Biological evaluation of porous degradable polyurethane scaffolds as meniscus replacement


Abstract

In the present study the influence of the chemical properties on the tissue regeneration in the implant is evaluated. The meniscus in the dog’s knee was replaced with either an aromatic 4,4-diphenylmethanediisocyanate based polyesterurethane implant (Estane) (n=2x6) or with an aliphatic 1,4-butanediisocyanate based polyesterurethane implant (PU1600) (n=6). Both foams had similar porous structure and comparable compression properties. After 3 and 6 months for the Estane and 6 months for PU1600 the knee joints were resected and the tissue in the different protheses was evaluated microscopically and mechanically. After 6 months, a meniscus-like distribution of the tissue phenotype was found with collagen type 1 in the peripheral fibrous area and collagen type 2 in the central more cartilaginous area. The compression and stress behavior of the implant-tissue construct was in between the stiffness of the polymer material and that of the native meniscus. The PU1600 implant seemed to provoke a 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 PU1600 implant was not significantly different compared to the degeneration after the Estane implants or after meniscectomy. The chemical differences between these two implants did not influence the tissue regeneration in the implant. However, PU1600 seemed to evoke less tissue reaction and thereby is thought to be less or even non-toxic as compared to the Estane implant. Therefore, for studies in the future, the authors prefer the PU1600 protheses for replacement of the meniscus.
Introduction

The menisci are C-shaped discs interposed between the femoral condyles and tibial plateau and have the function of shock absorption, stabilization and lubrication of the knee joint. Since the classical studies by Fairbank and King, it has been recognized that meniscectomy leads to articular cartilage degeneration [1,2]. Also, the degeneration of the cartilage seemed proportional to the amount of tissue removed and resections of lesions in the anterior and posterior horn had a worse prognosis [3,4]. 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 [5-8].

A recent study by Hoser et.al. showed that although early results of lateral meniscectomy may be satisfactory, the long-term outcome is not [9]. The patients that were studied had a high incidence of degenerative changes, a high rate of re-operation, and a relatively low functional outcome score. Therefore, a method to restore the meniscal function is still highly needed.

In different animal models, several groups replaced the meniscus with autologous materials [10-13]. However, the poor initial mechanical characteristics make long-term fixation problematic. Allograft transplantation is a rather successful technique and is clinically performed [14]. However, problems related to the availability, preservation techniques, the possible transfer of diseases, the individual shaping of the implant and possible immunological reactions to the implant are recognized worldwide [15]. An allograft transplantation may be the first choice but a total artificial implant based on Dacron and Teflon may be an attractive alternative but wear of the prosthetic material seemed to initiate severe synovial reactions [16,17].

Experiments were performed in dogs and rabbits using porous polymer scaffolds for repair of lesions in the avascular part of the meniscus [18-20], 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 [18].

In this study two different prostheses were implanted and evaluated after different times. These were either based on an aromatic 4,4’-diphenylmethanedisocyanate based polyesterurethane (Estane) and evaluated after 3 and 6 months or an aliphatic 1,4-butanediisocyanate based polyesterurethane (PU1600) which was evaluated after 6 months. Both foams had a comparable pore structure with similar pore sizes and interconnectivities and comparable initial mechanical properties.
Materials and methods

GPC, SEM, tensile and compression testing were performed as described in chapter 2 and 6.

Estane

Estane (5701-F1)(BF Goodrich Chemical, Belgium) is a polyesterurethane with a hard segment based on 4,4-methylene diisocyanate (MDI) and a soft segment based on poly(tetramethylene adipate). The polymer was purified once by precipitation from a 5% w/w polymer solution in dimethylformamide (DMF) into a six-fold volume of R.O. water. The number average molecular weight was 94.3 kg/mol with a polydispersity of 1.9.

1,4-Butanediisocyanate polyurethane

The polyurethane used here is based on a soft segment of poly(ε-caprolactone) initiated on 1,4-butanediol (PCL) with a length of 1600g/mol. The hard segment (HS) is based on 1,4-butanediisocyanate and 1,4-butanediol and has a uniform length. The synthesis is described in Chapter 2 [21]. The polymer is abbreviated as PU1600. The complete polymerization is carried out without the use of any catalyst to ensure a minimum amount of side reactions and prevent the use of any possible toxic products. The number average molecular weight was 86.2 kg/mol with a polydispersity of 3.2. Even though polydispersity has a major influence on the behavior of the system this is assumed not to influence the basic principles of this system [22,23].

