Meniscal Replacement in Dogs. Tissue Regeneration in Two Different Materials With Similar Properties


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Abstract: In earlier studies, meniscal replacement with a porous polymer implant led to regeneration of neo-meniscal tissue. To evaluate the influence of the chemical properties on the tissue regeneration in the implant, in the present study, the meniscus in the dog’s knee was replaced with either an aromatic 4,4-diphenylmethanediisocyanate based polyesterurethane implant (Estane) or with an aliphatic 1,4-butanediisocyanate based polyesterurethane implant (PCLPU). After 6 months, the knee joints were resected and the tissue behavior in the two different prostheses was evaluated microscopically. In both prostheses, a meniscus-like distribution of the tissue phenotype was found with collagen type I in the peripheral fibrous zones and collagen type II in the central, more cartilaginous zones. The compression-stress behavior of the implant-tissue construct remained in between the stiffness of the polymer material and that of the native meniscus. The PCLPU implant seemed to provoke less synovial tissue reaction. After meniscectomy solely, in 5 out of 6 cases, a meniscus-like regenerate was formed. Furthermore, the articular cartilage degeneration after placing a PCLPU implant did also not exceed the degeneration after the Estane implant or after meniscectomy. The differences between these two implants did not seem to influence the tissue regeneration in the implant. However, PCLPU seemed to evoke less tissue reaction and, therefore, is thought to be less or even nontoxic as compared with the Estane implant. Therefore, for studies in the future, the authors prefer the PCLPU prostheses for replacement of the meniscus.


Keywords: meniscus; replacement; polycaprolacton; implant; polyurethane

INTRODUCTION

The menisci are C-shaped discs interposed between the femoral condyles and tibial plateau and have the function of shock absorption, stabilization, load distribution, and lubrication of the knee joint. The meniscus contains fibrochondrocytes, with an abundant extracellular matrix. The peripheral regions of the meniscus contain much collagen type I to resist the tensile stresses. The central regions contain merely type II collagen to resist compressive forces between the femur and the tibia.

Since the classical studies by Fairbank and King,2,3 it has been recognized that meniscectomy leads to articular cartilage degeneration. Also, the degeneration of the cartilage seemed proportional to the amount of tissue removed4 and resection of lesions in the anterior and posterior horn had a worse prognosis.5 Today, the method of choice is partial meniscectomy to preserve as much meniscal tissue as possible. However, clinicians have noticed a high incidence of arthritic changes in the joint in mid- and long-term outcome studies of partial meniscectomy.6–9

In different animal models, several groups replaced the meniscus with autologous materials like fat tissue,10,11 perichondrium,12 and tendon.13 However, the poor initial mechanical characteristics resulted in poor outcomes. Our group performed experiments in dogs and rabbits, using porous polymer scaffolds for repair of lesions in the avascular part of the meniscus,14–16 which showed that a fast infiltration of fibrous tissue into the polymer scaffold depends on the presence of interconnected macropores. Furthermore, a higher stiffness (compression modulus) of the scaffold seemed to stimulate the differentiation of fibrous tissue into fibrocartilage.14 However, the Estane (an aromatic 4,4-diphenylmethanediisocyanate based polyesterurethane) material used in these studies contained an aromatic 4,4-diphenylmethane compound that may degrade into toxic degradation products.
after implantation. Also, a slower degradation pattern was required to enable the tissue to infiltrate into the prosthesis and to differentiate into new meniscal tissue, without losing the biomechanical properties of the prosthesis. (Ramrattan et al. publication in progress). Therefore, an aliphatic 1,4-butanediisocyanate based polyesterurethane (PCLPU) was developed. This material met with these requirements. In the present study, the native meniscus of the dog was replaced with either the PCLPU implant or an Estane implant. These materials had a similar stiffness, porosity, and pore size. The infiltrated tissue was analyzed histologically on synovial tissue reaction, several extracellular matrix components, the stiffness of the prosthesis explants, and finally, the consequences for the articular cartilage degeneration were assessed.

The hypothesis was that the PCLPU prosthesis enables a similar tissue infiltration rate as the Estane based on the comparable architecture and even less tissue reaction on the material considering the lower degradation rate. In that case, the PCLPU would be preferred, also because of the nontoxic degradation products.

