University of Groningen

Effect on tissue differentiation and articular cartilage degradation of a polymer meniscus implant - A 2-year follow-up study in dogs

Welsing, Roy T. C.; van Tienen, Tony G.; Ramrattan, Navin; Heijkants, Ralf; Schouten, Arend Jan; Buma, Pieter; Veth, René P.H.

Published in:
American Journal of Sports Medicine

DOI:
[10.1177/0363546508319900](https://doi.org/10.1177/0363546508319900)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2008

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Welsing, R. T. C., van Tienen, T. G., Ramrattan, N., Heijkants, R., Schouten, A. J., Buma, P., & Veth, R. P. H. (2008). Effect on tissue differentiation and articular cartilage degradation of a polymer meniscus implant - A 2-year follow-up study in dogs. *American Journal of Sports Medicine*, 36(10), 1978-1989.
<https://doi.org/10.1177/0363546508319900>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Effect on Tissue Differentiation and Articular Cartilage Degradation of a Polymer Meniscus Implant

A 2-Year Follow-up Study in Dogs

Roy T. C. Welsing,* MD, Tony G. van Tienen,* MD, PhD, Navin Ramrattan,* MD, Ralf Heijkants,[†] PhD, Arend Jan Schouten,[†] PhD, René P. H. Veth,* MD, PhD, and Pieter Buma,*[‡] PhD

*From the *Orthopaedic Research Laboratory, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands, and [†]Polymer Chemistry, Faculty of Mathematics and Natural Sciences, University of Groningen, Groningen, Netherlands*

Background: Replacement of the meniscus by an implant could potentially avoid cartilage degeneration.

Hypothesis: An implant of degradable polycaprolacton-polyurethane should act as a temporary scaffold enabling regeneration of a new meniscus by slow degradation of the polymer and simultaneous in-growth and differentiation of tissues into the typical cartilage-like tissue of the meniscus.

Study Design: Controlled laboratory study.

Methods: In 13 dogs' knees, the lateral meniscus was replaced with a porous polymer implant (6 and 7 for 6- and 24-month follow-up, respectively); in 7 knees only a meniscectomy was performed. In 6 knees, no surgery was performed. After 6 and 24 months, the implants and the articular cartilage were histologically evaluated. Compression-stress tests were performed on implant biopsy specimens.

Results: The implants were fully integrated into the tissue without formation of a capsule. The foreign body reaction did not exceed grade I. Differentiation from fibrous- to cartilage-like tissue was pronounced after 24 months. Viable cells were particularly absent after 24 months in central parts of the most anterior part of the scaffold. The mechanical properties of the implants were intermediate between the scaffold before implantation and native meniscus tissue and were not different between 6 and 24 months. After both 6 and 24 months, small areas of the implant were not covered with tissue. Cartilage degeneration was not prevented.

Conclusion: A final remodeling of tissue into neomeniscus tissue could not take place since the original structure of the polymer was still present after 24 months. The implant did not prevent cartilage degradation. Several factors are discussed that may be responsible for this.

Clinical Relevance: Although clinical application of a polymer implant for the replacement of the entire meniscus is not supported by this study, the authors strongly believe in the concept, but further improvements in the implant and surgical technique are needed before such an implant can be recommended for human clinical use.

Keywords: meniscus; replacement; tissue engineering; cartilage; implant; polyurethane

The knee menisci are wedge-shaped, semilunar discs that play a critical role in the normal biomechanics of the knee

[‡]Address correspondence to Pieter Buma, PhD, Orthopaedic Research Laboratory, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB, Nijmegen, Netherlands (e-mail: p.buma@orthop.umcn.nl).

No potential conflict of interest declared.

joint (eg, in load bearing and distribution, shock absorption, joint lubrication, and stabilization of the knee joint).^{10,11,46} The meniscus also plays an important role in the transduction of mechanical forces and in proprioception by nerve endings in the vascularized part of the meniscus.^{27,32} The function of the meniscus is reflected in its ultrastructural organization. The extracellular matrix of the fibrocartilage tissue of the meniscus consists mainly of collagen type I. Particularly in the peripheral two thirds of the meniscus, the collagen is oriented in radial and

circumferential bundles, providing resistance to tensile and radial forces.^{12,33} Most collagen type II is found in the inner region.¹⁷ Glycosaminoglycans (GAGs) are present in smaller quantities and are mainly localized with type II collagen. Glycosaminoglycans are particularly important in maintaining the viscoelastic properties, compression stiffness, and tissue hydration of the meniscus.^{12,33}

Substantial damage to the meniscus can make a (subtotal) meniscectomy inevitable, which, in time, induces cartilage degeneration of the tibia and femur.^{4,15,23} Clinically, the collagen meniscus implant can be used for meniscus tissue regeneration after a partial meniscectomy.^{30,36} In cases of a severely damaged meniscus, it can be replaced by an allograft meniscus,^{20,25,35,42,44} but this procedure should still be considered experimental.³¹ Replacement of the total meniscus by an autograft or an artificial implant could potentially protect the articular cartilage. So far, research was focused on the use of autologous tissue,^{2,5,21,45} synthetic materials,^{19,24,26} or on a combination of these 2 types of material.^{5,37,39}

A biodegradable porous polymer implant may be particularly promising. It acts as a temporary scaffold to enable the regeneration of new meniscus tissue in time by slow degradation of the polymer and simultaneous differentiation of the ingrown fibrovascular tissue into the typical avascular meniscus fibrocartilage. Commercially available polyurethanes have been used in meniscus replacement.^{6,18} After implantation, the implant was filled with tissue resembling cartilage-like tissue. Macroscopically, the cartilage degeneration was less severe than after total meniscectomy.^{7,9,34}

