Replacement of the Knee Meniscus by a Porous Polymer Implant

A Study in Dogs

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Background: Meniscectomy will lead to articular cartilage degeneration in the long term. Therefore, the authors developed an implant to replace the native meniscus.

Hypothesis: The porous polymer meniscus implant develops into a neomeniscus and protects the cartilage from degeneration.

Study Design: Controlled laboratory study.

Methods: In a dog model, a porous polymer scaffold with optimal properties for tissue infiltration and regeneration of a neomeniscus was implanted and compared with total meniscectomy. The tissue infiltration and redifferentiation in the scaffold, the stiffness of the scaffold, and the articular cartilage degeneration were evaluated.

Results: Three months after implantation, the implant was completely filled with fibrovascular tissue. After 6 months, the central areas of the implant contained cartilage-like tissue with abundant collagen type II and proteoglycans in their matrix. The foreign-body reaction remained limited to a few giant cells in the implant. The compression modulus of the implant-tissue construct still differed significantly from that of the native meniscus, even at 6 months. Cartilage degeneration was observed both in the meniscectomy group and in the implant group.

Conclusion: The improved properties of these polymer implants resulted in a faster tissue infiltration and in phenotypical differentiation into tissue resembling that of the native meniscus. However, the material characteristics of the implant need to be improved to prevent degeneration of the articular cartilage.

Clinical Relevance: The porous polymer implant developed into a polymer-tissue construct that resembled the native meniscus, and with improved gliding characteristics, this prosthesis might be a promising implant for the replacement of the meniscus.

Keywords: meniscus; animal study; prosthesis; polymer

The knee menisci are wedge-shaped, semilunar discs of fibrocartilage, interposed between the tibia and the condyles of the femur. Functions of the meniscus are load bearing and load distribution, shock absorption, joint lubrication, and stabilization of the knee joint.6,21

Lesions in the meniscus are frequently observed in today’s orthopaedic practice. The number of lesions will probably further increase owing to the increasing interest in a healthy lifestyle and interest in active sports participation at older ages. Only a few decades ago, it became clear that total meniscectomy leads to articular cartilage degeneration, and from then, it has been generally recognized that the amount of meniscal tissue removed should be minimized.7,26,30 In the lateral meniscus, the peripheral rim of the meniscus and its insertions play a particularly relevant role in the cartilage protective function.6 In the medial knee compartment, the degenerative changes appeared to be directly proportional to the amount of meniscus removed. Resection of the posterior one third in dogs has resulted in predisposition to poor radiologic result.4 In many cases, the large extent of meniscal damage makes a (sub)total meniscectomy inevitable. In these cases, replacement of the resected meniscal tissue by an implant might avoid the articular cartilage degeneration. A number of groups have tried to replace the meniscus with autologous materials such as fat tissue,13 perichondrium,2 and...
tendon.\textsuperscript{14} However, the poor initial mechanical properties make long-term fixation problematic. Synthetic permanent implants made of Dacron (in rabbits)\textsuperscript{19} and Teflon (dogs)\textsuperscript{27} have also been used to replace the meniscus, but wear of the prosthetic material seemed to initiate severe synovial reactions. Allograft transplantations are being performed in a clinical setting.\textsuperscript{26} However, problems related to the availability, preservation techniques, the possible transfer of diseases, the individual shaping of the implant, and possible immunologic reactions to the implant are recognized worldwide.\textsuperscript{24}

To avoid all problems related to the above-mentioned replacement techniques, our long-term aim was to generate a completely new meniscus by in vivo tissue engineering. This ambitious goal may be reached with the insertion of a biodegradable porous polymer that acts as a temporary scaffold for regenerating meniscal tissue. The regenerative capacity of the synovial tissue is well known from the formation of a neomeniscus after a meniscectomy\textsuperscript{9} and from experiments in which a partial meniscectomy was reconstructed with a polymer foam.\textsuperscript{10} Hence, we expect that the empty scaffold will be filled with regenerative tissue. During slow degradation of the polymer material and simultaneous differentiation of the ingrown tissue into the typical fibrocartilaginous tissue of the native meniscus, the original situation from before the trauma may be restored.

