JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 13 (2002) 351–359

Studies of polyurethane urea bands for ACL reconstruction

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The present report describes the mechanical tests, *in vitro* and *in vivo* studies of a poly(urethane urea) (PUUR) intended for clinical use in anterior cruciate ligament (ACL) reconstruction. In the mechanical tests, no evidence of severe fatigue was observed after repeated cyclic loading. Testings for mutagenicity and delayed contact hypersensitivity were found negative. Three *in vivo* studies were performed in rabbits and minipigs. Altogether 35 rabbits were operated upon in (1) an intraarticular implantation study, performed to evaluate the soft tissue response to woven bands and fiber bundles of PUUR and (2) a rabbit ACL study, examining the function of the PUUR ACL replacement and the tissue response to the material. In a third study, PUUR ACL replacement in minipigs was evaluated. Taken together, ingrowth of connective tissue in close contact with the PUUR fibers was detected both in rabbits and minipigs. The first clear histological signs of degradation of the polymer was detected after 24 months.

In conclusion, the evaluated mechanical properties of the PUUR band correspond to those of the mature, human ACL. Furthermore, both from a histological and functional point of view, the PUUR woven band show interesting properties for future clinical ACL reconstructions.

Introduction

The anterior cruciate ligament (ACL) is the primary and most important stabilizer of the knee [1]. Ruptures of the ACL are the most common serious ligament injuries, and with conservative treatment, the knee joint will progress into osteoarthritis in over 50% of the cases [2–5]. After surgical treatment, a number of patients have unsatisfactory long-term outcome with pain, loss of motion, progressive instability, graft failure and development of osteoarthritis [6–10].

Presently, the most used autografts for ACL reconstructions are the central third of the patella tendon and adjacent bone, multiple loops of the semitendinosus gracilis tendons or the quadriceps tendons [11–13]. The decreased stability over time, the donor-site morbidity in approximately 5–30% of the patients and reoperation rates of 30% [13–17], have resulted in a search for an ACL substitute duplicating both the structure and function of the human ACL.

Prosthetic non-degradable ligament bands, of for example, polytetrafluoroethylene, polyethyleneterepthalate [11, 17–21], polypropylene, polyethylene and carbon fibers [16, 22] were introduced three decades ago. However, they were abandoned after a short period due to inadequate mechanical properties.

Among the first synthetic materials used for degradable devices was polydioxanone (PDS), used as an augmentation to stabilize the autograft. However, the stiffness of the PDS led to stress shielding phenomena [23]. Currently, no satisfactory ACL device are available for clinical practice. The need of a degradable ACL substitute, for prosthetic or augmentation purposes, based on a biocompatible polymer with degradation times exceeding those of PDS to allow ingrowth and

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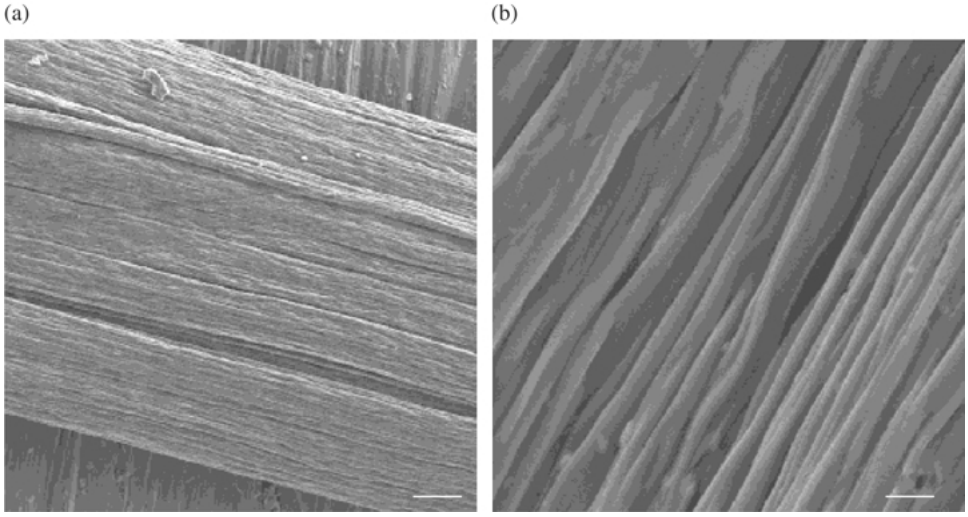