Scaffolds

Estane scaffold

35.14g Estane was dissolved in 45.3g 1,4-dioxane at 80°C. 7.2ml R.O. Water was added as non-solvent to induce liquid-liquid phase separation upon cooling. A certain amount of sucrose crystals (151g sucrose) sieved to a size of 150-355µm were added and mixed in with a mechanical stirrer. The mixture was transferred into a mould and frozen at -18°C. Dioxane and water were removed through freeze-drying. Sucrose was removed by washing the polymer/Sucrose mixture for 24 hours with 1l of water per gram of polymer. The foams were dried in a vacuum stove at 37°C for 24 hours. The obtained structure is shown in Figure 10-4. The procedure is described more extensive in Chapter 5.

1,4-Butanediisocyanate polyurethane scaffold

20.04g of polymer was dissolved in 33.3g DMSO at 80°C, after which 2.3ml R.O. water was added to adjust the quality of the solvent for the polymer. 156.6g Sodium chloride crystals (preheated to 130°C) sieved to a size of 150-355µm were added to
the solution at 80°C. The mixture was scooped into six glass molds and cooled –18°C. The NaCl and solvent were removed by washing at room temperature with a large excess of R.O. water containing 20% ethanol. One liter of solution was used per gram of polymer. The solution was renewed after 12 hours. After washing with the water/ethanol mixture for 20 hours, the foam was washed for 1 hour with 0.2L 96% ethanol per gram of polymer to remove the last traces of solvent. Finally the foam was dried under vacuum at 37°C for 24 hours. The obtained structure is shown in Figure 10-5. The procedure is described more extensive in Chapter 6.

Surgery

A lateral meniscectomy is performed on 24 legs of 24 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. In 2x6 knees, the meniscus was replaced by an Estane implant and in 6 knees by a PU1600 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 has a lower tear strength [21] and therefore it seemed necessary to lead two bonded non-degradable sutures through the Estane implant parallel to the inner and outer rim (Figure 10-1). In the PU1600 implant, however, the same sutures were pulled trough the implants horns only, due to 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 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.

![Estane implant and PU1600 implant](image)

Figure 10-1. Schematic presentation of the two operative procedures for either the Estane implant and the PU1600 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 non-resorbable sutures were pulled longitudinally through the implant (dotted lines (C)) and attached to the lateral proximal tibia. In the PU1600 implant the sutures were only pulled trough the horns. The periphery of the implant was attached to the capsule with resorbable sutures (D).
Histology

After sacrificing the dogs, the implants were resected and a 4mm full thickness biopsy was taken from the prosthetic posterior horn for the biomechanical testing. Routine histology was performed with Haematoxylin-Eosine and Toluidine blue. Monospecific monoclonal rabbit anti-collagen type I antibody [24] (PS-41, anti-raised in rabbit, Sanbio, Uden, The Netherlands) and mouse anti-collagen II antibody [25] (II-II6B3, anti-chicken raised in mouse, Developmental Studies Hybridoma bank, University of Iowa, USA) were applied and the samples were used for immunohistological analysis. Blocks of the tibial plateau and femoral condyles were fixed in a buffered formaldehyde solution (4%, pH 7.4) for two days and rinsed, dehydrated and embedded in methylmethacrylate for two 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 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 µm 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) [26].

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 Alcian Blue staining (grade 0) to no staining (grade 4) and an intact tidemark (grade 0) or a tidemark infiltrated with blood vessels (grade 1) [27]. The total score of each subcategory determined the Mankin score. The observer was blinded for the treatment.
Data analysis

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.

Compression tests

Compression tests were performed on cylindrical shaped specimens of about 3mm high and 2mm in diameter, cut manually from the foams. The experiments were performed at 21°C with a 100N load cell and a strain/compression rate of 2 mm/min using an Instron (4301) tensile tester.