Therefore, a meniscus-like substitute might be formed in a biodegradable porous polymer scaffold with the optimal compression modulus, porosity, and pore sizes. The properties of the scaffold should meet certain criteria. To enable rapid tissue infiltration, the volume fraction of the biomaterial should be as low as possible, and to enable complete infiltration, the scaffold should have a homogeneous distribution of large, interconnected pores. The degradation of the biomaterial should occur slowly to enable differentiation into fibrocartilage. Earlier studies have shown that the initial mechanical properties of the porous polymer determine the fate of the newly formed regenerative tissue; if the compression modulus of the starting material is below 150 kPa, only fibrous tissue is produced.\textsuperscript{5} Therefore, in this study, we used a new polymer that combines the optimal macroporosity of 78\% with a compression modulus of 300 kPa and a slow degradation rate.\textsuperscript{5,11}

The new scaffold material was evaluated and compared with total meniscectomy in the dog model. The analysis focused on the speed of the infiltration of tissue into the scaffold (histology), on the transformation of nondifferentiated ingrown tissue into fibrocartilage (immunohistochemistry), and on the comparison of the compression modulus of the explants with that of the native meniscus (mechanical testing). Finally, articular cartilage degeneration resulting from meniscectomy versus meniscal replacement was assessed.

**MATERIALS AND METHODS**

**Polymer**

Implants consisted of biodegradable Estane polymers (5701-F1, BF Goodrich Chemical, NV Westerlo-Oevel, Belgium).
group and 6 in the prosthesis group. The same occurred after 6 months.

Histology

After the dogs were sacrificed, the implants were resected, and a 4-mm full-thickness biopsy was taken from the prosthetic posterior horn for biomechanical testing. The implant was fixed in acetone (–20°C) for 6 hours, infiltrated in methyl methacrylate, and polymerized at –20°C for 2 days. Sections (7 µm) were cut in a transverse plane, dried at 37°C, deacrylated 3 times in chloroform-xylol (1:1) for 15 minutes, and stained with hematoxylin-eosin and toluidine blue (TB). The polymer was hardly visible on the sections; however, the presence of the polymer in the apparently void spaces was confirmed by staining the sections with Sudan black. For immunohistochemistry, 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 nonspecific 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). Monospecific polyclonal rabbit anticollagen type I antibody1 (PS-41, antihuman raised in rabbit, Sanbio, Uden, the Netherlands) and mouse anticollagen II antibody14 (II-II6B3, antichicken raised in mouse, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, Iowa) were applied, and the samples were incubated in a humidified chamber overnight at 4°C. Anticollagen antibodies were detected using a biotin-labeled antirabbit antibody (1/200 dilution, Dako, Carpinteria, Calif) and antimouse antibody (1/600 dilution, Dako) for 1 hour at room temperature. A biotin-streptavidin detection system (Vector 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 PBS with 12 µL H2O2 (30%) for 7 minutes. After rinsing, sections were dehydrated and mounted with DPX (BDH, Poole, England).

Blocks of the tibial plateau and femoral condyles were fixed in a buffered formaldehyde solution (4%, pH 7.4) for 2 days and rinsed, dehydrated, and embedded in methyl methacrylate for 2 days.

Microscopy

On an ordinal scale, sections were scored for integration between implant and capsule (percentage attachment) and tissue infiltration into the implant (percentage of pores filled with tissue). The amounts of proteoglycan staining (percentage positive toluidine blue staining on the total amount of ingrown tissue) and collagen type I and II labeling (percentage positive antibody labeling on the total amount of ingrown tissue) were determined by using the Quantimet 520 Image Analysis System (Cambridge Instruments, Cambridge, England) and compared with the mean distribution in 3 native menisci. The mean percentage of positive staining on the total amount of ingrown tissue was determined in 2 sections through the center of the polymer implant with 200 µm in between. Furthermore, the phenotypes of the cells in the implant were evaluated and classified as fibrous, as cartilage like, or as a combination of both. 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 [PMN] leukocytes, grade 2), moderate inflammation (many macrophages/giant cells with few PMN leukocytes, grade 3), and severe inflammation (abundant macrophages, giant cells, and PMN leukocytes, grade 4).29

