In vivo tissue engineering of the knee joint meniscus

T.G. van Tienen

Cover:

The meniscus is a semilunar piece of fibrous cartilage
(De meniscus is een stuk fibreus kraakbeen in de vorm van een halve maan)

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In vivo tissue engineering of the knee joint meniscus

T.G. van Tienen

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Contents

Chapter 1

General introduction and aims of the study.

Introduction

The menisci are wedge-shaped semilunar disks of fibrocartilage interposed between the condyles of the femur and tibia. Although they were once described as functionless remains of leg muscle, it has been realized that menisci are essential components in the complex biomechanics of the knee joint. This realization introduced new interest in the basic science of the meniscus in terms of anatomy, biochemistry, and function.

• Anatomy

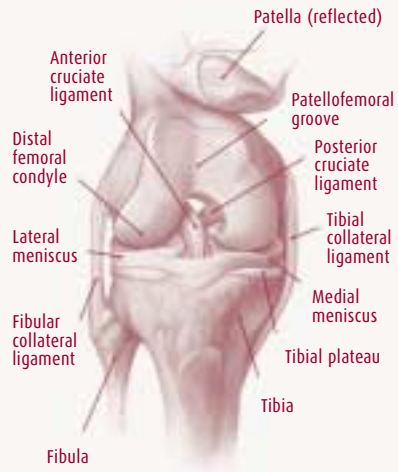
The medial meniscus is oval, matches the medial tibia plateau and covers approximately 30% of the plateau surface. It is securely fixed at its outer circumference. The peripheral borders of the medial meniscus are attached to the joint capsule throughout its length and therefore the medial meniscus has a limited mobility during knee motion. The anterior horn is attached to the tibia while the posterior fibers of the anterior horn are connected to the anterior horn of the lateral meniscus by the transverse ligament. The posterior horn is attached to the tibia at the intercondylar fossa, behind the posterior cruciate ligament. The lateral meniscus has no attachment to the fibular collateral ligament and only a loose peripheral attachment to the joint capsule, except in the region of the popliteal tendon.

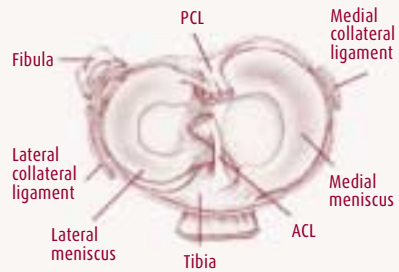
The anterior horn of the lateral meniscus has its origin behind the anterior cruciate ligament, while the posterior horn is attached to the tibia, in front of the posterior end of the medial meniscus. Also the anterior (in front of the posterior cruciate ligament) and the posterior (behind the posterior cruciate ligament) meniscofemoral ligaments originate from the lateral posterior horn to the medial tibial plateau (respectively ligament of Humphry and Wrisberg). The relative immobility of the medial meniscus may contribute to the increased prevalence of medial meniscal lesions.

• Biochemistry

60-70% of the dry weight of human meniscus consist in collagen fibers mainly collagen type I though other types have been identified (type II, III, V, VI) [2]. Within the collagen fiber network, histologically, the fibrocartilage-like tissue of the meniscus contains oval or fusiform cells in the superficial layer and round cells in the deeper layers. Though light microscopically they resemble fibroblasts, Ghadially stated that these cells more resemble Zone I chondrocytes of hyaline articular cartilage[25] based on sparse territorial matrix, which can be found in most instances. Besides collagen the extracellular matrix consists of proteoglycans (1% dry weight), non-collagenous proteins (8-13% dry weight) and water (70-75%)[2,26]. The proteoglycans (PG), which

figure 1





are aggregated by hyaluronic acid, consist of a protein core with many glycosaminoglycans. Dependent on the localization within the meniscus, the PG's contain different concentrations of glycosaminoglycans. The central rim and the superficial layers of the meniscus are more hyalinized and contain more chondroitine sulfate and keratan sulfate while the meniscal horns and the core of the meniscus contain more chondroitine and dermatan sulfate[26,49].

• Vascularity

The vascular supply to the menisci arises from the medial, lateral, and middle genicular arteries. A pre-meniscal capillary network, arising from branches of these arteries, originates within the synovial and capsular tissues of the knee along the periphery of the menisci. The postero-lateral aspect of the lateral meniscus adjacent to the popliteus tendon does not contain a penetrating vessel. Arnoczky and Warren have found that this arteriolar network nourishes the peripheral 20-30% of the meniscus[26,7]. Beginning with the fetal meniscus the vascular network extends to the most central zones and recedes to the periphery with aging. The central third of each meniscus in adults is avascular and receives nourishment by diffusion of synovial fluid into its superior and inferior surfaces.

• Meniscal structure and function

According to Bullough et al., the tensile strength of the meniscus is mainly determined by parallel fibers to the tensile axis, but is also positively influenced by the cross fibers[12]. In correspondence with cell orientation, they are organized into layers: the superficial layer consists in a mesh-like fashion while the deeper zones are organized in large circumferentially oriented collagen fibers with a few radially oriented fibers. Especially these circumferentially oriented fibers play an important role in load distribution. The wedge-shaped form of the meniscus causes a radial external force, which is developing hoop stress along the circumferentially arranged collagen fiber bundles. In this way the force is transferred towards the meniscal horns[9].
Menisci provide approximately 70% of the total joint contact area and the contact area after meniscectomy is half what it was prior to surgery[47]. Menisci are responsible for transmitting 45%-50% of the load during weight-bearing[45].
The deforming capacity of tissue is beneficial in protecting the bearing surfaces while by deformation during load the surface contact area increases. Also the joint lubrication minimizes wear by preventing direct surface-to-surface contact [18].
However, it remains unclear if the meniscus or the articular cartilage plays the major role in this process. In articular cartilage the fluid exudation may be the mechanism for fluid film generation for lubrication of the synovial joint[56]. In cartilage 70% of the total water amount is freely exchangeable while menisci are six times less permeable, so it remains unclear whether the meniscus plays a role in knee joint lubrication.

• Meniscectomy and cartilage degeneration

Clinical and animal studies have shown that preservation of the meniscus serves to maintain stability and distributes weight bearing forces in the knee, thereby reducing degenerating contact stresses across the articular cartilage surfaces[36,81,38,22,28,55].
Several studies have documented the altering effect of meniscectomy on knee biomechanics. Especially the 30 to 50% increase of knee contact pressure plays an important role[13,57,47], which results in increase of the stress concentration in the contact area. Changes in the knee joint after meniscectomy include ridge formation, narrowing of the joint space and flattening of the femoral condyle[22].
Partial meniscectomy leads to less severe degenerative changes, with the degree of change directly related to the amount of meniscus removed[14]. Partial meniscectomy of bucket-handle lesions and lesions in the anterior/posterior horn also have a worse prognosis than elsewhere in the meniscus[29]. Also the age of the patient, at which the procedure is performed, seems to play a role. Younger patients seemed to have longer delay in developing significant osteoarthritis after meniscectomy compared to older patients[58]. No relation could be demonstrated between the length of the follow-up period and the degree of Fairbank's changes but results seem better after medial than after lateral meniscectomy, and least satisfactory after removal of both menisci[81,38,3].

• Meniscal lesion repair

Knee joint meniscus lesions are very frequent in orthopaedic pathology. The degree of degenerative changes seemed to be directly proportional to the amount of meniscal tissue removed[15]. Therefore, nowadays it is good clinical use to preserve as much functional meniscal tissue as possible. However, only lesions in the vascular peripheral 10-30% of the meniscus are capable of repair[8]. Repair techniques traditionally have utilized a variety of suture methods, including inside-out and outside-in techniques[19,62]. Also, various bioabsorbable implants were developed which permit all-inside arthroscopic repairs of lesions in the vascular periphery[62]. However, these fixation techniques only succeed in well-vascularized tears. Lesions in the avascular central part of the meniscus, nowadays, partial meniscectomy is the golden standard. With the development of arthroscopical surgery this procedure is even more commonly utilized.
Several experimental techniques like creating access channels from the vascular periphery to the lesion, abrasion of the synovium, implanting non-vascularized and vascularized synovial flaps and implanation of fibrin clots with and without cells, have been successful in inducing repair of these lesions[31,10,61,59,32]. The basic principle is to improve the vascularity of the defect by stimulating infiltration of vascular tissue. Although repair of the lesion was achieved, only after implantation of the fibrin clots, the repair tissue did not resemble meniscal fibrocartilage. However, it remains to be determined whether these methods are applicable for repair of large and highly stressed lesions.
Creation of access channels between the vascular periphery towards the lesion was not very successful due to occlusion of this conduit[30].
Based on these studies, two decennia ago, Veth (University Medical Centre Groningen) and Pennings (Polymer Chemistry Department of the State University Groningen) initiated a research line on the healing of meniscal lesions in the meniscus. This research line was based on the hypothesis that a porous polymer scaffolds could initiate regeneration of meniscus-like tissue when implanted in the knee joint: "In-vivo Tissue Engineering of Meniscal Tissue". This thesis is the sixth in this research line. Veth et al. connected the lesion with the vascular periphery by implanting these polymer scaffolds into full thickness access channel in the meniscus

of dogs. These scaffolds were composites of polyurethanes, poly-L-lactides and carbon fibers[82]. After this procedure, respectively, vascular tissue infiltration occurred, repair of longitudinal lesions by fibrocartilage was observed and the polymer implants were replaced by repair tissue, containing fibrocartilage. Later, Klompmaker et al. used the same surgical technique with newly developed aromatic and aliphatic polymers with macropores which were interconnected by micropores [48,39,42,40,17]. Klompmaker already described the relevance of the appropriate pore-size for the rapid infiltration of fibrocartilaginous tissue[20,40]. A pore-size between the 150 and 500 microns provided the best infiltration of mesenchymal tissue and the least inflammatory response. A high porosity of the scaffold will induce a high infiltration tissue rate but also will decrease the stiffness of the material. Therefore, the macropores were interconnected by micropores or channels to retain a high stiffness. The minimal diameter of these channels was never defined and remained to be determined. The influence of these pore-sizes and the porosity on the tissue infiltration, the degradation rate and the host inflammatory response will be described in **chapter 2**.

Furthermore, the aromatic polyurethane might yield aromatic diamines upon degradation, which are very toxic and carginogenic[72]. Also, the full thickness defects interrupted all the peripheral longitudinal collagen fibers leading to an inadequate conduction of the load to the meniscal horns. A less traumatizing surgical technique and a better initial attachment between implant and native meniscal tissue might retain the structural integrity of the meniscus. This again might lead to more frequent healing of the lesions. The results of the new implants and a less traumatic implantation technique were evaluated and will be described in **chapter 3**.
The consequences of this technique for the underlying articular cartilage are discussed in **chapter 4**.

• Meniscal replacement

The main goal of meniscal substitution is re-establishment of a normal joint load distribution in order to prevent cartilage degeneration which is common after meniscectomy. Only a substitute which closely matches normal meniscal tissue properties can re-establish meniscal functions.

Autograft/ Autologous tissue

Immunologic factors and disease transmission are avoided by the use of autograft tissue. However, appropriate tissue is scarce because the tissue should contain certain initial biomechanical properties, which proved to play a significant role especially in the protection of the articular cartilage. Kohn et al. tried to replace the meniscus by a tendon autograft[44]. Histologically, after twelve months, the tendon showed signs of remodeling. Chondrocyte-like cells were seen with collagen fibers in all directions but the graft seemed not suitable as a meniscal substitute. While at earlier follow up the complete graft was vascularized, after twelve months vessels had withdrawn from the inner rim of the meniscus. Later the same group described a pediculated infrapatellar fat pad as meniscal replacement. Shrinkage occurred, the tissue was weak and not identical to that of a normal meniscus[43]. Only a temporary protective effect on the cartilage could be attributed to the autograft but the long-term results indicated that this was not permanent. These inferior properties were affirmed by a clinical experiment by Milachowski et al. who replaced the meniscus by a fat pad in combination with anterior cruciate ligament replacement[54]. Autologous rib-perichondrium showed similar collagen fiber orientation as native meniscal tissue but a superficial layer of synovial cells was present with hypertrophy in the periphery. All perichondral menisci had central areas of a cellular differentiation similar to hyaline-like cartilage but were associated with some calcification. Although less articular cartilage degeneration was observed, it could not compete with the biomechanical strength of native meniscal tissue[11]. All reports describing the results of autogenous grafts, mentioned spontaneously ingrown meniscal tissue including chondrocytes with their newly formed matrix. Nevertheless, the different autografts were all more prone for degeneration during the implantation period due to their minor tensile properties and the choice in available tissues is restricted. Also in case of an autograft, individual shaping of the meniscus, relevant in meniscal replacements, seemed a problem.

Allograft (homologous)

The meniscal allograft as homologous scaffold is already commonly used[13,76,79,16,24,64,60,46,37,1]. Initially an allograft was considered as replacement for the native meniscus when it was excised. However, DNA probe and Na--35SO4 analysis revealed that recipient cells replaced all of the donor meniscal cells in a freshly transplanted meniscus within weeks to months after transplantation. After 6 to 8 months host cells seem to replace the donor population[6,53,55]. However, preservation of the graft seemed to play a role in the remodeling rate of meniscal allografts. Although not proven biochemically, deep-frozen allogenic menisci histologically can remain fully functional without remodeling while lyophilized transplants at this term were completely remodeled[55]. Shrinkage of the transplant seems a common problem as different

preservation techniques influence structural integrity of the allograft[52,53,55]. Verdonk et al. reported favorable results about viable meniscal transplantation: the meniscal allograft is kept in an nutrient medium for approximately 2-3 weeks without apparent loss of viability, during which period the appropriate recipient can be selected and prepared, a laboratory screening can be conducted, and the culture results and disease transmission factors can be evaluated. In this way, live transplant hazards can be avoided, resulting in a higher success rate[80,79,78]. The long-term follow-up results, however, remain to be determined.
In conclusion, meniscal allograft can be considered as a spacer between in the femoral condyle and the tibial plateau to distribute the loading forces in the joint. There is much discussion if allografts really prevent degeneration of the articular cartilage. Also meniscal allograft may stimulate systemic and local immune responses despite different preservation techniques[27].

Synthetic permanent implant

The possibility to create a meniscal replacement in the desirable shape is one advantage of a synthetic prosthesis or scaffold. Toyonaga et al. introduced a teflon-net substitute meniscus, because of its flexibility, histocompatibility and rapid infiltration with cells[74]. Nine months after implantation in dogs, fibrocartilaginous cells and matrix formation were observed. However, besides foreign body responses, other studies also reported massive particle debris and reactive synovitis as a result of the prosthesis wear [65,66,51]. A polyesther-carbon fiber prosthesis had a cartilage protecting effect and was encapsulated by fibrous tissue but the expected tissue invasion into the prosthesis was not observed. Messner et al. implanted Teflon prostheses with a periosteal cover in rabbits to prevent prosthesis wear and enhance proliferation of fibrocartilage cells[65,50]. However, the prosthesis was insufficient and the expected biological coat was thin and fibrochondrocytes were not observed.
In conclusion, the uncoated permanent prostheses protected the cartilage for degeneration but seemed not appropriate because of wear and changes in shape. Biological coating did not improve the performance of the prosthesis.

Natural resorbable scaffold

Collagen fiber scaffolds may be a logical scaffold material considering that collagen is the main component of the meniscus[23]. In a canine study Stone et al. introduced meniscal regeneration after implantation with collagen type I scaffolds[71,69,70]. This porous matrix scaffold was obtained by purifying bovine collagen fibers and cross-linking these with glutaraldehyde. The scaffold was loaded with glycosaminoglycans.

The native meniscus was resected leaving a meniscal rim in-situ and the collagen scaffold was sutured in this meniscal rim. After 12 months the template regenerated menisci resembled normal native meniscus. Well-differentiated fibrochondrocytes were found. It was not clear if the repopulating cells in the scaffold originated from the meniscal rim or from synovial or capsular tissue. In later clinical studies biopsies were taken 12 months after surgery, which showed invasion and replacement of the collagen implant by fibrochondrocyte-like cells producing a new meniscus-like matrix [71,63,67].
Complete meniscal replacement by a collagen scaffold was not described.
By leaving the peripheral part of the native meniscal wedge in-situ and suturing the scaffold to this meniscal rim, better initial knee joint stability might be achieved while the circumferential forces could still be directed to the meniscal horns.
Question is if the minor biomechanical properties of the collagen scaffold, like stiffness, can resist the high loading forces in the knee joint and allow complete replacement of the meniscus.

Synthetic resorbable scaffold

Several authors described the desired properties of scaffolds to be able to induce regeneration of the meniscal tissue[41,65,68,4]. Both Arnoczky and Stone agree that the scaffold should provide in a matrix framework for restoration of vascular, cell and matrix elements of the tissue. Arnoczky described the different sources of cellular infiltration and questioned the need for growth factors to increase the amount of cells or to stimulate differentiation into fibrochondrocytes. According to these different cell sources Klompmaker described the importance of the porosity, pore size and compression modulus of the scaffold for the infiltration of tissue and differentiation into neo-fibrocartilage. An adequate fit of the scaffold may even improve knee stability and consequently initially protect the articular cartilage from damage[70,68]. In case of a degrading scaffold, the process of degradation should be in optimal relation with the tissue restoration[5,70,68].
Several sorts have been suggested in literature. Poly-glycolic acid (PGA) is described by several research groups as scaffold for meniscal cell infiltration[35,33,21]. This synthetic biodegradable polymer is derived from cartilage research and proved to be a useful template for attaching cartilage cells providing structural integrity as new tissue is created[75]. In all presented studies, after in vitro seeding of meniscal cells, new fibro-cartilage developed and after subcutaneous implantation, in vitro cultured meniscal cells on meniscus shaped scaffolds could form a cell-polymer construct[34].
After implantation in the knee, this resulted in new meniscal tissue[33,21]. Question

remained if the biomechanical characteristics of this scaffold material are sufficient to contribute to stability in the knee joint and prevent articular cartilage degeneration. Material properties like porosity and pore-sizes are, according to our information, not mentioned in the studies.
The present thesis is a result from the research line "In-vivo tissue engineering of the meniscus" of the University Medical Center Nijmegen, the Netherlands. This research line is based on the hypothesis that when a synthetic scaffold is implanted in the knee joint and attached to the synovium/capsule, tissue grows from the capsule into this scaffold. Under influence of biomechanical and biological factors this fibrous tissue differentiates into meniscus like tissue. In this research line, Klompmaker et al. tried to regenerate meniscal tissue from the synovium and capsule by attaching a temporary synthetic porous meniscus prosthesis to the peripheral knee joint capsule of dogs[41]. After six months, the prosthesis was completely filled with tissue and differentiation into fibrocartilage–like tissue seemed to occur. Although the consequences for the articular cartilage were only evaluated by gross inspection, less degenerative changes were observed in the knees with prosthesis than in knees after meniscectomy. Nevertheless, implantation of a prosthesis could not completely prevent degeneration of the cartilage. A fast tissue penetration into the prosthesis and differentiation into fibrocartilage seemed essential for the cartilage protective effect of the prosthesis. According to studies mentioned earlier, new scaffolds were developed with a higher material porosity and compression modulus to replace the meniscus. These newly developed scaffolds were implanted in the knees of dogs. The performance of the prosthesis and the consequences for the articular cartilage are described in **chapter 5**.

Furthermore, the influences of the polymer material on the tissue infiltration and differentiation were never determined. Differences in chemical composition might influence the tissue infiltration and differentiation in the prosthesis but also the reaction of the tissue on the implant.
Chapter 6 will describe how prostheses were implanted either consisting of an aromatic 4,4-diphenylmethanediisocyanate based polyesterurethane (Estane) or an aliphatic 1,4-butanediisocyanate based polyesterurethane (PCLPU). Both prostheses had similar geometrical properties. If this study reveals that the performance of the newly developed PCPLU prosthesis is at least similar as in the Estane prosthesis, then the PCLPU prosthesis has the preference because of its less toxic degradation products.
Considering the former studies, the biological performance of the prosthesis is promising. The biomechanical performance, however, still needed to be determined. The prosthesis has to prove that it mimics the behavior and deformation of the native meniscus in the knee joint during movement. Therefore, a study was performed to evaluate the behavior of a meniscus prosthesis in human cadaver knee joints. The movements of the prosthesis in a loading apparatus were determined by means of Roentgen Stereophotogrammetric Analysis (RSA) and were compared with the movements of the native meniscus in the same knee joint before replacement with the prosthesis (**chapter 8**). To validate this in vitro loading model, first the movements of the meniscus in the human cadaver knee joint were compared with the results of in-vivo and in-vitro MRI studies by Vedi and Thompson[73,77](**chapter 7**).

• Aims of the studies

1. To determine the degradation behavior and the role of different porosities, pore sizes, and compression modulus on the in vivo tissue infiltration and foreign body reaction in ectopic, non-loaded locations.
2. Based on this knowledge, new polymers were developed and the next aim was to determine whether these polymers could initiate meniscal lesion healing when placed in a partial-thickness access channel.
3. To determine the consequences of meniscal reconstruction for the underlying articular cartilage: meniscal reconstruction only has clinical relevance if articular cartilage degeneration can be prevented
4. To determine the performance of a newly developed polymer implant for menis-cal replacement with improved biomechanical and geometrical properties
5. To determine the influences of the polymer material on tissue infiltration and dif-ferentiation and the reaction of the tissue on the polymer material
6. To validate a newly developed in-vitro knee joint loading model with respect to former meniscal movement studies in-vivo.

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Chapter 2

Tissue ingrowth and degradation of two biodegradable porous polymers with different porosities and pore sizes...

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Abstract

Commonly, spontaneous repair of lesions in the avascular zone of the knee meniscus does not occur. By implanting a porous polymer scaffold in a knee meniscus defect, the lesion is connected with the abundantly vascularized knee capsule and healing can be realized. Ingrowth of fibrovascular tissue and thus healing capacity depended on porosity, pore sizes and compression modulus of the implant.
To study the lesion healing potential, two series of porous polyurethanes based on 50/50 ε-caprolacton/ L-lactide with different porosities and pore sizes were implanted subcutaneously in rats. Also in vitro degradation of the polymer was evaluated.
The porous polymers with the higher porosity, more interconnected macropores, and interconnecting micropores of at least 30μm showed complete ingrowth of tissue before degradation had started. In implants with the lower macro-porosity and micropores of 10-15 μm degradation of the polymer occurred before ingrowth was completed. Directly after implantation and later during degradation of the polymer, PMN cells infiltrated the implant. In between these phases the foreign body reaction remained restricted to macrophages and giant cells.
We can conclude that both foams seemed not suited for implantation in meniscal reconstruction while either full ingrowth of tissue was not realized before polymer degradation started or the compression modulus was too low. Therefor, foams must be developed with a higher compression modulus and more connections with sufficient diameter between the macropores.

Introduction

Longitudinal lesions in meniscus are among the most frequent orthopaedic affections of the knee.[16] Repair of meniscal lesions by simple suturing techniques is limited to the vascular outer 10-20% of the meniscus.[2,10] For repair of the more common meniscal lesions situated in the avascular part of the meniscus, we connected the lesion with the vascular periphery with soft tissue reconstruction or porous polymers that are able to guide the repair tissue into the avascular defect[12,13,14]. We observed healing of these lesions and fibrocartilaginous tissue developing inside the implants. For this purpose successful candidate implants must fulfill a number of requirements.[3] Among them are, complete degradability into non-toxic products, a sufficiently high compression modulus relevant for the formation of fibrocartilage, a high tear strength to prevent pulling out of the sutures, good adhesive properties, interconnected micropore structure and macropore sizes ranging from 150-355 μm. A variety of polymers have been used for this application. However, there were certain drawbacks. Some implants consisted of an aliphatic or aromatic polyurethane, which resulted in the production of toxic products during degradation[12,13,14]. Moreover, the initial adhesion between polymer and meniscal tissue was insufficient in some cases causing gap formation during load bearing, which impaired lesion repair[13]. Also materials with non-toxic degradation products were used but the degradation rate was too high and the compression modulus was limited[6]. Recently two new polyurethane foams were developed with a lower degradation rate, based on 50/50 ε-caprolacton-L-lactide, also with only non-toxic degradation products[5]. By varying the salt concentration we were able to create two new foams, foam 1 and foam 2, with different compression moduli and porosities.
Question is whether the tissue ingrowth rate differed between these two different polymer foams and whether full ingrowth occurred before degradation of the polymers started. Secondly we questioned if this polymer will initiate a foreign body reaction when implanted in vivo.
The aim of this study was to evaluate the degradation behavior *in vitro* of the newly developed polymers. Secondly we evaluated the role of different porosities, pore sizes, and compression modulus related to the two foams on the *in vivo* tissue ingrowth, polymer behavior and foreign body reaction in ectopic, non-loaded locations.

Materials and methods

Porous materials

Synthesis of the polyurethane was performed according to the procedure described by de Groot et al.[3] A random prepolymer of 50/50 ε-caprolactone/L-lactide (Mn=2000) was obtained by ring opening polymerization of the monomers with the required amount of 1,4-butanediol initiator at 130°C for 30 hours. The resulting macrodiol was end-capped with a six-fold excess of 1,4-butanediisocyanaat at 80°C and the excess was distilled off. Chain extension was performed in 50% (w/w) DMSO solution at 80°C using a BDO.BDI.BDO urethane block-extender. The resulting polymer was precipitated in water and dried under vacuum at 40°C.
The polyurethane was dissolved in 1,4-dioxane at a concentration of 20%.
The solution was mixed with 0.6 g of NaCl and 7.5 g NaCl per gram polymer for respectively foam 1 and 2 (NaCl crystals varying in size from 150-355 mm).
After addition of 4% water and mixing, the mixture was rapidly cooled to room

temperature where gel formation occurred. After cooling to -15°C the foam was freeze dried at 0°C under reduced pressure (0,05 mbar). NaCl crystals were removed by washing the polymer/salt mixture with water.

In vitro analysis of degradation

Porous polymer samples (40x6x6 mm) of foam 1 were subjected to degradation at 37±1°C in iso-osmolar phosphate buffer solutions at pH 6.9 [19]. To ensure a constant pH, the buffer solutions were refreshed regularly. In the first 15 weeks, samples were taken at the beginning of every week. After 15 weeks the interval was increased to 4 weeks.

In vivo analysis of tissue ingrowth and polymer behavior

The size of the foams was based on the size used when implanted in a meniscal defect (5x5x5 mm^3). Samples of foam 1 and 2 were disinfected with 70/30 vol.-% ethanol/water and one sample of both foams subcutaneously implanted bilaterally on the back of 24 Wistar albino rats. After follow up periods of 1, 4, 8 and 24 weeks, 6 rats were killed, samples removed and fixed by immersion in 4% buffered formaldehyde (pH 7,4), dehydrated in ethanol and embedded in polymethylmethacrylate for light microscopy. Sections (7µm) were stained with Haematoxylin-Eosine (HE) for histologic evaluation and tissue ingrowth measurement. To evaluate the behavior of the polymer and measure the polymer surface, adjacent sections were stained with Sudan Black, usually a stain for all kinds of lipid, but also showed to be an excellent histological stain for polymeric biomaterials[7].

Qualitative evaluation

Sections were obtained at equal intervals so that multiple areas of the implant and tissue could be assessed and inadequate sampling minimized. The sections were graded according to: (1) matrix staining; (2) the predominant nature of the ingrown tissue; (3) ingrowth of vascular tissue; (4) thickness of capsule formation; (5) inflammatory reaction and finally degradation of the polymer (6). (Table 1)
Tissue ingrowth into the porous polymer samples as a function of implantation time was determined on Haematoxylin-Eosine (HE) stained slides using the Quantimet 520 Image Analysis System. Three area fractions of tissue and porous polymer in the center and the periphery of every section were determined.
The percentage of tissue and polymer was measured using a magnification of two and a half. The tissue ingrowth and polymer surface in both periphery and center of the polymer sample were plotted as a function of the implantation time.

For each follow up and for both foams, the results of the ingrowth and polymer area fractions were tested for significance using a paired t-test.