Figure 1 SEM micrographs of the surface of a wet spun PUUR fiber, (a) bar = 50 μm, (b) bar = 5 μm.

adaptation of the autogenous tissue formed, and with mechanical properties similar to the human ACL is a high clinical priority. Further, the synthetic ACL device must be able to recapture its original length after loading in a similar manner as the human ACL does [24]. The polyurethane class of synthetic polymers has proven to be well tolerated by the human body when used as for example, catheters [25], lead insulation for pacemakers [26], vascular prostheses [27], artificial hearts [28, 29] and may constitute a suitable material for ACL prosthesis.

The aim of the present study was to evaluate new degradable polyurethane urea (PUUR) fibers [30] intended for ACL reconstruction. Initial *in vitro*, *in vivo* and mechanical data are presented.

Materials and methods

Chemical composition and fiber spinning

The poly(urethane urea) was synthesized by a two step method described earlier [31, 32]. In the first step polycaprolactone diol, (PCL) ($\bar{M}_n = 530\,\mathrm{g/mol}$) (Aldrich, Germany) was end-capped with 4,4′-diphenylmethane diisocyanate (MDI) (Bayer AB, Sweden) (NCO : OH = 2 : 1). In the second step the prepolymer was dissolved in N,N-dimethylformamide (DMF) (Fluka, Switzerland) to a concentration of 15–20% by weight followed by chain extension with 1,3-diaminopropane (Merck AG, Germany). PUUR fibers (Fig. 1(a), (b)) were prepared by wet spinning [32]. The PUUR solution was extruded through a spinneret (60 holes, 80 μm in diameter) into a precipitation bath of water. In a second bath the fibers were drawn 400–600% before they were collected on a final take up unit.

Band manufacturing

The wet spun multifilament fiber was converted by doubling and slight twisting to a coarse yarn, which was used as warp threads. The bands were woven with low weft tension in plain weave to utilize the yarn strength as much as possible combined with good stability. The PUUR bands were sterilized using ethylene oxide. The PUUR bands used in the rabbit ACL study had a thickness of 2.0 mm and a width of 4.0 mm. The corresponding dimensions of the PUUR bands used in the minipig ACL study was 4.0 mm and 6.0 mm.

Tensile testing

Three woven bands (rabbit ACL band) were tested in a wet state after equilibration with water at 20 °C in a tensile tester (Alwetron tensile tester, Stockholm, Sweden). The constant rate of extension was $900\,\mathrm{mm\,min^{-1}}$. The ultimate load (N) and the ultimate elongation (mm) were determined.

Cyclic tests

In order to study the elastic behavior during repeated elongation, 50 elongation cycles between the limits 10–30 mm elongation were performed at 1 Hz on the 100 mm long bands, in a wet state after saturation in water at 20 °C, using a tensile tester (UT 350, SDL International, England).

In vitro experiments

Extract preparation

Extracts for the mutagenicity and the hypersensibilization tests were prepared by cutting the bands in small pieces and subsequently putting them in sterile glass containers with the appropriate volume of extract medium under aseptic conditions. The extract mixture was incubated at approximately 80 °C for 14 days. Once daily, the extraction bottles were shaken carefully a few times.

Mutagenicity

Testing for mutagenicity of a 0.9% NaCl extract of the PUUR material was carried out according to Ames in *Salmonella typhimurium* strains [33], and as a mammalian cell gene mutation test using mouse lymphoma cells [34] by an accredited laboratory.