Articular Cartilage

Degenerative articular 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 Toluidine blue staining (grade 0) to no staining (grade 4), and an intact tidemark (grade 0) and a tidemark infiltrated with blood vessels (grade 1).16 The total score of each subcategory determined the Mankin score.
Biomechanical Analysis

After excision of the polymer-tissue construct from the dog’s knee, a 4-mm punch biopsy was taken of a specified region of the posterior horn of each implant and native meniscus. As a reference, punches were also taken from the porous polymer of which the implant was made. Compression testing was performed on the cylinder-shaped specimens in saline at room temperature using an Instron (4301) compression tester (Instron, Canton, Mass) equipped with a 100-N load cell. A compression rate of 2 mm/min was applied. The slope of the compression-stress curve was calculated for the native meniscus, the implant before implantation, and the implant at 3 months and 6 months after implantation.

Data Analysis

The results of the evaluation of cartilage degeneration were statistically evaluated using a 2-way analysis of variance (ANOVA). For the compression tests, differences between the meniscal tissue and the prosthesis after 3 and 6 months were analyzed with ANOVA and subsequent post hoc t tests (Tukey). P values were calculated, and values of less than .05 were considered significant.

RESULTS

Clinical Observations

The dogs had regained their normal gait patterns 14 days postoperatively. No infections were seen. All meniscectomized knees and knees with implants were available for evaluation. Postmortem, there were no signs of synovitis in the joint capsule, and the synovial fluid was clear.

Macroscopy (3 and 6 months)

After only 3 months, the implant was firmly attached to the peripheral capsule. In the popliteus tendon region, the tendon could freely move between the capsule and the peripheral rim of the implant. Also, the prosthetic horns were firmly attached to the tibial plateau. At this time, no tissue cover was visible on the surface of the implant. At gross inspection, variable damage of the articular cartilage was observed in both groups. The damage varied from an intact surface layer to degenerative lesions. No osteophyte formation had occurred, and the subchondral bone was never exposed. The damage in the prosthetic group did not evidently differ from that in the meniscectomy group.

At 6-month follow-up, the knee joints appeared similar. However, the popliteus tendon seemed to have entered the joint space and damaged the polymer implants in 3 cases. The tendon itself was never ruptured.

Microscopy (3 months)

Microscopically, the implant was intensively integrated with its periphery (75%-100% of the total peripheral prosthetic edge). All pores in the implants were completely infiltrated with vascularized fibrous tissue that had produced abundant extracellular matrix. This extracellular matrix showed abundant collagen type I antibody labeling throughout the implant. Proteoglycan staining and collagen type II labeling were absent.

Both in the meniscectomy group and in the implant group, the adjacent synovium did not show any signs of inflammatory reaction. In the implant, a slight inflammatory reaction was present with scarce macrophages and giant cells in the pores. These cells were organized in close contact with the surface of the polymer. Polymorphonuclear leukocytes were absent.

Implantation of an implant led to tibial cartilage lesions localized adjacent to the inner rim of the implant, whereas the tibial lesions after meniscectomy were spread over a greater area. In both groups, the damage on the femoral side was present over a broader area at the posterior curvature of the condyle. Degenerated areas showed varying degrees of surface fibrillation, cloning of the cells, and decreased toluidine blue staining, but the subchondral bone was never exposed in either of the groups. After 3 months, the Mankin score in the implant group did not differ from the score in the meniscectomy group, whereas the tibial lesions after meniscectomy were spread over a greater area. In both groups, the damage on the femoral side was present over a broader area at the posterior curvature of the condyle. Degenerated areas showed varying degrees of surface fibrillation, cloning of the cells, and decreased toluidine blue staining, but the subchondral bone was never exposed in either of the groups. After 3 months, the Mankin score in the implant group did not differ from the score in the meniscectomy group, for both the tibia and the femur. In both groups, more damage seemed to have occurred at the tibial side than at the femoral side (Figure 3), although the differences were not statistically significant (tibial vs femoral degeneration in meniscectomy group, P = .388; in implant group, P = .563).