Results

Porous materials

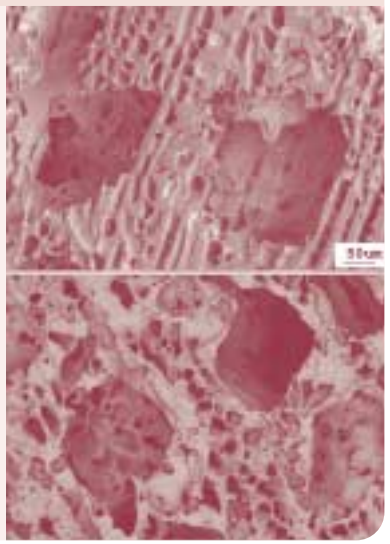
Scanning electron microscopy of the two porous polymers shows that the macropores are well interconnected by channel like micropores. A very open structure was obtained (Fig.1). At high magnification a bi-porous structure containing macropores

table 1

(1) HE matrix staining	Grade 1	Weakly red
	Grade 2	Red
	Grade 3	Deep red/pink
(2) the predominant nature of the ingrown tissue	Grade 0	Inflammatory tissue
	Grade 1	Inflammatory and fibrous
	Grade 2	Fibrous tissue
(3) ingrowth of vascular tissue	Grade 0	No vascular tissue
	Grade 1	Slight
	Grade 2	Fibrovascular tissue
(4) Thickness of capsule formation, cell layers	Grade 0	None observed
	Grade 1	Up to 5 layers
	Grade 2	Up to 10 layers
	Grade 3	Up to 15 layers
	Grade 4	> 15 layers
(5) Distribution of inflammatory cell types	Grade 0	No inflammation
	Grade 1	Slight inflammation with few macrophages and giant cells
	Grade 2	Well defined inflammation with many macrophages and giant cells but no PMN leucocytes
	Grade 3	Moderate inflammation as grade 2 but with few PMN leucocytes
	Grade 4	Severe inflammation, abundant macrophages, giant cells and PMN leucocytes
(6) Degradation of the polymer	Grade 0	No signs of degradation
	Grade 1	Decreased intensity SB staining
	Grade 2	Fragmentation of the polymer
	Grade 3	Regions without polymer

figure 1

SCANNING ELECTRON MICRO-GRAPH OF FOAM 1 (A) AND 2 (B) MADE BY FREEZE DRYING AND MIXED BY NACL CRYSTALS (100-300 MM). NOTICE THE DIFFERENCE IN POROSITY, PORE SIZE AND STRUCTURE.



of 150-300 µm. In foam 1 these macropores were interconnected only by micropores of 15 to 20 µm. Foam 2 showed interconnection by micropores with diameters of at least 30 µm. Polymer foams were produced with initial compression moduli of 460 kPa (foam 1) and 74 kPa (foam 2) and porosities of 73% (foam 1) and 86% (foam 2).

In vitro degradation

The samples only showed minor mass loss in approximately the first 5 weeks of degradation (Fig. 2). After this induction period, the mass loss as a function of time showed a linear relationship. After 10 weeks, the mass was still approximately 90% of its initial value.

In vivo

Gross appearance

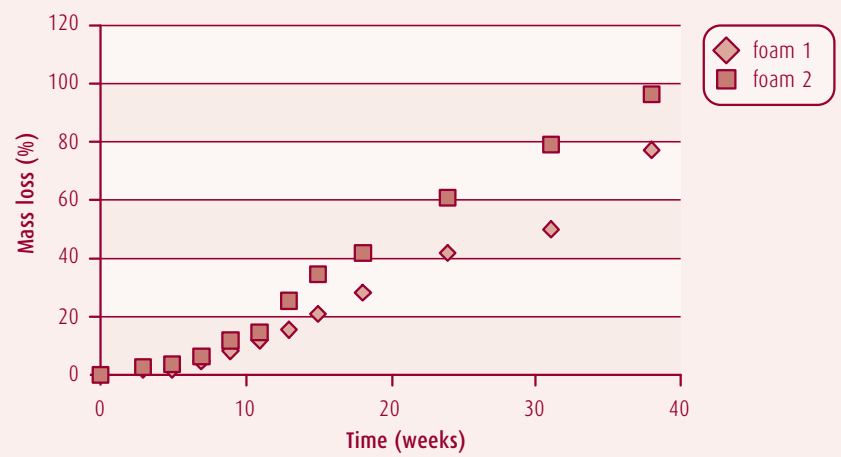
During follow up there were no signs of wound infection. All samples showed encapsulation by fibrovascular tissue. Macroscopically all foam 1 samples were enveloped in a thicker tissue capsule.

Histology

After 1 week, in foam 2, significant more tissue ingrowth was visible in the periphery

figure 2

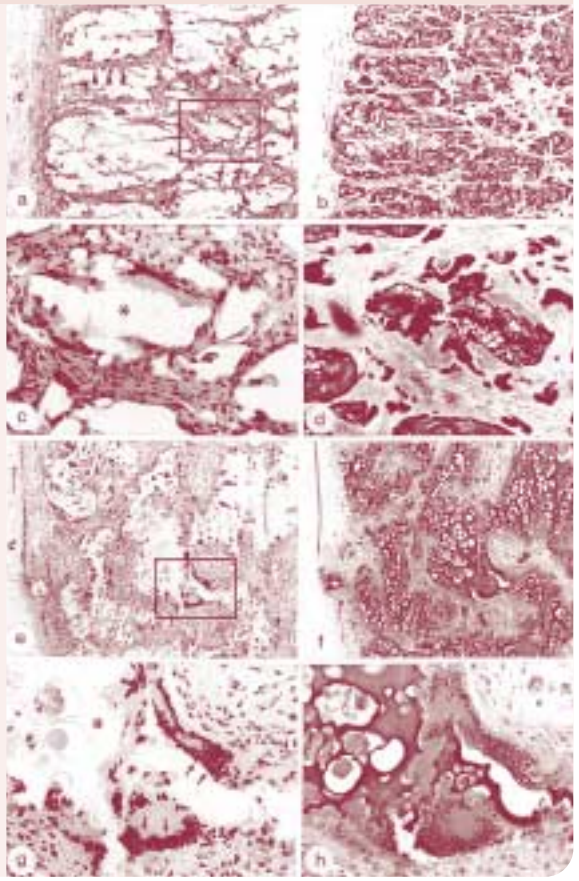
IN VITRO MASS LOSS (%) OF FOAM 1 AND 2 AS A FUNCTION OF THE DEGRADATION TIME



than in foam 1, containing 23% (versus 9%) of the peripheral area fraction (Fig. 4). Also in the center of the samples difference in ingrowth occurred although not significant. In the pores many fibroblasts already started matrix production which was expressed by weak HE staining (grade 1, table 1). All samples of foam 2 showed grade 1 capsule thickness while most samples of foam 1 showed a capsule thickness up to grade 2. Polymorphonuclear (PMN) leukocytes and few macrophages were seen in both foam types (grade 3). These cells initially dominated over the fibrous tissue ingrowth. Fibrovascular tissue was observed. In both foams, no signs of degradation were seen.

Again, after four weeks, there was more ingrowth in the periphery (significant) and center (not significant) of foam 2 than in foam 1 (Fig. 3 and 4). The predominant ingrown tissue had an obviously fibrous aspect (grade 2) which increased the HE staining intensity. Also the centre of foam 2 was infiltrated with fibrovascular tissue (grade 2) and showed a weakly red haemotoxylin-eosine (HE) staining (grade 1). The inflammatory reaction decreased (grade 2) with disappearing of the PMN leukocytes though the amount of macrophages increased. The macrophages became organized along the borders of the polymer pores and especially in foam 2 the macrophages seemed to organize in multiple giant cells (Fig. 3c). The capsule thickness

figure 3

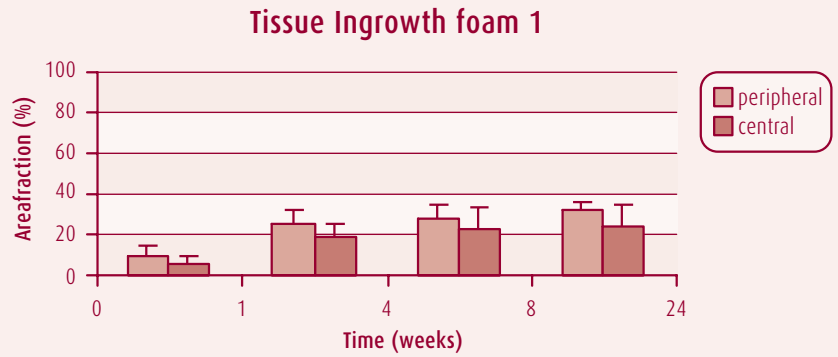


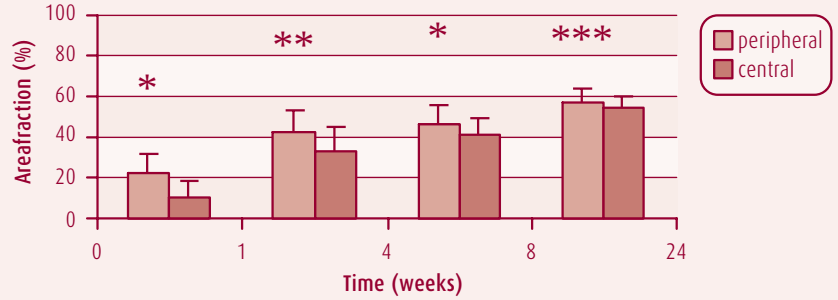
MICROGRAPHS OF SECTIONS Ł STAINED WITH HAEMATOXILINE EOSINE (LEFT COLUMN) AND SUDAN BLACK (ADJACENT SECTIONS) (RIGHT COLUMN) 24 WEEKS AFTER SUBCUTANUOUS IMPLANTATION. A,B INGROWTH FROM THE CAPSULE (CA) INTO THE NARROW INTER-CONNECTING MICROPORES Ł (ARROWHEADS) OF FOAM 1. 50X C,D ENLARGEMENT OF INGROWN TISSUE SURROUNDING THE POLYMER (ASTERIX). 100X E, F INGROWTH FROM THE CAPSULE (CA) INTO FOAM 2. NOTICE THE DIFFERENT STRUCTURE OF FOAM 2 (F) AND THE DIFFERENT ASPECT OF THE Ł INGROWN TISSUE COMPARED TO FOAM 1 (A). THE NACL CRYSTAL PORES, FILLED WITH TISSUE Ł (ARROWS), ARE INTERCONNECTED BY LARGER CHANNELS. 50X G, H ENLARGEMENT OF INGROWN TISSUE SURROUNDING THE POLYMER Ł (ASTERIX). NOTICE THE ACCUMULA-TION OF MACROPHAGES AND GIANT CELLS AROUND THE POLYMER Ł (ARROWS). 100X

remained unchanged.(grade 2) There were again no signs of degradation. After 8 weeks, all type 2 foams were completely penetrated with fibrovascular tissue. In foam 2 both in the periphery as in the center more tissue had infiltrated the pores than in foam 1 (resp. $p<0.05$ and $p<0.02$) (Fig. 4). Generally of all type 1 foams, the periphery was well penetrated but the centre was still devoid of ingrowth (Fig. 6). Consequently, in contrast to foam 2 (grade 2-3), the HE staining in the centre of foam 1 is still disappointing (grade 0-1). In both foams many giant cells were arranged along the borders of the polymer. Although in foam 2 the PMN leukocytes had disappeared (grade 1), in foam 1 these cells still did appear in central pores not infiltrated with fibrous tissue (grade 1). Again the capsule thickness remained unchanged. In both foam 1 and 2 (one and two cases, respectively)

figure 4

IN VIVO AREA FRACTION OF INGROWN TISSUE IN THE SUBCUTANEOUS IMPLANTED FOAMS EXPRESSED IN FRACTION (%) OF THE TOTAL ROI AREA IN THE PERIPHERAL AND CENTRAL REGION OF THE IMPLANT AS A FUNCTION OF THE IMPLANTATION TIME. THE DATA ARE EXPRESSED AS THE MEAN + 1 STANDARD DEVIATION AND VALUES FOR FOAM 1 ARE COMPARED WITH VALUES FOR FOAM 2. * P<0.05, ** P<0.02, *** P<0.001.

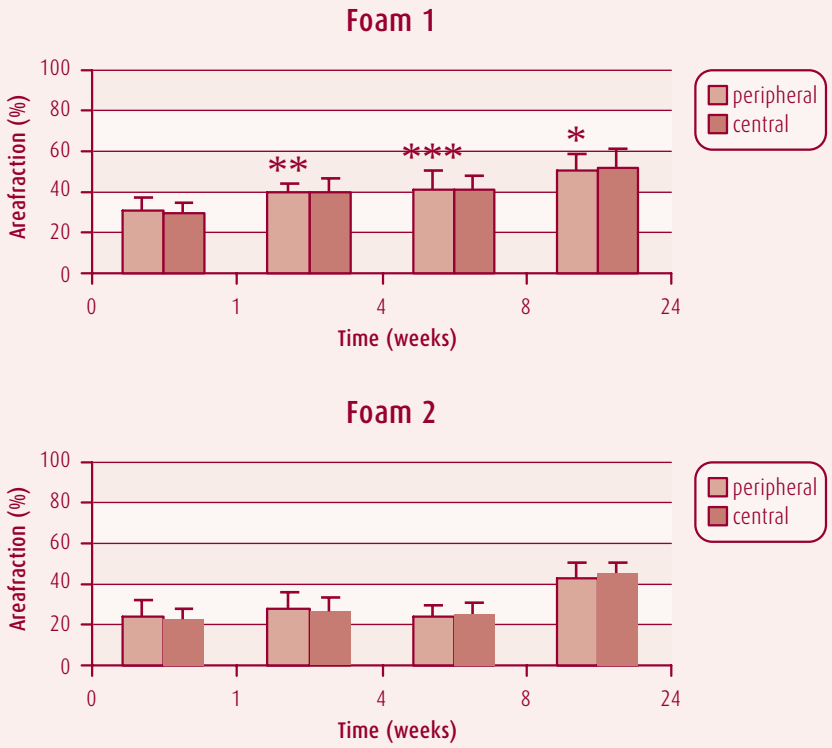




ragmentation of the polymer started which was the first visible sign of degradation. (grade 2) At the latest follow up in type 2 foams ingrowth was already completed. Macroscopically the staining of type 1 foams was more intensive (grade 2-3) (grade 2) implying more ingrowth and matrix formation had occurred since 8 weeks. In 4 samples of foam 1 the center was only filled up with fibrin without any ingrowth of fibrovascular tissue and showed weak HE staining. Both the amount of tissue infiltration in the periphery (57%) as the center (54%) of foam 2 differed significantly from foam 1 (resp. 32% and 23%) (resp. $p<0.001$ and $p<0.02$) (Figure 3a, 3e). In two samples of foam 2, PMN's appeared (inflammatory reaction grade 2-3)

figure 5

IN VIVO POLYMER AREA FRACTION IN THE SUBCUTANEOUS IMPLANTED FOAMS EXPRESSED IN FRACTION (%) OF THE TOTAL ROI AREA IN THE PERIPHERAL AND CENTRAL REGION OF THE IMPLANT AS A FUNCTION OF THE IMPLANTATION TIME. THE DATA ARE EXPRESSED AS THE MEAN + 1 STANDARD DEVIATION AND VALUES FOR FOAM 1 ARE COMPARED WITH VALUES FOR FOAM 2. * P<0.05, ** P<0.02, *** P<0.001.



(Figure 3e, 3g) while in other samples this reaction remained restricted to some macrophages (grade 1). Though in foam 1 this reaction remained at a higher level compared to foam 2. In three samples the predominant nature of the ingrown tissue was fibrous inflammatory tissue (grade 1).

The longer follow up did not influence the amount of cell layers in the capsule. So, capsule thickness in foam 1 samples remained thicker than in foam 2.

At 24 weeks Sudan Black staining showed signs of fragmentation of the polymer structure in 3 samples of foam 1 and 1 sample of foam 2.

The area fraction of the polymer in foam 1 did show an increase during follow up to a maximum of almost 50% both in the periphery as in the center compared to the initial area fraction of 27% (Figure 5). However in foam 2 up to 8 weeks no significant differences were seen between the different follow up terms. Though at 24 weeks the area fraction increased to 45% both in the periphery as in the center.

Discussion

In this study we investigated the in vitro mass loss and in vivo characteristics of two new developed polyurethane foams based on 50/50 ε-caprolactone/L-lactide with a special view to tissue ingrowth, degradation and foreign body reaction after subcutaneous implantation in rats. This simple in vivo experiment is relevant for extrapolation in the in situ model, where the same size samples are used as for biodegradable reconstruction of the knee meniscus. In order to assess the value as a model for in vivo degradation we also performed an in-vitro degradation.

It is already suggested that the repopulation of meniscal implants or allografts might occur through migration, proliferation and differentiation of fibroblasts. These cells should derive from the synovium and the joint capsule [1,2] and produce a fibrovascular scar tissue, which, under appropriate environmental conditions like vascularity and hydrostatic pressure, undergoes a process differentiation resulting in a modulation of the fibrous tissue into fibrocartilage.

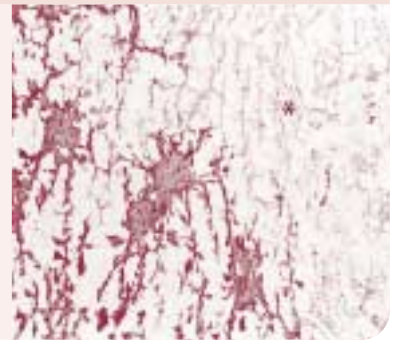
Earlier studies showed that fibrocartilage formation in polymer seemed positively affected by sufficient compression modulus of at least 150 kPa[4,1517].

The fibrovascular tissue ingrowth in foam 1 was significantly less than the ingrowth in the foam 2. In some samples the center was even devoid of ingrowth 24 weeks after implantation. Former studies revealed that microporous implants with macropore sizes of at least 100-300 μm allow fibrocartilage formation in the implant[11,13]. These macropores must be interconnected either directly or by micropores. According to literature, these micropores must have a minimal diameter since pore sizes less than 10-12 mm prevent cellular and capillary penetration[8,18]. However, supported by others studies, the present study showed that micropores of at least 30 μm (foam 2) enabled ingrowth of well vascularized cellular connective tissue while implants with smaller micropores (15-20 μm, foam 1) became filled with predominantly histiocytic tissue[14,20]. Also, we detected a higher ingrowth rate in samples with larger micropores (foam 2) than in samples with smaller micropores (foam 1). However, this difference in ingrowth might also be induced by more direct interconnection between the macropores in foam 2 caused by a higher salt

concentration per gram polymer during fabrication.
When fibrocartilage formation and healing of the longitudinal lesion is complete, degradation of the material is desired to obtain a completely healed meniscus. On the other hand, degradation has been found to affect the compression modulus and density of the implant. In former studies the induction time for fibrocartilage development was at least 8 weeks[14,15]. In order to allow differentiation of the fibro-vascular tissue into fibrocartilage, the degradation of the polymer should not start within this induction time. Till 8 weeks post-surgery, no samples of both foam 1 and 2 showed signs of degradation and we might assume that the structural integrity of the polymer foam remained intact. At later follow up, before full ingrowth was achieved in the center of the foam, degradation started as fragmentation occurred in some samples of foam 1. So, this early degradation not only prevented further ingrowth of tissue but might also inhibit fibrocartilage formation. On the other hand, all foam 2 matrices were completely filled with tissue already after 8 weeks.
The acute phase of a foreign body inflammatory reaction is hard to distinguish of the inflammatory reaction caused by the surgery. Normally this wound healing reaction extinguishes within one week[9]. In this experiment PMN leukocytes were abundantly present after one week of implantation. This cell infiltration seemed to precede ingrowth of the fibrovascular tissue. After four weeks the first signs of a chronic inflammatory reaction were visible with macrophages and giant cells getting organized around the edges of the porous polymer. Already after 4 weeks, PMN leucocytes were almost distinguished in both foams. However, after 24 weeks, besides fragmentation of the central parts of the polymer, in 3 samples of foam 1 new invasion of PMN leucocytes occurred. We assume this reaction starts with degradation of the polymer with detachment of L-lactides, which may cause a local decrease of pH.
By staining the polymer with SB and determining the area fraction of the polymer in the sections we could not confirm the degradation of the polymer by determining the area fraction of the polymer in the sections. The plots showed that in foam 1 even an increase of polymer area fraction seemed to occur both in the center as in the periphery. We assumed that this increase is probably due to swelling of the foam, which influences the area fraction, but not the mass measured in-vitro.
This swelling is a known feature of polymers[21]. Also embedding with PMMA might have caused swelling of the foams, which increased the polymer area fraction in the sections. Also, during the area fraction measurements, SB staining variations occurred within the different parts of the polymer that could hardly be distinguished by the Quantimet 520 Image Analysis System from the adjacent ingrown tissue.

figure 6

CENTER OF FOAM 1 24 WEEKS AFTER IMPLANTATION. NOTICE Ł THE EMPTY CENTER (ASTERIX) DEVOID FROM INGROWING TISSUE FROM THE PERIPHERY (LEFT) Ł AND EVENTUALLY DEGRADATION OF THE POLYMER.



This could have lead to area fractions that are higher than in reality. Therefor careful interpretation of this data is required and this method seemed not suitable for evaluating the degradation process.
This simple in vitro and in vivo experiment indicated that the presented polyurethanes based on 50/50 ε-caprolactone/L-lactide are promising matrices in conducting ingrowing tissue. However, to induce full ingrowth, besides a high porosity, a high interconnectivity between the pores seems required, either directly between the macropores or by interconnecting micropores. Moreover, these micropores should have a diameter of at least 30μm to conduct the fibrovascular tissue.
Both foams seemed not suited for implant in meniscal reconstruction while either full ingrowth of tissue was not realized before polymer degradation started (foam 1) or the compression modulus is too low (foam 2). Therefor, foams must be developed with a higher compression modulus and more connections with sufficient diameter between the macropores.

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Chapter 3

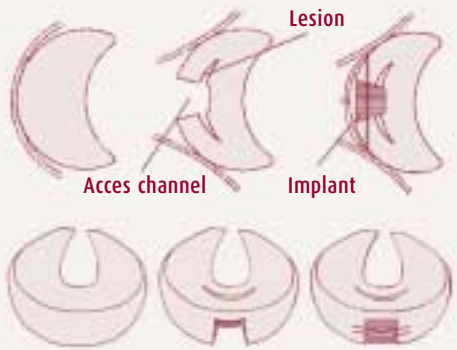
A porous polymer scaffold for meniscal lesion repair. A study in dogs.

TG van Tienen, RGJC Heijkants, P Buma, JH de Groot, AJ Pennings, RPH Veth

Biomaterials 24 :2541-2548, 2003

figure 1

DIAGRAM OF A LATERAL CANINE MENISCUS. ASPECT IN THE FRONTAL PLANE (A) Ł AND THREE DIMENSIONAL ASPECT (B) OF THE POSTERIOR HALF OF THE MENISCUS AFTER TRANSECTION TROUGH THE DOTTED LINE IN (A). THE DOTTED STRUCTURE REPRESENTS THE JOINT CAPSULE (OUTER LINE) AND THE SYNOVIAL LAYER (INNER LINE) OF THE KNEE JOINT. THIS WAS INCISED AND OPENED. A FULL-THICKNESS LONGITUDINAL LESION WAS CREATED IN THE AVASCULAR PART OF THE MENISCUS. Ł A PARTIAL-THICKNESS ACCESS CHANNEL WAS CREATED THAT CONNECTED Ł THE VASCULAR PERIPHERY WITH THE LESION IN THE AVASCULAR CENTRE OF THE MENISCUS. A POLYMER IMPLANT WAS SUTURED INTO THE ACCESS CHANNEL USING TWO SUTURES WITHOUT SUTURING THE LONGITUDINAL LESION

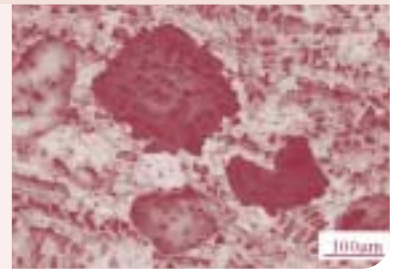


Abstract

Meniscal lesions often occur in the a-vascular area of the meniscus with little chance of spontaneous repair. An access channel in the meniscal tissue can function as an entrance for ingrowing repair tissue from the vascular periphery of the meniscus to the lesion in the avascular zone which again induced healing of the lesion. Implantation of a porous polymer in a full-thickness access channel induced healing. However, a better integration between meniscal tissue and the implant might be achieved with the combination of the newly developed porous polymers and a modified surgical technique. This might improve meniscal lesion healing and the repair of the access channel with neo-meniscal tissue.
Longitudinal lesions were created in the avascular part of 24 canine lateral menisci and a partial-thickness access channel was formed to connect the lesion with the meniscal periphery. In 12 menisci the access channel was left empty (control group), while in the remaining 12 menisci the polymer implant was sutured into

figure 2

SCANNING ELECTRON MICROSCOPIC ASPECT OF (50/50% L-LACTIDE-EPSILON-CAPROLACTON) POROUS POLYMER. BAR = 500 μm



the access channel. The follow up was 3 and 6 months.
Repair of the longitudinal lesions was achieved with and without polymer implantation in the partial-thickness access channel. Polymer implants induced fibrous ingrowth with cartilaginous areas, which resembled neo-meniscal tissue. Implantation did not prevent articular cartilage degeneration.

Introduction

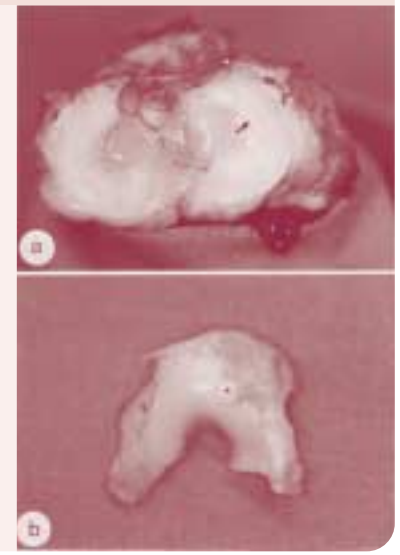
The central region of the meniscus is not supplied by any blood vessels and spontaneous repair of lesions outside the meniscal peripheral vasculature does not occur[1]. However, tears in the meniscus are commonly located in the non-vascularized part of the meniscus. Nowadays, partial meniscectomy is the standard treatment. Several reports documented the results of creating a radial perforation from the vascular periphery towards the lesion in the central a-vascular part of the meniscus[1,15]. This conduit allowed vascular tissue to grow into the lesion and healing of the lesion could be achieved but in many cases the conduit obstructed. Instead, Klompmaker and coworkers created full-thickness access channels in the meniscus of the dog and implanted a porous polymer into this access channel[7]. Healing of the longitudinal lesions in the avascular part of the meniscus was achieved. Furthermore, the access channels with the implants were repaired with fibrocartilage while in access channels without implant only fibrous tissue was observed. However, in some cases integration between the polymer and meniscal tissue was insufficient which led to impaired healing of the lesion. The polymer and the surgical technique still had to be improved.
Based on tissue ingrowth studies in rats[13], new scaffolds have been developed with a more optimal pore structure for fast ingrowth of tissue and integration with the

host meniscal tissue. Furthermore, the size of the access channel was minimized so that more of the meniscal structural integrity remained intact.
We hypothesized that the combination of the newly developed porous polymers and a modified surgical technique leads to a intensive integration between the polymer and the host meniscal tissue, which again leads to meniscal lesion healing and restoration of the access channels with neo-meniscal tissue.
The first aim of the present study on the dog was to determine whether the newly developed porous polymer placed in a partial-thickness access channel could initiate meniscal lesion healing compared to the access channel without implant. Second, we intended to determine the phenotype of the tissue formed in the newly developed implants compared to the tissue formed in the empty access channels. Third, we intended to evaluate the consequences for the articular cartilage.

Materials and Methods

We performed the experiments on 24 lateral menisci in 12 adult male and female Beagles, with an average weight of 13.1 kg (SD: ±1.6). 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 nitrousoxide (1:1) and isoflurane (0.5%). The right and/or left knee joint was entered using a lateral skin incision. We took great care not to injure the collateral and cruciate ligaments and the articular cartilage.
In 24 menisci a full thickness longitudinal lesion (further called "lesion") was created in the avascular part of the lateral meniscus at 1/3 from the radial width of the meniscus (Fig. 1). After creation of the lesion no blood did appear in the lesion, as a sign that the lesion was created in a non-vascularized area. Subsequently, a partial thickness access channel (further called "access channel") was created in the vascular periphery and the lesion. This access channel contained 50% of the height of the meniscus. By leaving 50% of the peripheral meniscal tissue intact, the meniscal function of transferring hoop stresses could still be exercised. A polymer implant was sutured into 12 access channels while the remaining 12 access channels were left empty. First, both sides of the polymer were sutured with two non-resorbent 3-0 Ethilon sutures (Ethicon, Johnson & Johnson, Amersfoort, the Netherlands) to the meniscus along the peripheral side of the implant. The lesions were stable and were not sutured. The capsule and skin were closed in layers using 3-0 Vicryl sutures

figure 3



A. MACROSCOPIC VIEW OF A Ł MENISCUS ON THE TIBIAL PLATEAU 3 MONTHS AFTER POLYMER Ł IMPLANTATION. THE SCAR OF THE LONGITUDINAL LESION IS STILL Ł VISIBLE (ARROW) BUT APPEARS TO HAVE HEALED.

