes for small intestinal submu-
cosa-treated meniscal defects in terms of amount and type of replacement tissue, limb function, and articular cartilage protection for up to 1 year after implantation through open arthroscopy in dogs. In fact, this biomaterial has recently received Food and Drug Administration (FDA) approval for use in an investigational device trial in humans. The technique for arthroscopic implantation of small intestinal submucosa into human knees has been developed using cadaver models.

In light of this, we believed it was important to specifically address methods for ensuring adequate hemarthro-
sis, access to pluripotent cells, and appropriate implant stabilization for arthroscopic application. To maximize the potential for access to initial clot and connective tissue progenitor cells for tissue regeneration, as well as optimize the surgical technique with respect to consistency and implant stability, the use of a tibial bone tunnel technique was investigated in a short-term (3 and 12 weeks) study (Cook JL, unpublished data, 2004). In addition, potential effects of the shape of the standardized menis-

cectomy defect used in previous studies on meniscal tissue regeneration were considered. Rather than using a standardized meniscal defect made using a template, a "taper cut" defect was used in this study to more closely resemble a clinical meniscectomy defect in humans. Results of the short-term study indicated that the tibial tunnel technique for implantation of the small intestinal submucosa meniscal scaffold allowed for meniscal tissue regeneration that was similar to that seen with small intesti-
nal submucosa scaffolds stabilized with suture alone (no tibial tunnel) with respect to presence and function of new meniscal-like tissue. In addition, no significant differences in primary outcome assessments were noted when comparing the template cut meniscectomy to the taper cut meniscectomy. Importantly, no adverse effects related to the tibial tunnel or the taper cut were noted over the 12-week study period.

The present study investigates the long-term (6-month) effects of small intestinal submucosa grafts placed in vas-
cular, posterior meniscal taper cut defects using the tibial tunnel technique on meniscal regeneration, limb function, and articular cartilage protection in dogs.

**Materials and Methods**

Preoperative Evaluation

All procedures were approved by the University Animal Care and Use Committee. Adult, conditioned dogs (n=8), weighing 22.0-28.5 kg were used. The dogs were judged healthy and free from orthopedic disease based on packed-cell volume, plasma total solids, blood urea nitrogen levels, heartworm test, complete physical and orthopedic examination, clinical lameness evaluation, and radiographs of the hips and knees. The dogs were housed individually in Association for Assessment and Accreditation of Laboratory Animal Care-approved runs and fed a commercially available maintenance diet.

Ultrasonographic evaluation of meniscal tissue was performed 1 week prior to surgery to ensure menisci were of sufficient size for inclusion in the study, and to ensure no meniscal pathology was present based on sonographic evaluation. Both knees were imaged using an ultrasound machine and 13 MHz transducer. Ultrasonographic evaluation of menisci included assessment of subjective appearance and determination of cross-sectional area. Cross-sectional images of the cranial, central, and caudal regions of the medial meniscus were obtained and recorded. Subjective assessment was based on size, shape, and echogenicity. Measurements of meniscal base (b) (proximal to distal extents) and height (h) (medial to lateral extents) were determined to the nearest 0.01 mm.
Surgical Instrumentation

On the day of surgery, the dogs were premedicated with xylazine (0.5 mg/kg intramuscular) and morphine (0.5 mg/kg intramuscular), anesthetized with thiopental (10-20 mg/kg intravenously [IV]), and maintained with halothane in oxygen. Using aseptic technique, a medial approach with osteotomy of the origin of the medial collateral ligament and medial arthrotomy was performed on one randomly assigned knee of each dog. Initially, a standardized, partial meniscectomy was created in the posterior portion of the medial meniscus using a cut template. The dimensions of the cut template were 10 mm in longitudinal length and 5 mm in radial depth. The dimensions of the meniscal resection matched the size of the wedge-portion of the Small Intestine Submucosa (SIS) Meniscal Implant (DePuy Orthopaedics Inc, Warsaw, Ind) and extended to the vascular zone of the meniscus. To create the taper cut, the anterior and posterior margins of the defect were trimmed to mimic a “typical clinical sculpted defect” (Figure 1). For the tibial tunnel, a bone tunnel (2-mm diameter) was drilled from the medial aspect of the proximal tibia to exit at the insertion of the medial posterior meniscotibial ligament (Figure 2).

The dogs were randomly assigned to one of the following treatment groups:

- SIS Meniscal Implant (SIS) (n=4): An SIS meniscal implant was sutured into the taper cut meniscal defect and passed through the tibial tunnel at its posterior aspect (Figure 3). The SIS meniscal implant was a 20-layer wedge with the inner edge consisting of folded layers of small intestine submucosa.