**MATERIALS AND METHODS**

**Polymer**

The Estane implants (5701-F1) (BF Goodrich Chemical N.V. Westerlo-Oevel, Belgium) consisted of a hard segment of 4,4-methylene-1,6-diphenyldiisocyanate. The content of hard segment mostly determines height of the compression modulus. The soft segment is based on poly(tetramethyl adipate). The porosity was 72.1%. This porosity was determined as follows. The mass of the polymer divided by the product of the density of the polymer and the volume of the scaffold. Hundred percent minus the amount of polymer results in the porosity of the polymer. The compression modulus at 20% compression was 260 kPa and the poresize 150–355 μm. The PCLPU polyurethanes consisted of a hard segment of 1,4-butanediisocyanate and butanediol and a soft segment of polycaprolactone initiated on 1,4-butanediol (PCL) with a length of 1600 g/mol (Aldrich Chemical) (Figure 1). The porosity of this material was 81.4%, the compression modulus 290 kPa, and the poresize 150–355 μm. Further characteristics are described by Heijkants et al. The synthesis of the Estane and PCLPU scaffolds were produced as described elsewhere: the Estane and PCLPU were dissolved at 80°C in either 1,4 dioxane and DMSO, respectively. Water was added as a non solvent to induce the liquid–liquid phase separation. Sucrose, 150–355 μm, was added to the Estane mixture to create the macropores in the scaffold. Then, the mixture was frozen at −18°C during which the water/dioxane was removed. The sucrose was washed out with water. In the PCLPU fabrication process, sodium chloride crystals were used instead of sucrose. Again, the scaffolds were washed with water and eventually with ethanol to wash out the DMSO. All pores were directly interconnected to achieve a high permeability of both polymers (Figure 2).

**Surgery**

We performed a lateral meniscectomy on 18 legs of 18 adult male and female Beagles. The average weight of the dogs was 13.2 kg (SD ± 2.6 kg). The institutional animal welfare committee approved all the procedures. Experiments were performed under aseptic conditions. Anesthesia was accomplished by intravenous administration of pentobarbital (30 mg/kg) and maintained after intubation with nitrous oxide (1:1) and isoflurane (0.5%). The knee joint was opened using a lateral skin incision. The capsule was opened longitudinally and dissected from the lateral attachment of the meniscus. We took great care not to injure the collateral and cruciate ligaments and the articular cartilage. Using the Beaver eyeblade (Waltham, Massachusetts, USA) the meniscus was separated from its anterior and posterior attachments. The prostheses were securely cut and modeled from a porous polymer block to the shape of the
ectomized native meniscus during the surgical procedure. In six knees, the meniscus was replaced by an Estane implant and in six knees by a PCLPU implant. Two drill holes were made in the lateral aspect of the proximal tibia, ending in the former anterior and posterior origin of the meniscal horns. The Estane material seemed to have a lower tear strength\(^{20}\) and therefore, it seemed necessary to lead two bonded nondegradable sutures through the Estane implant, parallel to the inner and outer rim (Figure 3). In the PCLPU implant, however, the same sutures were pulled through the implant horns only, because of the higher tear strength of this material. Further, the implantation procedures of both implants were identical. Subsequently, 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 The Netherlands) to realize close contact between synovial tissue and the meniscal implant. Afterwards, the capsule and skin were closed. The dogs were allowed to walk as soon as possible.

**Histology**

After 6 months, dogs were killed and the polymer tissue constructs were removed and fixed in acetone (−20°C) for 6 h, infiltrated in methylmethacrylate, and polymerized at −20°C for 2 days. Sections (7 microns) were cut, dried at 37°C, and stained with Haematoxylin–Eosine and Toluidine blue. In preparation for immunohistochemistry, sections were decarced three times in chloroform–xylol (1:1) for 15 min and subsequently treated with 1% testicular hyaluronidase (type I-S, EC 3.2.1.35; Sigma, St. Louis, MO, USA) and in PBS for 30 min 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, St. Louis, MO, USA). Monospecific monoclonal rabbit anti-collagen type I antibody (PS-41, anti-raised in rabbit, Sanbio, Uden, The Netherlands) and mouse anti-collagen II antibody (II-II6B3, anti-chicken raised in mouse, Developmental Studies Hybridoma bank, University of Iowa, USA)\(^{31}\) were applied, and the samples were incubated in a humidified chamber overnight at 4°C. Anti-collagen antibodies were detected using a biotin-labeled anti-rabbit antibody (1/200 dilution; Dako) and anti-mouse antibody (1/600 dilution; Dako) for 1 h at room temperature. A biotin–streptavidin detection system (Vectra elite kit, Vector, Burlingame, CA) was used according to the manufacturer’s recommendations. The peroxidase was detected using tablets containing 10 mg 3,3-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA) dissolved in 15 mL PBS with 12 μL H\(_2\)O\(_2\) (30%) for 7 min. After rinsing, sections were dehydrated and mounted with DPX (BDH, Poole, England).