TABLE I Summary of experimental outlines: rabbit intraarticular implication, rabbit ACL study and minipig ACL study

Study	No. of animals	Aim of study	Implantation site	PUUR material	Observation time, months	Tissue preparation	Withdrawn animals
Rabbit biocompatibility study	9 (total) Trial I; $n=4$ Trial II; $n=5$	Evaluate tissue response around PUUR material	Intraarticular implantation in knee joint	Bands and bundles	3, 9, 13, 18	Paraffin sections	1 (pulmonary infection)
Rabbit ACL study	26	Evaluate function and histology associated with PUUR ACL reconstruction	ACL reconstructions bilat/unilat	Woven bands	6, 12, 18, 24	Paraffin sections	3 (postop complications)
Miniping ACL study	2	Evaluate function and histology associated with PUUR ACL reconstruction	ACL reconstructions unilaterally	Woven bands	24	Ground sections	—

In vivo animal experiments

Hypersensibilization tests

Hypersensibilization tests were performed in guinea pigs by an accredited laboratory [35, 36]. The 0.9% NaCl extract of the PUUR material was used to test the dermal sensitisation potential.

Animal implantation

A total of 35 adult New Zealand White rabbits were used. In the intraarticular implantation study, nine rabbits, age 6–9 months (4.5–5.5 kg) of both sexes were used. The remaining 26 animals were included in the rabbit ACL study (castrated males and females of the same age and weight). After being kept in single cages two weeks postoperatively, the rabbits were housed together and allowed free movement in a separate room for the remaining observation period. The rabbits were fed a standard diet with free access to food and water.

In the minipig ACL study, two female Göttingen minipigs (Ellegaard Laboratory Pigs, Denmark), age two years, weighing approximately 35 kg at time of surgery were used. The pigs were kept in separate cages two weeks after surgery, and subsequently released together in a room. The food intake was controlled but the pigs had free access to water. The animal implantation experiments are summarized in Table I.

All animal experiments were approved by the Local Ethics Committee, Göteborg University.

Surgery

Rabbit intraarticular implantation

Nine rabbits were operated on in two series (trial I, $n=4$ and trial II, $n=5$). Surgery was performed under general anesthesia using i.m. injections of diazepam, 2.5 mg kg^{-1} b.wt. (Stesolid®; 5 mg ml^{-1}, Dumex A/S, Copenhagen, Denmark) and a combination of phentanyl and fluanizone, 1 mg kg^{-1} b.wt. (Hypnorm Vet®, Janssen Farmaceutika, Denmark). Both hind legs were shaved and washed with 4% chlorhexidine digluconate (Hibitane®, ICL, England) and 70% ethanol. Bilaterally, the knee-joints were opened by a 3–4 cm long medial parapatellar incision and the patella was dislocated laterally. In trial I, a 7 mm long piece of woven PUUR band was implanted in each knee-joint, and sutured to the synovial membrane in the medial part of the joint cavity, with size 4–0 Dexon (Davis and Geck, Hampshire, UK) sutures. In trial II, a 5-mm long PUUR band was sutured to the synovial membrane as described in trial I, while the right knee received a bundle of unwoven polymer fibers of a similar weight, sutured to the medial part of the synovial membrane with size 7–0 silk sutures (Davis and Geck, Hampshire, UK). After implantation, the knees were closed in separate layers and the synovial tissue and fibrous capsule sutured with size 6–0 polyester sutures (Ti-Crone®, Davis and Geck, Hampshire, UK). Finally, the skin was closed with size 3–0 monofilament polyester sutures (Novafil®, Davis and Geck, Hampshire, UK). Postoperatively, antibiotics (0.1 ml kg^{-1} b.wt. Intencillin, LEO®, Pharmacia, Sweden) and analgesics (0.05 mg kg^{-1} b.wt. Temgesic®, Rechkitt and Coleman, USA) were administered as single i.m. injections daily for three days.