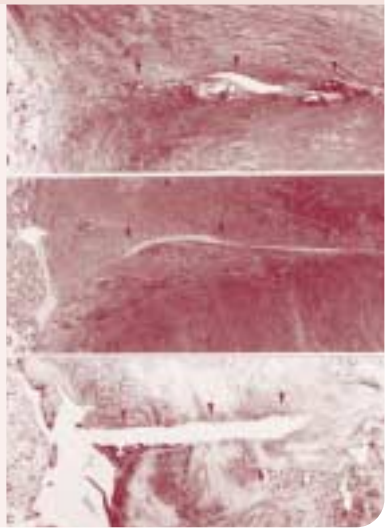
B. TIBIAL ASPECT OF A MENISCUS 3 MONTHS AFTER POLYMER Ł IMPLANTATION. POLYMER IS STILL VISIBLE AND THE CENTRAL REGION IS HARD TO DISTINGUISH FROM NATIVE MENISCAL TISSUE Ł (ASTERISK)

(Ethicon, Johnson & Johnson, Amersfoort, the Netherlands). The dogs were allowed to walk as soon as possible. Follow-up periods were three and six months.

Polymer

Implants consisted of biodegradable polyester urethanes based on L-lactide/ε-caprolactone as described previously[10]. A random prepolymer of 50/50 ε-caprolactone/L-lactide (Mn=2000) was obtained by ring opening polymerization of the monomers with the required amount of 1,4-butanediol initiator at 130°C for 30 hours using stannous octoate as catalyst. The resulting macrodiol was end-capped with a six-fold excess of 1,4-butanediisocyanaat at 80°C and the excess was distilled off. Chain extension was performed in 50% (w/w) DMSO solution at 80°C using a BDO.BDI.BDO urethane block-extender. The chain extender in the respective solvent was added slowly under vigorous mechanical stirring. When the reaction mixture became viscous, more solvent was added to keep the system homogeneous. After the addition of the chain extender was stirred for 10 hours and subsequently diluted to a 1-2 w/w % solution. The resulting polymer was precipitated in water and dried under vacuum at 40°C.
The polyester polyurethane was dissolved in 1,4-dioxane at a concentration of 20%. The solution was mixed with 2.5 g of NaCl per gram polymer (NaCl crystals varying in size from 150-355 mm). After addition of 4% water and mixing, the

figure 4



A. LONGITUDINAL LESION (INDICATED BY ARROWHEADS) ADJACENT TO THE POLYMER SCAFFOLD (LEFT ON THE MICROGRAPH), 3 MONTHS AFTER IMPLANTATION. NOTE THE PRESENCE OF FIBROBLASTIC TISSUE IN THE LESION. P = POLYMER, R = REPAIR TISSUE.

B. LESION PARTIALLY FILLED WITH FIBROBLASTIC TISSUE.

C. OPEN LESION ON THE FEMORAL SIDE OF THE MENISCUS (INDICATED BY ARROWHEADS) HE 40X

mixture was rapidly cooled (-15°C). The foam was freeze dried under reduced pressure (0,05 mbar). NaCl crystals were removed by washing the polymer/salt mixture with water. Upon degradation, this polymer yields L-lactic acid, hydroxyhexanoic acid, butanediamine and butanediol. Porosity was 80%. The compression modulus was 200 kPa.

Macropores were created by mixing the polymer solvent solution with salt crystals ranging in size from 155-355 µm. Micropores of at least 30 mm were created by freeze-drying the solvent (Fig. 2)[2]. Porosity was 80%.

Histology

After sacrificing the dogs, menisci were removed and fixed in acetone (-20°C) for six hours, infiltrated in methylmethacrylate and polymerized at -20°C for two days. Sections (7 µm) were cut, dried at 37°C and stained with Haematoxylin-eosine. Distinct to fibrous ligamentous tissue, fibrocartilage contains proteoglycans and collagen type II in its extra-cellular matrix. To differentiate between fibrous tissue and fibrocartilage, sections were stained with Alcian blue and an immunohistological detection of collagen type II was performed. In preparation for immunohistochemistry, sections were deacrylated three times in chloroform-xylol (1:1) for 15 minutes and subsequently treated with 1% testicular hyaluronidase (type I-S, EC 3.2.1.35; Sigma, St. Louis, MO, USA) in PBS for 30 min at 37°C. After washing and treatment with 10% normal horse serum in PBS with 1% bovine serum albumin (Sigma) to

table 1

HEALING RESPONSE OF LONGITUDINAL MENISCAL LESIONS WITH OR WITHOUT POLYMER IMPLANT

	Implant 3 months n=6	Implant 6 months n=6	Control 3 months n=6	Control 6 months n=6
Meniscus				
Healing lesion side (healed-partially-none)	3-2-0 [f]	2-4-0	0-3-3	0-4-2
Tibial cartilage				
Microscopy (Mankin score) Average (SD)	2.8 (±2.4)	1.7 (±0.8)	4.3 (±1.8)	1.5 (±1.2)

[f] poor-quality slices; could not be evaluated

table 2

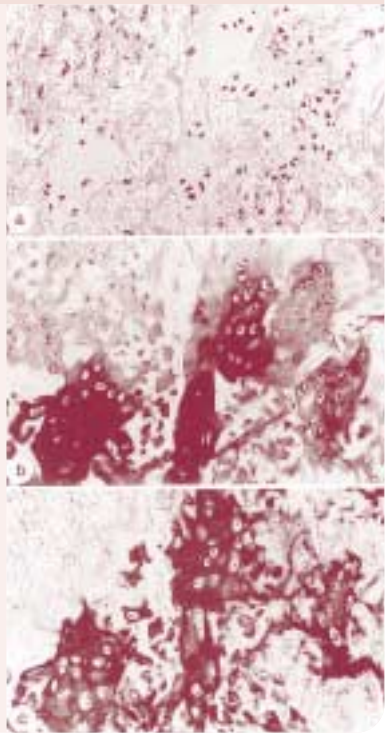
PHENOTYPE AND PERCENTAGE INGROWTH INTO IMPLANT/DEFECT

		Implant 3 months	Implant 6 months	Control 3 months	Control 6 months
		Number of cases			
Phenotype					
a) Fibroblastic cells		0	0	6	5
b) Cartilaginous cells		1	0	0	1
c) b + Collagen type II antibody labelling		2*	0**	0	0
d) b + Proteoglycan synthesis (Alcian Blue staining)		2	3	0	0
e) b + c + d		1	3	0	0
Percentage ingrowth					
Proportion	0–25%	1	0	0	1
	25–50%	2	0	0	1
	50–75%	2	0***	1	0
	75–100%	1	6	5	4

** Significantly different from results in control group after three months. * * Significantly different from results in control group after six months. ***Significantly different from implant group after three months*

block nonspecific labeling, monospecific monoclonal anti-collagen II antibody (II-II6B3, anti-chicken raised in mouse, Developmental Studies Hybridoma bank, University of Iowa, USA)[9] was applied and the samples were incubated in a humidified chamber overnight at 4°C. Anti-collagen II antibody was detected using a biotin-labeled anti-mouse antibody (1/600 dilution for one hour at room temperature;

figure 5



ADJACENT SECTIONS STAINED Ł WITH HAEMATOXYLIN-EOSINE (A), ANTI-COLLAGEN TYPE II LABELING (B) AND ALCIAN BLUE (C). A. Ł CARTILAGINOUS PHENOTYPE OF THE CELLS AND THEIR MATRIX. B AND C. COLLAGEN TYPE II ANTIBODY DETECTION AND ALCIAN BLUE IN THE CARTILAGINOUS CELL MATRIX IN ADJACENT SECTIONS. NOTE THE LABELLING AROUND THE CELLS. IMPLANT GROUP AT 3 MONTHS FOLLOW-UP. 200X

Dako). 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) dissolved in 15 ml PBS with 12 µl H_2O_2 (30%) for 7 min. After rinsing, sections were dehydrated and mounted with DPX (BDH, Poole, England).
Blocks of the tibial plateau were fixed in a buffered formaldehyde solution (4%, pH 7.4) for two days and decalcified in 10% EDTA (Titriplex III, Merck, Darmstadt, Germany) for 8 weeks at 4ºC. Tissue blocks were rinsed, dehydrated and embedded in methylmetacrylate for two days. Sections were made in the plane through the cartilage opposite the access channel.

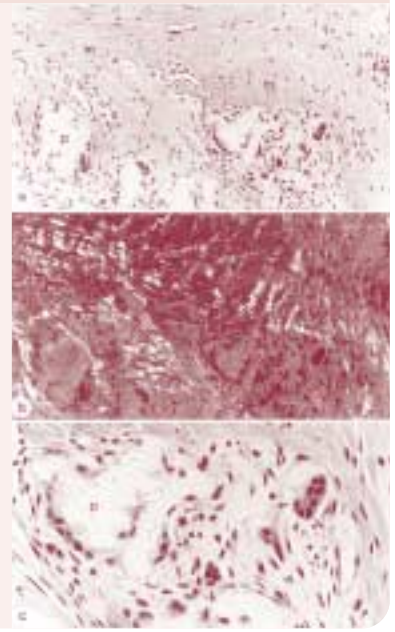
Macroscopy

We evaluated the appearance of the lateral meniscus, its attachment to the peripheral capsule on the access channel side and the incorporation and localization of the foam in relation to the native meniscal tissue.

Microscopy

On an ordinal scale, sections were scored for restoration of the access channel and healing of the longitudinal lesion. Healing of the lesion was defined as filling of this lesion with tissue. The healing was scored as none (no healing response), partial (at least 50% of the lesion was filled with repair tissue), or healed (at least 75% of the lesion was filled with repair tissue). We evaluated the phenotype of the tissue in the lesions, access channels and the attachment between the polymer implant and the native meniscal tissue.
The cell phenotype of the ingrown tissue was classified as fibroblastic or chondroblastic, combined with either proteoglycan synthesis (Alcian Blue staining) or collagen type II formation (collagen type II antibody labeling), or combined with both.
By using the Quantimet 520 Image Analysis System, the percentage of the total implant surface labeled with collagen type II antibody was determined quantitatively in two sections through the centre of the implant at a distance of 200 µm. Tissue ingrowth into the implant (percentage of tissue on the total implant surface) as well as the attachment of the implant to the meniscal tissue (the percentage of direct contact of the implant's total circumference) was determined on three sections at 200 µm intervals. Data were analyzed statistically by the Wilcoxon Rank Sum test.

figure 6



A. CLOSE INTEGRATION BETWEEN THE POROUS POLYMER (P) AND NATIVE MENISCAL TISSUE (M) AT 3 MONTHS AFTER IMPLANTATION. H&E 100X

B. SAME SECTION WITH POLARIZED LIGHT SHOWING OBVIOUS Ł INGROWTH OF COLLAGEN FIBRES INTO THE POROUS POLYMER.

C. POROUS POLYMER (P) Ł SURROUNDED BY NUMEROUS Ł GIANT CELLS (ARROWHEADS). Ł HE 250X

The inflammatory reaction in the implant group was compared to the reaction in the empty access channel control group.

Articular cartilage

Degeneration of the tibial articular cartilage was evaluated by its gross appearance and by the level of disruption of the normal architecture. 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)[10].
A total score for each tibial sample was obtained by adding together the scores in each of these subcategories. T-test p-values were calculated and values of less than 0.05 were considered to be significant.

Results

Clinical observations

The dogs had regained their normal gait pattern 10 days postoperatively. No infections were seen. All implant menisci and empty control access channel menisci were available for evaluation. Postmortem, there were no signs of synovitis in the joint capsule and the synovial fluid was clear.

Three months after surgery

One meniscus in the implant group (n=6) and two in the empty access channel group (n=6) showed degenerative aspects with fibrillation. All the polymer implants were firmly attached to the periphery and covered by a thin fibrous layer, but they could still be distinguished from the native meniscal tissue (Fig. 3b).
Microscopical examination of the sections revealed that healing of the lesions had occurred in both the implant group and the control group (no significant difference between the two groups: p=0.376 and p=0.537) (Fig. 4a and b) (Table 1). Healing was less in case of less integration between implant and meniscal tissue (Fig. 4c).
The repair tissue was fibrous in all the lesions. In one meniscus in the implant group, healing of the longitudinal lesion could not be evaluated due to poor quality of the sections.
The implants mainly contained cells with a fibroblast phenotype. Only the implant group showed areas with cartilaginous cells (Table 2) (Fig. 5). All six cases showed antibody labeling and Alcian Blue staining. One case showed clear-cut patches of cartilaginous cells surrounded by a matrix with positive staining for Alcian Blue and labeling with collagen type II (average 8% (6-16%). In the control group, the tissue in the access channels had a fibroblastic phenotype.
All the implants had become completely integrated into the native meniscal tissue (Fig. 6).

Six months

Macroscopically, all menisci in the implant group (n=6) were intact, whereas in the control group (n-6) two menisci had a degenerative appearance. The polymer had become completely incorporated, but remained distinguishable from the host meniscal tissue.
In the implant group, partial healing was observed was observed in five cases. One lesion had healed completely. The control group also showed healing, but none had healed completely (Table 1). The repair tissue was fibrous in all the lesions.
At least 75% of the access channel surface area in the implant group was filled with ingrown tissue (Table 2). In two cases the implant was dislocated peripherally.
All the implants contained cells with a cartilaginous phenotype that were lying in lacunae. Three cases showed evident collagen type II labeling over an average of 9% (2-22%) of the total implant surface area. These cases also showed positive staining for Alcian Blue. In one case, the patches with positive collagen type II labeling (22% of the total implant surface) matched identically with the Alcian Blue staining. All the empty control access channels were completely filled with fibroblastic tissue without any characteristics of (fibro)cartilage.
Five cases showed almost complete integration of the implant to the meniscal tissue. Degradation of the polymer implant had already started and the space was filled with repair tissue. Signs of inflammation were restricted to macrophages and giant cells, all arranged in the pores along the borders of the implant. No PMN leucocytes were observed at three or six months follow-up. The control access channels did not show any signs of inflammatory reactions.

Articular cartilage

At three months follow-up the control group the cartilage degeneration tended to be higher than the implant group (not significant (p=0.245)) (Table 1). Cartilage degeneration in the control group was significantly less at six months than at three months ($p<0.05$, t-test).

Discussion

In this study, meniscal lesions were created in the avascular part of the canine lateral meniscus and were connected with the vascularized periphery by suturing a porous polyurethane implant into a partial thickness defect. Subsequently, the repair of the meniscus was analyzed.
Although we minimized the depth of the access channel, our healing rates were comparable with those of Klompmaker et al.[7,8]. Although they found favorable healing rates, the repair rates were less in the posterior horns of several cases, probably because of an insufficient integration of the implant into the meniscal tissue. The full-thickness access channels interrupted the circumferentially-orientated collagen fibers. Which play an important role in transferring the axial loads via the meniscal horns to the tibial plateau[12]. Presumably, the radial extrusive forces during load bearing created a gap between the implant and the tissue in the posterior horn, which led to impaired healing of the lesion in those cases. In this study, some of the circumferential collagen fibers were left intact by creating partial thickness access channels. As a result, the implant had become almost completely integrated into the native meniscal tissue.
In the present study, newly developed biodegradable polyurethanes were used based on L-lactide, ε-caprolacton, butanediisocyanate and butanediamine. Degradation of this polymer yields L-lactic acid and hydroxy-hexanoic acid, which were found to be non-toxic in low concentrations as degradation products of nerve guides[3]. In vitro experiments, however, showed decreased monolayer chondrocyte activity in mediums with high concentrations of lactate[6]. However, in view of the slow degradation process of the polymer it is likely that the concentrations of degradation products were low[14]. Butanediisocyanate converts into butanediamine, which is also known as putrescine. This substance is present in the cells of mammals and plays an important role in cell division[5].
Ghadially, Webber and McDevitt described meniscal tissue as consisting of chondrocytic cells called fibrochondrocytes. These were surrounded by an abundant extra-cellular matrix containing mostly collagen type I, but also some collagen type II and proteoglycans, which are major components of hyaline cartilage[4,11,16]. In this study, all implant cases showed the features of (fibro)cartilage, i.e. cartilage-like cells, collagen type II and proteoglycans. However, matching patches of collagen type II antibody labeling and Alcian Blue staining were only observed in two cases. In a comparable experiment, Klompmaker et al. described only fibrous tissue in the implants at up to 12 weeks follow-up, but from 24 to 52 weeks follow-up there was increasing formation of fibrocartilage with collage types I and II. Therefore, in a longer follow-up study, more fibrocartilage formation might be expected.
Three months after implantation, the polymer scaffolds were slightly infiltrated with polymorphonuclear leucocytes, or not infiltrated at all. Nevertheless, in agreement with earlier studies, macrophages and giant cells were situated along the borders of the implant[7,8,17]. Apparently, the implanted polymer induced a foreign body reaction and these cells encapsulated the polymer. These cells not only play an important role in phagocytosis and the removal of material fragments[18], but they also release angiogenic factors that stimulate blood vessel development towards the implant[19]. All these aspects might be of relevance to integration of the implant.
Insertion of a polymer implant in the access channel may have had a favorable effect on the healing process of the meniscus, but it did not prevent articular cartilage degeneration. In the empty access channel group, it is possible that the edges of the access channel damaged the articular cartilage during meniscal movement. Despite close integration between the meniscal tissue and implant, cartilage degeneration was not prevented in the implant group, although it was less severe than in the control group. However, leaving the access channel empty might not be a suitable control method to evaluate cartilage degeneration. Nowadays, partial meniscectomy is the standard procedure in patients with a lesion in the avascular part of the meniscus. Therefore, to evaluate articular cartilage damage, it might be more justified to compare an implant group to a control group with partial meniscectomy.
We can conclude that creation of an access channel towards a meniscal lesion in the avascular region of the meniscus can initiate healing of this lesion. Implantation of the newly developed biodegradable porous polymer porous polymer scaffolds did not inhibit this healing process. The scaffold became intensively integrated with the host meniscal tissue and showed the potential to stimulate fast ingrowth of fibrovascular tissue into the created access channel but the scaffold also enabled the differentiation of this tissue into cartilage-like tissue as observed in native meniscus. The foreign body reaction to the scaffold remained restricted to macrophages aligned along the borders of the polymer. The initial goal of this study was to develop a new reconstruction technique to repair the lesion in the avascular zone of the meniscus without articular cartilage degeneration. However, degeneration could not be prevented.

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Chapter 4

Presence and mechanism of knee articular cartilage degeneration after meniscal reconstruction in dogs.

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Abstract

Partial meniscectomy is the golden standard for treating a bucket-handle tear in the meniscus of the knee, but it inevitably leads to articular cartilage degeneration. Surgical creation of an access channel between the lesion and the vascularized synovial lining is intended to induce ingrowth of repair tissue and thus avoid degeneration of articular cartilage.
The presence and mechanism of cartilage degeneration were evaluated in 24 canine menisci after a longitudinal lesion and access channel had been created in the avascular part of the meniscus. In 12 menisci the channel was implanted with a porous polymer scaffold, while the remaining 12 were left empty. Evaluation was performed using routine histology and antibodies directed against denatured type II collagen (Col2-3/4M). the follow-up was 3 and 6 months.
Articular degeneration was apparent in the polymer implant group and the empty channel group. This consisted of fibrillation, loss of chondrocytes and decreased proteoglycan content. Areas of fibrillated cartilage always showed positive labeling with the collagen degradation antibody Col2-3/4M. Collagen degradation was also visible in non-fibrillated areas. The upper zone of the cartilage showed swelling especially in the implant group, with empty cell lacunae and moderate levels of Col2-3/4M antibody labeling.
This reconstruction technique cannot be considered superior to partial meniscectomy. We propose that degradation of the collagen type II network is a result of cartilage fibrillation and vice versa.

Introduction

The mechanism of articular cartilage degeneration is not completely understood. Collagen type II degradation seems to play an important role in this degeneration process[2,16]. Disruption or disorganization of the highly structured fibril network in the superficial cartilage might be an initiating factor in the degeneration cascade and might eventually lead to weakening and fibrillation of the superficial cartilage layers. In mice and rats, Stoop et al. showed that degradation of collagen type II is involved in the first stages of OA, using immunolocalization [22,20]. Price et al. demonstrated the same feature in humans[19]. This raises the question as to whether collagen type II degradation also occurs after meniscal reconstruction and whether it is related to the presence of morphological cartilage damage.

As described in the previous chapter, in this study, a bucket-handle lesion was created in the avascular zone of 24 canine menisci. An access channel was opened between the lesion and the vascularized synovial lining to induce healing. In 12 cases a porous polymer scaffold was implanted in this channel with the aim of enhancing the healing.
The purpose of this study was to determine in detail the consequences of this reconstruction technique on the articular cartilage degeneration in dogs. The presence of cartilage degeneration and the role of collagen type II degradation was evaluated by means of routine histology and antibodies directed against denatured type II collagen (COL2-3/4M)[7].

Material and Methods

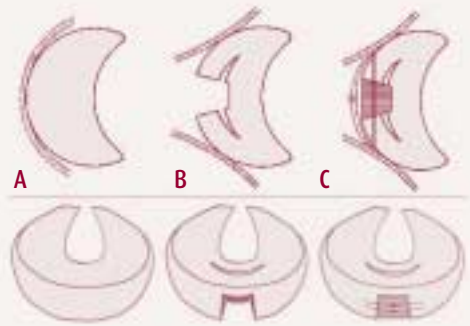
Experiments were performed under aseptic conditions on 24 lateral menisci in 12 adult male and female Beagles with an average weight of 13.1 kg (SD: +/- 1.6 kg). The institutional animal welfare committee approved all procedures.
In preparation for surgery, anesthesia was accomplished by intravenous administration of pentobarbital (30 mg/kg) and maintained after intubation with nitrous oxide (1:1) and isoflurane (0.5%). The right and/or left knee joint was entered using a lateral skin incision. Great care was taken to prevent damage to the collateral and cruciate ligaments and the articular cartilage.
To imitate a meniscal bucket-handle tear, a full thickness longitudinal lesion was created in the avascular part of the lateral meniscus (Fig. 1). In all cases, a partial thickness defect was created, occupying 30% of the meniscal length of the meniscal mid-substance to form an access channel between the vascular synovial lining and the lesion in the avascular zone of the meniscus.
In 12 dogs, a polymer implant was sutured into the partial thickness defect in the meniscus (Access Channel with Implant, ACI), while on the contralateral side the meniscal defects were left empty (Empty Access Channel, ACE). Both sides of the polymer implant were sutured to the meniscus with two 3-0 Ethilon sutures along the peripheral side of the implant. The longitudinal lesion was not sutured. The capsule and skin were closed in layers using 3-0 vicryl sutures. The dogs were allowed to walk as soon as possible. Cartilage degeneration was evaluated after three and six months.

Polymer

Implants consisted of biodegradable polyester urethanes based on L-lactide/ε-

figure 1

DIAGRAM OF A NORMAL LATERAL MENISCUS IN THE DOG: UPPER VIEW AND THREE DIMENSIONAL PRESENTATION (A). THE DOTTED STRUCTURE REPRESENTS THE JOINT CAPSULE (OUTER LINE) AND THE SYNOVIAL LAYER (INNER LINE) OF THE KNEE JOINT. THIS WAS INCISED AND OPENED (B). A FULL THICKNESS LONGITUDINAL LESION WAS CREATED IN THE AVASCULAR PART OF THE MENISCUS (B). Ł
A PARTIAL THICKNESS CHANNEL WAS CREATED CONNECTING THE VASCULAR PERIPHERY TO THE LESION IN THE AVASCULAR CENTRE OF THE MENISCUS. A POLYMER IMPLANT WAS SUTURED INTO THE CHANNEL USING TWO SUTURES; THE LONGITUDINAL LESION WAS NOT SUTURED (C).



caprolactone as described previously, which only yield non-toxic degradation products[6]. The properties of the polymers were improved compared to the polymers used in the earlier study[13]. The new polymers express many reactive carboxylic groups at the surface during degradation, which may increase the attachment between the polymer and the meniscal tissue. The polymer contained macropores with the size of 155-355 µm. To increase the ingrowth of repair tissue, these pores were interconnected with micropores of at least 30 µm, as suggested in earlier studies[14,25]. The porosity was 80% and the compression modulus was 200 kPa.

Histology

Two sets of 12 knee joints were dissected after killing a equal proportions of dogs at 3 and 6 months post-surgery. Directly opposite the defect region in the meniscus the lateral tibia plateau was divided into an anterior half and a posterior half. The anterior part was processed for routine histology and the posterior part for immunohistochemistry.

The femur condyles and anterior halves of the lateral tibial plateau cartilage were fixed in 4% formaldehyde buffered with 0.1M phosphate buffer (pH 7.4) and decalcified in 10% EDTA (Titriplex III, Merck, Darmstadt, Germany). After extensive rinsing with tap water, tissue blocks were dehydrated in alcohol and embedded in polymethylmethacrylate for sectioning. Sections (7 µm) were made in the plane through the cartilage opposite the defect and stained with Haematoxiline/Eosine (HE) and Toluidine Blue.

Immunohistochemistry

The posterior halves of the tibial plateau cartilage were decalcified without previous chemical fixation in 10% EDTA (Titriplex III, Merck, Darmstadt, Germany) and 7.5% polyvinylpyrolidone (PVP, Mr 29,000, Serva, Brinswich, Amsterdam, the Netherlands) in 0.1M Tris buffer for eight weeks at 4ºC[21,23]. After extensive rinsing with 7.5% PVP in 0.1M Tris buffer, tissue blocks were rapidly frozen in liquid nitrogen and stored at -70ºC. Coronal sections were cut (7 µm) on a Bright 3050 cryostat and mounted on glass microscope slides pre-coated with 3-aminopropyltriethoxysilan (Sigma, St. Louis. MO). Sections were made in the plane through the cartilage opposite the defect and were dried for one hour and stored at -80ºC until required for further use. After thawing, the sections were fixed in freshly prepared paraformaldehyde (5 minutes) and washed extensively in 0,1 M phosphate buffered saline (pH 7.4; PBS) for 15 minutes. To enhance the permeability of the extracellular matrix, glycosaminoglycans were removed by incubating the sections with 1% hyaluronidase (testicular, type I-s, EC 3.2.1.35, Sigma, St. Louis, MO) in PBS, for 30 minutes at 37ºC.
Non-specific staining was blocked by incubation of the sections with 10% normal horse serum (Col2-3/4M) in PBS with 1% bovine serum albumin (Sigma).
Sections were incubated over night with the Col2-3/4M antibody (1/800) against denatured type II collagen at 4ºC in a humidified chamber. Biotin-labelled horse anti-mouse antibodies and goat anti-rabbit antibodies (DAKO, Glostrup, Denmark, 1/400) were used as secondary antibodies (1 hour, room temperature). A biotin streptovidin detection system (Vectra elite kit, Vector, Burlingame, CA) was used according to the manufacturers recommendations. Peroxidase was detected using tablets containing 10 mg 3',3'diaminobenzidine (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). Adjacent sections were stained with toluidine blue to demonstrate glycosaminoglycans[21].

Evaluation

The healing response of the meniscus was scored as none (no healing response), partial (at least 50% of the lesion was filled with repair tissue), or healed (at least 75% of the lesion was filled with repair tissue). The phenotype of the tissue in the empty access channels and the polymer implants was evaluated.

Macroscopically, all joint surfaces were evaluated directly after dissection for cartilage damage, which was graded on an ordinal scale as no degeneration (deg-), minor degeneration (discolored cartilage areas, deg+), mild degeneration (roughened cartilage surface, deg++), and presence of depressions or craters in the cartilage (crater)[29].

During microscopical evaluation the observer (TvT) was blinded to the treatment. Microscopically, the femoral and tibial articular cartilage degeneration was evaluated on the basis of disruption of the normal architecture of the articular cartilage as could be observed on sections stained with Toluidine blue and Hematoxyline-eosine. These 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 3), normal Toluidine blue staining (in stead of Safronine O staining, as used in the original Mankin score(17))(grade 0) to no staining (grade 4) and an intact tide mark (grade 0) or a tidemark infiltrated with blood vessels (grade 1).

Toluidine blue staining was scored with an ordinal scale from no staining (-) to normal staining (++++) as in non-damaged articular cartilage.

Collagen type II degradation was evaluated on immunohistological sections. The presence of denatured collagen type II (positive staining Col2-3/4M) was assessed on an ordinal scale as absence of labeling (-), labeling in the cartilage surface layers (+), labeling down to the middle zone (++) and labeling down to the deeper zone of the cartilage (+++).

Data were statistically analyzed by means of the t-test; p-values of less than 0.05 were considered significant.