Blocks of the complete 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 methylmetacrylate for 2 days. The synovium biopsies were from the most lateral area of directly next to the prosthesis. The samples were fixated in the same way.

**Figure 3.** Schematic presentation of the two operative procedures for either the Estane implant and the PCLPU implant. After resection of the native meniscus, two drill holes were created originating from the lateral tibial side (A) to the former attachments of the anterior and posterior horns of the native meniscus (B). In the Estane implant two nonresorbable sutures were pulled longitudinally through the implant (dotted lines) (C) and attached to the lateral proximal tibia. In the PCLPU implant, the sutures were only pulled through the horns. The periphery of the implant was attached to the capsule with resorbable sutures (D).
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 amount 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) was determined by using the Quantimet 520 Image Analysis System. The average percentage of positive staining on the total amount of ingrown tissue was determined in two sections through the center of the implant, with 200 microns in between. Further, 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 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).16

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) or a tidemark infiltrated with blood vessels (grade 1).22 The total score of each subcategory determined the Mankin score. The observer was blinded for the treatment.

After excision of the polymer-tissue construct from the dog’s knee, 4 mm biopsies were punched out from 5 out of the 6 explanted menisci/implants. These were taken from a specified region of the posterior horn of both the implants, as from the native meniscus. Also, biopsies were taken from the porous polymer before implantation. Nonconfined compression testing was performed on the cylinder shaped specimens in saline at room temperature, using an Instron (4301) compression tester, equipped with a 100 N load cell. A compression rate of 2 mm/min was applied.

Differences in cartilage degeneration were statistically evaluated by using the Kruskal–Wallis one way analysis of variance of ranks (ANOVA). Differences in tissue reaction between the two prostheses were evaluated using the Mann–Whitney rank sum test. p-Values were calculated, and values of less than 0.05 were considered to be significant.

RESULTS

The dogs had regained their normal gait pattern 14 days postoperatively. No infections were seen. All meniscectomized knees and knees with implant were available for evaluation. Postmortem analysis showed no signs of synovitis, and the synovial fluid was clear.

Macroscopically, both the Estane group as well as the PCLPU prostheses were completely integrated with the peripheral capsule and the prosthetic horns were firmly attached to the tibial plateau. All prostheses seemed to be completely covered by a transparent layer. In both the Estane group (3 cases) as well as the PCLPU implant group (4 cases), the popliteus tendon seemed to have entered the joint space and damaged the polymer implants. Macroscopically, it could not be determined if this interpolating tendon also damaged the articular cartilage.

In the group with meniscectomy solely, in 5 out of 6 cases tissue had formed in between instead of the resected meniscus. This tissue resembled the native meniscal tissue. However, the tissue was more flexible and softer than native meniscal tissue.

Most of the samples still have to be analyzed, but preliminary histological analysis only showed fibrous scar tissue instead of the fibrocartilage that is observed in native meniscal tissue.

Microscopically, the Estane prostheses and the PCLPU prostheses were completely infiltrated with tissue and covered the whole prosthetic surface with a thin tissue layer. In the PCLPU prostheses, small pores several microns in size were also observed, which were connected with the macropores and filled with a-cellular fibrin. These pores were not observed in the Estane prostheses. Only mechanical damage to the prosthesis seemed to have occurred as a result of the popliteus tendon entering the joint space. In none of the prostheses the degradation of the material had already started.

In all cases, the tissue in the peripheral areas of the prostheses had a fibrous phenotype, with an abundant extracellular matrix [Figure 4(A)]. The extracellular matrix in these areas contained many fiber bundles, which also penetrated the connections between the pores [Figure 4(B)]. These bundles positively stained with collagen type I antibodies. In 5 cases in the Estane group and in 4 cases in the PCLPU group, the tissue in areas near the inner rim of the prostheses showed less collagen type I antibody labeling than in the peripheral zones. The cells in these areas had a cartilaginous phenotype. The round cells were lying in their lacunae and were surrounded by an abundant extracellular matrix, which showed less H&E staining and evident positive Toluidine Blue staining. These areas of positive Toluidine Blue staining exactly matched with the areas of positive collagen type II antibody labeling in the adjacent sections [Figures 4(C,D)].