Rabbit ACL study

The anaesthesia and surgical preparation of the 26 rabbits were performed as described above for the rabbit intraarticular implantation study. The PUUR bands were pre-soaked in sterile saline for 30 min before surgery. The knee-joints were opened by a medial parapatellar incision and the patella was dislocated laterally. The ACL was identified and surgically removed. A tunnel, diameter 2.7 mm, was made by a pneumatic drill (minidriver, 3M Health Care, MN, USA) from the medial proximal part of the tibia through the tibial plateau. A second tunnel was made from the natural intracondylar femoral insertion of the ACL ending at the lateral part of the femur. The bands were pulled through the tunnels in the tibia and femur and were fixed with a knot in the tibial end, and a firm tension was applied before two staples fixated the band outside the femoral tunnel. Finally, the patella was relocated, the wound closed in separate layers and postoperative medication administered as described above for the rabbit intraarticular implantation study. All hard tissue preparation was performed under generous irrigation with sterile saline (NaCl 9 mg/ml; ACO, Sweden).

Minipig ACL study

The animals were anesthetized with an injection of Ketamine hydrochloride i.m. (Ketalar® 50 mg ml^{-1}, 10 ml), diazepam (2.5 mg kg^{-1} b.wt. Stesolid®; 5 mg ml^{-1}, Dumex A/S Copenhagen, Denmark) and a combination of phentanyl and fluanizone i.v. 1 mg kg^{-1} b.wt. (Hypnorm Vet®) along with endotracheal ventilation (N_2O, 5 l and O_2, 2 l). The right knee was shaved and washed with 4% chlorhexidine digluconate (Hibitane®, ICL, England) and 70% ethanol. The bands were pre-soaked in sterile saline 30 min before surgery. The right

knee was opened by a medial parapatellar incision and the patella was dislocated laterally. The ACL was identified and surgically removed. Using a trephine drill, diameter 7.0 mm, a tunnel was made from the medial proximal part of the tibia through the tibial plateau. A second tunnel was made from the natural intrachondylar femur insertion of the ACL ending at the lateral part of the femur. A PUUR ACL band was inserted and fixed with a stainless steel staple at both ends. The patella was relocated and the wound was closed in separate layers. The synovial membrane and the fibrous capsule were sutured with 3–0 Ti-Crone (Davis and Geck, Hampshire, UK) and the skin was closed with 3–0 Novafil (Davis and Geck, Hampshire, UK). The left knee was left without surgery to function as a control.

Preparation of specimens

Rabbit intraarticular implantation

In trial I, one rabbit had to be killed after two months due to a pulmonary infection and was withdrawn from the study. The remaining eight animals were killed (two at each observation time) after 3, 9, 13, and 18 months, respectively, with an overdose of barbiturate (Mebumal®, ACO Läkemedel AB, Solna, Sweden). The implants with surrounding tissue were removed *en bloc*, and immersed in buffered formalin (4%) for 24 h. In two animals (one animal at nine months and one at 18 months), the implant bands were found detached from the synovial membrane and only the tissue was embedded and sectioned. Additionally, tissue from the lungs, kidneys, and liver was obtained from six rabbits after 3, 9, and 18 months. All specimens were embedded in paraffin and sections of $\sim 5\,\mu m$ thickness were prepared and stained with hematoxylin and eosin for light microscopic evaluation.

Rabbit ACL study

One animal died postoperatively owing to the anesthesia. After one month, one animal was killed due to luxation of the patella, and another one due to pneumonia. The remaining animals were killed after 6 months ($n = 2$), 12 months ($n = 3$), 18 months ($n = 6$) and 24 months ($n = 12$). The implants with surrounding tissue were removed *en bloc* and immersed in buffered formalin (4%) for 24 h. The specimens were decalcified in EDTA, dehydrated in ethanol and subsequently embedded in paraffin. Sections of $\sim 5\,\mu m$ thickness were prepared and stained with hematoxylin and eosin for light microscopic evaluation.