However, the used polyurethanes may release toxic components and are therefore not suitable for human use.¹³ Therefore a polycaprolacton-based polyurethane (PCLPU) with nontoxic degradation products was developed.^{7,9,13,34} The PCLPU consists of well-defined hard segments in combination with slowly degrading polyester soft segments.¹³ The hard segments determine the stiffness of the polymer (compression modulus of 300 kPa at 20% compression). To facilitate the process of ingrowth the polymer was provided with a porosity of ~80% and with a homogeneous distribution of larger interconnected pores (pore size, 150–355 μ m). In 2 studies we found that after 6 months the ingrown tissue was fibrous-like in the peripheral zones of the meniscus prosthesis, but in the central areas, cartilage-like tissue had developed.^{14,40,41} Given the loss of all mechanical properties of the PCLPU between 6 and 12 months in an *in vitro* degradation study,¹³ we hypothesized that the resulting increase of load on the tissue in the scaffold could be a trigger for further remodeling into a more organized, meniscus-like extracellular matrix. To study this, we implanted PCLPU implants in dogs and analyzed the implants after 6 and 24 months. Total meniscectomy and native menisci served as control groups. Implants were made in a mold to minimize variation as much as possible. In addition to long-term tissue differentiation, changes in stiffness of the implants, the foreign body reaction, and articular cartilage degeneration were studied in this long-term study.

Figure 1. Meniscus implant produced in mold before implantation. A, the PCLPU polymer. Note the interconnective pore structure. B, the meniscus implant.

MATERIALS AND METHODS

Implant

The PCLPU consists of a hard segment of 1,4-butanediisocyanate and butane diol and a soft segment of poly(ϵ -caprolactone). The pores were created by mixing the polymer solvent solution with salt crystals ranging in size from 150 to 355 microns, which were washed away after complete polymerization of the scaffold.⁸ The macropores were directly interconnected to achieve a high permeability of the polymer. The porosity was 81.4%, and the compression modulus at 20% compression was 300 kPa. We used a Teflon mold (Teflon PTFE; DuPont, Wilmington, Del) to produce the implants to create implants in a form similar to the native meniscus. The implants were also smooth and were provided with an interstice for the popliteus tendon (Figure 1).

TABLE 1
Interventions Performed on 26 Knees^a

Dog	Left Knee	Right Knee
1	Unoperated	Meniscectomy 24 mo
2	Unoperated	Meniscectomy 24 mo
3	Unoperated	Implant for 24 mo
4	Unoperated	Implant for 24 mo
5	Unoperated	Meniscectomy 24 mo
6	Unoperated	Implant for 24 months
7	Implant for 6 mo	Meniscectomy 24 mo
8	Implant for 6 mo	Implant for 24 mo
9	Implant for 6 mo	Meniscectomy 24 mo
10	Implant for 6 mo	Meniscectomy 24 mo
11	Implant for 6 mo	Implant for 24 mo
12	Implant for 6 mo	Implant for 24 mo
13	Meniscectomy	Implant for 24 mo

^amo, months.

Surgery

Thirteen adult male and female beagle dogs were operated on bilaterally but not in one session. Their average weight was 12.5 kg (SD, ±0.2 kg). A total lateral meniscectomy was performed on 20 legs. Six knees were left intact to analyze the effect on the contralateral side of the meniscectomy and of implantation of the scaffold (Table 1). The local institutional animal welfare committee approved all procedures.

The lateral meniscus was approached by a lateral incision of the knee joint capsule without detachment of any ligament. Using a Beaver eyeblade (Waltham, Mass) the meniscus was separated from its anterior and posterior attachments. Two drill holes were created originating from the lateral tibial side to the former attachment of the anterior and posterior horns of the native meniscus. Two bonded nondegradable sutures were led through the 2 horns of the implant. The sutures were pulled through the drill holes in the tibia. The periphery of the implant was sutured to the peripheral knee joint capsule using 2-0 resorbable bonded sutures (Vicryl 2/0; Ethicon, Amersfoort, Netherlands) to achieve close contact between synovial tissue and the implant. Afterward, the capsule and skin were closed. The dogs were not immobilized but allowed to walk as soon as possible.

Histology

After 6 or 24 months, the dogs were sacrificed. Radiographs were taken of the joints, and thereafter the joints were opened and the femur was dissected from the tibial plateau. The noncovered part of the tibial plateau was stained with india ink and photographed (Figure 2A). Then the meniscus, the tissue that had been formed in the meniscectomy group, or the polymer implant was photographed and carefully removed. Again, the femoral condyles and the tibial plateau were stained with india ink (Figure 2B, C). After 30 seconds, the nonadhering ink was carefully removed by running tap water and the femoral condyles and tibial plateaus were photographed to enable

Figure 2. Tibia and femur specimens of 24-month implant group. A, ink staining of noncovered part of tibial plateau. B, same specimen but after dissection of the implant (laterally) and meniscus (medially) from the plateau. C, ink-staining pattern on femoral condyle of same knee.

visualization and analysis of the fibrillated area on femur and tibia.¹⁶ The diameter of the heavily fibrillated area on the femur was measured by using an interactive, computer-controlled, image-analysis system (analySIS Soft Imaging System, GmbH, Münster, Germany). The implants in the meniscectomy group were resected, and a 4-mm full-thickness biopsy specimen was taken from the posterior part of the tissues for biomechanical analysis. The femoral condyles and the tibial plateau were dissected (see Figure 3 for details of the procedure).