Results and discussion

Chemical evaluation

Polymer properties

One of the main differences between Estane and the PU1600 polyurethane is that the hard segment is based on different diisocyanates: In case of Estane it is based on 4,4’-diphenylmethanediisocyanate (MDI), while PU1600 is based on 1,4-butanediisocyanate (BDI). The major disadvantage of MDI is that it might be converted into the toxic and carcinogenic methylenedianiline on degradation [28-30]. The aliphatic PU1600 prostheses, however, contains a hard segment based on BDI instead, which degrades into 1,4-diaminobutane (putrescine) upon degradation, a non-toxic polyamine that is essential for cell growth and differentiation [31-33]. The difference in chemical structure between Estane and PU1600 will also influence the chemical surface characteristics of the prosthesis, which might play an important role in the cell attachment [34,35]. However as will be shown later, 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 (as was done during operation). Several blood proteins seemed to intermediate between the cells and the polymer surface and this process should increase the cell affinity to the polymer [36].

The different soft segments from both polymers also influence the degree of phase separation between the hard and soft segments, but even more important also influence the speed of degradation. It is known that Estane looses its mechanical
properties within several months, while PU1600 retains its mechanical properties for almost one year [37,38]. These differences in chemical composition will also be expressed in the thermal and mechanical properties. Figure 10-2 shows the DSC thermograms of PU1600 and Estane. Table 10-1 gives the accompanying data. While the $T_g$’s are comparable the hard segment melting points differ with 25°C. An additional difference is the presence of crystalline soft segment. PU1600 shows some crystalline PCL as is explained in Chapter 6.

![DSC thermogram of PU1600 and Estane](image)

**Figure 10-2. DSC thermogram of PU1600 and Estane**

<table>
<thead>
<tr>
<th></th>
<th>$T_g$ (°C)</th>
<th>$T_{m,HS}$ (°C)</th>
<th>$T_{m,SS}$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PU1600</td>
<td>-52.1</td>
<td>94.0</td>
<td>70.8</td>
</tr>
<tr>
<td>Estane</td>
<td>-47.7</td>
<td>120.7</td>
<td>-</td>
</tr>
</tbody>
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-: not found

The difference in thermal properties is expressed in the mechanical properties (Figure 10-3 and Table 10-2). A major difference is found in the Young’s modulus due to the different melting enthalpy, which also causes the difference in tear energy [39]. The stress strain curves also show a major difference in tensile strength and strain at break, PU1600 even shows some strain hardening due to strain induced crystallization, which is not seen for Estane.
Figure 10-3. Stress-strain behavior of Estane and PU1600

Table 10-2. Mechanical properties of PU1600 and Estane

<table>
<thead>
<tr>
<th></th>
<th>Young’s modulus [MPa]</th>
<th>Strain at break [%]</th>
<th>Tensile strength [MPa]</th>
<th>Tear energy [kJ/m²]</th>
<th>Yield stress [MPa]</th>
<th>Yield strain [%]</th>
<th>Toughness [MPa]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PU1600</td>
<td>64.6</td>
<td>1081</td>
<td>43.6</td>
<td>136.9</td>
<td>6.4</td>
<td>19.2</td>
<td>229.2</td>
</tr>
<tr>
<td>Estane</td>
<td>20.2</td>
<td>755</td>
<td>14.2</td>
<td>47.0</td>
<td>1.6</td>
<td>9.3</td>
<td>59.4</td>
</tr>
</tbody>
</table>

Scaffold properties

As described in Chapter 5 and 6 the two different foams are made via two different methods.

Table 10-3. Properties of the Estane and PU1600 foams.

<table>
<thead>
<tr>
<th></th>
<th>Porosity (%)</th>
<th>Compression modulus (kPa)</th>
<th>Pore size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PU1600</td>
<td>81.4</td>
<td>290</td>
<td>150-355</td>
</tr>
<tr>
<td>Estane</td>
<td>72.1</td>
<td>260</td>
<td>150-355</td>
</tr>
</tbody>
</table>

The major differences between these methods are the solvents that are used. In the case of Estane toxic dioxane is used of which it is even known that it is not always completely removed after freeze drying and only has limited solubility problems [40]. The PU1600 foam method does not make used of toxic solvents. In this case DMSO is used as solvent, which is also used in the medical field as a radical scavenger and generally accepted as biocompatible [41-43]. Even though two different methods are used to obtain a porous structure one can see that the foams that are obtained are very similar with respect to interconnectivity and
pore size. The porosity of the two foams was different in order to obtain comparable compression moduli. On micrometer scale there is a minor difference. As one can see in the SEM pictures the PU1600 foam is built up from micrometer size spheres due to the crystallization of the polymer while the Estane foam does not show this structure [44].