Microscopy (6 months)

In all cases, the implant was integrated with the peripheral capsule and completely filled with tissue. In the peripheral half of the implant, the infiltrated tissue had a fibrovascular phenotype with spindle-shaped cells surrounded by extracellular matrix that showed an abundant labeling with collagen type I antibody labeling (Figures 4 A and B). These collagen bundles penetrated the channels between the macro pores. In these areas, no toluidine blue staining and collagen type II antibody labeling were observed.

The central half of the implant had a more fibrocartilage-like phenotype with characteristic round cells lying in their
lacunae. Their extracellular matrix showed positive staining for toluidine blue and positive labeling for collagen type II antibodies (Figure 4C). The areas of collagen type II labeling completely matched the areas of proteoglycan staining in the adjacent sections (Figure 4D). In areas where collagen type II antibody labeling was present, the labeling of collagen type I antibodies was evidently less, which resulted in a nonsignificant decline of the total amount of collagen type I labeling after 6 months (Figure 5).

In the native meniscus, abundant collagen type I labeling was especially visible in the peripheral regions (mean, 69.6% of the total tissue area in the implant), whereas 32.9% of the total tissue area showed positive labeling with collagen type II antibodies and toluidine blue, especially near the inner rim of the meniscus. This collagen type II labeling and proteoglycan staining were especially localized in the inner rim of the meniscus (Figure 5).

After 6 months, the inflammatory reaction tended to be increased compared with that at 3 months but was still classified as slight. More giant cells were observed along the surface of the pores in the polymer than after 3 months.

The histologic aspect of the articular cartilage had not deteriorated after 6 months, which was confirmed by the Mankin score (Figure 3). In the implant group, more damage seemed to have occurred on the tibial side, whereas the femoral damage had increased in the meniscectomy group. However, these differences were not statistically significant.

Compression Tests

The slope of the compression-stress curves increased after implantation in the knee joint (Figure 6). There was still a great difference between the meniscal tissue and the prosthesis after 3 and 6 months ($P < .001$). From 3 until 6 months, the stiffness did not increase significantly because of the high SDs (prosthesis at 3 vs 6 months, .927).

**Figure 5.** Percentage labeling with collagen type I and II or TB of the total amount of tissue in the meniscus and implant. Collagen type II antibody labeling and TB staining were observed after 6 months. Note that the amounts of collagen type I and II and proteoglycans in the implants approached the amounts in the native meniscus. Column 1, collagen type I antibody labeling; column 2, collagen type II antibody labeling; TB, toluidine blue staining.

**Figure 4.** Micrographs of sections of the implant-tissue construct 6 months after implantation. The void spaces are filled with polymer. A, implant labeled with collagen type I antibodies. Note abundant staining in the peripheral regions of the meniscus and less labeling near the inner rim. B, magnification of a region in the peripheral zone of the implant: tissue with many blood vessels and intensive labeling of the collagen bundles. Collagen type I antibody labeling, ×100. C and D, adjacent sections of tissue near the inner rim of the implant. Note the cartilage-like phenotype of the cells and the abundant collagen type II antibody labeling (C) and toluidine blue staining (D) of their matrix (×100).
DISCUSSION

In the present study, the native lateral meniscus in the dog’s knee was replaced by an improved porous polymer implant. The tissue infiltration and differentiation in the implant and the consequences for the articular cartilage were evaluated.