The harvested implants were divided into 4 equal parts. The 2 anterior and the most posterior part were fixed in acetone (4°C) for 6 hours, infiltrated in glycolmethacrylate monomer (GMA), and polymerized at room temperature for 1 day. The part in the medial-posterior region (part II, Figure 3B) was treated the same way except that it was infiltrated with methylmethacrylate monomer (MMA) and polymerized at -20°C for 2 days for histological and immunohistochemistry analysis. Sections (7 microns) were cut in the transverse plane, deacrylated (polymethylmethacrylate [PMMA]-embedded parts of the meniscus), dried at 37°C, and stained with hematoxylin and eosine (H&E), safranin O, Sudan Black (Merck, Darmstadt, Germany), and Picrosirius Red (PSR) (Chroma, Kongen, Germany).²⁹ For PSR staining, sections were stained for 10 minutes with Weichert Haematoxiline (Merck) and then stained for 30 minutes in a PSR solution (10 mL of 1% Sirius Red [Chroma] in 90 mL in a saturated Picric acid solution [Chroma]). All sections of PMMA-embedded tissues were deacrylated in a mixture of xylene and chloroform (1:1) for 3 × 15 minutes before staining. In preparation for immunohistochemistry, PMMA sections were subsequently treated with 1% testicular hyaluronidase (type I-S, EC 3.2.1.35; Sigma, St Louis, Mo) in phosphate-buffered saline (PBS) for 30 minutes at 37°C. To block non-specific labeling, sections were treated with 10% normal goat serum (for collagen type I antibody labeling) and normal horse serum (for collagen type II antibody labeling) in PBS with 1% bovine serum albumin (Sigma). Polyclonal anticollagen type I antibodies (1/600, PS-41, antihuman raised in rabbit; Sanbio, Uden, Netherlands) and monoclonal anticollagen II antibodies¹ (1/300, II-II6B3, antichick raised in mouse; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, Iowa)²² were applied and the samples were incubated in a humidified chamber overnight at 4°C. Anticollagen antibodies were detected using a biotin-labeled goat antirabbit antibody (1/200 dilution; Dako) and horse antimouse antibody (1/200 dilution; Dako) for 90 minutes at room temperature. A biotin-streptavidin detection system (Vectra Elite Kit; Vector, Burlingame, Calif) was used according to the manufacturer's recommendations. The peroxidase was detected using tablets containing 10 mg 3,3-diaminobenzidine tetrahydrochloride (Sigma) dissolved in 15 mL aqua dest with 12 microliters H₂O₂ (30%) for 7 minutes. After rinsing, sections were dehydrated and mounted with DPX (BDH, Poole, England).

The tibial plateaus and femoral condyles were dissected in equal proportions. The tibial plateaus were divided into a lateral and a medial part, which were subsequently divided into an anterior and posterior half. The anterior half was processed for routine histology and the posterior half for immunohistochemistry (Figure 3B). The femoral condyles were also divided in a medial half for immunohistochemistry and a lateral half for routine histologic examination. Sections of the tibia were cut in midtransverse plane and sections of the femur in midsagittal plane. The anterior halves of the tibial plateaus and the lateral halves of the femoral condyles were fixed in buffered formaldehyde (4%, pH 7.4) and decalcified in 10% ethylene diamine tetraacetic acid (EDTA) (Titriplex III; Merck, Darmstadt,

Figure 3. A, all meniscus implants were divided into 4 parts after dissection from the tibial plateau. The location of the biopsy for mechanical analysis is denoted by the letter B in a circle. B, planes of sectioning of the femoral condyles and tibial plateaus.

Germany). After extensive rinsing with tap water, tissue blocks were dehydrated in alcohol and embedded in PMMA for 2 days at 37°C. Two synovium biopsies were taken from each knee from the most lateral area and from the synovium directly next to the implant. The samples were frozen in liquid nitrogen and stored at -80°C.

Microscopy

The implant with ingrown tissue in the microscope was grossly divided into 3 zones (eg, peripheral, central, and inner zone). The phenotype of the tissue in the scaffold was

TABLE 2
Width of the Articular Cartilage Damage^a

Knee	Macroscopic Cartilage Damage, mm (mean + SD)			
	Femur Medial	Femur Lateral	Tibia Medial	Tibia Lateral
Native meniscus	0	1.47 (3.28) ^{b,c}	3.85 (1.25)	4.64 (0.72) ^d
Implant knee after 6 mo	0	5.06 (1.63)	4.77 (1.31) ^d	7.80 (1.86) ^d
Implant knee after 24 mo	0	6.40 (0.53) ^b	4.13 (0.65)	5.69 (0.56)
Meniscectomy	0	5.40 (3.23) ^c	5.48 (0.87)	6.39 (2.17)

^aSD, standard deviation.

^bSignificant difference, $P = .01$.

^cSignificant difference, $P = .04$.

^dSignificant difference, $P = .02$.

scored as vascularized fibrous tissue or as avascular fibrocartilage tissue. The safranin O staining for labeling proteoglycans was scored on an ordinal scale from no staining (–) to normal staining (++++). Collagen orientation was analyzed with polarized light on the Picrosirius Red–stained sections.²⁹ The foreign body reaction in the synovium and in the pores of the implant was scored according to an ordinal scale as no inflammation (grade 0), slight inflammation (few macrophages/giant cells, grade 1), well-defined inflammatory reaction (many macrophages/giant cells, no polymorphonuclear leucocyte (PMN) leucocytes, grade 2), moderate inflammation (many macrophages/giant cells with few PMN leucocytes, grade 3), and severe inflammation (abundant macrophages, giant cells, and PMN leucocytes, grade 4).⁴³ The exposure of the implant to the surface, for example, not covered with tissue, was scored on a scale as no exposure (no), just a few particles exposed to the surface (minimal), less than a quarter of the surface area exposed (<1/4), less than a half (<1/2), less than 3 quarters (<3/4), or almost total exposure of the surface area (total).

Articular Cartilage

The articular cartilage of femoral condyles and tibial plateaus was photographed and inspected for gross degenerative changes. Degenerative changes were scored according to the Mankin grading system from normal structure (grade 0) to complete disorganization (grade 6), normal cells (grade 0) to hypocellularity (grade 6), normal safranin O staining (grade 0) to no staining (grade 4), and an intact tidemark (grade 0) or a tidemark infiltrated with blood vessels (grade 1). The total score of each subcategory determined the qualitative Mankin score.