- Meniscectomy (n=4): The taper cut meniscal defect received no implant (Figure 4). A tibial tunnel was drilled. The implant was stabilized in the defect site with sutures of 5-0 prolene. Sutures of 5-0 prolene were also placed at the margin of resection in the meniscectomy group. The medial collateral ligament was reattached by means of 2.7-mm screw fixation of the osteotomy site. Nonweight-bearing slings were placed on the operated limb of each dog. Analgesics (morphine or aspirin) were administered to the dogs at the time of extubation, and then as necessary to control signs of pain. The dogs were recovered and returned to their individual kennels.

Clinical Evaluation

The dogs were observed daily and rectal temperature, pulse rate, and respiratory rate as well as appetite, attitude,
and activity level were recorded. The dogs were restricted to cage rest postoperatively. The slings were maintained based on daily observation and changed as needed until they were removed 3 weeks postoperatively. Soft-padded, bivalved casts were placed on the operated limbs after sling removal and were maintained for 3 weeks. Clinical lameness evaluation was performed by two veterinary orthopedic surgeons, blinded to dog number and treatment group, every 4 weeks after surgery by observing the dogs at a trot and scoring their operated limb function based on the following scale\textsuperscript{11}: 0 = no observable lameness; 1 = intermittent, mild weight-bearing lameness with little if any change in gait; 2 = consistent, mild weight-bearing lameness with little change in gait; 3 = moderate weight-bearing lameness, obvious lameness with noticeable change in gait; 4 = severe weight-bearing lameness, “toe-touching” only; and 5 = nonweight-bearing.

The dogs were sacrificed 6 months postoperatively by IV overdose of phenobarbital/phenytoin.

**Gross Evaluation**

After euthanasia, both knees of each dog were examined. The tibial plateau and femoral condyles were photographed. The entire medial meniscus from both knees of each dog was collected and photographed, prior to placement in formalin for histologic processing. After removal of the medial meniscus, the medial femoral and tibial condyles of both knees from each dog were painted with India ink, washed after 60 seconds with tap water, and photographed.

Unexposed radiographic film was placed over each condyle and plateau, and cut to match the surface area of the condyle. The areas of India ink staining were outlined using a permanent marker. Tracings of the India ink-stained tibial and femoral condyles were evaluated without knowledge of dog number or treatment group. The tracings were scanned using a computer software program and percentage of the total area of the stained tibial and femoral condyles was calculated and recorded as percentage area of cartilage damage. The percentage area of cartilage damage was determined for the tibial and femoral condyles, separately and together, for each dog.

**Area of Replacement Tissue**

Cross-sectional area measurements were calculated using the direct measurements of operated and nonoperated (contralateral) medial menisci, and the formula for an isosceles triangle \((b^2h/2)\). Cross-sectional percentage-original area was calculated using the formula: \((\text{Replacement cross-sectional area)/(Contralateral cross-sectional area})\times100\).

Photographs of the operated menisci obtained at the time of surgery and at postmortem examination were outlined to obtain a value for total surface area of each. Total surface area percentage-original area (total surface area percentage) was determined using the formula: \((\text{Replacement total surface area)/(Original total surface area})\times100\).

**Histologic Assessment**

After routine histologic processing, 5-\(\mu\)m sections were cut from each meniscus, and stained with hematoxylin-eosin. Histologic assessment was performed by one investigator (I.L.C.) who was blinded to dog number and treatment group. Hematoxylin-eosin stained sections were subjectively evaluated for the amount and character of meniscal replacement tissue.
Biomechanical Testing

All dogs were evaluated for relative compressive stiffness of the medial femoral and tibial condyles of both the operated and contralateral knees. Compressive stiffness of the cartilage was determined for each condyle using an arthroscopic cartilage stiffness testing device (Artscan 1000; Artscan Oy, Helsinki, Finland). Multiple readings (minimum of 8) were obtained at consistent locations on the weight-bearing surface of each condyle. Artscan software was used to choose three compressive stiffness measurement curves in which the applied force was consistently held at 10 N for a minimum of 2 seconds as recommended to determine the mean relative compressive stiffness value for each of the three curves. The mean of the three readings was determined and used as the relative compressive stiffness value for each condyle. Mean values are reported as relative compressive stiffness (reaction force in N).

Statistical Analyses

All statistical analyses were performed using a computer software program (SigmaStat, San Rafael, Calif). Data from each group at each sacrifice time point were combined and standard errors (SE) were determined. A t test was used to determine significance between groups for lameness scores, cross-sectional area and total surface area measurements, and amount of articular cartilage damage. A one-way analysis of variance (ANOVA) was used to test for significant differences in relative compressive stiffness values. Significance was set at P < .05.

RESULTS

All dogs survived and no complications were noted. Dogs in the meniscectomy group were significantly (P = .002) more lame than dogs in the small intestinal submucosa group 6 months after implantation (Figure 5).