The inflammatory reaction within the Estane implant did not differ from the reaction in the PCLPU implant. A slight inflammatory reaction was present in the pores, with scarce macrophages and giant cells. These cells were organized in close contact with the surface of the polymer. However, particles of the polymer in the macrophages could not be observed. PMN leucocytes were absent. In the synovium, however, the tissue adjacent to the Estane implant showed significantly more macrophages and giant cells than the tissue near the PCLPU implant (p = 0.041). In all Estane explants, the synovium scored a grade 2 reaction. In the PCLPU
prosthesis the synovium scored only two times grade 2 and the other four samples scored grade 1. Despite the difference in tissue reaction, the tissue reaction near both implants never exceeded grade 2 (many macrophages/giant cells, no PMN leucocytes). Luxation of the popliteus tendon into the joint space seemed not to have influenced the degree of tissue reaction.

At gross inspection, both in the groups with prostheses as well as in the meniscectomy group, the damage of the cartilage varied from an intact surface layer to degenerative le-

**Figure 4.** Micrographs of sections of an Estane implant (left column) and a PCLPU implant (right column) 6 months after implantation. (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 (arrowheads) ×4. (B) Magnification of a region in the peripheral zone of the implant. Tissue with many blood vessels (arrows) and intensive labeling of the collagen bundles (arrowheads). 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 (TB) staining (D) of their matrix. ×100.
macropores in the range of 150–355 µm. This was confirmed in the present study, in which both implants contained directly interconnected macro pores only (155–355 µm), which might be responsible for the relatively low recruitment of these cells, as new prostheses were developed, with a higher compression modulus. This may have led to the location specific phenotypical differentiation of the ingrown tissue.

One of the differences between the two prosthetic materials is the degree of phase separation between the hard and soft segment. The PCLPU polymer seemed to be more phase-separated than the Estane polymer. The hydrophobic segment of the polymer is mainly located in the periphery of the implant where in the Estane polymer, the phase separation is less, resulting in a greater exposure of the more hydrophilic elements of the polymer, which again might play an important role in the cell attachment. However, the differences between the two prostheses seemed to be too small to evoke a difference in tissue infiltration. The difference might even be smaller when the implant becomes filled with blood, and a layer of proteins will be deposited on the implant surface. Several blood proteins seemed to intermediate between the cells and the polymer surface and this process should increase the cell affinity to the polymer. The porous structures were made using a different method, which might have an effect on amount solvent that remains in the implant after washing. Compared to the DMSO solvent the dioxane is a more harmful solvent for the cells. Nevertheless, the difference in solvent did not play an important role in the tissue ingrowth. Another important difference between the two prosthetic materials consists in the presence of an aromatic 4,4-diphenylmethylene compound in the Estane polymer. This is important in the degradation phase of the material. The aromatic 4,4-diphenylmethylene diisocyanate (MDI) may be converted into the toxic and carcinogenic methylenedianiline after degradation. The aliphatic PCLPU prostheses, however, contains butanediisocyanate (BDI) instead, which degrades into 1,4-diaminobutane upon degradation. This is also known as putrescine, which is suggested to act as a growth factor for mammalian cells.

The reaction of the host to the polymers remained restricted to macrophages and multinucleated giant cells (MNGC’s). Polymorphonuclear leucocytes were never observed. Nevertheless, the amount of macrophages and MNGC’s was higher in the synovium near the Estane implant, than in the synovium near the PCLPU implant. The higher recruitment of cells may be due to the difference in chemical composition between the prostheses or may also be a result of degradation of the Estane implant and the exposure of the tissue to the degradation products. As stated in an earlier study, the Estane seemed to be degraded after 40 weeks, while the PCLPU material remains in situ longer than a year. The macrophages and giant cells may also exhibit a higher affinity for the Estane degradation products than for the products of the PCLPU implant. Currently, studies are being performed for further evaluation of the differences in tissue-host reaction between the two polymers. In a former study, the amount of MNGC’s seemed higher, which contributed to the relatively large amount of small pores in the polymer. In the present study, polymers were used with macro pores only (155–355 microns), which might be responsible for the relatively low recruitment of these cells, as

DISCUSSION

In the present study, the meniscus was replaced with a porous polymer implant in the dog’s knee. In earlier studies, the ideal pore sizes for a fast infiltration of tissue seemed to be in the range of 150–500 µm. This was confirmed in the present study, in which both implants contained directly interconnected macropores in the range of 150–355 µm and showed complete tissue infiltration within 3 months after implantation. Considering that the cartilage-like tissue formation seemed to increase with a higher initial compression modulus of the scaffold, new

Figure 5. Articular cartilage degeneration according to the Mankin score. The cartilage degeneration in the meniscectomy group is significantly less than in the PCLPU implant group.
described in a study by White et al. When cells are not able to infiltrate and integrate the material, in case of small pores, reaction cells are recruited to phagocyte the material.