Biomechanical Analysis

After excision of the meniscus-like tissue of all dogs' knees, 4-mm punch biopsy specimens (see Figure 2 for location) were taken of a specified region of the posterior horn of the implants, the native menisci, and the regenerated tissue of the meniscectomy group. As a reference, punches also were taken from polymer that was not implanted. All punches were immediately frozen in liquid nitrogen and thereafter

stored at -80°C . Before compression testing, punches were thawed at room temperature. Samples were then cut with a sharp blade in a hollow cylinder to a height of 3 mm with a flat surface perpendicular to the long axis of the cylinder, and directly afterward loaded with an Instron (4301; Norwood, Md) compression tester, equipped with a 100-N load cell, with a compression rate of 2 mm/min in an unconfined way at room temperature (22°C). The maximum compression was 50%.

The results of the cartilage degeneration were analyzed by analysis of variance (ANOVA) test (ANOVA on ranks for the Mankin scores of the medial sites) and post hoc t test (Tukey). For the compression test data, the ANOVA and post-hoc t test (Tukey) were used. Statistical significance was set at P values less than .05.

RESULTS

Clinical and Macroscopic Observations

Two dogs had a delay of 3 to 4 days after the surgery in loading the knee. A similar gait pattern was observed in all dogs postoperatively and in the long term. Postoperatively, 1 dog was successfully treated for a suspected infection. While harvesting the menisci the surgeon noticed that in all knees the popliteus was in a medial position. At the same location, the implants were severely damaged. The original implant, including the inner part of it, was cut into 2 parts. All implants appeared well connected to the peripheral synovial tissue with minimal synovial overgrowth. All implants had a rigid peripheral attachment to the capsule and appeared in a near anatomic position. All tissues were available for analysis.

Analyzing the india ink–staining patterns of the uncovered part of the tibial plateaus, it appeared that staining patterns were quite similar in meniscectomy and implant groups (Figure 2A, B). The diameter of the uncovered surface area of the tibial plateau measured in the midtransverse plane, however, was different (Table 2). Uncovered areas tended to be wider on the lateral plateau than on the (nonoperated) medial side, also in the native meniscus group (Figure 2B). A clear significant difference was found between the lateral and medial uncovered area of the

6-month implant group ($P = .02$). The uncovered area on the lateral side appeared to be wider in the implant and meniscectomy groups compared with the native meniscus group, but only at 6 months was this difference significant ($P = .02$). The width and location of the staining pattern on the femur corresponded to the uncovered area of the lateral part of the tibia plateau (Figure 2B, C).

Six-Month Histology

The whole scaffold was filled with fibrovascular tissue with spindle-shaped cells surrounded by extracellular matrix (Figure 4A). Abundant collagen type I labeling was found throughout the scaffold (Figure 5). Irrespective of the location in the implant, the bundles of collagen completely filled the pores and were, in each pore, oriented toward the larger and smaller interconnecting channels between the pores (Figure 6). Along the surface of the pores, some giant cells were present, but the inflammatory reaction was scored as grade I. The synovium was normal or slightly hypertrophic.

In 2 of the 6 harvested implants, the central part of the scaffold contained some areas of cartilage-like tissue with fibroblasts with a more chondrocyte-like phenotype (Figure 4B; Figure 7, area III). These areas were less vascularized, contained fewer cells and a positive collagen type II antibody labeling (Figure 5C), and proteoglycan staining was present. However, in 4 of 6 implants, this differentiation had not occurred and the tissue had a fibrovascular phenotype (areas I and II, Figure 7).

Like the pores of the scaffold, the surface was mostly covered with tissue of a fibrovascular phenotype. However, in all knees, implants were not completely covered with tissue; in some of the implants, one quarter of the polymer surface was exposed to the opposite cartilage surface (Figure 8 and Table 3). In some of the microscopic images on cross-section, the distance between thick fibrous tissue layers not containing any polymer was seen in the periphery of implants. These layers separated those parts of the implant from the bulk of the implant, suggesting also fragmentation of the implants on a more microscopic scale (Table 3).

Histology at 24 Months

After 24 months, the cross-sectional areas of anterior cross-sections tended to be slightly larger than the posterior cross-sections. Two distinct areas were present in all the specimens. First, there was a thin outer zone of fibrovascular tissue with spindle-shaped cells and positive staining using Picrosirius Red and positive immunohistochemical staining using collagen type I antibodies (Figure 5B; area I, Figure 7). Second, there was a much larger (compared with the 6-month specimens) inner zone containing mainly cartilage-like tissue with chondrocyte-like cells (area III and IV, Figure 7). The extracellular matrix in this area stained positively with antibodies to both collagen types I and II (Figure 5B, C). Collagen type I had a homogeneous distribution throughout the entire implant. Collagen

Figure 4. Histological sections of the porous polymer implant 6 months in vivo (200× magnification). A, fibrovascular tissue with abundant cells filled the macropores and micropores (stained with H&E). B, different section of the same implant. The tissue differentiated into cartilage-like tissue. Note the decreased number of cells (stained with H&E). C, same sections as B stained with safranin O. The extracellular matrix, ie, glycosaminoglycans, is well stained.

Figure 5. A, collagen type II labeling of the native meniscus is depicted. Collagen type I (B) and type II (C) antibody labeling in adjacent sections in a central region of the implant after 24 months in vivo (200× magnification). The localization of the collagen type II antibody labeling matched the localization of the collagen type I antibody labeling.

type II localization was confined to areas II and IV (Figure 7). No collagen type II staining was observed in the peripheral transitional zone (area I, Figure 7) to the synovium.