<table>
<thead>
<tr>
<th>Group</th>
<th>Small Intestinal Submucosa (n=4)</th>
<th>Meniscectomy (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defect Fill</td>
<td>Excellent</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>1</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>Poor</td>
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<td>Tissue Appearance</td>
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<td>3</td>
</tr>
<tr>
<td></td>
<td>Immature</td>
<td>1</td>
</tr>
</tbody>
</table>

Gross Evaluation

Small intestinal submucosa-treated meniscal defects had more replacement tissue present with a more mature appearance than seen in meniscectomy defects (Table 1). New meniscal tissue appeared firmly attached to remaining meniscus in small intestinal submucosa-treated defects, and the margin of initial resection was not distinct. New tissue was noted to extend into the tibial tunnel in the small intestinal submucosa grafted tibial tunnel dogs. Little to no new tissue was present in the meniscectomy defects. The tissue that was present appeared immature and friable. Representative images of menisci from each group are shown in Figure 6.

Area Measurements

The cross-sectional percentage-original area values for the small intestinal submucosa group (mean ± SE, 77.4 ± 7.3) and the meniscectomy group (mean ± SE, 69.6 ± 10.1) were not significantly different (P = .226, t test, power = 0.51). However, total surface area percentage values were significantly (P = .001, t-test) lower in the meniscectomy group compared to the small intestinal submucosa grafted group (Table 2).

Articular Cartilage Damage

Six months postoperatively, the meniscectomy group had significantly (P = .010, t test) more articular cartilage damage than the small intestinal submucosa group (Figure 7).

Histologic Assessment

Based on subjective evaluation of histologic sections, meniscal defects treated with small intestinal submucosa were more consistent in terms of amount, type, and integration of new tissue compared to meniscectomy defects. Small intestinal submucosa-treated menisci had moderate
to large amounts of new tissue present. The new tissue appeared meniscal-like with respect to cellularity and matrix composition and architecture. There was evidence of very good to excellent integration of new tissue in small intestinal submucosa treated menisci. In contrast, little new tissue was seen in menisci treated by meniscectomy. The tissue that was present was not well organized or meniscal-like. The tibial tunnel could not be delineated in these sections. Representative photomicrographs of menisci from each group are shown in Figure 8.

Articular Cartilage Compressive Stiffness

In the control (nonoperated) knees, femoral condylar cartilage was significantly ($P < .001, t$-test) more stiff than tibial condylar cartilage. Similarly, for operated knees in both treatment groups, femoral condylar cartilage was significantly ($P = .007$ [small intestinal submucosa], $P = .006$ [meniscectomy]), $t$-test) more stiff than tibial condylar cartilage. No statistically significant differences (one-way ANOVA) were noted among groups when comparing femoral condylar cartilage stiffness or tibial condylar cartilage stiffnesses, respectively (Figure 9).

**DISCUSSION**

Small intestinal submucosa scaffolds placed in posterior, vascular meniscal defects resulted in production of meniscal-like replacement tissue. The meniscal replacement tissue in small intestinal submucosa grafted meniscal defects was consistently superior to meniscectomy in terms of amount, type and integration of new tissue, chondroprotection, and limb function. Based on subjective assessment, the tibial tunnel technique used in this animal model appeared to provide secure fixation and consistent placement of the small intestinal submucosa meniscal implant with potential advantages over previous techniques.$^5,10,11$ In theory, the tibial tunnel technique also would allow for greater potential access to connective tis-

**TABLE 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cross-Sectional</th>
<th>Total Surface Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small intestinal submucosa</td>
<td>77.4±7.3</td>
<td>88.9±4.2*</td>
</tr>
<tr>
<td>Meniscectomy</td>
<td>60.0±10.1</td>
<td>59.0±2.6*</td>
</tr>
</tbody>
</table>

*Denotes statistically significant difference between groups.
sue progenitor cells and significant blood clot formation associated with the small intestinal submucosa meniscal implant when performing this technique arthroscopically. These potential technical and biological advantages of the tibial tunnel technique deserve careful consideration when determining the recommended implantation and fixation protocol for clinical use in human patients. It is important to note that no untoward effects related to the use of this technique were recognized. In addition, the taper cut had no apparent influences on new tissue regeneration or integration. Because this taper cut technique was modeled after the “typical” sculpted defect created by surgeons performing partial meniscectomy, these data suggest that surgeons can continue to use their current technique for partial meniscectomy prior to placement of this small intestinal submucosa meniscal implant.