Minipig ACL study

After 24 months, the animals were killed by a lethal injection of Ketalar® $50\,mg\,ml^{-1}$ and potassium chloride. The knee joints were removed and immediately immersed in buffered formaline (4%) for two days. In order to obtain thin sections, an initial attempt to demineralize the tissue blocks with EDTA was undertaken. However, the process was abandoned due to an unacceptable long time to complete the demineralization process. Instead, the specimens were dehydrated in ethanol, embedded in plastic resin (LR White, The London Resin Co, Hampshire, UK), and divided longitudinally by sawing (Exakt® cutting and grinding equipment, Exakt Apparatebau, Norderstedt, Germany). Four ground sections (one from each joint) of ~ 15–$20\,\mu m$ thickness were prepared and stained with 1% toluidine blue [37].

Results

Tensile testing

The results of the tensile tests are presented in (Fig. 2) Three bands were tested. The mean elongation at rupture was 89% at a mean breaking load of 207 N.

Cyclic loading

The elastic properties of one woven band of three tested are demonstrated in (Fig. 3). The elongation vs. cycle curves did not show any tendency to excessive relaxation during the 50 repeated cycles tested.

In vitro experiments

Mutagenicity

The PUUR extract was found to be non-mutagenic in the Ames test and the *in vitro* mammalian cell gene mutation test.

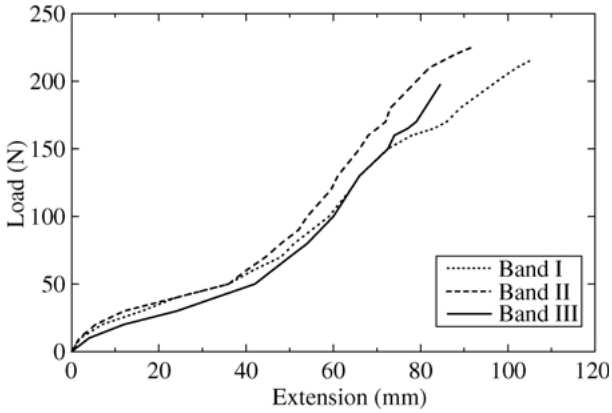


Figure 2 Stress–strain behavior of three PUUR ACL bands.

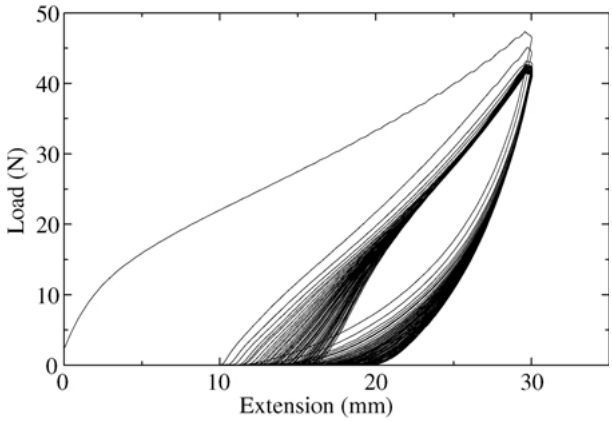


Figure 3 Strain controlled cyclic test behavior of a PUUR ACL Band.

In vivo animal experiments

Hypersensibilization tests

No evidence of delayed contact hypersensitivity was observed in the guinea pig experiment after treatment with the PUUR extract.

Rabbit intraarticular implantation

General clinical evaluation. The eight animals were in good health with unaffected knees during the observation period.

Macroscopical evaluation. The cartilage of all facets of both the femur and the tibia was intact. No synovial reaction was noticed in the knees.