Bundles of collagen filled the pores completely and were oriented toward the larger and smaller pores (Figure 6). In anterior specimens (area I, Figure 7), most safranin O staining was lacking in the center of the scaffold (area IV, Figure 7). In these areas, vital cells were scarce or absent (Figure 9). Only around vital cells was safranin O–stained matrix present (Figure 9B). Hardly any signs of an inflammatory reaction were seen in the implant or synovium,

Figure 6. Collagen bundles run through the scaffold. Note the orientation toward the structure of the scaffold; for example, the bundles run in the direction of and through the larger (arrow) and smaller pores (arrowhead). Polarized light on Picrosirius Red–stained scaffold, 200× magnification.

Figure 7. Schematic representation of the differentiation areas in the implant after 6- and 24-month follow-up periods.

which showed a normal cellularity. The surface of the implant was almost completely covered with a layer of variable thickness (50–400 μ M) fibrovascular tissue as described above (area II, Figure 7). All knees showed some extent of surface exposure of the PCLPU, but this was far less than the exposure in the 6-month group (Table 3).

After 24 months, the polymer seemed to fragment within the newly formed tissue; however, it was not degraded noticeably. Disintegration by fragmentation of the PCLPU scaffold was found in 6 of the 7 implants (Table 3). This disintegration was seen mainly at the peripheral parts of the scaffold. In the central part, the microstructure of the scaffold remained unchanged. In one knee we found some small fragmented parts of the implant in nearby synovial tissue. At the same location, some synovial hypertrophy was found (Figure 10).

Figure 8. Surface exposure of the PCLPU scaffold. Sudan Black stained the polymer dark. The scaffold is not completely covered with tissue, leaving some scaffold (arrows) exposed to the opposite articular cartilage of tibia or femur (Sudan Black staining, 100× magnification).

Figure 10. Loss fragments of the polymer scaffold (arrows) found at a distance from the implant, which indicates that fragmentation of the scaffold had occurred. The fragments are located in fibrous, loose, synovial-like tissue (section stained with Sudan Black and H&E, 200× magnification).

Figure 9. Histological sections of the porous polymer implant 24 months in vivo, central region (200× magnification). A, fibrocartilage-like tissue (stained with H&E). Note the absence of most chondrocytes, for example, central necrosis and a cluster of vital chondrocytes (arrow). B, adjacent sections to A but stained with safranin O. In locations with central necrosis, some safranin O staining is seen around the vital clustered chondrocytes.

Figure 11. Safranin O-stained section of native meniscus (A) and newly formed tissue (B) in 24-month meniscectomy group (200× magnification).

TABLE 3
Fragmentation and Surface Exposure
of the Porous Polymer Scaffold^a

# of Dog (L or R)	Fragmentation ^b	Surface Exposure ^c
6 months		
7 L	Yes	<1/4
8 L	No	<1/4
9 L	Yes	<1/4
10 L	No	<1/4
11 L	No	<1/4
12 L	Yes	Minimal
24 months		
3 R	Yes	Minimal
4 R	Yes	Minimal
6 R	Yes	Minimal
8 R	Yes	<1/4
11 R	No	Minimal/<1/4
12 R	Yes	Minimal
13 R	Yes	<1/4

^aL, left; R, right.

^bFragmentation of the scaffold: the scaffold lost its original microscopic morphologic characteristics.

^cSurface exposure: minimal, almost completely covered with tissue except very small areas; <1/4, less than one quarter of the surface area uncovered with tissue.

Native Meniscus

In general, the patterns in the native meniscus control group were different from the patterns in the implant groups. In the native menisci, a positive collagen type I labeling was found in the outer rim (peripheral one third of a coronal section). In the inner rim (central one third of a coronal section), more collagen type II and proteoglycans were found. In the peripheral transitional zone to the synovium, no collagen type II staining was observed. Staining with Picrosirius Red showed an equally distributed staining throughout the meniscus. The circumferential fibers were well stained and located toward the periphery of the meniscus. Radial tie fibers were seen with H&E staining and polarized light on Picrosirius Red staining. The tie fibers stained equally for collagen type I and type II antibodies (Figure 5A).

In the meniscectomy group, a fibrous tissue had developed. Occasionally, the collagen was more oriented, and some diffuse safranin O-positive areas were present, which were mainly dense fibrous with some focal areas of a tissue resembling native meniscus tissue (eg, oriented collagen bundles, collagen type I and II labeling, and some proteoglycans staining by safranin O) (Figure 11).

Cartilage Degeneration

The Mankin scores of the tibia and femur (Table 4) did not differ significantly between 6 and 24 months ($P = .65$, $P = .64$, respectively). No significant difference was found

TABLE 4
Articular Cartilage Degeneration in the Femoral
Condyles and Tibial Plateaus According
to Mankin Scores^a

# of Dog (L/R Knee)	Mankin Femur		Tibia	
	Medial	Lateral	Medial	Lateral
Unoperated				
1 L	0	0	1	1
2 L	1	1	2	1
3 L	2	2	3	2
4 L	1	0	1	3
5 L	0	6	5	1
6 L	2	0	2	2
Average	1.0	1.5 ^b	2.1	1.3 ^b
Implant knee after 6 months				
7 L	2	3	2	9
8 L	5	5	5	6
9 L	4	9	2	11
10 L	2	5	2	7
11 L	2	7	10	6
12 L	2	10	8	2
Average	2.8	7.2 ^b	5.0	7.0 ^b
Implant knee after 24 months				
3 R	1	15	2	12
4 R	3	7	5	4
6 R	2	8	2	7
8 R	2	7	2	11
11 R	2	9	8	10
12 R	2	8	2	6
13 R	6	9	3	9
Average	2.6	9.0 ^b	3.4	8.4 ^b
Meniscectomy				
13 L	1	2	1	4
1 L	2	7		11
2 L	0	8	2	7
5 L	4	2	2	7
7 L	1	11	1	10
9 L	0	9	1	9
10 L	5	6	1	7
Average	1.9	6.4 ^b	1.1	7.6 ^b

^aL, left; R, right.