![Figure 10-4. Estane foam.](image1)

![Figure 10-5. PU1600 foam.](image2)

### Medical evaluation

#### Clinical observations

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. Post mortem, there were no signs of synovitis in the joint capsule and the synovial fluid was clear.

**Gross inspection**

Macroscopically, the 3 and 6 months Estane as the PU1600 prostheses were completely integrated with the peripheral capsule and the prosthetic horns were firmly attached to the tibial plateau. After 6 months all prostheses seemed to be completely covered by a transparent layer, while after 3 months this was only partially found. In both the 6 months Estane group (three cases) as the PU1600 implant group (four
cases), the popliteus tendon seemed to have entered the joint space and damaged the polymer implants, although this was not the case after 3 months of implantation. 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 groups did not evidently differ with that in the meniscectomy group.

In the group with meniscectomy solely, in 5 out of 6 cases a meniscus like regenerate had formed in the joint space, although this was smaller in diameter than the native meniscus and was more flexible and softer.

**Tissue infiltration**

Microscopically, the Estane prostheses and the PU1600 prostheses were completely infiltrated with tissue and covered the whole prosthetic surface with a thin tissue layer. As can be seen in Figure 10-5 does the PU1600 prostheses, besides macropores, also contain pores of several micrometers originating from the solvent that was present in the polymer rich phase. These pores are too small to be incorporated by cells and were only filled with a-cellular protein rich precipitate, probably fibrin. Due to the different method of foam production these pores were not observed in the Estane prostheses.

**Tissue phenotype**

In all cases, the tissue in the peripheral areas of the prostheses had a fibrous phenotype, with an abundant extracellular matrix (Figure 10-6a). The extracellular matrix in these areas contained many fiber bundles, which also penetrated the connections between the pores (Figure 10-6b). These bundles positively stained with collagen type I antibodies. In these areas no Toluidine blue staining and collagen type II antibody labelling was observed.

In 5 cases in the 6 months Estane group and in 4 cases in the PU1600 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 HE 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 (Figure 10-6c and d). After quantitative determination, the amounts of Toluidine Blue, collagen type I and II staining did not significantly differ between the native meniscus and the prostheses. Also, no significant differences were observed between the two different prostheses (Figure 10-7).

In the native meniscus, abundant collagen type I labeling was especially visible in the peripheral regions (average 69.6% of the total tissue area in the implant) while 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 10-6. Micrographs of sections of an Estane implant (left column) and a PU1600 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) 4X. (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, 100X. (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. 100X.
Figure 10-7. Percentage labeling with collagen type I and II or TB of the total amount of tissue in the meniscus and implant. Only after 6 months collagen type II antibody labeling and TB staining was observed. Note that the amounts of collagen type I and II and proteoglycans in the implants approached the amounts in the native meniscus. Col I: collagen type I antibody labeling; Col II: collagen type II antibody labeling; TB: Toluidine blue staining.

**Foreign body reaction**

After 3 months the foreign body reaction tended to be decreased compared to 6 months but was still classified as slight. More giant cells were observed along the surface of the pores in the polymer than after three months. The inflammatory reaction within the 6 month Estane implant did not differ from the reaction in the PU1600 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. PMN leucocytes were absent. In the synovium, however, the tissue adjacent to the 6 month Estane implant showed significant more macrophages and giant cells than the tissue near the PU1600 implant (p=0.041). However, the tissue reaction near both implant never exceeded grade 2 (many macrophages/giant cells, no PMN leucocytes). The higher recruitment of cells may be due to the difference in chemical composition between the prostheses but 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 PU1600 material remains in situ longer than a year [37,38]. The macrophages and giant cells may also exhibit a higher affinity for the Estane degradation products than for the products of the PU1600 implant.

**Cartilage degeneration**

Compared to the 3 months Estane, the histological aspect of the articular cartilage had not deteriorated after six months, which was confirmed by the Mankin score (Figure 10-8). At gross inspection, both in the groups with 6 month prostheses as in the
meniscectomy group, the damage of the cartilage varied from an intact surface layer to degenerative lesions. Neither meniscectomy nor implantation of a implant resulted in osteophyte formation.