Light microscopic morphology

Lung, kidney, and liver. The sections of the vital organs showed normal histology, without any indication of inflammatory reaction.

Knee joints. At all observation times, the interface between the PUUR fibers and the tissue was characterized by a mild foreign body reaction and a mild inflammatory response (Fig. 4(a)–(c)). In some of the sections containing fiber bundles (Fig. 5(a)), lymphocyte collections were seen in a perivascular and implant-adjacent position. Even after 18 months, no clear signs of degradation of the polymer was evident.

Fibrous tissue ingrowth, mainly a non-vascularized collagenous tissue was detected after three months close to the surface of the implants. After 13 months, vascularized, fibrous tissue ingrowth was seen at several locations, even in the core of the implant (Fig. 4(b)).

The tissue response to the fibers was characterized by an encapsulation of the implant by a relatively thin fibrous tissue. After 18 months, a more well defined fibrous capsule was surrounding the material than at earlier time periods (Fig. 4(a)). In general, the fibrous capsule was separated from the PUUR band by macrophages and foreign body multinuclear giant cells (FBMGCs). Overall, there are only few, scattered inflammatory cells in the stromal tissue (Fig. 5(b)), mainly macrophages and plasma cells. The synovial membrane had a normal appearance but did sometimes present an attenuation or slight thickening (Fig. 5(c)).

Rabbit ACL

General clinical evaluation. Apart from the three animals withdrawn from the study, the animals were all in good health with no signs of discomfort or instability of the knees during the observation period.

Macroscopical evaluation. The animal killed after one month with patella dislocation showed synovial reactions and exudation, while the animal with pulmonary infection had normal joints without signs of pathology. In the remaining animals, the cartilage of all facets of both the femur and the tibia was intact with no signs of osteoarthritis. No synovial reaction was noticed in the knees.

At 6, 12, 18, and 24 months all rabbits had intact knees and no signs of joint damage. At six months the overall appearance revealed an integrated, intact PUUR band in both the tibia and the femur without any adverse macroscopic reactions in neither the bone nor the joints (Fig. 6).