Results

Three months

Lesion healing and fibrocartilage formation

Data concerning the healing of the lesion are presented in Table 1. In the ACI group, in three cases the lesion was healed, while in the ACE (Empty Access

table 1

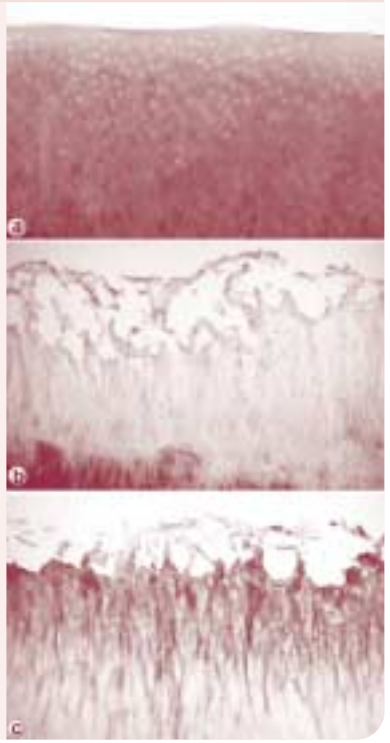
TIBIAL CHARACTERISTICS OF THE MACROSCOPICAL AND MICROSCOPICAL RESULTS IN THE INDIVIDUAL CASES IN THE FOUR GROUPS. ACI: ACCESS CHANNEL WITH IMPLANT, ACE: EMPTY ACCESS CHANNEL, * SIGNIFICANT DIFFERENCE IN AVERAGE MANKIN SCORE BETWEEN THE ACE GROUPS AT 3 AND 6 MONTHS.

ACI		6 months						
dog nr.	Side	Phenotype tissue	Lesion healing	Degeneration	Mankin score	TB staining	Collagen degradation	Swelling
				Macroscopy		Microscopy		
1	R	fibrocart	?	crater	7	+	+	-
2	R	fibrous	healed	deg ++	3	++	?	+
3	R	fibrous	partially	deg +	3	+++	-	+
4	R	fibrous	healed	deg -	0	+++	++	+
5	R	fibrous	healed	crater	3	++	+++	-
6	R	fibrous	partially	deg +	1	+++	-	++
				Average	2.8			
ACE								
7	L	fibrous	partially	crater	7	++	+++	-
8	L	fibrous	partially	crater	4	++	++	-
9	L	fibrous	partially	deg -	2	+++	-	-
10	L	fibrous	not healed	crater	5	++	+++	-
11	L	fibrous	not healed	crater	3	+++	+	+
12	L	fibrous	not healed	deg +	5	+++	+++	+
				Average	4.3*			

?: damaged sections; could not be evaluated

ACI		6 months						
dog nr.	Side	Phenotype tissue	Lesion healing	Degeneration	Mankin score	TB staining	Collagen degradation	Swelling
				Macroscopy		Microscopy		
1	L	fibrocart	partially	deg ++	3	++	+++	-
2	L	fibrous	partially	deg +	1	++	+	-
3	L	fibrocart	partially	deg -	2	++	-	+
4	L	fibrocart	healed	deg -	2	+	++	-
5	L	fibrous	partially	deg -	1	++	-	+
6	L	fibrous	healed	deg -	1	++	++	-
				Average	1.7			
ACE								
7	R	fibrous	partially	deg +	1	++	++	-
8	R	fibrous	not healed	deg +	1	++	++	-
9	R	fibrous	partially	crater	3	++	++	-
10	R	fibrous	partially	deg +	0	+++	+	-
11	R	fibrous	partially	crater	3	++	+++	+
12	R	fibrous	not healed	deg -	1	+++	-	+
				Average	1.5*			

figure 2



A. HISTOLOGICAL SECTION OF NON-DAMAGED ACE GROUP Ł CARTILAGE.

B. THE UNDERLYING TIBIAL Ł CARTILAGE IN THE ACE GROUP, 3 MONTHS AFTER SURGERY. TOLUIDINE BLUE. FIBRILLATION OF THE TIBIAL ARTICULAR CARTILAGE IS APPARENT. THIS ASPECT WAS ALSO VISIBLE IN THE ACI GROUP. 100X.

C. ADJACENT SECTION WITH ABUNDANT COL2-3/4M ANTIBODY LABELING REACHING THE DEEPER CARTILAGE LAYERS. 100X.

Channel) group no complete healing was observed. In the ACI group (Channel with Implant) only one polymer contained fibrocartilage-like tissue (dog nr. 1). The ACE group only showed fibrous tissue in the access channel.

Surface changes in the articular cartilage

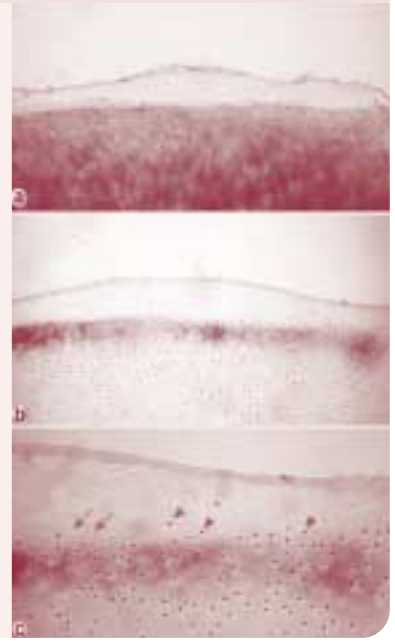
Macroscopically, the lateral femoral condyle in the ACI group and in the ACE group seemed unaffected, while the aspect of the lateral tibial cartilage varied from absence of degeneration to craters in the cartilage. The core of degeneration was located directly opposite the access channel in the meniscus. Further away from this area, the amount of cartilage damage decreased. No evident difference in macroscopic degeneration was found between the ACE and ACI group at this time point (Table 1).

Microscopically, varying stages of cartilage degeneration were observed in the ACE and ACI group. Cases with less degeneration showed evident proteoglycan staining (dog nr. 4 and 9). Extensive fibrillation with clefts perpendicular to the surface was apparent in two cases in the ACI group compared to four in the ACE group (Fig. 2a and b). In these fibrillated areas, no flat superficial chondrocytes were seen and there was evident less proteoglycan staining in the pericellular matrix than in non-damaged areas of the cartilage. The only case with an intact smooth cartilage surface was seen in the ACI group. The Mankin degeneration tended to be higher in the ACE group than in the ACI group, however, this difference was not significant (p=0.245) (Table 1).

All cases with fibrillated sites showed intense positive labeling of Col2-3/4M antibodies (Fig. 2c). Cartilage cells in the surface layers were completely surrounded by labeled epitopes and labeling reached the deep zones of the articular cartilage. The degree of degradation in the different cases did not differ between the ACE and ACI group (p = 0.330).

Besides evidence of fibrillation, four cases in the implant group showed loosening and swelling of the lower cartilage matrix, covered by an intact superficial layer (Table 1). These areas of swelling showed minor PG staining and moderate collagen type II degradation (Fig. 3a and b). However, in all cases with swelling, proteoglycan staining was present in the adjacent cartilage. Only a few round cells were observed but there were abundant empty cell lacunae in these swollen superficial areas (Fig. 3c). This aspect of swelling was not visible around fibrillated areas.

figure 3



A. HISTOLOGICAL SECTION OF THE UNDERLYING ARTICULAR TIBIAL CARTILAGE IN THE ACI GROUP, 3 MONTHS AFTER SURGERY. TOLUIDINE BLUE. LOOSENING OF THE SURFACE LAYER IS VISIBLE WITH UNDERLYING SWELLING OF THE CARTILAGE. 40X.

B. ADJACENT SECTION AFTER Ł LABELING WITH COL2-3/4M. ONLY MODERATE DEGRADATION OF COLLAGEN TYPE II IS VISIBLE. 40X.

C. MAGNIFICATION OF (B). NOTE THE EMPTY CELL LACUNAE Ł (ARROWS) AND FEW REMAINING CELLS (ARROW HEADS) IN THE TRANSITION ZONE. 200X.

figure 4

A. HISTOLOGICAL SECTION OF CARTILAGE IN THE ACE GROUP WITH SLIGHTLY DECREASED STAINING OF TOLUIDINE BLUE. 40X.

B. ADJACENT SECTION OF (A). A NON-FIBRILLATED ZONE WITH POSITIVE LABELING OF COL2-3/4M 6 MONTHS AFTER SURGERY. 100X.

Six months

Lesion healing and fibrocartilage formation

In the ACI group, in two cases the lesion was healed, while in the ACE group again no complete healing was observed. In three cases in the ACI group the polymer contained fibrocartilage-like tissue (dog nr. 1,3 and 4). Again, the ACE group showed only fibrous tissue in the access channel.

Surface changes in the articular cartilage

Macroscopically, again no cartilage degeneration of the lateral femoral condyles was found, while degeneration of the tibial articular cartilage was apparent. However, after this time, no craters were present in the articular cartilage in the ACI group.

Microscopically, damage was generally restricted to surface irregularities in the ACE and ACI group. Although the superficial cell layers were damaged and proteoglycan staining was decreased in these zones, extensive cell death had not occurred. The decrease in Mankin score between three and six months was only significant in the ACE group ($p<0.05$) (Table 1).

Again, degradation of collagen type II was detected in the ACE and ACI group in areas of fibrillation in the upper layers. The degree of degradation in the different cases did not differ between these two groups ($p = 0.330$). However, at this time, degradation was also especially apparent in these groups in areas where fibrillation had not occurred (Fig. 4). Labeling of Col2-3/4M in the upper layers of the articular cartilage was combined with an absence of proteoglycan staining, although chondrocytes still had a normal distribution and appearance (Fig. 4a and b). Only two cases in the ACI group showed loosening of the cartilage surface layers with swelling and empty cell lacuna. This aspect was not visible in the ACE group.

Discussion

In this study, we evaluated the presence and mechanism of articular cartilage degeneration after partial thickness access channels had been created between a lesion in the avascular area of the meniscus and the vascularized synovial lining, to determine the value of this technique as a alternative to partial meniscectomy.

The ingrowth of tissue into a polymer implant and its effect on healing have been described elsewhere[24]. In short, after three months, the peripheral part of the polymer scaffold was invaded by fibrovascular tissue. After six months, fibrocartilage-like tissue was present in the polymer.

The authors already showed that an access channel between a meniscal lesion in the avascular area and the vascularized synovial lining leads to healing of the lesion[24]. If healing of the lesion can be achieved, partial meniscectomy will not be necessary. Various other reconstruction techniques have also been found to induce healing of lesions in the avascular part of the meniscus[3,9,8,10,18,26,27,28,30]. However, the consequences on articular cartilage degeneration have not been evaluated.

Meniscal reconstruction as applied in this study showed a high incidence of degenerative changes in the underlying cartilage, which consisted of fibrillation, loss of chondrocytes and decreased proteoglycan content. According to the literature, these changes also occur after partial meniscectomy[5,11,12]. The degeneration in the implant group mainly seemed to occur during a short phase after surgery, in which the implant had not yet become incorporated into the meniscus. After three months, the degenerative effect of the reconstruction might be less as the implant becomes better incorporated[24]. This assumption corresponds with the observation in the present study that the severity of cartilage degeneration did not increase from three to six months. Craters in the articular cartilage, observed after three months follow up, were found after six months follow up. Therefore, we speculate that in the longer-term, articular cartilage damage after reconstruction that leads to a healed meniscal lesion will be less severe than after partial meniscectomy. However, also various ACLT models in dogs (according to Pond-Nuki) did not show a progress of the OA over time[17]. This might imply that this animal model is not

appropriate for evaluation of the consequences of meniscal reconstruction for the articular cartilage, or that the evaluation period is not long enough.
Six months after surgery, the ACE group even showed a significant decrease in Mankin score with less surface damage and more proteoglycan staining than after three months. This evident decrease in articular cartilage degeneration during longer follow-up, might suggest a reparative capacity of the canine articular cartilage.
The reparative capacity in dogs was already suggested by Adams et al, who reported an active synthetic response by the chondrocytes after anterior cruciate ligament transection (ACLT) resulting in hypertrophic cartilage repair[1]. 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 OA[15]. Moreover, 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. When extrapolating these results to a human situation, all these factors should be taken into account.
ACLT models in dogs (according to Pond-Nuki)[2,17] and smaller animals[22,20] showed that weakening of the collagen network played an important role in the early morphological changes that lead to osteoarthritic cartilage. In these studies, it was hypothesized that mechanical influences after ACLT caused fibrillation, which in turn induced degradation of the underlying collagen network. If this were true, then collagen degradation would never be detected before fibrillation has occurred. In the present study, fibrillated cartilage areas always indeed showed strong positive degradation antibody labeling. However, degradation was also observed in morphologically non-damaged areas of cartilage. Although the surface layers were still intact, the underlying cartilage matrix network may have become weakened, which might eventually lead to fibrillation of the articular cartilage.
Swollen areas in the underlying cartilage were especially apparent in cases with a polymer implant. In these areas, it can be considered that cell death had occurred in view of the decreased number of cells and the empty lacunae in the swollen regions. Also, proteoglycan staining was decreased; only moderate collagen degradation was visible in the underlying layers, while the upper surface layer was intact.
This was not observed in the empty access channel group, in which the sharp edges of the meniscal defect might have caused direct damage to the articular cartilage. In the implant group, the cartilage was not exposed to the edges of the defect, but to a bare polymer surface. This might have caused different kind of strain and shear stresses in the underlying cartilage. The upper superficial layers were better protected against these stresses by the parallel oriented collagen fibers while the underlying matrix network, which might be more vulnerable, was weakened by these external forces[4].
In conclusion, creation of an access channel with or without porous polymer scaffold implantation led to degeneration of the articular cartilage. In this short-term experiment, creation of an access channel led to fibrillation of the underlying articular cartilage; in the implant group, the cartilage showed swollen areas with empty cell lacunae in the matrix beneath an intact surface layer. The damage did not increase between 3 and 6 months post-surgery. Degradation of collagen type II was observed in fibrillated areas, as shown in smaller animals, and also in non-fibrillated areas of the cartilage. We not only propose that degradation of the collagen type II network is the result of superficial mechanical cartilage damage, but also that weakening of the deeper collagen network might lead to morphological damage of the cartilage.

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Chapter 5

Replacement of the knee meniscus by porous polymer implants. A study in dogs.

TG van Tienen, RGJC Heijkants, JH de Groot, AJ Pennings, AJ Schouten, RPH Veth, P Buma

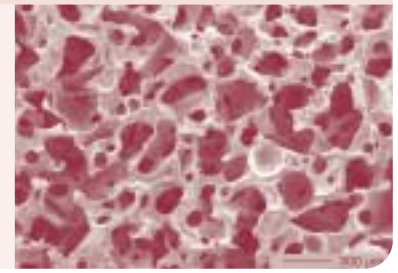
Abstract

Meniscectomy will lead to articular cartilage degeneration in the long term. Therefore, we developed an implant to replace the native meniscus. The porous polymer meniscus implant develops into a neo-meniscus and protects the cartilage for degeneration.
In a dog model, a porous polymer scaffold with optimal properties for tissue infiltration and regeneration of a neo-meniscus was implanted and compared with total meniscectomy. The tissue infiltration and re-differentiation in the scaffold, the stiffness of the scaffold and the articular cartilage degeneration were evaluated. Three months after implantation the implant was completely filled with fibrovascular tissue. After six 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 stiffness of the implant increased from 3 to 6 months and approached that of the native meniscus. Cartilage degeneration was observed both in the meniscectomy group as in the implant group.
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.
The porous polymer implant developed into a polymer-tissue construct which resembled the native meniscus and with improved gliding characteristics this prosthesis might be promising implant for the replacement of the meniscus.

Introduction

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 may avoid the articular cartilage degeneration. A number of groups tried to replace the meniscus with autologous materials like fat tissue[12], perichondrium[2] and tendon[13]. However, the poor initial mechanical properties make long-term fixation problematic. Synthetic permanent implants made of Dacron and Teflon[18,27] 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[28]. However, problems related to the availability, preservation techniques, the possible transfer of diseases, the individual shaping of the polymer implant and possible immunological reactions to the implant, are recognized worldwide[25].
To avoid all problems related with the above mentioned replacement techniques our long term aim is to generate a completely new meniscus by in vivo tissue engineering. This ambitious goal may be reached by 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 neo-meniscus after a meniscectomy[3] and from experiments in which a partial meniscectomy was reconstructed with a polymer foam[9]. Hence, we expect that the empty scaffold will be filled with regenerative tissue. During slow degradation of the polymer material and simultaneously differentiation of the ingrown tissue into the typical fibro-cartilaginous tissue of the native meniscus, the original situation from before the trauma may be restored.
Therefore, we hypothesize that a new meniscus can be recreated with a biodegradable porous polymer scaffold with optimal biological properties like compression modulus, porosity and pore-sizes. To test the hypothesis, the properties of the scaffold should meet certain criteria. To enable a rapid tissue ingrowth, the volume fraction of the biomaterial should be as low as possible, and to enable complete ingrowth the scaffold should have a homogenous distribution of large interconnected pores. The degradation of the biomaterial should occur slowly to enable the differentiation into fibro-cartilage. Earlier studies showed that the initial mechanical properties of the porous polymer determines the fate of the newly formed regenerative tissue; if the compression modules of the starting material is below 150 kPa, only fibrous tissue is produced[5]. Therefore in this study we used a new polymer that combines the optimal macro-porosity of 78% with a compression modulus of 300 kPa and a slow degradation rate[5,10].

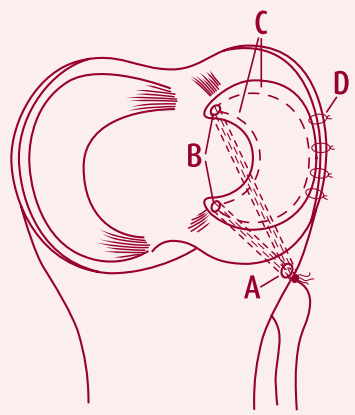
figure 1



Scanning electron micrograph of the Estane implant. Note the interconnectivity of the pores.

figure 2

SCHEMATIC PRESENTATION OF THE OPERATIVE PROCEDURE. AFTER Ł RESECTION OF THE NATIVE Ł MENISCUS, TWO DRILL HOLES Ł WERE CREATED ORIGINATING FROM THE LATERAL TIBIAL SIDE (A) TO THE FORMER ATTACHMENTS OF THE ANTERIOR AND POSTERIOR HORNS OF THE NATIVE MENISCUS (B). SUBSEQUENTLY, TWO NON-Ł RESORBABLE SUTURES WERE PULLED LONGITUDINALLY THROUGH THE IMPLANT (DOTTED LINES (C) AND ATTACHED TO THE LATERAL PROXIMAL TIBIA. THE PERIPHERY OF THE Ł IMPLANT WAS ATTACHED TO THE CAPSULE WITH RESORBABLE Ł SUTURES (D).



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 non-differentiated ingrown tissue into fibro-cartilage (immunohistochemistry) and on the comparison of the compression modulus of the explants with that of the native meniscus (mechanical testing). Finally, the 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 N.V.Westerlo-Oevel, Belgium). Pores were created, as described earlier[4], by mixing the polymer solvent solution with salt crystals ranging in size from 150-355 microns. These macropores were directly interconnected (Fig.1). Porosity was 78%. The compression modulus at 20% compression was 300 kPa.

Surgery

We performed total lateral meniscectomy on 24 legs of 12 adult male and female Beagles, with an average weight of 14.4 kg (SD: ±1.9 kg). 6 dogs with an implant and 6 meniscectomized dogs were available for 3 and 6 months follow up (n=6). 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 entered using a lateral skin incision. Great care was taken not to injure the articular cartilage and the collateral and cruciate ligaments. Using the Beaver eyeblade (Waltham, Massachusetts, USA) the meniscus was separated from its anterior and posterior attachments. In 12 knees the meniscus was replaced by an 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. Two bonded non-degradable sutures were leaded through the implant parallel to the inner and outer rim (Fig. 2).
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 to realize close contact between synovial tissue and the implant. Afterwards the capsule and skin were closed. The dogs were allowed to walk as soon as possible.

Histology

After killing the dogs, the implants were resected and a 4 mm full thickness biopsy was taken from the prosthetic posterior horn for the biomechanical testing.
The implant was fixed in acetone (-20°C) for six hours, infiltrated in methylmeth-acrylate and polymerized at –20°C for two days. Sections (7 microns) were cut in a transversal plane, dried at 37°C and stained with Haematoxylin-Eosine and Toluidine blue. In preparation for immunohistochemistry, sections were deacrylated three times in chloroform-xylol (1:1) for 15 minutes and subsequently treated with 1% testicular hyaluronidase (type I-S, EC 3.2.1.35; Sigma, St. Louis, MO, USA) 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). Monospecific monoclonal rabbit anti-collagen type I antibody[1] (PS-41, anti- raised in rabbit, Sanbio, Uden, The Netherlands) and mouse anti-collagen II antibody[14] (II-II6B3, anti-chicken raised in mouse, Developmental Studies Hybridoma bank, University of Iowa, USA) 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 one hour 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) dissolved in 15 ml PBS with 12 microliters H2O2 (30%) for 7 min. 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 two days and rinsed, dehydrated and embedded in methylmetacrylate for two days.

Microscopy

On an ordinal scale, sections were scored for integration between implant and capsule (percentage attachment) and tissue ingrowth 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) were determined by using the Quantimet 520 Image Analysis System and compared with the average distribution in three native menisci. The average percentage of positive staining on the total amount of ingrown tissue was determined in two sections through the center of the polymer 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)[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 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)[15]. The total score of each subcategory determined the Mankin score.

Biomechanical analysis

After excision of the polymer-tissue construct from the dog's knee, 4 mm punch biopsies were taken of a specified region of the posterior horn of both the implant as the native meniscus. As a reference, also punches were 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, 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 native meniscus, the implant before implantation, and the implant at 3 months at 6 months after implantation.

Data analysis

Differences in cartilage degeneration were statistically evaluated by using the two-way analysis of variance (ANOVA). P-values were calculated and values of less than 0.05 were considered to be significant.

Results

Clinical observations

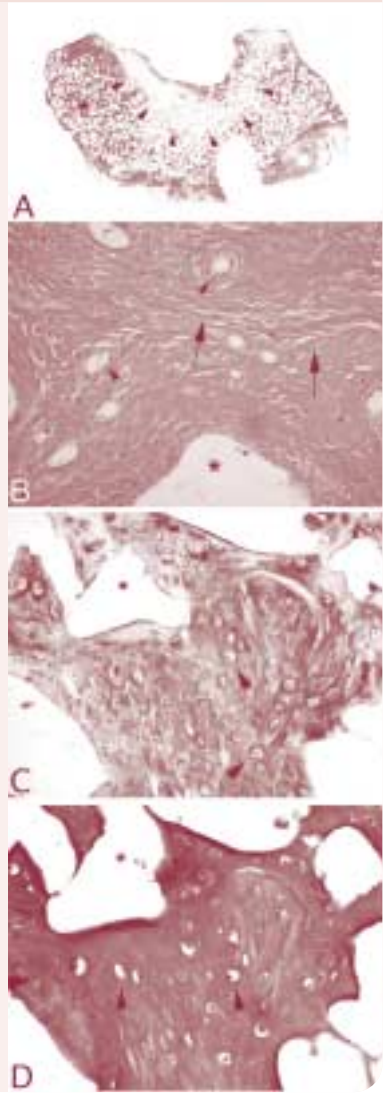
The dogs had regained their normal gait pattern 14 days postoperatively. No infections were seen. All meniscectomized knees and knees with implant were available for evaluation. Post mortem, there were no signs of synovitis in the joint capsule and the synovial fluid was clear.

Three months

Macroscopy (3 and 6 months)

Already after three months, the implant was firmly attached to the peripheral capsule. Only in the popliteal 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 term, 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 with that in the meniscectomy group.
After 6 months the knee joints showed similar aspects. However, after this term the

figure 3



MICROGRAPHS OF SECTIONS OF THE IMPLANT-TISSUE CONSTRUCT Ł 6 MONTHS AFTER IMPLANTATION. (A) IMPLANT LABELED WITH Ł COLLAGEN TYPE I ANTIBODIES. NOTE ABUNDANT STAINING IN THE PERIPHERAL REGIONS OF THE Ł MENISCUS (ASTERISK) AND LESS LABELING NEAR THE INNER RIM (ARROWHEADS) 4X. (B) MAGNIFICATION OF A REGION IN THE PERIPHERAL ZONE OF THE IMPLANT. TISSUE WITH MANY BLOODVESSELS (ARROWHEADS) AND INTENSIVE LABELING OF THE COLLAGEN BUNDLES(ARROWS). 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 (ARROWHEADS) AND THE ABUNDANT COLLAGEN TYPE II ANTIBODY Ł LABELING (C) AND TOLUIDINE BLUE STAINING (D) OF THEIR Ł MATRIX. 100X.

popliteus tendon seemed to have entered the joint space and damaged the polymer implants in three cases.

Microscopy

Also 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 as 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. PMN leucocytes were absent.

Implantation of an implant led to tibial cartilage lesions, merely localized adjacent to the inner rim of the implant while 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 dorsal 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 three months, the Mankin score in the implant group did not differ from the score in the meniscectomy group, for both tibia and femur. In both groups, more damage seemed to have occurred at the tibial side than at the femoral side (table 1), although the differences were not statistically significant (tibial versus femoral degeneration in meniscectomy group p = 0.388, in implant group p = 0.563).

Six months

Microscopy

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, which showed an abundant labeling with collagen type I antibody labeling (Fig.3a and b). These collagen bundles penetrated the channels between the macropores. In these areas no Toluidine blue staining and collagen type II antibody labeling was observed.

The central half of the implant had a more fibrocartilage-like phenotype with characteristic round cells lying in their lacunae (Fig. 3d). Their extracellular matrix showed positive staining for Toluidine blue and positive labeling for collagen type II antibodies. The areas of collagen type II labeling completely matched the areas of proteoglycan staining in the adjacent sections. In areas where collagen type II antibody labeling was present, the labeling of collagen type I antibodies was

figure 4

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.

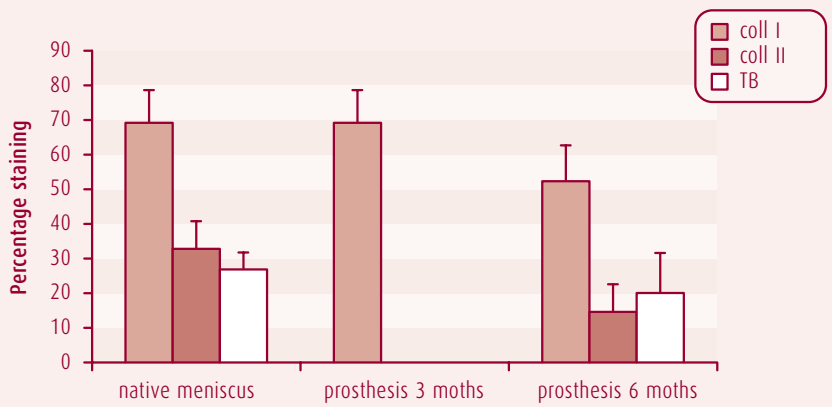
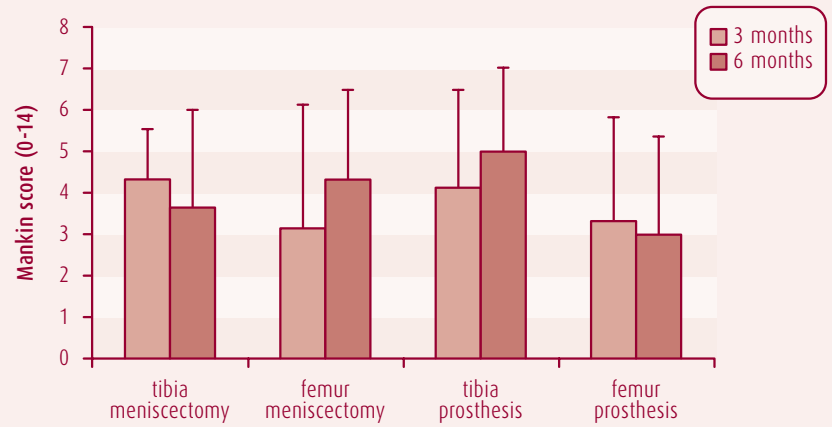


figure 5

MANKIN-SCORES OF THE TIBIAL LATERAL COMPARTMENT AND THE FEMORAL CONDYLES. NOTE THE HIGH VARIATION IN RESULTS.



evidently less (Fig. 3a), which resulted in a non-significant decline of the total amount of collagen type I labeling after six months (Fig.4).
In the zones with collagen type II and proteoglycan staining sporadic islands of a-cellular areas were observed. If necrosis had occurred or if the cells never reached this area could not be determined. This was not observed at three months after implantation.
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.
After 6 months the foreign body reaction tended to be increased compared to 3 months but still was classified as slight. More giant cells were observed along the surface of the pores in the polymer than after three months.
The histological aspect of the articular cartilage had not deteriorated after six months, which was confirmed by the Mankin score (Fig. 5). In the implant group, more damage seemed to have occurred on the tibial side, while the femoral damage had increased in the meniscectomy group.
However, these differences were statistically not significant.