In the early 1980s, a study described regeneration of meniscal tissue after total meniscectomy and mentioned the importance of the synovium as a source for the newly formed tissue. In several animal studies and even clinically, collagen-based biomaterials were used as a scaffold for the regenerating tissue. However, this technique depends on a remaining native meniscal rim as a source for the neomeniscal tissue. Until now, total meniscus replacement with a collagen scaffold has not been described. It is likely that the mechanical properties of this scaffold are insufficient for this application. Klompmaker et al. were the first to replace the entire meniscus with similar porous polymer implants as used in the present study. These implants seemed to provide an appropriate surrounding for mesenchymal tissue infiltration and for differentiation into meniscus-like tissue. The low compression modulus of the implant (150 kPa vs 400 kPa of the normal meniscal tissue) could still have been insufficient to withstand the high loading forces in the knee joint. This factor might have impaired the stability of the knee joint. Also, a higher stiffness seemed to stimulate the differentiation of the infiltrated fibrovascular tissue into fibrocartilage. Therefore, for the present study, implants were developed with a higher compression modulus (300 kPa). Furthermore, the pore size in the newly developed implants was increased to improve the infiltration rate of tissue (155-355 µm). In the former study, tissue infiltration depended on micropores (< 90 µm) combined with macropores (150-300 µm), and the tissue infiltration was incomplete until 18 weeks, whereas in the present study, the implant was completely filled 3 months after implantation. The change in pore interconnectivity in the polymer might have been responsible for this increased tissue infiltration rate.

Distribution

Ghadially et al., Webber, and McDevitt and Webber described meniscal fibrocartilage as a tissue containing chondrocytic cells called fibrochondrocytes, which are surrounded by an abundant extracellular matrix. This matrix mostly contains collagen type I, but near the central rim of the meniscus, type II collagen and proteoglycans are also present, which are major components of hyaline cartilage. This distribution of extracellular matrix appeared to represent its function. The collagen type I in native peripheral meniscal tissue provides the circumferential tensile strength to resist the hoop stresses during loading of the joint. The type II collagen is able to resist compressive forces and therefore is found in the central rim of the meniscus, where the force transduction between the femur and tibia is highest. In the present study, a similar distribution of fibrous and cartilage-like tissue with their matrix products was observed in the native meniscus but also in the implants 6 months after implantation. The similarity of location-specific differentiation of tissue between the native meniscus and the implant could suggest that the implant approached the functional behavior of the native meniscus in the knee joint. The authors expect that during degradation of the implant, the amount of collagen type I bundles and their orientation will further adapt under influence of the load in the knee joint. The polymer implant is expected to start degrading and to lose its mechanical characteristics approximately 40 weeks after implantation. This action enables the tissue to complete the process of infiltration and differentiation into neomeniscal tissue.

Compression Modulus

The curves as presented in Figure 6 show the compression behavior of the materials compared with the native meniscal tissue. By presenting the complete curves, the compression modulus (stiffness) of the material can be determined by calculating the slope of the curves at the different levels of stress that can be present in the knee joint. These compression moduli can subsequently be compared with those of the native meniscal tissue. In this experiment, the slope (compression modulus) of the prosthesis, 3 and 6 months after implantation, was greater than before implantation. There was no significant difference between the stiffness at 3 and 6 months. However, the meniscal tissue tended to reach the final slope in an earlier...
stage, which means that the prostheses were compressed more to reach that certain stress level. This factor might be attributed to lower stiffness of the polymer directly after implantation in the knee joint. During the filling of the void spaces in the polymer implant, the stiffness and further maturation of the tissue into fibrocartilage might be beneficial for stiffness and, thus, the mechanical functioning of the implant in the knee joint. Especially in the role of stabilization and alignment of the knee joint, a high compression modulus is important for the functioning of the implant.21 In physiologic circumstances, the high compression modulus of the meniscus leads to a restricted compression of the meniscus during axial loading. The remaining forces extrude the wedge-shaped meniscus from the knee joint and are transduced via the meniscal horns to the tibial plateau. Consequently, when the meniscus is absent, the forces in the subchondral bone seemed to be 2 to 5 times higher than with the meniscus present.8