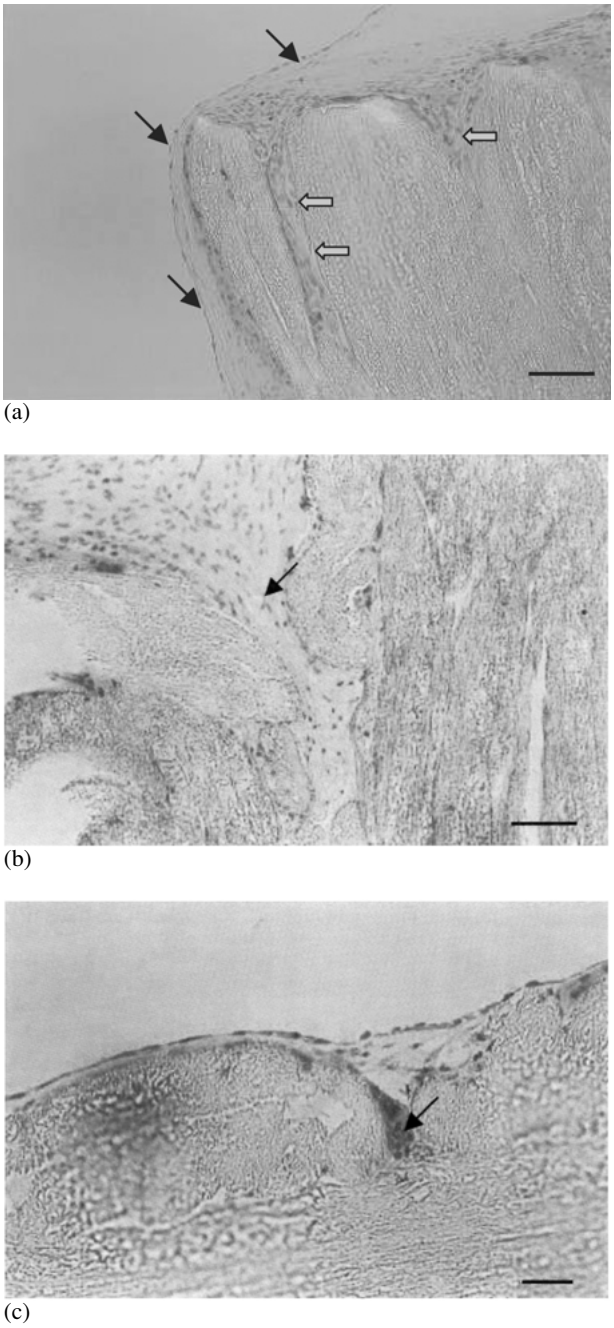
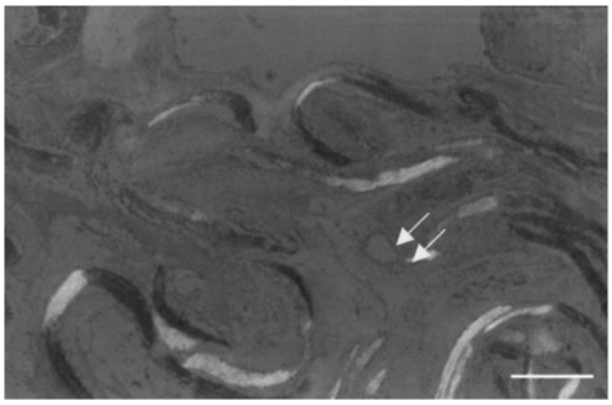
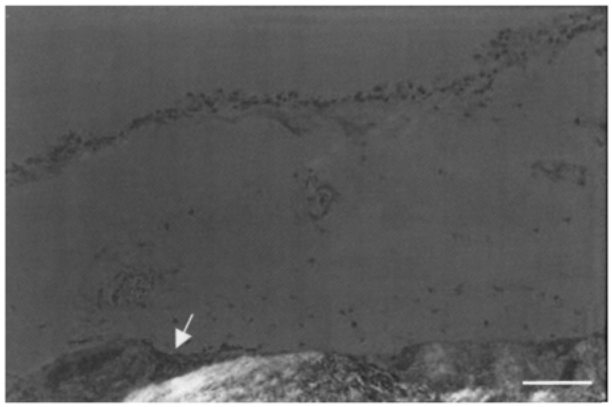


Figure 4 Mount of light micrographs from the rabbit intraarticular implantation study, (a) 18 months. Thin synovial membrane (black arrows) and an essentially inflammation-free tissue surrounding the band. Ingrowth of tissue seen at the end of the band (gray arrows), with a few giant cells close to the implant surface. Bar = 100 μm, (b) thirteen months. Ingrowth of loose connective tissue within the central portion of the PUUR band. Small blood vessel denoted by arrows Bar = 100 μm, (c) 18 months. Thin and reaction free synovial membrane close to the fiber. Some foreign body multinuclear giant cells (arrow) close to the implant. Bar = 70 μm.

Light microscopic morphology. LM morphology at all stages revealed an integrated PUUR band in both the bone and in the soft tissue (Fig. 7(a), (b)). At 6 and 12 months, bone formation was observed in close contact with the PUUR fibers at the outer surface of the bands within the cortical passage, without signs of interfering



(a)



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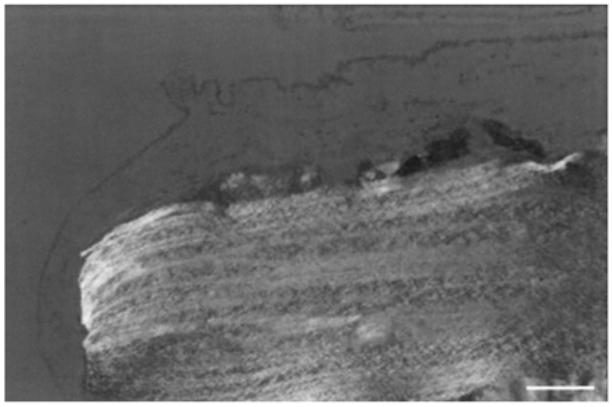