Compression tests

Up till three months, the slope of the compression stress curves evidently increased and seemed to approach that of the native meniscus from 3 to 6 months (Fig. 6).

Discussion

In the present study, the native lateral meniscus in the dog's knee was replaced by an improved porous polymer implant. The tissue ingrowth and differentiation in the implant and the consequences for the articular cartilage were evaluated.
In the early eighties several studies described regeneration of meniscal tissue after total meniscectomy and mentioned the importance of the synovium as source for the newly formed tissue[19]. In several animal studies and even clinically, collagen based biomaterials were used as scaffold for the regenerating tissue [26,23]. However, this technique depends on a remaining native meniscal rim as a source for the neo-meniscal tissue. Until now, total meniscus replacement with a collagen scaffold

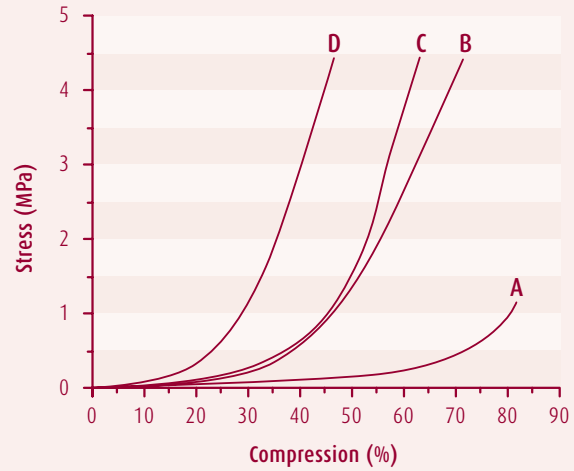
has not been described. Probably, the mechanical properties of this scaffold are inferior for this application. Klompmaker et al. were the first to replace the entire meniscus with similar porous polymer implants as used in the present study[11]. These implants seemed to provide an appropriate surrounding for mesenchymal tissue infiltration and for differentiation into meniscus-like tissue. The low compression modulus of the implant (150 kPa versus 400 kPa of the normal meniscal tissue[24]) could still have been insufficient to withstand the high loading forces in the knee joint. This 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[5]. 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 ingrowth rate of tissue (155-355 microns). In the former study, tissue infiltration depended on micropores (<90 microns) combined with macropores (150-300 microns) and the tissue ingrowth was incomplete until 18 weeks while in the present study the implant was completely filled three months after implantation[11]. The change in pore-interconnectivity in the polymer might have been responsible for this increased tissue infiltration rate.

Distribution

Ghadially, Webber and McDevitt described meniscal fibrocartilage as a tissue containing chondrocytic cells called fibrochondrocytes, which are surrounded by an abundant extra-cellular matrix. This matrix mostly contains collagen type I, but near the central rim of the meniscus also type II collagen and proteoglycans are present, which are major components of hyaline cartilage[8,17,30]. This distribution of extracellular matrix appeared to represent its function[20]. The collagen type I in native peripheral meniscal tissue provides the circumferential tensile strength to resist the hoop stresses during loading of the joint[24]. The collagen type II is able to resist compressive forces and therefore is rather found in the central rim of the meniscus where the force transduction between the femur and tibia is highest[6]. 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 six 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 loose its mechanical characteristics approximately

figure 6

AVERAGE COMPRESSION-STRESS CURVES OF SAMPLES FROM THE POSTERIOR HORN OF THE PROSTHETIC MATERIAL BEFORE IMPLANTATION (A) AND AFTER 3 MONTHS (B) AND 6 MONTHS (C) FOLLOW UP. NOTE THAT AT A STRESS LEVEL OF 1.25 MPA THE SLOPES OF THE IMPLANT AT SIX MONTHS AND THAT OF THE MENISCUS (D) ARE APPROXIMATELY SIMILAR.



40 weeks after implantation[5]. This enables the tissue to complete the process of infiltration and differentiation into neo-meniscal tissue.
After six months we observed several sporadic islands of a-cellular areas with collagen type II and proteoglycans. We assumed that these are necrotic areas considering that after 3 months these areas were not observed and in an earlier stage a cartilaginous matrix was generated. The process of differentiation process into cartilage-like tissue together with the retracting of the blood vessels might have went to fast to enable to tissue tot adjust to the new rather avascular circumstances. The long term studies have to reveal whether these areas will become repopulated with cells or whether this process will proceed.

Compression modulus

The compression modulus of the implant-tissue construct increased with time after implantation. The increase during the first 3 months can probably be attributed to the filling of the void spaces in the polymer implant. Already six months after

implantation, the compression modulus of the implant approached that of the meniscus. Further maturation of the tissue into fibrocartilage might be expected in the long term, which might be beneficial for 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[20]. In physiological 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 2-5 times higher than with the meniscus present[7].

Foreign body reaction

Both after 3 and 6 months, the foreign body reaction to the polymer implant remained restricted to multinuclear macrophages, which were aligned to the borders of the polymer. Polymorphonuclear leucocytes 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[16]. However, in addition to the phagocytic capacity of giant cells, these cells are also capable to release lysosomal acid hydrolases and these enzymes may provide a method for the extracellular degradation of any opposed uningested material[21]. The polyester polyurethane (Estane) is susceptible to enzymes released during this foreign body reaction in addition to its own hydrolytic and oxidative degradation[22]. Further, these cells also release angiogenic factors that stimulate blood vessel development towards the polymer implant[31]. Therefore, in our view, the presence of this mild foreign body reaction might even be beneficial.

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 as after implantation of the implant. The severity of degeneration was highly variable between the cases, also after meniscectomy. Consequently, no significant differences in cartilage degeneration could be observed between the meniscectomy and implant group and between the follow up periods. Thus, the porous polymer implant could not prevent cartilage degeneration.
This might be due the relatively rough polymer surface. The implants 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 implants with a mold may provide scaffolds with a smooth surface and with the desired standard form. In this way, also all implants will be identical, which may decrease the variance in cartilage degeneration. Further, the authors speculate that degeneration merely had taken place 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. Between three and six months after implantation the whole implant was covered with a tissue layer and longer term studies have to prove whether further cartilage degeneration will be prevented.
A popliteus tendon which 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 was 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, regeneration of new meniscus seemed to be possible by in vivo tissue engineering. The optimal properties of these polymer implants resulted in a fast ingrowth 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 approached that of the native meniscus at 6 months follow up. 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 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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Chapter 6

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) (n=6) or with an aliphatic 1,4-butanediisocyanate based polyester-urethane implant (PCLPU) (n=6). 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 1 in the peripheral fibrous zones and collagen type 2 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 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 PCLPU implant did also not exceed the degeneration after the Estane implant or after meniscectomy.
The differences between these two implants did not seem of influence on the tissue regeneration in the implant. However, PCLPU 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 PCLPU prostheses for replacement of the meniscus.

Introduction

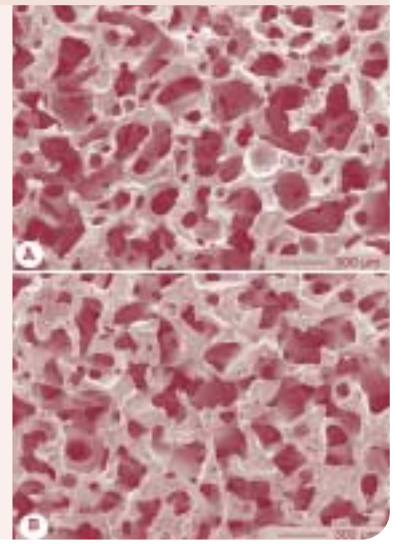
In consequence of the previous chapter, in this study, we focused on the influence of different chemical composition of the polymer material on the tissue infiltration and foreign body reaction.
New prostheses were developed either consisting of an aromatic 4,4-diphenylmethane-diisocyanate based polyesterurethane (Estane) or an aliphatic 1,4-butanediisocyanate based polyesterurethane (PCLPU). Both implant materials had a similar stiffness, porosity and had similar pore sizes. In the present study, the native meniscus of the dog was replaced with either the PCLPU implant or an Estane implant.
The infiltration of tissue into the two kinds of prostheses was analyzed and compared between the two kinds of prostheses as well as the synovial tissue reaction by means of histology. Also, several extracellular matrix components were assessed qualitatively and quantitatively to evaluate the differentiation of the infiltration tissue into neo-meniscal tissue. Furthermore, the compression modulus of the explants was compared with native meniscus. Finally, the articular cartilage degeneration resulting from meniscectomy versus meniscal replacement was assessed.

Materials and Methods

Polymer

The Estane implants (5701-F1)(BF Goodrich Chemical N.V.Westerlo-Oevel, Belgium) consisted of 4,4-methylenediphenyldiisocyanate. The PCLPU polyurethanes consisted of a hard segment of 1,4-butanediisocyanate and butanediol and a soft segment of polycaprolactone (Aldrich Chemical). Synthesis of PCLPU and formation of the porous structure is described elswhere[1], All pores were directly interconnected to achieve a high permeability of both polymers (Fig.1). Porosity was 78%. The compression modulus of both implants at 20 % compression was 300 kPa.

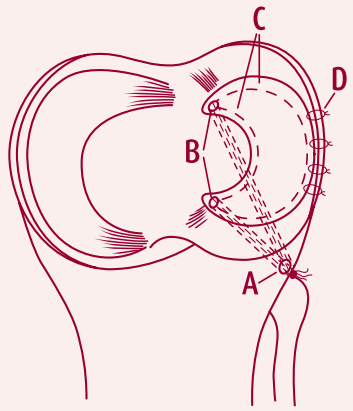
figure 1



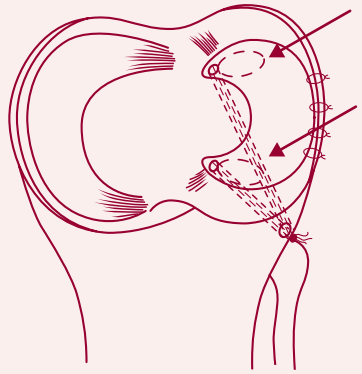
SCANNING ELECTRON MICROGRAPH OF THE ESTANE (A) AND PCLPU (B) IMPLANT. NOTE SIMILARITY OF THE POLYMERS AND THE INTERCONNECTIVITY OF THE PORES.

figure 2

SCHEMATIC PRESENTATION OF THE TWO OPERATIVE PROCEDURES Ł FOR EITHER THE ESTANE IMPLANT AND THE PCLPU IMPLANT. AFTER Ł 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 Ł LONGITUDINALLY THROUGH THE IMPLANT (DOTTED LINES (C) AND ATTACHED TO THE LATERAL Ł PROXIMAL TIBIA. IN THE PCLPU IMPLANT THE SUTURES WERE ONLY PULLED TROUGH THE HORNS. Ł THE PERIPHERY OF THE IMPLANT WAS ATTACHED TO THE CAPSULE WITH RESORBABLE SUTURES (D).



Estane implant



PCLPU implant

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. Surgery was performed as described in **Chapter 5**. In 6 knees, the meniscus was replaced by an Estane implant and in 6 knees by a PCLPU implant. The Estane material seemed to have a lower tear strength[1] and therefore it seemed necessary to lead two bonded non-degradable sutures through the Estane implant parallel to the inner and outer rim (Fig. 2). In the PCLPU 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.

The process of histology and microscopy of the polymer and articular cartilage, the compression testing and the statistical analysis are as described in the previous chapter.

Results

Clinical observations

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

Gross inspection

Macroscopically, both the Estane 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 (three cases) as the PCLPU implant group (four cases), the popliteus tendon seemed to have entered the joint space and damaged the polymer implants. In the group with meniscectomy solely, in 5 out of 6 cases a meniscus like regenerate had formed in de joint space. The tissue was smaller in diameter than the native meniscus and was more flexible and softer.

Tissue infiltration

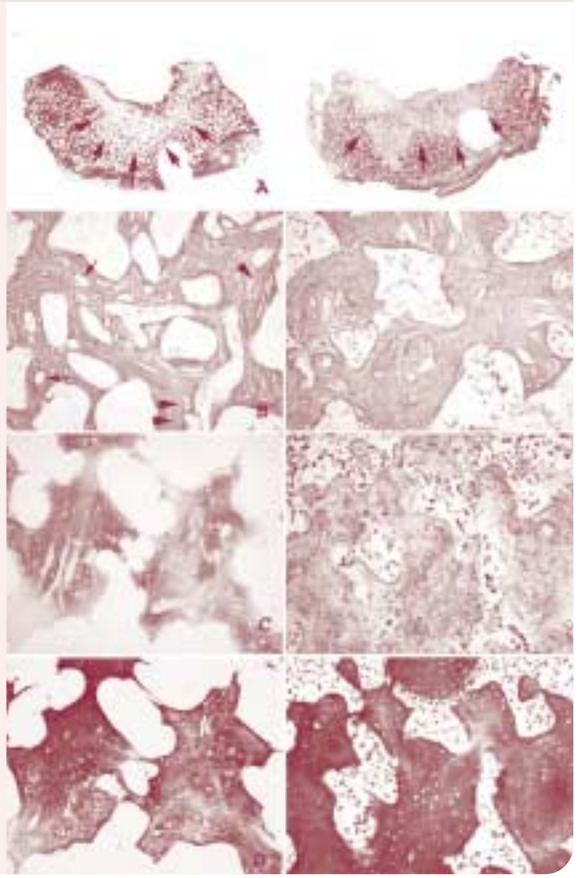
Microscopically, the Estane prostheses and the PCLPU prostheses were completely infiltrated with tissue and covered the whole prosthetic surface with a thin tissue layer. In the PCLPU prostheses also several microns small pores were observed, which were connected with the macropores and filled with a-cellular fibrin. 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 (Fig 3a). The extracellular matrix

in these areas contained many fiber bundles, which also penetrated the connections between the pores (Fig. 3b). 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

figure 3



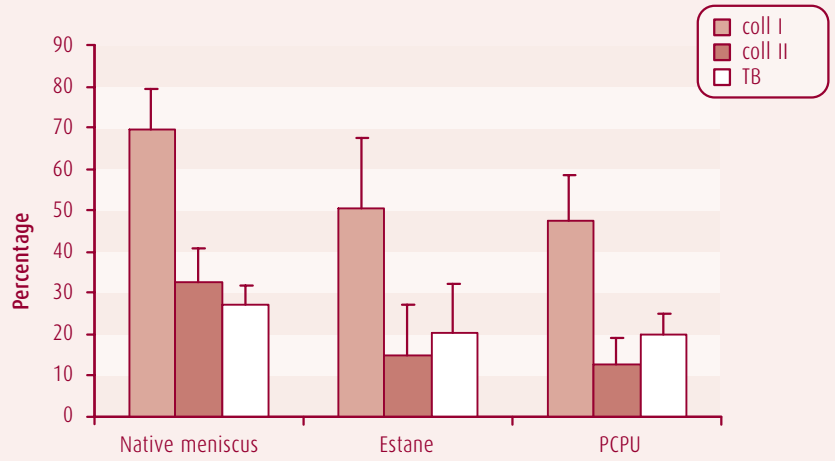
MICROGRAPHS OF SECTIONS OF AN ESTANE IMPLANT (LEFT Ł COLUMN) AND A PCLPU IMPLANT (RIGHT COLUMN) 6 MONTHS Ł AFTER IMPLANTATION. (A) IMPLANT LABELED WITH COLLAGEN TYPE I ANTIBODIES. NOTE ABUNDANT STAINING IN THE PERIPHERAL Ł REGIONS OF THE MENISCUS AND LESS LABELING NEAR THE INNER RIM (ARROWHEADS) 4X.(B) Ł MAGNIFICATION OF A REGION IN THE PERIPHERAL ZONE OF THE Ł IMPLANT. TISSUE WITH MANY BLOOD VESSELS (ARROWS) AND INTENSIVE LABELING OF THE Ł COLLAGEN BUNDLES (ARROW-HEADS). COLLAGEN TYPE I ANTI-BODY LABELING, 100X. (C AND D) ADJACENT SECTIONS OF TISSUE Ł NEAR THE INNER RIM OF THE Ł 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.

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 (Fig. 3c 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 (Fig. 4).

figure 4

PERCENTAGE LABELING WITH COLLAGEN TYPE I AND II OR TB OF THE TOTAL AMOUNT OF TISSUE IN THE MENISCUS AND THE TWO DIFFERENT IMPLANTS. 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

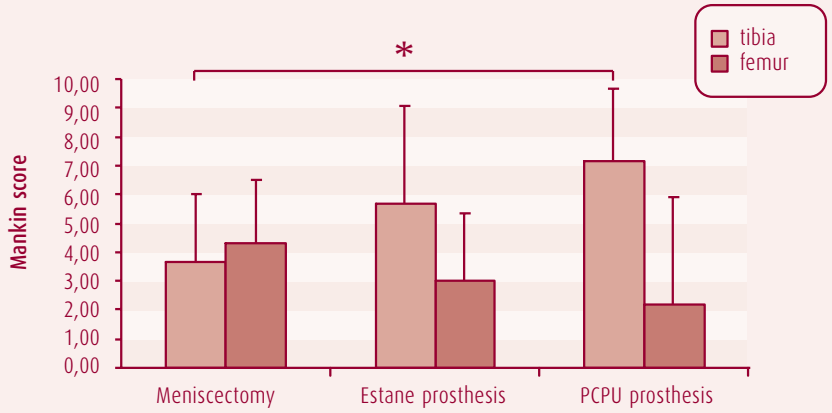
The inflammatory reaction within the Estane implant did not differ from the reaction in the PCLPU implant. A slight inflammatory reaction was present in the pores with scarce macrophages and giant cells. These cells were organized in close contact with the surface of the polymer. PMN leucocytes were absent. In the synovium, however, the tissue adjacent to the Estane implant showed significant more macrophages and giant cells than the tissue near the PCLPU implant (p=0.041). However, the tissue reaction near both implant never exceeded grade 2 (many macrophages/giant cells, no PMN leucocytes).

Cartilage degeneration

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

figure 5

ARTICULAR CARTILAGE DEGENERATION ACCORDING TO THE MANKIN SCORE. THE CARTILAGE DEGENERATION IN THE MENISCECTOMY GROUP IS SIGNIFICANTLY LESS THAN IN THE PCPU IMPLANT GROUP.



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. (Fig. 5). 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.

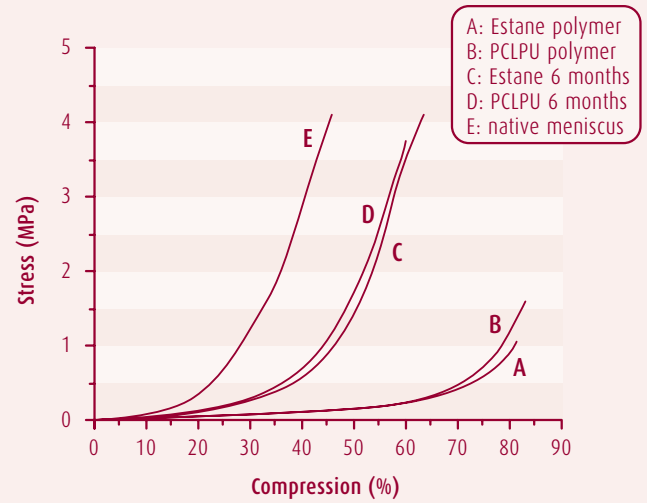
In dogs with an implant, more degeneration tended to occur at the tibial side than at the femoral side. In the meniscectomy group the degeneration on these sides was comparable. In both groups with a implant, the tibial cartilage was more damaged than at the femoral side, although the Mankin scores were not significant. After meniscectomy this difference was not observed. The Mankin score did not significantly differ between the three groups (p=0.124).

Compression tests

Six months after implantation, the Estane implant showed approximately similar compression curve as the PCLPU implant. In both the Estane prostheses as the PCLPU prostheses, the slopes of the compression curve evidently increased from implantation to 6 months follow-up (Fig. 6).

figure 6

AVERAGE COMPRESSION-STRESS CURVES OF SAMPLES FROM THE POSTERIOR HORN OF THE PROSTHETIC MATERIAL BEFORE IMPLANTATION (A) AND AFTER 3 MONTHS (B) AND 6 MONTHS (C) FOLLOW UP.



Discussion

In the present study, the meniscus was replaced with a porous polymer implant in the dog's knee. The prostheses consisted either of Estane or PCLPU but had similar geometrical and biomechanical properties. The tissue infiltration into the implant and the tissue reaction to the prostheses were studied. The consequences for the articular cartilage were evaluated and compared with the cartilage after meniscectomy.

Literature

Although other materials such as collagen may be useful for regeneration of the meniscus[16], our group focused on porous polymer scaffold material[7]. In earlier studies, the ideal pore sizes for a fast infiltration of tissue seemed to be in the range of 150-500 um[6]. This was confirmed in the present study, in which both implants

contained directly interconnected macropores in the range of 150-355 microns 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[2], new prostheses were developed with a higher compression modulus. This may have led to the location specific phenotypical differentiation of the ingrown tissue.

Polymer chemical properties

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 the more hydrophilic elements of the polymer[10], which again might play an important role in the cell attachment[9]. 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[8]. The porous structures were made using a different method which might have an effect on amount solvent that remains in the implant after washing. Another important difference between the two prosthetic materials consisted in the presence of an aromatic 4,4-diphenylmethane compound in the Estane polymer. The presence of this chemical compound may influence the tissue reaction but the aromatic 4,4-diphenylmethane diisocyanate (MDI) may also be converted into the toxic and carcinogenic methylenedianiline after degradation[17]. The aliphatic PCLPU prostheses, however, contains butanediisoccyanate (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[13,14].

Host tissue reaction

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 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 PCLPU material remains in situ longer than a year[1]. 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 was contributed to the relatively large amount of small pores in the polymer[5]. In the present study, polymers were used with macropores only (155-355 microns), which might be responsible for the relatively low recruitment of these cells[19].

Distribution

Meniscal fibrocartilage is described as a tissue containing fibrochondrocytes, which are surrounded by an abundant extra-cellular matrix[4,11,18]. The collagen type I in native peripheral meniscal tissue provides the circumferential tensile strength to resist the hoop stresses during loading of the joint[15]. 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[3]. In the present study, the tissue distribution in both prostheses was similar to that in the meniscus. 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[12] 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.

Compression modulus

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[12]. 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[1] 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 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.

Cartilage degeneration

In the present study, both the Estane implant 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 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. 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 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 implant and the cartilage was 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

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Chapter 7

Displacement of the medial meniscus within the passive motion characteristics of the human knee joint. An RSA study in human cadaver knees.

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Knee Surgery, Sports Traumatology, Arthroscopy, accepted

Abstract

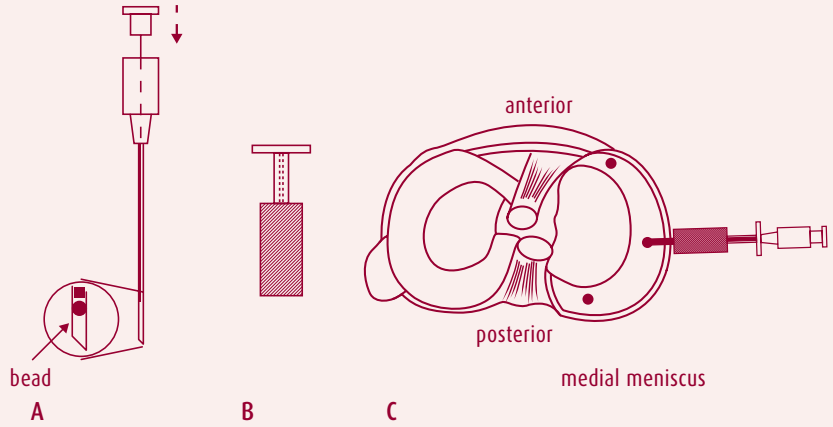
Objective of this study was to validate an in-vitro human cadaver knee joint model for the evaluation of the meniscal movement during knee joint flexion. Question was if our model showed comparable meniscal displacements as shown in earlier meniscal movement studies in-vivo. Furthermore, we determined the influence of tibial torque on the meniscal displacement during knee joint flexion.
Three tantalum beads were inserted in the medial meniscus of 6 human cadaver joints. The knee joints were placed and loaded in a loading apparatus and the movements of the beads were determined by means of RSA during knee joint flexion and extension with and without internal (IT) and external tibial torque (ET). During flexion without tibial torque, all menisci moved in posterior and lateral direction. The anterior horn showed significantly greater excursions than the posterior horn in both posterior and in lateral direction. Internal tibial torque caused an anterior displacement of the pathway on the tibial plateau. External tibial torque caused a posterior displacement of the pathway. External tibial torque restricted the meniscal displacement during the first during the first 30 degrees of knee joint flexion.
The displacements of the meniscus in this experiment were similar to the displacements described in the in-vivo MRI studies. Furthermore, the application of tibial torque confirmed the rather immobility of the posterior horn of the meniscus.
During external tibial torque, the attached knee joint capsule or the femoral condyle might restrict the posterior displacement of the pathway on the tibial plateau during the first 30 degrees of flexion.
This model revealed representative meniscal displacements during simple knee joint flexion but also during the outer limits of passive knee joint motion.

Introduction

The promising results of the prosthesis in the knee joints of dogs are described in the previous chapters. The performance of the prosthesis still needed to be determined in the human knee joint and it should be proven that the prosthesis mimics the behavior and deformation of the native meniscus in the knee joint during movement. To study the behavior of a newly developed prosthesis and compare it with the native meniscus, an experimental protocol needed to be developed in which the displacements of the meniscus and the prosthesis could be determined subsequently within the same knee.
Two groups already developed a model to study the displacements of the meniscus in human knee joints by means of MRI scans. Thompson et al.[6] performed a study in human cadaver knee joints and Vedi et al.[8] studied the behavior of the meniscus in vivo in a loaded and non-loaded situation. These studies supplied valuable data about the displacements of the meniscus during simple knee joint flexion. However, during flexion of the knee joint the different parts of the meniscus displace separately which results in deformation of the meniscus. The exact direction of movement of different parts was not examined and in our view is difficult to quantify accurately by means of MRI imaging. Therefore, markers within the different parts of the meniscus are required. We were also interested in the behavior of the meniscus during flexion in combination with tibial torque. Especially in this extreme range of knee joint motion, the behavior of the meniscal prosthesis is of great importance also because the combination of flexion and tibial rotation play an important role in the etiology of meniscal tearing[5].
Blankevoort et al. developed a passive knee joint loading model in which the displacements of the meniscus can be determined during knee joint flexion in a very

figure 1

SCHEMATIC VIEW OF THE INSERTION DEVICE AND PROCEDURE OF INSERTION OF THE BEADS INTO THE MENISCUS. BEADS WERE PLACED IN THE INSERTION NEEDLE(A) AND THE NEEDLE WAS AGAIN PLACED INTO A DEVICE (B), WHICH SECURED AN INSERTION DEPT OF 10 MM IN THE MENISCAL STROMA THROUGH THE KNEE JOINT CAPSULE. AS SHOWN IN (C) 3 BEADS OF 0.8 MM DIAMETER WERE INSERTED UNDER ARTHROSCOPIC CONTROL.



accurate manner by means of RSA. In this model the movements of the meniscus could also be determined during knee joint flexion in combination with internal and external tibial torque so that the complete range of passive knee joint motion is addressed[1].

Objectives of this study

The first objective of this study was to validate our experimental protocol with respect to earlier meniscal movement studies in-vivo[6,8]. The second objective was to evaluate the meniscal movements during knee joint flexion in combination with internal and external tibial torque.

Materials and methods

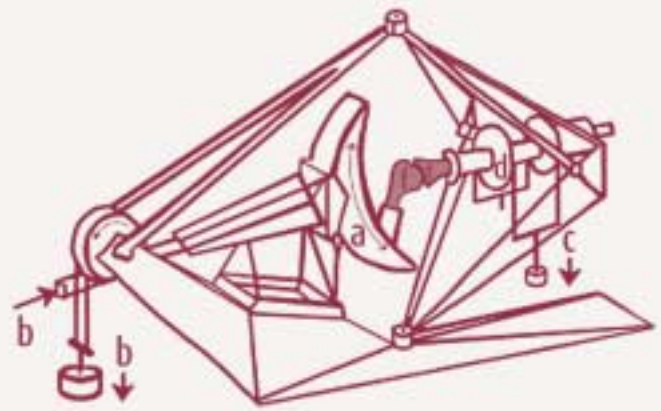
6 fresh knee joints (3 female and 3 male knee joints, age 68-92 years) were obtained from autopsy and kept frozen until the time of use. At manual and arthroscopic examination of the 6 knees, no abnormalities were found in soft tissue, cartilage, bony structures, ligaments or menisci.