Foreign-Body Reaction

After 3 months and 6 months, the foreign-body reaction to the polymer implant remained restricted to multinuclear macrophages, which were adjacent to the borders of the polymer. The PMN leukocytes were never observed. The macrophage affinity for the rough surfaces of the polymer pores and the first release of polymer degradation products could encourage the formation of giant cells through constant recruitment of newly arriving macrophages.17 However, in addition to the phagocytic capacity of giant cells, these cells are capable of releasing lysosomal acid hydrolases, and these enzymes may provide a method for the extracellular degradation of any opposed undigested material.20 The polyester polyurethane (Estane) is susceptible to enzymes released during this foreign-body reaction in addition to its own hydrolytic and oxidative degradation.21 Furthermore, these cells also release angiogenic factors that stimulate blood vessel development toward the polymer implant.30

Cartilage Degeneration

The main function of a meniscal replacement is to prevent severe long-term articular cartilage damage. In the present study, articular cartilage damage was observed both after meniscectomy and after implantation of the implant. The severity of degeneration was highly variable among the cases. This factor was also true after meniscectomy. Consequently, no significant differences in cartilage degeneration could be observed between the meniscectomy and implant groups and between the follow-up periods. Thus, the porous polymer implant could not prevent cartilage degeneration. This factor might be owing to the relatively rough polymer surface. The implants were cut and modeled from a porous polymer block to the shape of the native meniscus during the surgical procedure. Nevertheless, scanning electron microscope examination of the prosthetic surface revealed the inevitable irregularities on the prosthetic surface. Producing these implants with a mold may provide scaffolds with a smooth surface and with the desired standard form. In this way, all implants will be identical, which may decrease the variance in cartilage degeneration. Furthermore, the authors speculate that degeneration merely had taken place during the first months, when the prosthetic surface, not covered with tissue, was in direct contact with the articular cartilage. A tissue layer between the polymer material and the articular cartilage might have more gliding capacity than would the bare polymer surface itself. Between 3 and 6 months after implantation, the whole implant was covered with a tissue layer, and longer term studies will be needed to prove if further cartilage degeneration will be prevented. It is likely that the prosthesis can be coated with tissue by means of in vitro tissue engineering before implantation in the knee joint. Currently, these studies are being performed.

When extrapolating these results to a human situation, several factors should be taken into account. For example, Adams and Brandt1 suggested a reparative capacity of the articular cartilage in dogs. This study reported an active synthetic response by the chondrocytes after ACL transection, resulting in hypertrophic cartilage repair. In this way, the response of the canine cartilage seemed to differ from that of human articular cartilage, in which loss of cartilage mass and proteoglycan synthesis is recognized as characteristic end-stage osteoarthritis.17 Also, it should be emphasized that the axial loading pattern in the rather extended knees of man differs from the loading pattern in the flexed knees of dogs. Furthermore, the small size of the Beagle knee joints made the surgery difficult and therefore more sensitive for iatrogenic damage. These factors all influence the results of these studies.

A popliteus tendon that enters the knee joint space might also contribute to cartilage degeneration. During surgery, a vertical arthrotomy is performed, and the dorsal flap is completely mobilized to obtain exposure of the knee joint. By dissecting the dorsal flap from the tibia and meniscus, the tendon sheath of the popliteus tendon might have been damaged, which eventually led to loosening of the tendon from the periphery. It is likely that the popliteus tendon lying in the knee joint damaged the implant and the cartilage. In future studies, we need to limit the dissection in the dorsal flap to prevent dissection of the tendon sheet.

In conclusion, regeneration of new meniscus seems to be possible by in vivo tissue engineering. The optimal properties of these polymer implants resulted in fast infiltration of fibrovascular tissue into the implant and in a location-specific phenotypic differentiation of this tissue. Only a very mild foreign-body reaction was observed in and around the polymer. The compression modulus of the implant-tissue construct still significantly differed from that of the native meniscus, even at 6 months. In this short-term study, cartilage degeneration could not be prevented. However, the authors speculate that in the long term, when the implant is completely infiltrated and surrounded with tissue, the gliding characteristics of the construct will improve. This situation might end the progression of the degeneration. Nevertheless, in the development of an implant for total replacement of the heavily damaged meniscus, the results of this experiment are very promising.
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REFERENCES