In the present study, small intestinal submucosa grafting of vascular posterior meniscal defects resulted in significant differences in limb function after surgery. Dogs receiving small intestinal submucosa meniscal implant had significantly better limb function as assessed by lameness scores 6 months postoperatively than dogs that had meniscectomies. Small intestinal submucosa grafting of meniscal defects also resulted in increased tissue regeneration in the defects as assessed by total surface area measurements, gross appearance, and histologic assessment. Previous studies have reported that retaining or replacing as much meniscal tissue as possible is critical for maintaining or re-establishing the biomechanical properties of the meniscus, limb function, and chondroprotection, while preventing osteoarthritic changes in the tissues.1,2,5,6,12,26-28

Figure 9. Relative compressive stiffness values for all operated and nonoperated medial femoral and tibial condyles 6 months after surgery.

The primary clinical objective for meniscal grafting is to provide long-term function of the limb while preventing or retarding the progression of osteoarthritis. Studies suggest that increasing the amount of functional meniscal tissue in a defect will allow achievement of that objective.1,2,5,6,12,27 Small intestinal submucosa grafting accomplished the objective of chondroprotection in this study. Small intestinal submucosa-grafted dogs had significantly less articular cartilage damage than dogs treated by partial meniscectomy. In addition, although no statistically significant differences were noted, the relative compressive stiffness of femoral and tibial condylar articular cartilage in small intestinal submucosa-treated knees more closely matched that of control knees than did meniscectomized knees. Both the amount of tissue in the defect and the nature (maturity) of the tissue in the defect likely influenced these outcomes. Not only was the amount of tissue in small intestinal submucosa-grafted defects greater than in meniscectomy defects, but the tissue morphology was superior as well. Tissue in small intestinal submucosa-grafted defects was more mature (amount, type, and organization of cells and extracellular matrix), better integrated to remaining meniscus, and achieved a more normal shape compared to tissue in meniscectomy defects.

These data support previous findings with respect to small intestinal submucosa grafts for treatment of vascular meniscal defects in a dog model.5,10,11 The four requirements for optimizing meniscal regeneration and knee function when using small intestinal submucosa in this model appear to still hold true:

1. For the small intestinal submucosa meniscal implant to promote significant, meniscal-like tissue regeneration in the defect, the meniscal defect must extend to the vascular zone of the meniscus.

2. Sufficient hemarthrosis, for initial clot formation associated with the small intestinal submucosa meniscal implant, should be established.

3. The initial stability of the small intestinal submucosa meniscal implant in the meniscal defect needs to be ensured.
4. Biomechanical protection of the small intestinal submucosa meniscal implant, and associated early regenerative tissue, needs to be maintained for a minimum of 6 weeks.

A source of blood for clot formation via hemarthrosis from open arthrotomy, hemorrhage from meniscal vessels, or induction of hemorrhage via trephination of meniscus or subchondral bone appears to be necessary for successful small intestinal submucosa-enhanced meniscal regeneration. The tibial tunnel technique is another method for providing a source of blood for clot formation while concurrently allowing potential access to connective tissue progenitor cells from bone marrow that have the capacity to participate in meniscal tissue regeneration. Importantly, it appears that the need for the blood clot is a separate issue from the requirement that the meniscal defect treated must extend to the vascular portion of the meniscus for small intestinal submucosa grafting to be consistently successful in accomplishing functional meniscal-like tissue regeneration. Therefore, both of these requirements must be specifically addressed when attempting to apply data from the dog model studies to human application.

Although the efficacy of the tibial tunnel technique cannot be fully assessed for arthroscopic application with respect to hemarthrosis and blood clot formation on the small intestinal submucosa meniscal implant using this model in dogs, there is evidence that intra-articular bone tunnels are a source of blood clot for tissue healing. 20,23 Grafts used for reconstruction of the anterior cruciate ligament received a blood supply from bone tunnels used for fixation. 25 In addition, meniscal repairs are reported to have a higher success rate when concurrent ACL reconstruction with bone tunnels is performed. 20 This increase in successful meniscal healing is hypothesized to be the result of increased hemarthrosis associated with bleeding bone tunnels.

Compared to historical data from our previous studies using the small intestinal submucosa meniscal implant, no advantages with respect to measured outcome variables were noted for the tibial tunnel technique compared to small intestinal submucosa grafting alone at 6 months after surgery. 8 However, subjective advantages seemed apparent with respect to initial stability of the small intestinal submucosa implant and intimate association of the implant with vascularized meniscus (ie, posterior horn and meniscotibial ligament). In addition, the tibial tunnel technique may augment meniscal tissue healing and regeneration associated with small intestinal submucosa implants in arthroscopically treated meniscal defects in the human knee by providing access to bleeding and cells from the bone tunnel. Because no adverse effects were noted using the tibial tunnel technique and the taper cut meniscectomy, these factors deserve consideration for use in clinical trials testing this biomaterial. Further research investigating the use and importance of the tibial tunnel technique with respect to technical, biomechanical, and biological factors in clinical application is warranted.

ACKNOWLEDGEMENTS

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