The bones were transected approximately 15 cm above and 15 cm below the joint line. The tendon of the rectus femoris muscle was left intact for joint loading. All soft tissues, including the skin were left intact. Three tantalum beads (0.8 mm diameter) were fixed into the cortex of the tibia and femur. Five beads were inserted through the capsule in the periphery of the medial meniscus by means of an insertion device, which placed the bead at exactly 10 mm from the peripheral side of the knee joint capsule into the meniscus stroma (Fig. 1).

Each specimen was then fixed in a specially developed motion and loading apparatus (Fig 2)[1,7], allowing the tibia six degrees of freedom-of-motion relative to the femur. Flexion of the knee joint (axis 1), was performed by changing the angle of the femur relative to the tibia. This flexion angle was prescribed in steps of 5 degrees. The other two rotation axes of the apparatus were tibial rotation (axis 3) and varus-valgus rotation (axis 4), of which flexion and varus-valgus rotation occur in mutually perpendicular planes. Axial translation (axis 6) was possible through a four bar linkage mechanism on the femoral side. The anterior-posterior (axis 2) and medial-lateral (axis 5) translations were possible via rotations around two axes located distally on the tibial side, relatively far from the joint. The femur was positioned with the intercondylar notch in the intersection of axes 1 and 4. The anterior edge of the tibial shaft was leveled horizontally and aligned with rotation axis 3 of the

figure 2

KNEE JOINT LOADING APPARATUS. ON THE LEFT SIDE THE FEMUR (F), WHICH IS RIGIDLY FIXATED IN THE SEMILUNAR DEVICE (A). DIFFERENT KNEE JOINT FLEXION ANGLES ARE REALIZED ROTATING THE SEMI-LUNAR DEVICE THEREBY CHANGING THE ANGLE OF THE FEMUR RELATIVELY TO THE TIBIA, AS INDICATED IN (A). THE AXIAL LOAD (B) IS APPLIED TO THE FEMUR. ON THE RIGHT SIDE THE TIBIA (T). ON THIS SIDE OF THE APPARATUS, FREEDOM OF MOVEMENTS ARE INTERNAL AND EXTERNAL ROTATION, VARUS-VALGUS ROTATION, MEDIAL-LATERAL TRANSLATION AND ANTERIOR POSTERIOR TRANSLATION. INTERNAL AND EXTERNAL TORQUES (D) WERE APPLIED THROUGH A PAIR OF SHEAVES (C).

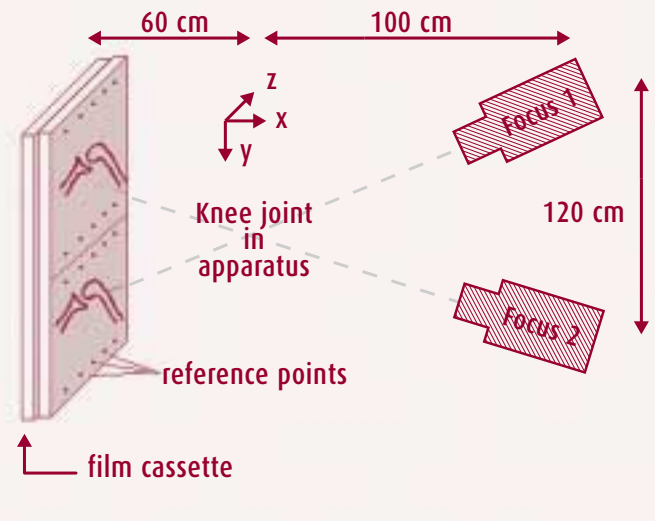


apparatus. Due to its freedom in rotation, tibial alignment in the rotational axe was not necessary.

Loading of the knee was realized by applying weights to those parts of the apparatus that correspond with the respective motions. The axial force (200 N) and a pulling force on the rectus femoris muscle tendon (90N) was applied through a pair of sheaves and wires at the femoral side of the loading apparatus where the flexion mechanism was located. Consequently, for each specific flexion angle prescribed, the axial force was applied on the femur, but remained directed approximately along the tibial axis. The limits of internal and external tibial rotation are defined at torques of 3 Nm, which were applied through a pair of sheaves on the tibial side. The motion pathway along these limits are defined as the envelopes of passive knee joint motion as described by Blankevoort et al.[1]. Beyond these limits damage to the knee joint might occur. It must be stressed that the apparatus was designed to accommodate passive range of knee joint laxity at moderate loading, rather than to test knee joint behavior at high loads.

figure 3

POSITIONING OF THE LOADING APPARATUS, THE ROENTGEN TUBES AND THE FILM CASSETTE. TWO SEPARATE X-RAYS WERE TAKEN WITH TWO TUBES ON THE SAME ROENTGEN FILM. NOTE THE REFERENCE POINTS ON ŧ THE FILM CASSETTE AND THE POSITIONING OF THE KNEE JOINT IN THE COORDINATE SYSTEM: THE X-DIRECTION REPRESENTS ML-DISPLACEMENTS ON THE TIBIAL PLATEAU AND THE Z-DIRECTION REPRESENTS THE AP DISPLACEMENT ON THE TIBIAL PLATEAU.



The loading apparatus was positioned at 60 cm from the filmcassette and at 100 cm from the x-ray tubes. The distance between the X-ray tubes was 120 cm (Fig. 3). Biplanar x-rays exposures were made.
After the experiments, the joint was dissected and the most posterior insertion of the anterior cruciate ligament on the tibia was marked with an additional tantalum bead to define the origin of the coordinate systems in extension[1,7].
Each knee was tested without tibial torque (neut), with internal tibial torque (IT) and external tibial torque (ET) in different degrees of flexion (0°, 15°, 30°, 60°, 90°, 60°, 30°, 15°, 0°). At each flexion position, two roentgen exposures of the bones and meniscus with the beads were taken.
The roentgen exposures were evaluated on a two-dimensional digitizer (Aristo 104-S, equipped with an Aaton video camera), measuring the marker images with an accuracy of 20 microns according to the descriptions of the manufacturer. A computer program calculated the kinematic parameters, which described the motions in z-axis (anterior-posterior direction (AP)) and x-axis (medial-lateral direction (ML)) of different parts of the meniscus during flexion of the knee joint. The displacements of the meniscal beads relative to the beads in the tibia were determined. Then, the translation of the different meniscal beads was determined during flexion without rotational torque with an internal and external rotational torque relative to the position of the bead in 0° knee flexion without tibial torque.

Data analysis

Univariate Analysis of Variance tests were applied for statistical analysis of the displacements of the different meniscal beads with respect to the beads in the tibia. Each specimen served as its own control to study the effects of internal and external tibial torque relative to the position of the bead at zero rotational torque.
The knee joints and the knee joint flexion angles were considered as independent variables and the displacement of the different meniscal parts as dependent variables. Any interactions between the flexion angle and the tibial torque were also determined. Statistical significance was set at $p<0.05$.

Results

Without rotational tibial torque

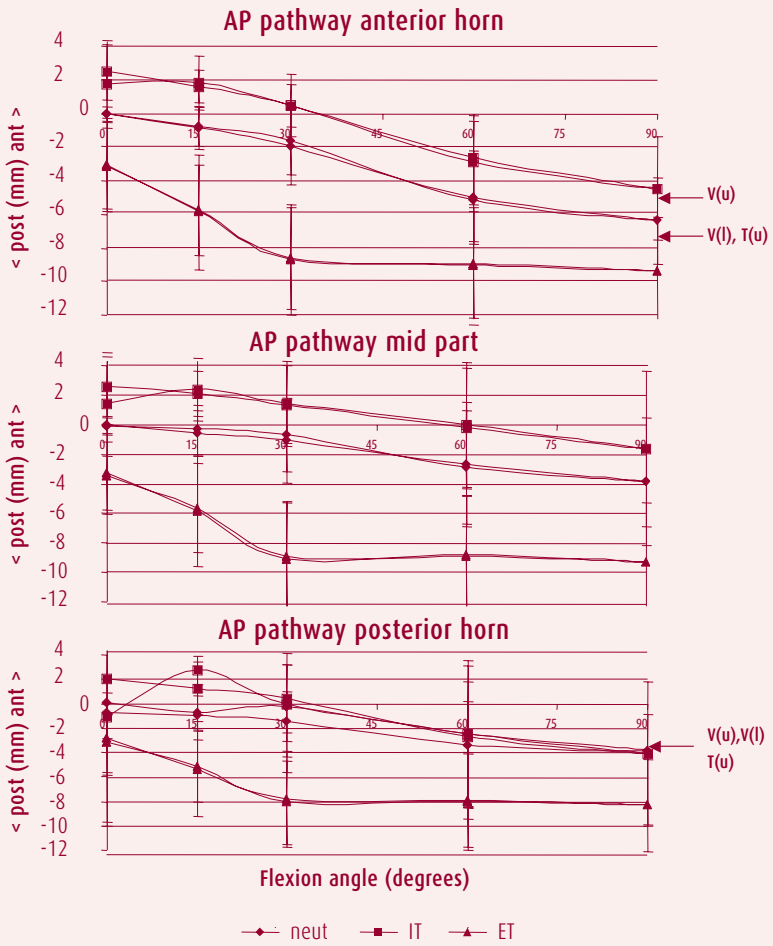
All beads in the meniscus followed different pathways on the tibial plateau during flexion and returned to their origin via approximately the same pathway during the extension phase, both in AP (Fig. 4) as in ML direction (Fig. 5). All menisci moved in posterior and lateral direction, however, displacements in ML direction were significantly less than in AP direction. Maximal displacement was reached at 90 degrees flexion. The anterior horn showed significantly greater excursions in AP direction than the posterior horn both in posterior as lateral direction (average 6.4 and 3.2 mm, respectively) ($p< 0.001$). Without tibial torque the mid part of the menisci followed merely the posterior excursions of the posterior horn. Also in ML direction, the anterior horn tended to be more mobile than the mid part and the posterior horn (1.8 mm, 0.4 mm and 1.3 mm, respectively).

Internal tibial torque

Again, all different beads followed different pathways and returned to their origin during extension of the knee joint via their flexion pathway. After applying tibial torque the pathways of the different parts of the meniscus were located more anteriorly on the tibia plateau (Fig. 4). However, only for the anterior horn and the

figure 4

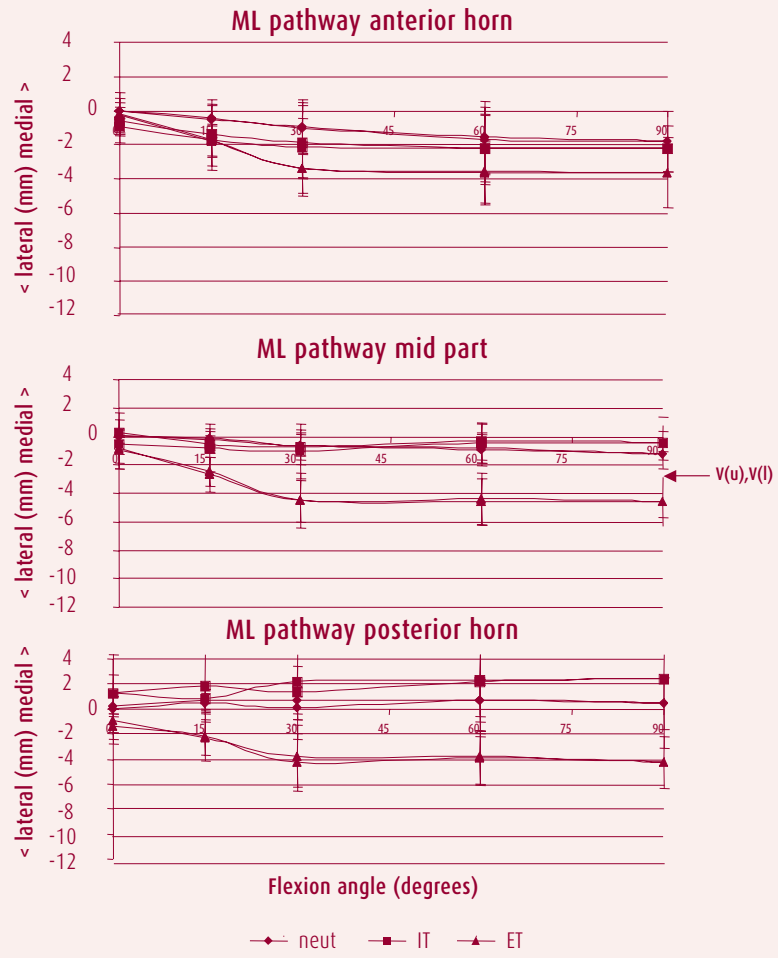
PATHWAYS OF THE DIFFERENT MENISCAL PARTS IN ANTERIOR (ANT) AND POSTERIOR (POST) DIRECTION ON THE TIBIA PLATEAU. NOTE THE LINEAR POSTERIOR DISPLACEMENT DURING FLEXION AND THE ANTERIOR AND POSTERIOR SHIFT DURING IT AND ET RESPECTIVELY. (NEUT = WITHOUT TIBIAL TORQUE)



mid part of the meniscus this difference was significant (p<0.001). The anterior displacement of the pathway of the posterior horn was not significant (p=0.264). The different parts of the meniscus showed a parallel pathway without and with internal tibial torque. Consequently no interaction was observed between the flexion angle of the knee and the internal tibial torque on the posterior displacement

figure 5

PATHWAYS OF THE DIFFERENT MENISCAL PARTS IN MEDIAL AND LATERAL DIRECTION ON THE TIBIA PLATEAU. EVIDENTLY LESS DISPLACEMENT WAS OBSERVED IN ML DIRECTION THAN IN AP DISPLACEMENTS. ESPECIALLY WITH ET DISPLACEMENT WAS OBSERVED. (NEUT = WITHOUT TIBIAL TORQUE)



of the meniscus. The displacement of the anterior horn was more than the displacement of the posterior horn (p<0.001).
With tibial torque, the displacements in ML direction were also smaller than in AP direction. Application of internal tibial torque did not result in significant lateral or medial displacement of the pathway on the tibial plateau (Fig. 5). The anterior horn

showed a lateral displacement on the tibial plateau while the posterior horn showed a medial displacement. During IT, the mid part tended to be the least moving part of the meniscus. Statistically, no interactions were observed between the flexion angle and the internal tibial torque on the displacement in ML direction.

External tibial torque

The different parts of the meniscus chose different pathways and again returned to their origin via the same pathway during extension. The pathway of all different meniscal parts was located significantly more posteriorly and laterally on the tibial plateau than during neutral knee joint flexion (Fig. 4 and 5). In all three parts of the meniscus, the displacement of the meniscus reached a plateau phase at 30 degrees of knee joint flexion, which suggested an interaction between the flexion angle and the tibial torque. This was confirmed statistically ($p<0.001$). There were no significant differences between displacements of the anterior and posterior horn. With application of external tibial torque, a significant displacement of the pathway was observed in lateral direction. Also in the ML direction, an interaction was present between the flexion angle and the tibial torque on the displacement of the different meniscal parts ($p=0.001$). No significant effect was observed between the displacement of the anterior and posterior horn.

Discussion

With this in vitro model in the human cadaver joint, it was possible to evaluate displacements of the different parts of the meniscus up to 90° of flexion. Also, the meniscal displacements with application of internal and external tibial torque could be determined.

For the present study the behavior of the meniscus was analyzed by means of RSA. We chose for this technique because of its accuracy and the broad experience with this technique in our laboratory. Also, we used a loading apparatus that already proved its value in earlier biomechanical studies of the knee joint[1].

With this experimental protocol all motions could be measured, and controlled accurately either directly during testing from the apparatus or later after calculation of the kinematical parameters acquired by RSA[4].

It should be realized that the number of specimens was limited and the study was performed in-vitro, which implicated that the influence of most muscles was absent. An active muscle complex around the knee joint with forces up to 4 times body weight would have caused a different deformation pattern of the meniscus during flexion and tibial torque[9]. Furthermore, it is difficult to uniquely identify natural landmarks in the knee, enabling a precise and consistent definition of the anatomical reference system. The alignment procedure adopted in this study depended on the visual confirmation of the investigator. The arbitrary nature of this procedure may also produce slightly different anatomical orientations in the motion rig and the laboratory coordinate system for each specimen.

The first objective of this study was to validate this experimental model with respect to earlier meniscal in-vitro and in-vivo movement studies[6,8]. With MRI the behavior of the different parts of the knee could be visualized in a non-invasive manner and provided valuable data about the movement of the meniscus during knee joint flexion.

The results in the present study approached those of the MRI imaging studies performed by Vedi et al.[8] who found displacements in AP direction of 5.4 mm (anterior horn) and 3.8 mm (posterior horn), in an in-vivo non-weight-bearing situation. In a weight bearing situation especially the displacement of the anterior horn increased (anterior horn: 7.1 mm and posterior horn: 3.9 mm). These results suggest that the application of load induces an increase of anterior horn displacement on the tibia. However, in an in-vitro non-loaded experiment, Thompson et al. found higher anterior horn displacements (respectively 7.0 mm and 3.2 mm displacement of the anterior and posterior horn)[6] which compared rather well with the displacements during weight-bearing in the in-vivo experiment by Vedi et al.[8]. The unexpected difference between the results of the two non-loaded studies remained unexplained. The similarities of results in the present study with those of the non-loaded experiment by Vedi et al. might suggest that, with respect to meniscal displacement, the applied constraint of the knee in the loading rig in the present study resembles the non-loaded in-vivo state of the knee-joint. This confirms the role of the small axial forces used in the present model, which seemed to mimic the function of the physiologic active stabilizers (muscles) in combination with geometric and ligamentous constraints in non-weight bearing situations[1].

To our knowledge, only Vedi et al. also determined ML displacements, although only of the outer inferior edge of the meniscus[8]. Their results and the results in the present study evidently show less movement in ML than in AP direction (Fig. 4). These meniscal displacements might be partly determined by the movements of the femoral condyle on the tibial plateau: these movements merely occur in AP direction while in ML direction the meniscus only should adjust to the declining diameter of the femoral condyle during joint flexion. Only during application of ET, the mid part

and the posterior horn of the menisci showed significantly more displacement into the joint cavity. During tibial rotation the medial tibial plateau might have been placed more medially with respect to meniscus and femoral condyle. As a result, the circumferential hoop stresses and tension on the meniscal horns might be reduced and the meniscus moved into the joint to stay in close contact with the femoral condyle, which indicates that menisci play a role in retaining the congruency as stated earlier[3].

Application of tibial torque

In the present study, the extra influencing factor of tibial torque was applied because the combination of flexion and tibial rotation play an important role in the movement of the knee joint but also in the etiology of meniscal tearing. Bylski-Austrow et al. already showed a linear relationship between degree of tibial rotation and tibio-meniscal displacement although only during 0-30° knee joint flexion [2]. Addition of internal and external tibial torque during knee joint flexion indeed resulted in more anterior and posterior pathway location on the tibia plateau, respectively. However, the relationship between tibial rotation and the meniscal displacement could not be confirmed because the tibial rotation angle was not measured. The anterior horn displacement during internal tibial torque showed a pathway parallel to the curve without internal tibial torque. This indicated that the anterior horn started from a point more anterior on the tibial plateau further proceeded with its pathway as without tibial torque, not hindered by any ligamentous or bony restrictions. The curve of the posterior horn seemed not to react on the application of internal tibial torque. This again indicated the rather immobility of this part of the meniscus, which might be responsible for the common tears in this area of the meniscus, especially after anterior cruciate ligament rupture[5].
External tibial torque caused a posterior displacement of the horns on the tibial plateau. However, the pathway was not parallel to the pathway without tibial torque: during the first 30 degrees of flexion an interaction was observed between the external tibial torque and the flexion angle. This was observed both in the anterior as in the posterior horn. This might imply that the external tibial torque attempted to pull the meniscus backwards but that it is hindered by its firm attachment to the peripheral capsule. According to Blankevoort et al.[1], the joint capsule laxity increases during this first range of flexion , which enabled the meniscus to move in posterior direction. Another explanation might be that, due to its fixation on the tibial plateau, the meniscus might be forced to move anteriorly during external tibial rotation and pushed against the femoral condyle. In this position the meniscus might follow the contours of the femoral condyle, which could also explain the interaction of flexion and external tibial torque.

Conclusion

In this study, we evaluated the displacement pattern of the meniscus within the passive motion pathway of the human knee joint. The displacements of the meniscus during knee joint flexion were similar to the displacements in earlier studies performed in-vivo with MRI. Consequently, this knee joint loading and motion model is appropriate for the evaluation of the displacement of the meniscus during knee joint flexion. Furthermore, the application of tibial torque confirmed the rather immobility of the posterior horn of the meniscus. With external tibial torque, movement of the meniscus seemed to be constraint by other structures in the knee.

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Chapter 8

Prosthetic replacement of the medial meniscus in human cadaver knees. Does the implant mimic the functional behavior of the native meniscus?

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American Journal of Sports Medicine, accepted

Abstract

Meniscal replacement by a polymer meniscus implant in dogs resulted in regeneration of neo-meniscal tissue and less articular cartilage degeneration compared to total meniscectomy.
Optimal functioning of the implant would involve realistic deformation and motion patterns of the implant during flexion-extension of the knee joint.
The native medial meniscus displacements and subsequently the implant were determined in 6 human cadaver knee joints during knee joint flexion.
Three tantalum beads were inserted in the medial meniscus. The knee joints were placed and loaded in a loading apparatus. The movements of the beads were determined during knee joint flexion and extension with and without internal and external tibial torque by means of RSA. Subsequently, the meniscus was replaced by a meniscus implant and the same measurements were performed.
All different parts of the meniscus showed a posterior displacement during knee joint flexion. The anterior horn was more mobile than the posterior horn.
The implant mimicked the movements of the meniscus. However, the excursions of the implant on the tibial plateau were less. The knee joint laxity was not significantly higher after replacement with the meniscal implant.
These results suggest that the implant approximated the behavior of the native meniscus during knee joint motion. Improvement in both the gliding characteristics of the prosthetic material and the fixation technique of the implant may improve the function.
The meniscus implant needs to be optimized to achieve a better initial function in the knee joint.

Introduction

As described in chapter 5 and 6, ingrowth of fibrovascular tissue into the prosthesis occurred after implantation in the knee joint of the dog. The tissue differentiated into meniscal fibrocartilage. In this dynamic biological process, the neo-meniscal tissue may be able to adjust to the functional requirements of its surroundings to which it is subjected in the long term. Prior to these biological interactions, the implant should already mimic the behavior of the native meniscus directly after implantation. After validation of the experimental protocol for the study of meniscal movement in the previous chapter, the movement of the native meniscus and the implant could be evaluated subsequently within the same human cadaver knee joint. Therefore, material characteristics of the implant, like stiffness, tear strength and gliding capacity are of great influence to the performance of the synthetic implant. Furthermore, size and fixation of the implant may also be very important as stated in meniscal allograft transplantation studies[1,2,10].

Aim of the study

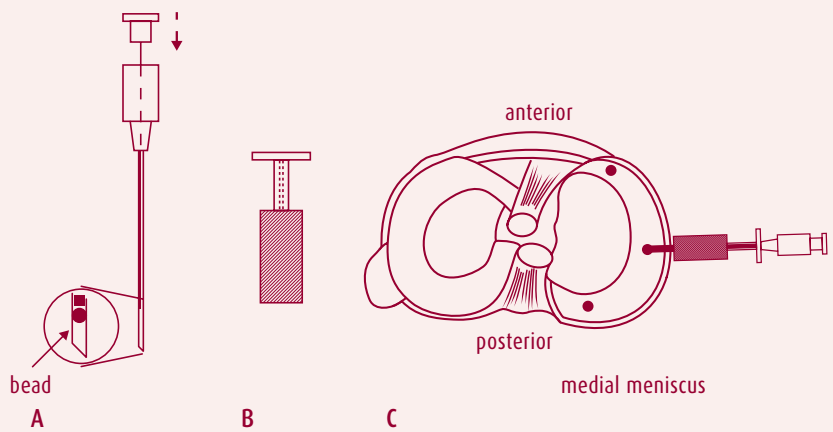
The purpose of this study was to determine how close the implant mimicked the functional behavior of the native meniscus. Therefore, the displacements of the native meniscus and subsequently the implant were measured during knee joint flexion in human cadaver knee joints with or without an additional tibial torque.

Materials and methods

6 fresh knee joints (3 female and 3 male knee joints, age 68-92 years) were obtained from autopsy and kept frozen until the time of use. At manual and arthroscopic

figure 1

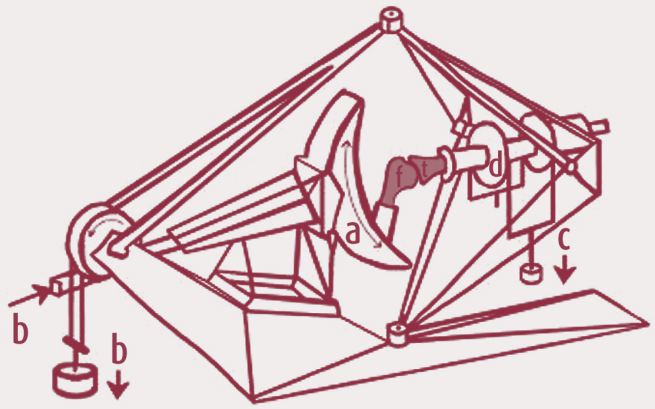
SCHEMATIC VIEW OF THE INSERTION DEVICE AND PROCEDURE OF INSERTION OF THE BEADS INTO THE MENISCUS. BEADS WERE PLACED IN THE INSERTION NEEDLE (A) AND THE NEEDLE WAS AGAIN PLACED INTO A DEVICE (B), WHICH SECURED AN INSERTION DEPT OF 10 MM IN THE MENISCAL STROMA THROUGH THE KNEE JOINT CAPSULE. AS SHOWN IN (C) 3 BEADS OF 0.8 MM DIAMETER WERE INSERTED UNDER ARTHROSCOPIC CONTROL.



examination of the 6 knees, no abnormalities were found in soft tissue, bony structures, ligaments or menisci.
The bones were transected approximately 15 cm above and 15 cm below the joint line. The tendon of the rectus femoris muscle was left intact for joint loading. All soft tissues, including the skin were left intact. Five tantalum beads (0.8 mm diameter) were fixed into the cortex of the tibia and femur. Under arthroscopic control, three beads were inserted through the capsule in the periphery of the medial meniscus by means of an insertion device, which placed the bead at 10 mm from the peripheral side of the knee joint capsule into the meniscal stroma (Fig. 1). Each specimen was then fixed in a specially developed motion and loading apparatus (Fig 2)[4], allowing the tibia six degrees of freedom-of-motion relative to the femur.

figure 2

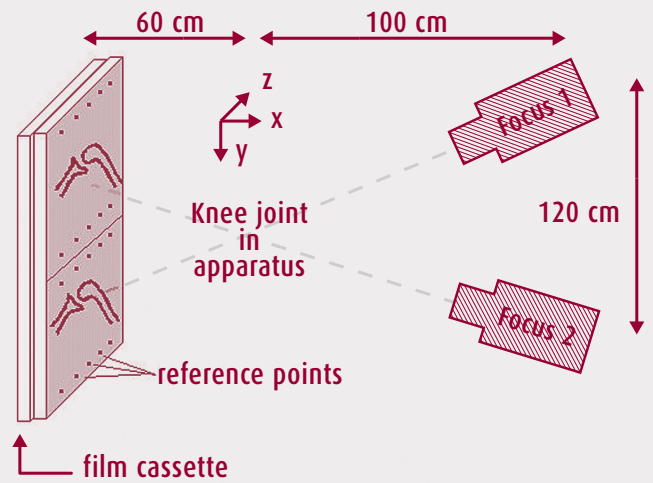
KNEE JOINT LOADING APPARATUS. ON THE LEFT SIDE THE FEMUR (F), WHICH IS RIGIDLY FIXATED IN THE SEMI LUNAR DEVICE (A). DIFFERENT KNEE JOINT FLEXION ANGLES ARE REALIZED ROTATING THE SEMI-LUNAR DEVICE THEREBY CHANGING THE ANGLE OF THE FEMUR RELATIVELY TO THE TIBIA, AS INDICATED IN (A). Ł THE AXIAL LOAD (B) IS APPLIED TO THE FEMUR. ON THE RIGHT SIDE THE TIBIA (T). ON THIS SIDE OF THE APPARATUS, THE DEGREES OF FREEDOM-OF-MOTION ARE INTERNAL AND EXTERNAL ROTATION, VARUS-VALGUS ROTATION, MEDIAL-LATERAL TRANSLATION AND ANTERIOR POSTERIOR TRANSLATION. INTERNAL AND EXTERNAL TORQUES (D) WERE APPLIED THROUGH A PAIR OF SHEAVES (C).