^bSignificant difference between the native meniscus group and the implant at 6 months, 24 months, and meniscectomy group.

between the implant groups (6 and 24 months) and the meniscectomy control group (tibia: $P = .88$ and $P = .97$, respectively; femur: $P = .96$ and $P = .33$, respectively). Significant differences were found between the native meniscus control group and the implant at 6 months, 24 months, and meniscectomy group of the tibial cartilage degeneration ($P = .01$, $P < .01$, and $P < .01$, respectively). For the femoral cartilage degeneration, significant differences also were found in relation to the native meniscus control group ($P = .01$, $P < .01$, and $P = .03$, respectively). On the medial, nonoperated side, no significant differences between groups were found.

Figure 12. Compression-stress curves of the implant after 6 and 24 months, the native meniscus, and the PCLPU-scaffold before implanting. No significant differences were found between groups.

Biomechanical Evaluation

The slope of the compression-stress curves increased from 6 to 24 months (Figure 12). There was no significant difference between the implants after 6 and 24 months in vivo ($P = .29$) and between the implants and the native meniscus ($P = .86$).

DISCUSSION

In this long-term in vivo follow-up study, we analyzed a porous polymer meniscus implant after 6 and 24 months in adult beagle dogs. The main questions addressed were if the implants would evoke a foreign body reaction, if the ingrown tissue in the implant would differentiate into the typical meniscus fibrocartilage, and if cartilage degeneration of the tibial plateau and femoral condyles could be prevented.

This is the first report of 2-year results of tissue differentiation in scaffolds for meniscus replacement after total meniscectomy. The results of tissue ingrowth and differentiation in PCLPU scaffolds after 3 and 6 months in vivo have been previously described.^{40,41} Kobayashi et al¹⁹ reported 2-year results of articular cartilage degeneration and mechanical tests of a polyvinyl alcohol-hydrogel implant in rabbits, but no evaluation of tissue ingrowth and differentiation in the implant was performed. In the present study, we again found complete ingrowth of fibrovascular tissue into the scaffold after 6 months with abundant collagen type I labeling. Similar to previous studies, a remodeling into a cartilage-like tissue with type II and GAGs was found in the inner higher loaded region of the scaffold.^{40,41} However, the amount of differentiation after 6 months in this study was less than in previous studies with the same polymer.^{40,41} Tissue differentiation from fibrovascular to cartilage-like had progressed in the 24-month implants, particularly in the central region of each scaffold. In the peripheral transitional zone to the synovial tissue, collagen type II staining was absent, similar as the distribution in the native meniscus.^{3,17} A homogeneous distribution of type I collagen was found

throughout the scaffold. However, despite this differentiation toward cartilage-like tissue, the typical organization of meniscus tissue, as found in the native meniscus, was not found in the scaffolds.

One of the reasons for this lack of tissue differentiation into typical meniscus tissue could be that the scaffold was not degraded after 24 months. The general microscopic structure of the scaffolds after 24 months was not changed compared with the 6-month follow-up. Only at the microscopic level did the Sudan Black stain reveal more local fragmentation after 24 months in the specimens.

A second reason might be the differentiation toward cartilage-like tissue in the scaffolds, which is normally not present in meniscus tissue. If the remodeling of the initial vascularized fibrous tissue into this avascular cartilage-like tissue is an end stage of differentiation, further differentiation into the typical meniscus tissue may be blocked. Therefore, scaffolds with a shorter degradation period might be an option, which could enable the differentiation toward the more fibrocartilaginous tissue. This might also prevent the relatively unwanted differentiation into cartilage-like tissue.

A third reason for a lack of differentiation might be inadequate nutrition of the ingrown tissue by the remaining polymer. The damage of the implants might have resulted in suboptimal loading conditions, which would have had an additional role in the insufficient nutrition but also might have played a role in the lack of remodeling toward the typical meniscus fibrocartilage.

Although a differentiation into the typical organization of the native meniscus was not observed, the compression modulus of the implants was significantly improved by the tissue ingrowth. A high compression modulus of a meniscus or of a meniscus implant is important to resist the high loading forces and to distribute these loads across a greater surface and is therefore important in the role of stabilization and alignment of the knee.²⁸ The mechanical analysis in this follow-up study showed no difference in compression modulus between the implants between 6 and 24 months, and the curves were similar to those previously described in the short-term studies.^{40,41} The mechanical properties were intermediate between native meniscus and scaffold without tissue ingrowth. On the basis of in vitro degradation studies, it can be expected that the PCLPU has lost all mechanical strength after 1 year of implantation.¹⁴ Therefore, we assume that a stiffer tissue had been formed between 6 and 24 months, which partly compensates for the loss of mechanical strength of the polymer. The strength in the circumferential direction was not sufficient. The collagen architecture had a more mesh-like appearance instead of the circumferential orientation in the native meniscus. Further studies should work to prove if this circumferential strength can compete with the native situation if optimized implants are developed.

The previous 6-month studies showed that cartilage degeneration had started during the first months in vivo.^{40,41} This was again observed in the present study. We speculated that the improved surface properties of the implant would have a beneficial effect on chondroprotection, but that was not found in this study. After 24 months, no

difference was observed between the meniscectomy group and the implant group. Although the ink staining did not show the extrusion of the implant, it might be speculated that the implant was unable to resist the extrusion forces in the knee joint during in vivo loading. This might have led to comparable peak stresses in implant and meniscectomy joints with comparable damage to the articular cartilage.