Meniscal fibrocartilage is described as a tissue containing fibrochondrocytes, which are surrounded by an abundant extracellular matrix. 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 collagen type II is able to resist compressive forces and, therefore, is merely found in the central rim of the meniscus where the force transduction between the femur and tibia is highest. In the present study, 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. However, the high anisotropic orientation of the collagen type I fibers, which is observed in the native meniscus, was not observed in the implant. The authors expect that during degradation of the implant, the amount of collagen type I bundles and their orientation will further adjust under influence of the load in the knee joint.

Especially in the role of stabilization and alignment of the knee joint, a high compression modulus is important to resist the high loading forces and to distribute these loads over a greater surface. In this study, the compression curves of the two prostheses did not differ and approached that of the native meniscus. How these curves will develop during degradation of the polymer, remained to be determined. The degradation of the Estane starts earlier and proved to have a higher degradation rate than the PCLPU polymer and consequently will faster loose its biomechanical properties. Therefore, over time, the stiffness of the implant-tissue construct will progressively depend on the characteristics of the ingrown tissue rather than on the prosthetic material. Ideally, a perfect balance exists between polymer degradation rate and maturation of the tissue so as to maintain the material stiffness. The authors speculate that the polymer should retain its biomechanical characteristics for at least 1 year to enable the tissue to complete the maturation process.

In the present study, both the Estane implant as well as the PCLPU implant induced articular cartilage degeneration. More degeneration was seen on the tibia than on the femur. During knee joint flexion, the femur rolls over the meniscus while the meniscus glides backwards over the tibia. The latter movement may have caused higher stresses on the cartilage. The exact cause for the degeneration could not be determined. In our laboratory, the dog used for animal experiments is the Beagle, which has relatively small knee joints. This makes the trauma after resection of the meniscus alone relatively lower than after replacement of the implant. This might have played a role in the degeneration process. In the human situation, the joint is much bigger, which makes the surgery easier. Probably, there is an arthroscopic way for implantation of the prosthesis, which also might lead to less damage to the knee joint. Next experiments will be performed in bigger animals to resolve this problem. Another explanation might be material characteristics of the implant, which had a relatively rough polymer surface. The prostheses were securely cut and modeled from a porous polymer block to the shape of the ectomized native meniscus, during the surgical procedure. Nevertheless, scanning electronic microscopical examination of the prosthetic surface revealed the inevitable irregularities on the prosthetic surface. Producing these prostheses with a mold may provide scaffolds with a smooth surface and with the required standard form. Furthermore, in an earlier study we observed that the degeneration did not increase from 3 to 6 months (unpublished results). Degeneration might have taken place merely during the first months while 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 the bare polymer surface itself. Seeding autologous meniscal cells in the implant previous to the implantation procedure might solve this problem. In this way, the cells are able to produce their extracellular matrix so that the polymer surface will be covered with a tissue layer at the time of implantation.

In the meniscectomy group, a meniscus like regenerate was observed, which also might have protected the articular cartilage from damage. However, the tissue had a fibrous appearance and seemed less stiff than native meniscal tissue. A more detailed characterization of this tissue is being performed at this moment.

A popliteus tendon that enters the knee joint space might also contribute to the 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 sheet of the popliteus tendon might have been damaged, which eventually led to loosening of the tendon from the periphery. Probably, the implant and the cartilage were damaged by the popliteus tendon lying in the knee joint. In future studies, we need to limit the dissection in the dorsal to prevent dissection of the tendon sheet.
In conclusion, implantation of these prostheses led to fast infiltration of tissue and differentiation into cartilage-like tissue. In both prostheses, a meniscus-like distribution of the tissue phenotype was found. Also, the stiffness of the implant-tissue construct approached that of the native meniscus. Furthermore, neither implant in its current shape could prevent articular cartilage degeneration. Further improvements to the prostheses might change this finding.

Even though both prostheses seemed suitable for tissue regeneration, the PCLPU implant had some advantages over the Estane implant. Firstly, the PCLPU is thought to be less or even nontoxic as compared with the Estane implant. Furthermore, the PCLPU implant seemed to provoke a less synovial tissue reaction.

REFERENCES