Flexion of the knee joint (axis 1), was performed by changing the angle of the femur relative to the tibia. This flexion angle was prescribed in steps of 5 degrees. The other two rotation axes of the apparatus were tibial rotation (axis 3) and varus-valgus rotation (axis 4), of which flexion and varus-valgus rotation occur in mutually perpendicular planes. Axial translation (axis 6) was possible through a four bar linkage mechanism on the femoral side. The anterior-posterior (axis 2) and medial-lateral (axis 5) translations were possible via rotations around two axes located distally on the tibial side, relatively far from the joint.
Loading of the knee was realized by applying weights to those parts of the apparatus that correspond with the respective motions. The axial force (200 N)(Fig. 2) and a pulling force on the rectus femoris muscle tendon (90N) was applied through a pair of pulleys and wires at the femoral side of the loading apparatus where the flexion mechanism was located. Consequently, for each specific flexion angle prescribed, the axial force was applied on the femur, but remained directed approximately

figure 3

POSITIONING OF THE LOADING APPARATUS, THE ROENTGEN TUBES AND THE FILM CASSETTE. TWO SEPARATE X-RAYS WERE TAKEN WITH TWO TUBES ON THE SAME ROENTGEN FILM. NOTE THE REFERENCE POINTS ON Ł THE FILM CASSETTE AND THE POSITIONING OF THE KNEE JOINT IN THE COORDINATE SYSTEM: THE X-DIRECTION REPRESENTS ML-DISPLACEMENTS ON THE TIBIAL PLATEAU AND THE Z-DIRECTION REPRESENTS THE AP DISPLACEMENT ON THE TIBIAL PLATEAU.



along the tibial axis. The limits of internal and external tibial rotation were defined at torques of 3 Nm, which were applied through a pair of sheaves on the tibial side. The motion pathway along these limits were defined as the envelopes of passive

knee joint motion as described by Blankevoort et al.[4]. Beyond these limits damage to the knee joint might occur. It must be stressed that the apparatus was designed to accommodate passive range of knee joint laxity at moderate loading, rather than to test knee joint behavior at high loads.

The loading apparatus was positioned at 60 cm from the film cassette and at 100 cm from the x-ray tubes. The distance between the X-ray tubes was 120 cm (Fig. 3). Biplanar x-rays exposures were made as shown in Fig. 3.

Each knee was tested without tibial torque (neut), with internal tibial torque (IT) and external tibial torque (ET) in different degrees of flexion (0°, 15°, 30°, 60°, 90°, 60°, 30°, 15°, 0°, respectively). At each flexion position, two roentgen exposures of the bones and meniscus with the beads were taken.

The roentgen exposures were evaluated on a two-dimensional digitizer (Aristo 104-S, equipped with an Aaton video camera), measuring the marker images (accuracy of 20 microns according to the descriptions of the manufacturer). A computer program calculated the kinematical parameters, which described the finite motions in z-axis (anterior-posterior direction (AP)) and x-axis (medial-lateral direction (ML)) of different parts of the meniscus during flexion of the knee joint.

Then, the movements of the beads between its location at 0 degrees knee joint flexion and the actual location at the different flexion angles were determined and defined as "displacement". The "pathway" was defined as the route on the tibial plateau during knee joint flexion and extension.

As described by Blankevoort et al. the rotation of the tibia relative to the femur increases as a result of tibial torque during knee joint flexion[4]. The degree of tibial rotation relative to the femur and thus the the outer limits of internal and external tibial rotations can be measured as a degree of the knee joint laxity.

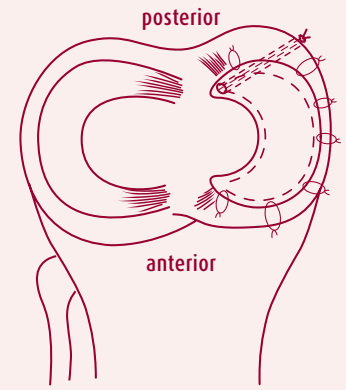
Prosthetic material

The implants consisted of biodegradable aromatic polyester urethanes based on Estane (5701-F1)(BF Goodrich Chemical N.V.Westerlo-Oevel, Belgium). Macropores were created, as described before[7], by mixing the polymer solvent solution with salt crystals ranging in size from 150-355 microns. Freeze-drying the solvent created micropores of at least 30 microns. Porosity was 80%. The compression modulus was 200 kPa.

Meniscal transplantation

Next, we performed an arthrotomy via a medial parapatellar incision of the skin and performed a bony release of the medial collateral ligament from its insertion at the medial epi-condyle of the femur. By turning down the ligament, an exposure of the joint was obtained. Then, the medial meniscus was excised. One drill hole was made in the posterior-medial side of the proximal tibia, ending in the posterior area of the intercondylar eminence (Fig. 4). One monofilament non-absorbable suture was attached to the properly sized porous polymer meniscus implant and was pulled through the drill hole. The anterior horn together with the entire peripheral rim of the implant was sutured to the capsule using vertical 2/0 absorbable braided sutures. The MCL was reattached at its insertion on the epi-condyle using a 3.5 mm trabecular screw. The capsule and skin were closed and 20 ml of saline was injected in the joint space. Then the same test procedure was performed as for native meniscus. After the experiments, the joint was dissected and the most posterior insertion of the anterior cruciate ligament on the tibia was marked with an additional tantalum bead to define the origin of the coordinate systems in extension[4,13].

figure 4



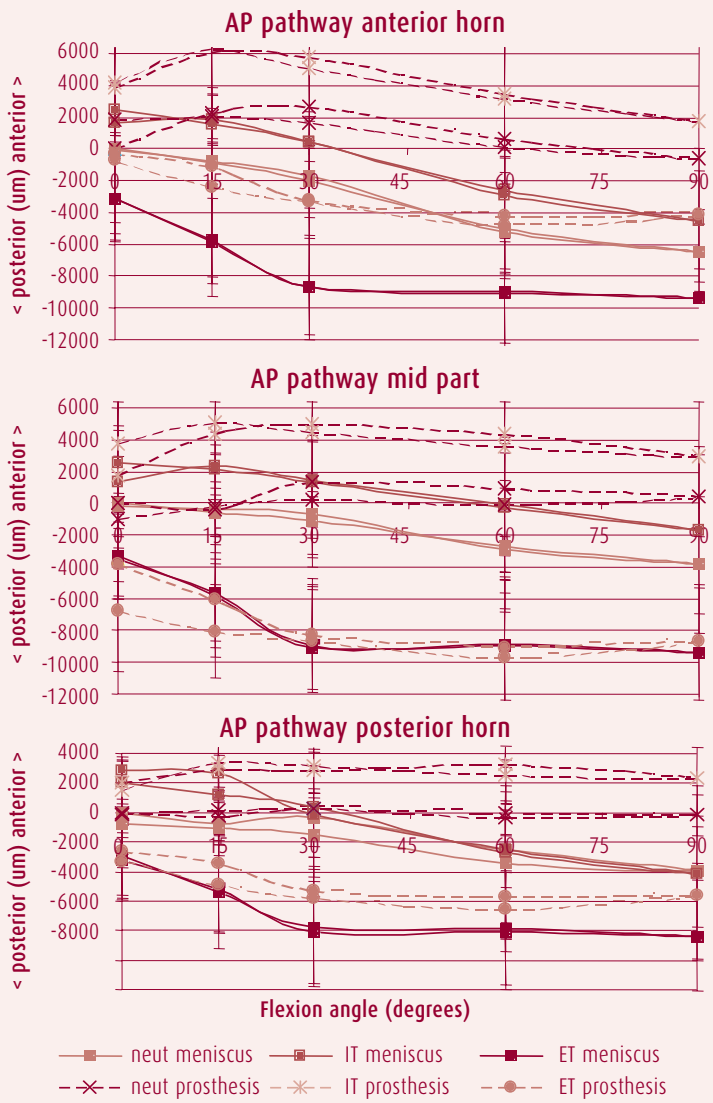
SCHEMATIC PRESENTATION OF THE OPERATIVE PROCEDURE. AFTER Ł RESECTION OF THE NATIVE MENISCUS, ONE DRILL HOLE (DOTTED LINE) WAS CREATED ORIGINATING FROM THE POSTERIOR TIBIAL SIDE TO THE FORMER ATTACHMENT OF THE POSTERIOR HORN OF THE NATIVE MENISCUS. SUBSEQUENTLY, A Ł MONOFILAMENT SUTURE WAS PULLED THROUGH POSTERIOR HORN OF THE IMPLANT AND ATTACHED TO THE TIBIA. THE PERIPHERY OF THE IMPLANT WAS ATTACHED TO THE CAPSULE WITH 2/0 RESORBABLE BRAIDED SUTURES.

Data analysis

Univariate Analysis of Variance tests were applied for statistical analysis of the displacements of the different meniscal beads with respect to the beads in the tibia. Each specimen served as its own control to study the effects of internal and external tibial torque relative to the position of the bead at zero rotational torque. The knee

figure 5

PATHWAYS OF THE DIFFERENT MENISCAL AND PROSTHETIC PARTS IN ANTERIOR (ANT) AND POSTERIOR (POST) DIRECTION ON THE TIBIA PLATEAU. BOTH WITH AND WITHOUT TIBIAL TORQUE, THE SLOPE OF THE CURVES OF THE IMPLANT IS LESS STEEP THAN THOSE OF THE MENISCUS, RESULTING IN LESS DISPLACEMENT ON THE TIBIAL PLATEAU.



joints and the knee joint flexion angles were determined as independent variables and the displacement of the different parts of the meniscus and the implant as dependent variables. Any interactions between the flexion angle and the tibial torque were also determined. Statistical significance and correlation was set at $p<0.05$.

Results

Without tibial torque

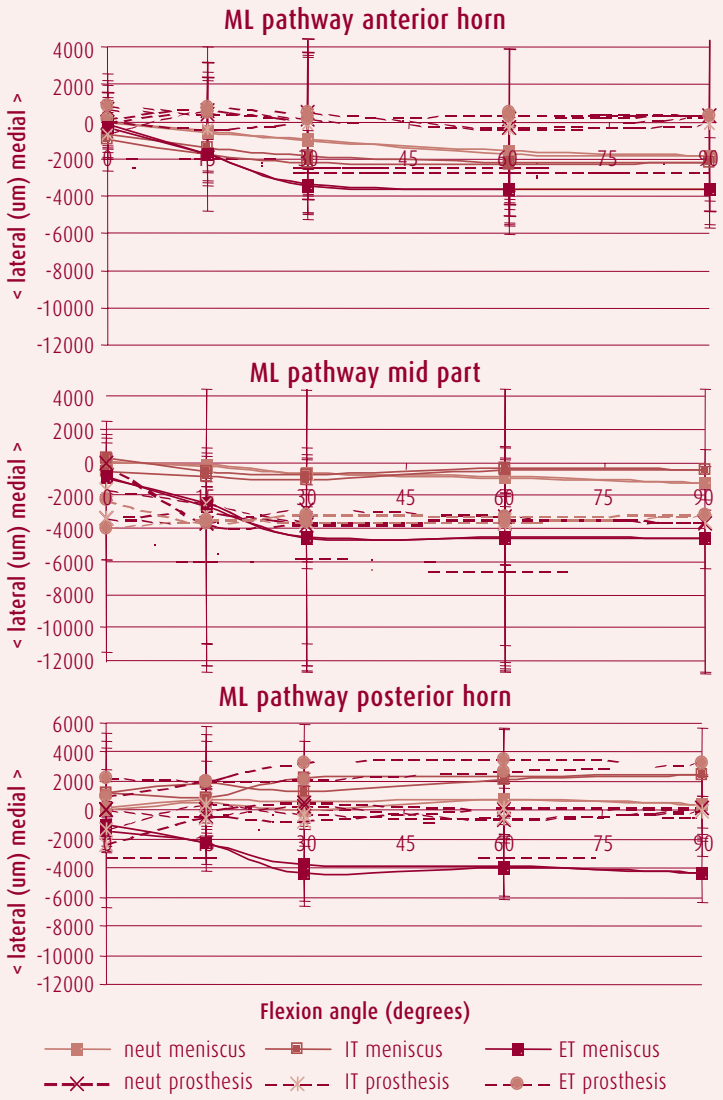
During flexion, all menisci and implants moved in posterior direction (Fig. 5). Both in AP as in ML direction, the displacement of the prosthetic anterior horn was smaller than that of the meniscus (AP: $p = 0.023$ and ML: $p = 0.03$). In AP direction, the prosthetic posterior horn showed a trend to displace less than in the native meniscus (AP: $p = 0.059$). In ML direction, all parts of the menisci and the implants showed less movement than in AP direction. The maximal displacements of the meniscal and prosthetic posterior horns did not differ significantly (Fig. 6). Maximal posterior displacement was reached at 90 degrees knee joint flexion. The anterior horn of the meniscus showed significantly greater maximal posterior displacements (average 6.4 mm) than its posterior horn (3.2 mm, $p< 0.001$). The displacements of the prosthetic horns were smaller (anterior horn of the implant 0.6 mm and 0.2 mm for the posterior horn, $p = 0.410$). In ML direction, the displacement of the native meniscal anterior horn was not significantly greater than the posterior horn. (average 1.8 and 0.2 mm, respectively).

Internal tibial torque

When internal tibial torque (IT) was applied to the knee joint, the pathways of all different meniscal parts shifted anteriorly on the tibial plateau. Subsequently, all parts of the menisci and implants again moved in posterior direction on the tibial plateau during knee joint flexion. Only for the anterior horn this shift was significant compared to the situation in neutral knee joint flexion ($p<0.001$). The anterior horn of the meniscus showed a parallel pathway without and with internal tibial torque. The same trend was observed for the mid part and posterior horn. No significant interaction was observed between the flexion angle of the knee and the internal tibial torque on the posterior displacement of the meniscus. The displacement of the anterior horn was more than the displacement of the posterior horn ($p<0.001$). Maximal displacement was reached at 90 degrees of flexion. The average displacement of the meniscal anterior horn was 2.2 mm in lateral direction while the average

figure 6

PATHWAYS OF THE DIFFERENT MENISCAL PARTS IN MEDIAL AND LATERAL DIRECTION ON THE TIBIA PLATEAU. BOTH THE MENISCUS AND THE IMPLANT SHOWED EVIDENTLY LESS DISPLACEMENT IN ML DIRECTION THAN IN AP DISPLACEMENTS. ONLY IN THE MENISCI MORE DISPLACEMENTS WERE OBSERVED DURING WITH ET.



displacement of the posterior horn was 2.4 mm in the opposite medial direction. The implant showed the same behavior after applying internal tibial torque.
The pathway of the prosthetic anterior horn remained more anteriorly on the tibia plateau than the pathway of the meniscus ($p=0.036$, 0.003 and $p<0.001$ for the anterior horn, midpart and posterior horn, respectively). Also during internal tibial torque, all different parts of the implant were evidently less mobile than the parts in the meniscus.
In ML direction, the posterior horn in the meniscus and in the implant did not show any displacement. The anterior horn and midpart in the meniscus showed more displacement than in the implant ($p<0.001$ and 0.003, respectively).

External tibial torque

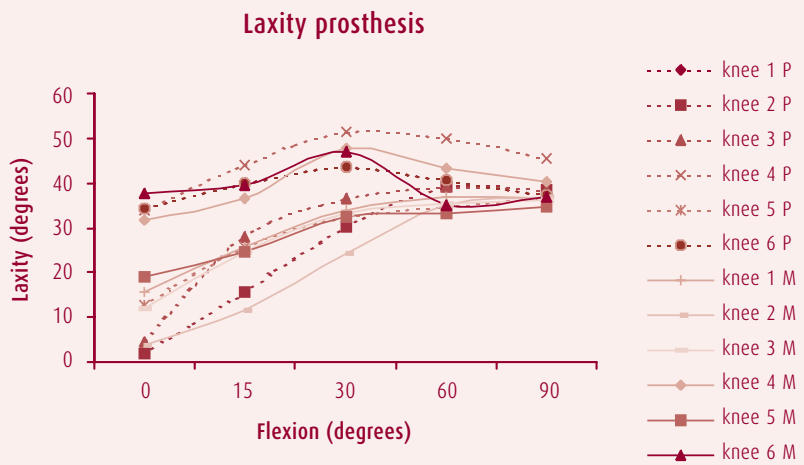
The pathway of all different meniscal parts was located significantly more posteriorly and laterally on the tibial plateau than during neutral knee joint flexion (Fig. 5 and 6). In all three parts of the meniscus, the displacement of the meniscus reached a plateau phase at 30 degrees of knee joint flexion, which suggested an interaction between the flexion angle and the tibial torque. This was confirmed statistically ($p<0.001$). There were no significant differences between displacements of the anterior and posterior horn.
As during the absence of tibial torque, the implant again showed less displacement than the native meniscus. Especially the anterior horn of the meniscus was more mobile in posterior and lateral direction than that of the implant ($p=0.010$ and 0.013, respectively)
Also the implant showed a similar interaction pattern between the flexion angle of the knee joint and the displacements. Again this was confirmed statistically ($p=0.006$ and 0.034 for the anterior and posterior horn, respectively).

Laxity

Minimal laxity was found at a knee flexion angle of 0 degrees in all knees (Fig. 7). Maximal increase of laxity was observed during the first 30 degrees of flexion, which remained to be observed after implantation of the meniscal implant. Although less steep, laxity increased during further flexion in the knees with the native meniscus and reached a maximum at 90 degrees of flexion in all knees, except for 2 cases (knees 4 and 6), which reached their maximum at 60 degrees. After implantation of the meniscal implants, the laxity of the knee was not significant different ($p= 0.304$, Univariate analysis of variance).

figure 7

TIBIAL ROTATORY LAXITY OF THE DIFFERENT KNEE JOINTS AS FUNCTION OF FLEXION. IN MOST KNEES MAXIMAL LAXITY WAS OBSERVED AT 90 DEGREES OF FLEXION. KNEE JOINT LAXITY TENDED TO BE LOWER AFTER IMPLANTATION OF THE IMPLANT (DASHED CURVES) THAN WITH NATIVE MENISCUS STILL IN SITU.



Discussion

In this study, the displacements of the meniscal implant on the tibial plateau were quantified and compared with the behavior of native meniscus during knee joint flexion. To compare the function of the implant with that of a native meniscus within the same knee, an in vitro model was developed with an accurate measurement system for three-dimensional kinematics and a loading and motion apparatus. With the provided data we were able to reconstruct the meniscal and prosthetic behavior on the tibia plateau.

The meniscus implant was made of biodegradable polyestherurethane (Estane). The characteristic porosity, pore-sizes and biomechanical properties of this polyester-urethane proved to be an appropriate for ingrowth and regeneration of neo-meniscal tissue in animal studies[7,8]. Also in the present study, the polymer material could easily be modeled into the specific shape of the medial meniscus and the material provided a high tear strength, which made it strong enough for suturing into the peripheral capsule and fixation of the horns on the tibial plateau.

As suggested earlier in the literature[6,12,14], the present study showed that all parts of the native meniscus moved posteriorly on the tibia plateau during knee joint flexion. These studies suggest that the femoral condyles move in the same direction during flexion, it may be assumed that the meniscus stays in close contact with the femoral condyles, thereby maintaining a large contact surface between the femoral condyles and the tibia. The meniscus implant also showed a posterior movement on the tibia plateau. This suggests that, due to its form and its fixation in the knee joint, the implant was forced to stay in contact with the femoral condyles during knee joint flexion, as is the native meniscus. However, the degree of displacement was less, resulting in a smaller displacement at 90° of knee joint flexion.

Also during flexion with addition of rotational tibial torque, the implant showed less displacement than the native meniscus. This could negatively influence the congruity between the implant and the femoral condyles, which again may decrease the function of the implant. However, this function may improve on the long-term in-vivo during the infiltration of tissue into the porous implant. As stated before, animal studies showed cartilage-like tissue formation in the implant which might improve the gliding capacity of the implant and thereby the displacements of the implant.

The relatively mobile anterior horn seemed to be responsible for the adjustment to the decreasing diameter of the femoral condyle during knee joint flexion[6,12,14]. This was confirmed in the present study, in which the anterior horn of the meniscus showed more AP displacement than the posterior horn. In the implant, however, no difference was observed between displacement of the anterior and posterior horn. The fixation of the implant, in our view, may play a dominant role. In the anatomical situation, the anterior horn fixation is realized by the combination of partly the attachment to the anterior capsule, the transverse ligament and the attachment on the tibial plateau[3]. This combination may enable the anterior horn to move more in AP direction than in ML direction. Therefore, we decided to attach the anterior horn of the implant only to the anterior capsule to achieve maximal mobility without a chance of dislocation of the horn. However, due to this attachment, full adjustment of the anterior horn to the posterior movement and decreasing diameter of the femoral condyles might not have been possible. Therefore, in future experiments, fixation of the anterior horn on the tibial plateau may be preferable. The posterior horn of the native medial meniscus firmly attaches to the posterior intercondylar fossa and is integrated into the different layers of the posterior capsule, which combination functionally leads to restricted movements of the posterior horn[9]. Based on the anatomical situation, the posterior horn of the implant was fixated to

the tibial plateau and sutured into the posterior knee joint capsule. Probably, this fixation was too rigid which impaired the movement of the prosthetic posterior horn. The material characteristics of the polymer implant may also have influenced the movement on the tibial cartilage. Due to its porous structure, initially, the bare polymer affects the gliding surface on the surrounding tibial and femoral articular cartilage. Also, the absence of synovial fluid after an arthrotomy does not improve this situation. The initial performance may be improved by injection of an elastic and viscous hyaluron base fluid. This lubricant can at least temporarily replace de synovial fluid lost after surgery[5].

In the absence of the anterior cruciate ligament (ACL), the menisci have been shown to enhance the stability of the knee in the AP, varus-valgus, and internal-external directions in vitro[12]. Although the menisci transmit load, they do not contribute to the primary stability of the knee when the ACL is intact[11]. Nevertheless, determining the laxity of the knee joint before and after replacement of the native meniscus provided us information about the consequences of the meniscus implant on the knee joint function. In the first flexion angles, there was no difference in knee joint laxity between native meniscus and implant. However, although not significant, the laxity of the different knee joints with implant seemed to be higher at higher flexion angles than in the knees with the native meniscus in situ. This may imply that, with the same tibial torques, greater internal and external tibial rotations were possible after implantation of a meniscus implant. This suggests a minor contribution of the implant to the restraint of knee joint motions. However, sham operations were not performed and thus it cannot be excluded that this increase in laxity was evoked by the arthrotomy and the release and reattachment of the MCL from the epicondyle during the implantation of the implant. With the beads in the femur and tibia, it would be possible to determine the influence of the implantation procedure on the kinematics of the knee joint itself. These factors may be subjects for future investigation.

In this phase of the research project we decided to implant the implant by means of an arthrotomy to enhance the exposure and to ensure a good fixation of the implant in the joint. Arthroscopical implantation probably would less harm the capsule of the knee joint and therefore would have less influence on the laxity. Furthermore, the chance on clinical implementation of this implant would also be increased when implantation occurs in an arthroscopical way. In the next phase we want to implant the implant arthroscopically.

In this in vitro model, the performance of a meniscus implant could be assessed and compared to the behavior of the native meniscus on the tibial plateau within the same knee in a very accurate manner. All different parts of the meniscus showed a posterior displacement during knee joint flexion, which pathway showed an anterior and posterior shift on the tibial plateau during application internal and external tibial torque, respectively. The anterior horn was evidently more mobile than the posterior horn, probably to retain the congruity with the femoral condyles. The meniscus implant showed the same pathways, although the different parts of the implant showed lower excursions on the tibial plateau and the implantation procedure resulted in a higher knee joint laxity.

This experiment provided valuable new information for improvement of the surgical technique and the polymer material characteristics. Considering the results of the earlier in vivo animal experiments and the results in this study, replacement may be a promising technique in the prevention of knee articular cartilage degeneration after meniscectomy.

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Chapter 9

Summery, address to the aims, closing remarks and prospects to the future.

Aims of the thesis

1. The first goal of this thesis was to determine the degradation behavior and the role of different porosities, pore sizes and compression modulus on in-vivo tissue infiltration and foreign body reaction in a ectopic, non-loaded location. Which combination of geometrical characteristics of the polymer provides for the fastest infiltration of tissue?
2. To develop new polymers for implantation into a partial-thickness access channel to initiate meniscal tissue infiltration and healing of an artificially made tear in the meniscus' non-vascularized zone.
3. To determine the consequences of meniscal reconstruction for the underlying articular cartilage: meniscal reconstruction only has clinical relevance if articular cartilage degeneration can be prevented.
4. To evaluate the performance of a newly developed polymer implant for total meniscus replacement with improved biomechanical and geometrical properties.
5. To determine the influences of the polymer material on the tissue infiltration and differentiation and the reaction of the tissue on the polymer material.
6. To evaluate the movements of the meniscus prosthesis in a human cadaver knee joint.

Summary and address to the aims

Chapter 1

Nowadays it is beyond any doubt that menisci are important components of the normal knee joint. Meniscectomy leads to abnormal high stresses on the articular cartilage in the affected compartment, which might lead to articular cartilage degeneration on the longer term. This chapter is an introduction in the world of the knee joint meniscus and considers the anatomy, function, pathology and (experimental) therapeutical options. Furthermore, the aims of the thesis were presented.

Chapter 2

This chapter focuses on the microscopical architecture of the porous polymer scaffolds: which geometrical characteristics are required for fast infiltration of tissue and differentiation into fibrocartilage? An in vitro degradation study was performed and the foams were subcutaneously implanted in the spinal subcutaneous tissue of rats. The porous polymers with the higher porosity, more interconnected macropores, and interconnecting micropores of at least 30 mm showed complete infiltration of tissue before degradation had started. In implants with the lower macro-porosity and micropores of 10-15 mm, degradation of the polymer occurred before infiltration was completed. Directly after implantation and later during degradation of the polymer, polymorphonuclear cells infiltrated the implant. In between these phases the foreign body reaction remained restricted to macrophages and giant cells.
We concluded that both the foam with the high compression modulus and low porosity and the foam with a low compression modulus and high porosity seemed not suitable for meniscal reconstruction: one foam started degradation before full infiltration of tissue was realized while the compression modulus of the other foam was too low. Therefore, foams must be developed with a higher compression modulus and more connections with sufficient diameter between the macropores.

Address to Aim 1:
For the purpose of meniscal reconstruction the degradation rate of the polymers should be low to achieve full infiltration of fibrovascular tissue in the scaffold. Therefore, the porosity should be high and the connecting channels between the macropores should have a diameter of at least 30 micron.

Chapter 3

This chapter is a continuation on the studies by Klompmaker and Veth et al.[4,5,13]. This study focused on the healing of meniscal lesions in the avascular zone of the meniscus. According to the studies by Klompmaker et al.[6], slowly degrading polymers were developed with a higher porosity and compression modulus in order to initiate a faster infiltration. An access channel was created in the meniscus to connect the artificially made tear with the peripheral capsule. A polymer implant was sutured into this access channel as a scaffold for the infiltrating repair tissue to reach the lesion in the avascular zone of the meniscus. Repair of the longitudinal lesions was achieved with and without polymer implantation in the partial-thickness access channel. The polymer implants induced fibrous infiltration with cartilaginous areas, which resembled neo-meniscal tissue. The initial goal of this study was to develop a new reconstruction technique to repair the lesion in the avascular zone of the meniscus without articular cartilage degeneration. However, articular cartilage degeneration could not be prevented, which was a drawback in the search for an alternative for partial meniscectomy, which is normally performed in case of a symptomatic meniscal tear in the avascular region of the meniscus. However, the degeneration may be a result of the surgical trauma and the rough surface of the

polymer scaffold. Nevertheless, an arthrotomy is required for this procedure, which seems not attractive in the era of arthroscopical surgery.

Address to Aim 2:

The newly developed polymers performed well when sutured into the meniscal defect: they were completely infiltrated by fibrous tissue before degradation had started. The implants showed complete integration with the native meniscal tissue, contained tissue with a cartilage-like phenotype and initiated healing of lesions in the avascular part of the meniscus. Question remained if this technique would become an alternative for partial meniscectomy.

Chapter 4

Partial meniscectomy is the golden standard for treating a bucket-handle tear in the meniscus of the knee but inevitably leads to articular cartilage degeneration. This chapter further evaluates the consequences of the reconstruction technique for the articular cartilage as presented in the previous chapter. Articular degeneration was apparent and consisted of fibrillation, loss of chondrocytes and decreased proteoglycan content. Collagen degradation was apparent in areas of fibrillated cartilage but also in non-fibrillated areas. The implants also seemed to evoke swelling in the upper zone of the cartilage, with empty cell lacunae and moderate levels collagen degradation. We concluded that this reconstruction technique should not be considered superior to partial meniscectomy. Furthermore, degradation of the collagen type II network seemed to be a result of cartilage fibrillation and vice versa.

Address to Aim 3

Macroscopical and microscopical damage to the cartilage were observed after meniscal reconstruction. Damage to the collagen architecture seemed to be an ominous sign. We concluded that this reconstruction technique should not be considered superior to partial meniscectomy.