The results on cartilage degradation should also be considered in light of the small beagle knee in which complicated open surgery is inevitably associated with a relatively large trauma reaction. This trauma is induced by the meniscectomy itself and by creating the drill holes in the tibial plateau. Also, the effect of the popliteus tendon needs to be considered in future animal knee models. We have to adjust the surgical procedure to protect the tendovaginal sleeve, thereby preventing the tendon from entering the knee joint. Clinically, it could be considered to treat patients with a polymer implant but with an operation technique similar to that of the collagen meniscus implant, in which the outer rim of the meniscus is left in situ.^{37,38} In such a procedure, the outer rim protects the implant from overloading and will prevent the implant from being pushed into a peripheral position, which will lead to almost normal contact stresses between femur and tibia.

CONCLUSION

The PCLPU meniscus implant is a good scaffold for ingrowth of fibrovascular tissue. The implants were fully integrated into the tissue without formation of capsule. The foreign body reaction was very mild and did not exceed grade I. Even in cases where loose particles were shown in the synovium, foreign body reaction was very mild (grade I). The process of differentiation into the typical meniscus-like tissue, that is, tissue with a specific structural organization and a fibrocartilage phenotype, was lacking after both 6 and 24 months. The porous polymer scaffold was histologically not noticeably degraded after 24 months, and cell death was observed in the central region of the anterior parts of the implant. The stiffness of the implants after 24 months was intermediate between scaffolds before implantation and native meniscus tissue. Articular cartilage degeneration in knees containing a meniscus implant was similar to that in knees that underwent meniscectomy only. Further improvements in the implant, model, and surgical technique are essential for the adequate protection of articular cartilage.

ACKNOWLEDGMENT

The authors gratefully acknowledge Natasja Lieuwes for her technical assistance. This research was funded by the Dutch Technology Foundation.

REFERENCES

1. Amenta PS, Gay S, Vaheri A, Martinez-Hernandez A. The extracellular matrix is an integrated unit: ultrastructural localization of collagen types I, III, IV, V, VI, fibronectin, and laminin in human term placenta. *Coll Relat Res*. 1986;6(2):125-152.
2. Bruns J, Kahrs J, Kampen J, Behrens P, Plitz W. Autologous perichondral tissue for meniscal replacement. *J Bone Joint Surg Br*. 1998;80(5):918-923.
3. Cheung HS. Distribution of type I, II, III, and V in the pepsin solubilized collagens in bovine menisci. *Connect Tissue Res*. 1987;16(4):343-356.
4. Cox JS, Nye CE, Schaefer WW, Woodstein IJ. The degenerative effects of partial and total resection of the medial meniscus in dogs' knees. *Clin Orthop Relat Res*. 1975;109:178-183.
5. Cummins JF, Mansour JN, Howe Z, Allan DG. Meniscal transplantation and degenerative articular change: an experimental study in the rabbit. *Arthroscopy*. 1997;13(4):485-491.
6. de Groot JH, de Vrijer R, Pennings AJ, Klompmaker J, Veth RP, Jansen HW. Use of porous polyurethanes for meniscal reconstruction and meniscal prostheses. *Biomaterials*. 1996;17(2):163-173.
7. de Groot JH, de Vrijer R, Wildeboer BS, Spaans CJ, Pennings AJ. New biomedical polyurethane ureas with high tear strengths. *Polymer Bulletin*. 1997;11(38):211-218.
8. de Groot JH, Nijenhuis AJ, Bruin P, et al. The use of biodegradable porous polymer implants in meniscus reconstruction. 1. Preparation of porous biodegradable polyurethanes for the reconstruction of meniscus lesions. *Colloid & Polymer Science*. 1990;268:1073-1081.
9. de Groot JH, Spaans CJ, Dekens FG, Pennings AJ. On the role of aminolysis and transesterification in the synthesis of ϵ -caprolactone and L-lactide-based polyurethanes. *Polymer Bulletin*. 2007;41:299-306.
10. DeHaven KE. The role of the meniscus. In: Ewing JW, ed. *Articular Cartilage and Knee Joint Function: Basic Science and Arthroscopy*. New York, NY: Raven Press Ltd; 1990:103-115.
11. Fithian DC, Kelly MA, Mow VC. Material properties and structure-function relationships in the menisci. *Clin Orthop Relat Res*. 1990;252:19-31.
12. Ghosh P, Taylor TK. The knee joint meniscus: a fibrocartilage of some distinction. *Clin Orthop Relat Res*. 1987;224:52-63.
13. Heijkants RG, Calck RV, van Tienen TG, et al. Uncatalyzed synthesis, thermal, and mechanical properties of polyurethanes based on poly(ϵ -caprolactone) and 1,4-butane diisocyanate with uniform hard segment. *Biomaterials*. 2005;26(20):4219-4228.
14. Heijkants RG, van Calck RV, De Groot JH, et al. Design, synthesis, and properties of a degradable polyurethane scaffold for meniscus regeneration. *J Mater Sci Mater Med*. 2004;15(4):423-427.
15. Jaureguito JW, Elliot JS, Lietner T, Dixon LB, Reider B. The effects of arthroscopic partial lateral meniscectomy in an otherwise normal knee: a retrospective review of functional, clinical, and radiographic results. *Arthroscopy*. 1995;11(1):29-36.
16. Kahn AR, Kahane JC. India ink pinprick assessment of age-related changes in the cricoarytenoid joint (CAJ) articular surfaces. *J Speech Hear Res*. 1986;29(4):536-543.
17. Kambic HE, McDevitt CA. Spatial organization of types I and II collagen in the canine meniscus. *J Orthop Res*. 2005;23(1):142-149.
18. Klompmaker J, Veth RP, Jansen HW, Nielsen HK, de Groot JH, Pennings AJ. Meniscal replacement using a porous polymer prosthesis: a preliminary study in the dog. *Biomaterials*. 1996;17(12):1169-1175.
19. Kobayashi M, Toguchida J, Oka M. Preliminary study of polyvinyl alcohol-hydrogel (PVA-H) artificial meniscus. *Biomaterials*. 2003;24(4):639-647.
20. Kohn D, Verdonk R, Aagaard H, Seil R, Dienst M. Meniscal substitutes—animal experience. *Scand J Med Sci Sports*. 1999;9(3):141-145.
21. Kohn D, Wirth CJ, Reiss G, et al. Medial meniscus replacement by a tendon autograft: experiments in sheep. *J Bone Joint Surg Br*. 1992;74(6):910-917.
22. Linsenmayer TF, Hendrix MJ. Monoclonal antibodies to connective tissue macromolecules: type II collagen. *Biochem Biophys Res Commun*. 1980;92(2):440-446.
23. Maletius W, Messner K. The effect of partial meniscectomy on the long-term prognosis of knees with localized, severe chondral damage: a 12- to 15-year follow-up. *Am J Sports Med*. 1996;24(3):258-262.