The cartilage degeneration could be confirmed microscopically. The tibial cartilage lesions were merely localized adjacent to the inner rim of the implant while the tibial lesions after meniscectomy were spread over a greater area, while on the femoral side the degeneration had spread over a more broad area at the dorsal curvature of the condyle, both in the meniscectomy and the implant groups. (Figure 10-8). Degenerated areas showed varying degrees of surface fibrillation, cloning of the cells and decreased Toluidine Blue staining. The subchondral bone was never exposed in either of the groups.

![Figure 10-8. Mankin-scores of the tibial lateral compartment and the femoral condyles.](image)

In dogs with an implant, more degeneration tended to occur at the tibial side than at the femoral side. In the meniscectomy group the degeneration on these sides was comparable. In both groups with an implant, the tibial cartilage was more damaged than at the femoral side, although the Mankin scores were not significant. After meniscectomy this difference was not observed. The Mankin score did not significantly differ between the three groups (p=0.124). 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 less than after replacement of the implant. This might have played a role in the degeneration process.
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. 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 for 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 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 to prevent dissection of the tendon sheet.

**Compression tests**

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 [45]. Moreover, meniscal fibrocartilage is described as a tissue containing fibrochondrocytes, which are surrounded by an abundant extracellular matrix [46-48]. The collagen type I in native peripheral meniscal tissue provides the circumferential tensile strength to resist the hoop stresses during loading of the joint [49]. 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 [50].

The Estane implant showed approximately similar compression curve after three or six months after implantation. Compared to the scaffold before implantation, the slope of the compression curve evidently increased from implantation to 6 months follow-up and approached that of the native meniscus (Figure 10-9).

Approximately a similar compression curve is found for the PU1600 implant. Also for the PU1600 prostheses, the slope of the compression curve evidently increased from
implantation to 6 months follow-up and approached that of the native meniscus (Figure 10-9). How these curves will develop during degradation of the polymer, remained to be determined. Compared to PU1600 will Estane lose its mechanical properties earlier [37,38]. 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 in order to maintain the material stiffness. The authors speculate that the polymer should retain its biomechanical characteristics for at least 1 year in order to enable the tissue to complete the maturation process.

![Compression curves of scaffold before implantation, native meniscus tissue, Estane after 3 and 6 months and PU1600 scaffold after 6 months of implantation.](image)

**Conclusions**

In the present study, the meniscus was replaced with a porous polymer implant in the dog’s knee. The prostheses consisted of either Estane or PU1600 but had similar geometrical and mechanical properties (a compression modulus of 300kPa and a pore size of 155-355µm and a high interconnectivity). The tissue infiltration into the implant and the tissue reaction to the prostheses were studied. The Estane scaffold has been evaluated after 3 and 6 months of implantation and the PU1600 after 6 months. The consequences for the articular cartilage were evaluated and compared with the cartilage after meniscectomy.
Both scaffolds could be produced with a broad variation of mechanical properties, although the method, which is used to for the PU1600 scaffold is far more versatile. A major improvement compared to scaffolds made in the past is the increase in interconnectivity. This was always the bottleneck of the scaffolds.

In earlier studies, the ideal pore sizes for a fast infiltration of tissue seemed to be in the range of 150-500 µm [19]. This was confirmed in the present study, in which both implants contained directly interconnected macropores in the range of 150-355 µm showed complete tissue infiltration within three months after implantation. Considering that the cartilage-like tissue formation seemed to increase with a higher initial compression modulus of the scaffold [18], new prostheses were developed with a higher compression modulus. This may have led to the location specific phenotypical differentiation of the ingrown tissue.

The tissue distribution in the prostheses was similar to that in the meniscus. However, the high anisotropic orientation of the collagen type I fibers, which is observed in the native meniscus [45] was not observed in the implant. The authors hope 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.

In conclusion, implantation of these prostheses led to fast infiltration of tissue and differentiation into cartilage-like tissue. In the 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. Most probably due to the popliteus tendon that seemed to have entered the joint space and damaged the implants. Further improvements to the prostheses might change this finding.

Even though the prostheses seemed suitable for tissue regeneration, the PU1600 implant had some advantages over the Estane implant. Firstly, the PU1600 is thought to be less or even non-toxic as compared to the Estane implant. Furthermore, the PU1600 implant seemed to provoke a less synovial tissue reaction and will degrade much slower and retain its mechanical properties for a longer period. The PU1600 implant is assumed to be superior in the long term compared to the Estane implant.

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