Chapter 5

We also continued the meniscus replacement studies as started by Klompmaker et al.[7]. In a dog model, a meniscal scaffold prosthesis with optimal properties for tissue infiltration and regeneration of a neo-meniscus was implanted and compared with total meniscectomy. The tissue infiltration and re-differentiation in the prosthesis, the stiffness of the prosthesis and the articular cartilage degeneration were evaluated.

Regeneration of new meniscus tissue seemed to be possible by in-vivo tissue engineering. The optimal properties of these polymer implants resulted in a fast infiltration of fibrovascular tissue and 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 increased toward that of the native meniscus at 6 months follow up. In this short-term study, cartilage degeneration could not be prevented. This might be explained by the rather rough surface of the implant due to the porous structure of the polymer implant. Whether the arthrotomy and the dissection in the knee joint played a role in this process is not known. Furthermore, the replacement was performed on a very small joint, which makes this a technically demanding procedure. Together with the rather vulnerable articular cartilage of this dog, obviously the joint is susceptible for damage and degeneration. At first, we see this replacement technique as an alternative for allograft transplantation in symptomatic osteoartritic knee joints as described by Verdonk et al.[12]. In these cases the implant should serve as a spacer to distribute the forces over the tibial compartment to decrease the pain rather than preventing further cartilage damage[8]. Nevertheless, we aimed to reduce or prevent articular cartilage damage as seen after meniscectomy. Question remained how the prostheses and the cartilage would perform on the longer term.
These long-term experiments with improved prostheses and surgical technique are being performed at this moment.
However, in the development of a implant for total replacement of the heavily damaged meniscus, the results of this experiment appeared to be very promising.

Address to Aim 4:

The polymer-tissue construct seemed to develop meniscus-like phenotypical and biomechanical characteristics. The improvements to the geometrical properties of the implant induced a fast infiltration of tissue and fast differentiation of this tissue into fibrocartilage. However, the polymer implant did not prevent articular cartilage degeneration and this aspect needs further attention in the future.

Chapter 6

The Estane implant as described in the previous chapter produces potentially toxic degradation products. Therefore, prostheses were produced of a non-toxic alternative polymer material (polycaprolactonpolyesterurethane) and a comparison was made between these two prostheses after implantation in the knees of dogs. 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, both prostheses in their current shape could not prevent articular cartilage degeneration. The newly developed PCLPU implant seemed to provoke less intensive tissue reaction.

Address to Aim 5

The influence of the two polymers in this study on the tissue infiltration and differentiation into meniscus-like tissue was minimal, except for the slight tissue reaction by the PCLPU implant. Thus, both the nontoxic degradation products and the less foreign body reaction are reasons to prefer the PCLPU implant for meniscal transplantation studies in the future.

Chapter 7 and 8

Chapter 5 and 6 showed the favorable results of the meniscus implant in dogs with the formation of fibrocartilage. In this dynamic biological process, the neo-meniscal tissue may be able to adjust to the functional requirements of its surroundings to which it is subjected in the long term. Prior to these biological interactions, the implant should already mimic the behavior of the native meniscus directly after implantation. Therefore, material characteristics of the implant, like stiffness, tear strength and gliding capacity are of great influence to the performance of the synthetic implant. Furthermore, size and fixation of the implant may also be very important as stated in meniscal allograft transplantation studies[1,2,8]. In these chapters a loading apparatus for human cadaver knee joints was described as developed by Blankevoort et al.[3] in which the joint was subjected to load and passive motion and the menisco-tibial displacements were determined by means of Roentgen Stereophotogrammetric Analysis (RSA). In chapter 7 this model was validated based on MRI meniscus movement studies by Vedi et al. and Thompson et al.[10,11].
In chapter 8, the meniscus of the human cadaver knee joint was replaced by a porous polymer implant and the behavior of the implant was evaluated within the limits of passive knee joint motion.
In this in vitro model, the performance of a meniscus implant could be assessed in a very accurate manner. All different parts of the meniscus showed a posterior displacement during knee joint flexion. The meniscus implant showed the same pathways, although the different parts of the implant showed lower excursions on the tibial plateau, which might be explained by the absence of synovial fluid or the fixation technique of the implant in the knee joint. Another factor may be the rather rough surface of the prosthetic material. This experiment provided valuable new information for improvement of the surgical technique and the polymer material characteristics. Obviously, the gliding characteristics of implant need to be improved which might be achieved in a polymer chemical manner or/and by the addition of artificial synovial fluids. Furthermore, the fixation seemed to be to rigid. Probably, peripheral attachment to the capsule solely is sufficient as described in the allograft transplantation literature. And lastly, the implantation procedure needs to be performed arthroscopically: not only to make the procedure more attractive for future clinical implantation but also to prevent additional laxity to the knee joint as a result of the arthrotomy and the temporary detachment of the medial collateral ligament.

Address to Aim 6:

This model proved to be a valid method to analyze meniscal behavior during knee joint flexion. Furthermore, it seemed helpful in the development of an implant for complete meniscal replacement and the implantation technique. The analysis revealed that especially the gliding characteristics of the prosthesis need to be improved and that the fixation technique of the prosthesis to the capsule might have been too rigid.

Closing remarks and future directions

Meniscal lesions are very common, particularly in the young more active patient and in many cases degeneration of the knee articular cartilage is inevitable. Partial arthroscopic meniscectomy became the treatment of first choice although even this treatment will lead to changes in biomechanical loading patterns onto the articular cartilage, with degeneration and osteoarthritis as end result.

Many articles described the results of meniscal repair techniques. However, until now no clinical applicable solution has been presented for the lesions in the non-vascularized zone of the meniscus. The results as presented in this thesis were also disappointing. Despite the fast infiltration of tissue into the scaffolds, either the arthrotomy itself or the scaffold induced cartilage degeneration. Furthermore, the arthrotomy, necessary for the reconstruction of the meniscus, seems obsolete in the era of less traumatic arthroscopic partial meniscectomy. These factors make clinical implementation in the near future less likely.
The results of meniscal replacement were more hopeful. The tissue phenotype distribution within the implant resembled the distribution in the native meniscus.

However, cartilage degeneration was not completely prevented although the degeneration was not progressive on the longer term. The degeneration was probably due to the implantation technique and the rough material surface. At this moment long-term studies are being performed with prostheses which are produced with a mold to create a smoother surface (Fig. 1). At this moment also gait analyses is performed in dogs to obtain more information about the functional results of this implantation procedure versus the meniscectomy.

Furthermore, in vitro culture studies are performed to load the implant with meniscal cells prior to implantation. We hypothesize that in this way the implant will be covered with a biofilm in an earlier stage so that the gliding characteristics are improved.

For the clinical practice, we have more confidence in the replacement technique than in the reconstruction technique, especially if we would be able to develop an arthroscopical way to implant the implant as described by Stone et al. with the collagen meniscal implant[9].

In the past two decades this meniscus research line has led to promising polymers for meniscal tissue replacements and highly developed analysis methods for the in-vivo and in vitro evaluation of these polymers. At the moment we are developing a technique to perform the meniscal replacement arthroscopically. Eventually, we hope that this may lead clinical implementation of this technique on the long term.

figure 1

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Chapter 10

Samenvatting, beschouwing van de doelstellingen en afsluitende opmerkingen.

Aims of the thesis

Doelstellingen

1. Vaststellen van het degradatie gedrag en de invloed van porositeit, porie grootte en compressie modulus op de in-vivo weefsel infiltratie en vreemd lichaamreactie wanneer de scaffolds geplaatst worden op een ectopische niet belaste locatie, in dit geval subcutaan op de rug bij ratten.
2. Plaatsen van nieuw ontwikkelde polymeren scaffolds in een gecreëerd defect in de meniscus om contact te maken tussen de nagebootste scheur in het avasculaire gebied van de meniscus en het synovium/kapsel van de knie. Ontstaat er nieuw meniscus weefsel in de scaffold en vindt er heling van de scheur plaats?
3. Bepalen van consequenties voor het onderliggende kraakbeen van deze procedure.
4. Totale vervanging van de meniscus met nieuw ontwikkelde implantaten. Hebben de verbeterde biomechanische en geometrische eigenschappen een positieve invloed op de infiltratie van weefsel en differentiatie naar nieuw meniscus weefsel?
5. Bepalen van de invloed van het gebruik van twee verschillende polymeren op de infiltratie van weefsel. Welk polymeer is het meest geschikt als vervanging van de meniscus?
6. Vervangen van de meniscus door een polymeer implantaat in humane kadaver knieën en bepalen van de bewegingen van het implantaat ten opzichte van de normale meniscus.

Samenvatting en beschouwing van de doelen

Hoofdstuk 1

Tegenwoordig wordt algemeen erkend dat de meniscus een belangrijke component is van het kniegewricht en dat meniscectomie kan leiden tot kraakbeenslijtage op de langere termijn. Dit hoofdstuk leidt u rond in de wereld van de meniscus en beschrijft de anatomie, functie, pathologie en (experimentele) therapeutische mogelijkheden hiervoor. Vervolgens worden de doelen van dit proefschrift beschreven.

Hoofdstuk 2

Uit eerder onderzoek bleek dat de geometrische eigenschappen van het polymeer zoals o.a. porie grootte en porositeit veel invloed hebben op de infiltratie van weefsel in het polymeer drager-materiaal. Dit hoofdstuk beoogt een verfijning van deze eigenschappen te geven en bekijkt de degradatiesnelheid zowel in-vitro als in-vivo. Twee scaffolds met verschillende porie grootte en porositeit werden subcutaan bij ratten op de rug geplaatst. Dit onderzoek toonde aan dat infiltratie van weefsel alleen mogelijk is indien de poriën minimaal 30 micron groot zijn. Bij de polymeren met kanalen van een kleinere diameter was de degradatie van het materiaal reeds begonnen voordat volledige infiltratie met weefsel was voltooid. De vreemd-lichaam reactie bleef beperkt tot enkele macrofagen en reuscellen. Een hogere porositeit verhoogde ook de infiltratie snelheid in het polymeer maar had weer een lagere compressiemodulus tot gevolg wat weer nadelig is voor de differentiatie van het weefsel richting kraakbeen[5]. De conclusie van deze studie was dat zowel het geteste schuim met de hoge compressie modulus en een lage porositeit als het schuim met de lage compressie modulus en hoge porositeit niet geschikt waren voor de meniscus reconstructie zoals beschreven in hoofdstuk 3.

Beschouwing doelstelling 1:

Voor gebruik voor de meniscus reconstructie operatie moet de degradatiesnelheid omlaag om een volledige infiltratie van weefsel te kunnen realiseren. Daarvoor moet de porositeit hoog zijn bij een acceptabele compressie modulus van het materiaal en de diameter van de kanalen tenminste 30 micron zijn.

Hoofdstuk 3

Als vervolg op de studies van Klompmaker en Veth et al.[7,13] focust deze studie op de heling van een scheur in het avasculaire gedeelte van de meniscus. Naar aanleiding van de studie van Klompmaker et al. werden langzaam degraderende polymeren ontwikkeld met een hoge porositeit en compressiemodulus (stijfheid) om de infiltratie van weefsel in het polymeer te versnellen en het herstel van de scheur in de meniscus te bespoedigen. Een defect werd gecreëerd in de meniscus om een toegangsweg te vormen voor herstel weefsel vanuit het kapsel naar de meniscus scheur. In de ene groep werd het polymeer hierin gehecht. In de andere groep werd het defect leeg gelaten om te zien wat het effect van het polymeer was. In beide groepen werd een herstel van de scheur gezien. Echter in het polymeer had zich fibreus kraakbeen gevormd wat leek op nieuw meniscus weefsel terwijl in de groep zonder polymeer slechts fibreus weefsel werd gevonden. Tevens trad er gewrichtskraakbeen slijtage op. Gezien de hypothese dat de reconstructie techniek een alternatief zou moeten worden voor de partiële meniscectomie (met de kans op kraakbeenslijtage op de langere termijn) was dit resultaat teleurstellend. Ook is het gegeven dat een arthrotomie zou moeten gebeuren niet aantrekkelijk in het tijdperk van de arthroscopie.

Beschouwing van doelstelling 2:
De ontwikkelde polymeren induceerden volledige infiltratie van nieuw meniscusweefsel voordat de degradatie van het polymeer was begonnen maar de reconstructie leidde ook tot kraakbeen degeneratie. Heling van de scheur in het avasculaire gebied van de meniscus trad op. Niettemin blijft de vraag of deze techniek een alternatief kan zijn voor partiële meniscectomie.

Hoofdstuk 4:
Dit hoofdstuk evalueert uitgebreid de consequenties voor het onderliggende kraakbeen van de hierboven beschreven reconstructie techniek. Kraakbeen degeneratie was aanwezig en bestond uit fibrillatie, verlies van chondrocyten en een afname van proteoglycanen in het kraakbeen. Door middel van immunohistochemie werd zichtbaar dat er tevens sprake was van afbraak van het collageen netwerk in de gebieden met fibrillatie maar ook (!) in de, bij routine histologie, onbeschadigde gebieden. In het gebied waar zich het implantaat op het kraakbeen bevond trad tevens zwelling op van de bovenste kraakbeenlaag met lege cel lacunes en enige collageen degradatie. Conclusie was dat kraakbeenslijtage niet kon worden voorkomen en dat deze reconstructie techniek dus niet superieur lijkt aan partiele meniscectomie. Verder bleek dat degradatie van collageen type II een gevolg van kraakbeenfibrillatie was en vice versa.

Beschouwing van doelstelling 3:
Macro- en microscopische schade aan het kraakbeen werd gezien na meniscus reconstructie. Schade aan het collageen netwerk bleek een omineus teken. Conclusie is dat deze reconstructie techniek tot op heden niet superieur lijkt aan partiele meniscectomie.

Hoofdstuk 5
Dit hoofdstuk is ook een vervolg op de studies van Klompmaker et al. waarin de volledige meniscus werd vervangen door een polymeer scaffold[4,6] . In knieën van honden werd de meniscus vervangen door vernieuwde implantaten met optimale eigenschappen voor de infiltratie van weefsel en regeneratie van meniscusachtig weefsel. Kraakbeen slijtage na meniscusvervanging werd vergeleken met slijtage na meniscectomie. Verder werd de weefselinfiltratie, weefselfenotype in het polymeer en de stijfheid van het polymeer na infiltratie met weefsel geëvalueerd. Regeneratie van meniscus weefsel in deze implantaten bleek mogelijk door middel van in-vivo tissue engineering. De optimale eigenschappen van deze implantaten resulteerden

in een snelle infiltratie van fibrovasculair weefsel en een locatie specifieke differentiatie van dit weefsel zoals in de oorspronkelijke meniscus gezien wordt. Slechts een milde vreemd lichaamreactie werd gezien in en rond het polymeer. 6 maanden na operatie benaderde de compressie modulus van het implantaat de stijfheid van de normale meniscus. Kraakbeen degeneratie werd gezien in beide groepen.
Het implantaat had een ruwe oppervlakte als gevolg van de poreuze structuur. Dit zou slijtage mede hebben kunnen veroorzaakt. Verder is het kniegewricht van de Beagle klein wat de operatie gecompliceerd maakt en tezamen met het kwetsbare kraakbeen van de hond maakt dat de kans op kraakbeen schade groter.
In eerste instantie dient meniscusvervanging met het polymeer gezien te worden als een alternatief voor allograft transplantatie in symptomatische knieën met osteoarthritis zoals beschreven door Verdonk et al.[12]. Na deze procedure hebben de patiënten minder klachten waarschijnlijk vanwege een betere verdeling van de krachten over het gewrichtskraakbeen[2]. In de literatuur blijft het onduidelijk of kraakbeen slijtage daadwerkelijk ook wordt voorkomen. Niettemin stellen we ons ook ten doel om de kraakbeen slijtage te verminderen in vergelijking met meniscectomie. Daarvoor zijn nieuwe implantaten ontwikkeld en deze worden nu in de lange termijn studies getest. Niettemin zijn de resultaten van het hier gepresenteerde experiment veelbelovend.

Beschouwing van doelstelling 4:
Het weefsel in het implantaat lijkt op fibreus kraakbeen en de compressie modulus benadert die van de meniscus. De verbeteringen aan porositeit en porie grootte leidde tot een snelle infiltratie van weefsel en een snelle differentiatie van dit weefsel in de richting van fibreus kraakbeen. Niettemin kon het implantaat de kraakbeenslijtage niet voorkomen. Dit verdient meer aandacht in toekomstige studies.

Hoofdstuk 6
Zoals in eerder hoofdstukken al beschreven, geeft het Estane implantaat potentieel toxische degradatie producten af. Er werden nieuwe implantaten (polycaprolacton-polyesterurethane) ontwikkeld met niet toxische afbraakproducten en deze implantaten werden vergeleken met de Estane implantaten zoals beschreven in het hoofdstuk 5. Daarvoor werd weer hetzelfde hondenmodel gebruikt. In beide implantaten werd eenzelfde fenotype en distributie gevonden als in de normale meniscus. Wederom werd kraakbeenslijtage gezien.

Beschouwing van doelstelling 5

Het verschil tussen de twee polymeren leidde niet tot verschillen in infiltratie van weefsel en de differentiatie in de richting van nieuw meniscus weefsel. Er werd slechts een klein verschil in vreemd lichaam reactie gevonden in het voordeel van het PCLPU polymeer. Dus naast de niet-toxische afbraakproducten van het PCLPU, is dit een extra reden om voorkeur te geven aan dit materiaal voor toekomstige meniscus vervangingen .

Hoofdstuk 7 en 8

Hoofdstuk 5 en 6 laten de veelbelovende resultaten zien van meniscusvervanging in honden en de vorming van meniscusachtig weefsel in het implantaat. Dit weefsel in het implantaat kan zich op langere termijn gaan aanpassen aan de omgevings-omstandigheden. Echter het is van belang te weten hoe het implantaat zich direct na implantatie gedraagt. Dit gedrag wordt bepaald door materiaaleigenschappen van het implantaat, zoals stijfheid en gladheid maar natuurlijk ook door de maat van het implantaat en fixatie in het kniegewricht zoals ook wel beschreven in de allograft studies[1,2,8]. In de hoofdstukken 7 en 8 wordt het belastingsapparaat beschreven als gebruikt door Blankevoort et al.[3]. Hierin wordt een humane kadaver knie belast en passief bewogen en ondertussen worden de bewegingen van de meniscus ten opzichte van de tibia bepaald met behulp van Röntgen Stereophotogrammatic Analysis (RSA). In hoofdstuk 7 valideren we dit model aan de hand van MRI studies door Vedi et al. en Thompson et al.[10,11]. In hoofdstuk 8 werd de meniscus vervangen door een Estane implantaat en het gedrag van het implantaat werd geëvalueerd.

Voor deze doeleinden bleek dit een zeer accuraat model. Alle delen binnen de meniscus bewogen in posterieure richting over het tibia plateau tijdens buiging van het knie gewricht. Na meniscus vervanging liet het implantaat dit ook zien maar de excursies waren kleiner dan die van de oorspronkelijke meniscus. De afwezigheid van synoviale vloeistof na de arthrotomie zou hierbij een rol hebben kunnen spelen. Een te rigide fixatie van het implantaat in de knie of te stroef materiaal zouden ook een verklaring kunnen zijn. Dit experiment gaf veel nieuwe informatie ter verbetering van het materiaal en van de operatietechniek. In de toekomst dient de gehele implantatie procedure ook arthroscopisch te gebeuren teneinde de schade aan het gewricht door de arthrotomie te voorkomen. Dat zou de techniek attractiever maken voor gebruik in een klinische setting in de toekomst.

Beschouwing van doelstelling 6:

Dit model bleek geschikt als methode om de bewegingen van de meniscus tijdens een kniebuiging te analyseren. Dit levert waardevolle informatie bij de ontwikkeling van het implantaat en de chirurgische techniek. Het lijkt dat de glij-eigenschappen van het materiaal en de operatie techniek dienen te worden verbeterd.

Afsluitende opmerkingen

Scheuren in de meniscus komen frequent voor, met name in de jonge actieve patiënt. Partiele meniscectomie is de behandeling in deze geworden ondanks de grote kans op kraakbeen degeneratie in de toekomst.

Veel studies beschreven allerlei herstel technieken. Tot op heden zijn er geen technieken bruikbaar voor laesies in het avasculaire gebied van de meniscus.

De resultaten van de reconstructie techniek voor dit soort laesies zoals gepresenteerd in dit proefschrift waren ook teleurstellend. Ondanks de snelle infiltratie in de implantaten kon kraakbeen slijtage niet worden voorkomen. Daarbij komt dat bij deze techniek er aanzienlijk trauma wordt aangebracht aan de meniscus wat de functie ook niet ten goede zal komen. Dit alles maakt een klinische implementatie in de nabije toekomst onwaarschijnlijk.

De resultaten van totale meniscus vervanging waren meer hoopvol. De verdeling van de verschillende soorten weefsel door het implantaat leek op dat van de normale meniscus. Echter kraakbeenslijtage kon niet worden voorkomen hoewel de slijtage niet doorzette tussen 3 tot 6 maanden postoperatief. Bovendien leek enige kraak-beenslijtage inherent aan opereren in het kleine gewricht van de Beagle hond.

Echter de ruwheid van de oppervlakte van het implantaat heeft wellicht ook een rol gespeeld. Fabricage van de nieuwe implantaten met behulp van een mal zou tot een gladder oppervlak kunnen leiden (Fig. 1). Dit is in volle gang. Momenteel worden ook gangbeeld analyses gemaakt om het functionele verschil tussen meniscectomie en het implantaat te kunnen beoordelen. Tevens worden er in-vitro kweekstudies verricht om de implantaten vooraf te vullen met cellen. Op deze manier zou de prothese sneller bedekt kunnen zijn met een fibreuze laag die het kraakbeen weer beschermt tegen slijtage.

Voor de klinische praktijk hebben we meer vertrouwen in vervanging van de gehele meniscus dan in de reconstructie techniek, zeker als we in staat blijken om de complete meniscus vervanging arthroscopisch te kunnen verrichten zoals Stone et al. reeds deed met de “Collagen Meniscal Implant”[9].

In de afgelopen 20 jaar heeft deze meniscus onderzoekslijn geleid tot veelbelovende polymeren voor vervanging van meniscus weefsel en ver ontwikkelde analyse methode voor in-vivo en in-vitro evaluatie van deze materialen. We hebben alle vertrouwen dat dit uiteindelijk leidt tot een klinische implementatie van deze technieken, met name de totale meniscus vervanging.

figure 1

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Dankwoord

Zo, het ligt er. Dit was mijn laatste proefschrift, denk ik. Tijdens de vervaardiging ervan ben ik er achtergekomen dat een groot deel in mijn leven wordt bepaald door mensen die mij enthousiast maken en houden. Enthousiasme is dan ook een sleutelwoord in de totstandkoming van dit boekwerk. De drie jaar dat ik hier full-time mee bezig was heb ik met veel mensen gewerkt die dit enthousiasme wisten over te brengen. En dat houdt je in moeilijke tijden dat elk proefschrift met zich meebrengt, op de been. Enkele van deze mensen wil ik specifiek danken:

Allereerst Prof. Veth: ik had de eer de derde in de rij te zijn om aan dit project te mogen werken. De meniscuslijn heeft ondertussen zijn 4de lustrum gehad en leeft nog steeds. Dit getuigt van de waarde van dit onderzoek hoewel ik menigmaal mensen daarvan moest overtuigen. Met de professor in de auto naar Groningen en dan die ellenlange vergaderingen met de hele groep: het was toch een bijzondere ervaring. Dank voor het vertrouwen en voor de snelle correcties van al die manuscripten die de afgelopen tijd gepasseerd zijn. Iemand moet de “helicopter-view” bewaren!

En dan Dr. Buma, maar zo heb ik je nooit genoemd. Alleen als ik snel iets van je moest hebben... Beste Pieter, de tijden dat we het over andere dingen hadden dan over werk waren me ook veel waard. Je liet me volledig vrij in mijn werkzaamheden en af en toe wat sturende tikjes als ik als bal de weg in de flipperkast even kwijt was.

Jacqueline, wat een drive, dat is het eerste wat me aan je opviel. Als ondersteuning aan de kant van de polymeerchemie heb je een heel belangrijke bijdrage geleverd aan het project. Jij zorgde dat er schuimen kwamen... Ralf mag je dankbaar zijn. Deze drive bracht je tot jullie eigen bedrijf, Orteq. En daar mag je trots op zijn. Jammer dat uiteindelijk de wegen moesten scheiden...

Prof. Pennings,
over drive gesproken, pensioen of niet....aangekleed door mooie anekdotes met een cynische ondertoon werden allerlei nieuwe ideeën aangeboden waar wij dan weer een nieuwe richting uit moesten destilleren. Uw bijdrage voor het project is van grote waarde geweest.

Prof. Schouten,
Prof. Pennings had de ideeën en aan u de taak om ze te verwezenlijken. Als nieuweling in het meniscus project en als nieuw hoofd van de afdeling was dit geen gemakkelijke taak maar uiteindelijk is het allemaal goed gekomen.

Ralf, ik begreep in het begin helemaal niets van polymeerchemie. Nu nog niet eigenlijk maar je hebt me door die brei van fasescheidingen, vriesdrogen en katalysatoren heengetrokken. Dank daarvoor. Tja, ik had echt die schuimen nodig, en snel...

Beste Nico,
In afwachting van de polymeerschuimen hebben we samen twee mooie studies opgezet die enthousiast zijn ontvangen in de buitenlandse tijdschriften. Je bent van niet te onderschatten waarde voor het lab: altijd positieve en enthousiast. Soms is de commercie ook mooi…

Joep, Richard, en Marloes,
Tjonge wat ben ik jullie dankbaar. Jullie hebben me veel (monnikken)werk uit handen genomen. Jullie weten als geen ander dat de resultaten niet altijd zijn zoals je hoopt. En al jullie werk heeft uiteindelijk geleid tot publicaties in goede tijdschriften. Het was ook gewoon gezellig, ook niet onbelangrijk. Op het feest drinken we er nog eentje op!!

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Diny en Natasja,
Als dokter moest ik mijn best doen om niet aan dat stereotype te voldoen. “Ik wil graag het weefsel in aceton gefixeerd en in plastic gegoten. Wanneer zijn de coupes klaar denken jullie”. Ik denk dat jullie vaak gedacht hebben: "daar heb je weer zo’n dokter, die heeft makkelijk praten". Jullie weten dat ik jullie heel dankbaar

ben. Diny als leermeester en Nastasja als gezel, jullie waren een productief koppel. Diny, ik ben blij dat je zo van je pensioen geniet.

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Navin,
Ik heb het stokje aan je doorgegeven. We houden er verschillende manieren van werken op na maar er leiden meerdere wegen naar Rome. Heel veel succes bij de laatste loodjes van je periode als onderzoeker, want over een paar maanden wacht de volgende taak, de vooropleiding. Je zal met weemoed terugdenken aan je onderzoekstijd.

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Het is een heftig jaar geweest, zowel sociaal als op mijn werk. Teveel veranderingen tegelijk is niet goed, ben ik achter. Maar misschien moest het zo zijn. Soms lopen dingen anders dan je vooraf dacht... “If it doesn’t kill you, it makes you stronger”. Ik heb veel steun gehad van veel mensen en één in het bijzonder. Diegene weet dat ik haar daar heel dankbaar voor ben.

Een kroeg, straaljagerpiloot, al die onrust, van wie zou ik dat nu hebben?
Pap, mam bedankt voor de rust.

Curriculum vitae

16 april 1973, Tony van Tienen werd geboren in Helmond. Hij groeide op in Beek en Donk en bracht de middelbare schooltijd door op het Dr. Knippenberg College te Helmond. In 1991 behaalde hij zijn atheneum diploma. In datzelfde jaar begon hij met de studie geneeskunde aan de Medische Faculteit van de Katholieke Universiteit Nijmegen. In 1998 werd het arts-examen gehaald. Na een jaar proeven aan het "dokter zijn" als AGNIO in het St Anna Ziekenhuis in Oss en Elkerliek Ziekenhuis in Helmond begon hij aan het "meniscus onderzoek" waarvan het resultaat voor u ligt. Op 1 juli 2002 begon hij met de vooropleiding Algemene Heelkunde in het Elisabeth Ziekenhuis Tilburg onder de opleiders Dr. J.A. Roukema, Dr. J.F.Hamming en Dr. C.J.H.M. van Laarhoven. Per 1 juli 2004 keert hij weer terug naar Nijmegen om daar eindelijk te beginnen met de opleiding Orthopaedie bij de opleiders Prof.dr. R.P.H. Veth (UMC St.Radboud, Nijmegen), Dr. A.B. Wymenga (St. Maartenskliniek, Nijmegen) en Dr. W.J. Rijnberg (Rijnstate Arnhem).