24. Messner K. Meniscal substitution with a Teflon-periosteal composite graft: a rabbit experiment. *Biomaterials*. 1994;15(3):223-230.
25. Messner K, Kohn D, Verdonk R. Future research in meniscal replacement. *Scand J Med Sci Sports*. 1999;9(3):181-183.
26. Messner K, Lohmander LS, Gillquist J. Cartilage mechanics and morphology, synovitis, and proteoglycan fragments in rabbit joint fluid after prosthetic meniscal substitution. *Biomaterials*. 1993;14(3):163-168.
27. Mine T, Kimura M, Sakka A, Kawai S. Innervation of nociceptors in the menisci of the knee joint: an immunohistochemical study. *Arch Orthop Trauma Surg*. 2000;120(3-4):201-204.
28. Mow VC, Ratcliffe A, Chern KY, Kelly MA. Structure and function relationships of the menisci of the knee. In: Mow VC, Arnoczky SP, Jackson DW, eds. *Knee Meniscus: Basic and Clinical Foundations*. New York, NY: Raven Press Ltd; 1992:37-57.
29. Puchtler H, Meloan SN, Waldrop FS. Are picro-dye reactions for collagens quantitative? Chemical and histochemical considerations. *Histochemistry*. 1988;88(3-6):243-256.
30. Reguzzoni M, Manelli A, Ronga M, Raspanti M, Grassi FA. Histology and ultrastructure of a tissue-engineered collagen meniscus before and after implantation. *J Biomed Mater Res B Appl Biomater*. 2005;74(2):808-816.
31. Rijk PC, Tigchelaar-Gutter W, Bernoski FP, Van Noorden CJ. Functional changes in articular cartilage after meniscal allograft transplantation: a quantitative histochemical evaluation in rabbits. *Arthroscopy*. 2006;22(2):152-158.
32. Saygi B, Yildirim Y, Berker N, Ofluoglu D, Karadag-Saygi E, Karahan M. Evaluation of the neurosensory function of the medial meniscus in humans. *Arthroscopy*. 2005;21(12):1468-1472.
33. Setton LA, Guilak F, Hsu EW, Vail TP. Biomechanical factors in tissue-engineered meniscal repair. *Clin Orthop*. 1999;367:S254-S272.
34. Spaans CJ, de Groot JH, Dekens FG, Pennings AJ. High molecular weight polyurethanes and a polyurethane urea based on 1,4-butanediisocyanate. *Polymer Bulletin*. 1996;41:131-138.
35. Stone KR. Meniscus replacement. *Clin Sports Med*. 1996;15(3):557-571.
36. Stone KR, Rodkey WG, Webber R, McKinney L, Steadman JR. Development of a prosthetic meniscal replacement. In: Mow VC, Arnoczky SP, Jackson DW, eds. *Knee Meniscus: Basic and Clinical Foundations*. New York, NY: Raven Press Ltd; 1992:165-173.
37. Stone KR, Rodkey WG, Webber R, McKinney L, Steadman JR. Meniscal regeneration with copolymeric collagen scaffolds: in vitro and in vivo studies evaluated clinically, histologically, and biochemically. *Am J Sports Med*. 1992;20(2):104-111.
38. Stone KR, Rodkey WG, Webber RJ, McKinney L, Steadman JR. Future directions: collagen-based prostheses for meniscal regeneration. *Clin Orthop*. 1990;252:129-135.
39. Stone KR, Steadman JR, Rodkey WG, Li ST. Regeneration of meniscal cartilage with use of a collagen scaffold: analysis of preliminary data. *J Bone Joint Surg Am*. 1997;79(12):1770-1777.
40. Tienen TG, Heijkants RG, de Groot JH, et al. Replacement of the knee meniscus by a porous polymer implant: a study in dogs. *Am J Sports Med*. 2006;34(1):64-71.
41. Tienen TG, Heijkants RG, de Groot JH, et al. Meniscal replacement in dogs: tissue regeneration in 2 different materials with similar properties. *J Biomed Mater Res B Appl Biomater*. 2006;76(2):389-396.
42. van Arkel ER, de Boer HH. Survival analysis of human meniscal transplantations. *J Bone Joint Surg Br*. 2002;84(2):227-231.
43. Van Tienen TG, Heijkants RG, Buma P, de Groot JH, Pennings AJ, Veth RP. Tissue ingrowth and degradation of 2 biodegradable porous polymers with different porosities and pore sizes. *Biomaterials*. 2002;23(8):1731-1738.
44. Verdonk R, Kohn D. Harvest and conservation of meniscal allografts. *Scand J Med Sci Sports*. 1999;9(3):158-159.
45. Veth RP, den Heeten GJ, Jansen HW, Nielsen HK. An experimental study of reconstructive procedures in lesions of the meniscus: use of synovial flaps and carbon fiber implants for artificially made lesions in the meniscus of the rabbit. *Clin Orthop*. 1983;181:250-254.
46. Walker PS, Erkman MJ. The role of the menisci in force transmission across the knee. *Clin Orthop*. 1975;109:184-192.