(c)

Figure 5 Mount of compensated polarized light micrographs of H and E stained paraffin sections from the rabbit intraarticular implantation study, (a) three months. Survey of PCL fiber bundle surrounded by fibrous tissue including blood vessels. Normal synovial membrane. Bar = 1000 μm (b) nine months. Some FBMGCs close to the implant surface. Essentially inflammation free tissue outside the implant material with synovial membrane in a normal appearance. Bar = 100 μm (c) Survey micrograph 13 months. PUUR band with some macrophages and FBMGCs lose to the surface. Almost inflammation-free synovial tissue with a normal synovial membrane. Bar = 500 μm.

soft tissue (Fig. 7(d)). Moreover, connective tissue ingrowth was frequently observed between PUUR fibers along the course of the band with ingrowth of blood vessels at several locations (Fig. 8). At the earlier times, no signs of material degradation was detected. After 24 months, the first signs of degradation of the polymer was evident, when the continuity of the fibers were occasionally broken (Fig. 9).

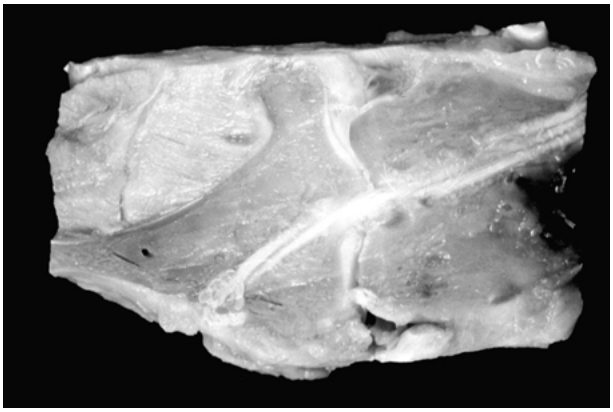


Figure 6 Macroscopic appearance of PUUR band in rabbit knee joint, six months after operation. Note the intact ligament prosthesis with normal articular cartilage in joints.

Minipig ACL study

General clinical evaluation. During the two year observation period, the knees were stable and no clinical signs of discomfort was reported.

Macroscopical evaluation. The cartilage of all facets of both the femur and the tibia was intact. No synovial reaction was noticed.

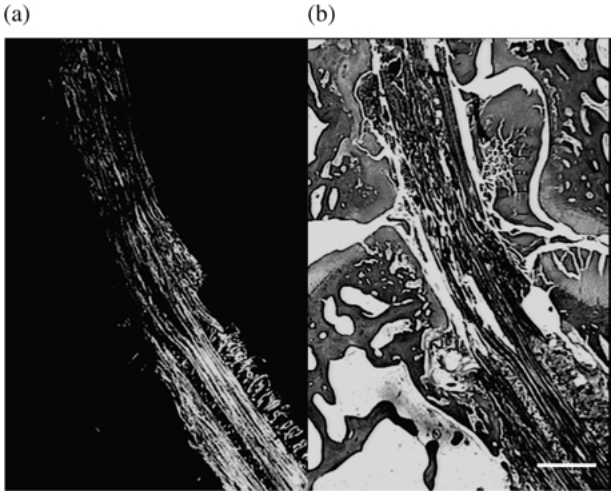
Light microscopic morphology. The PUUR bands were well incorporated in the osseous host tissue. At the ends of the band (in the cortical passage), the band had a discontinuous appearance, with bone present at the periphery of the band (Fig. 10(a)).

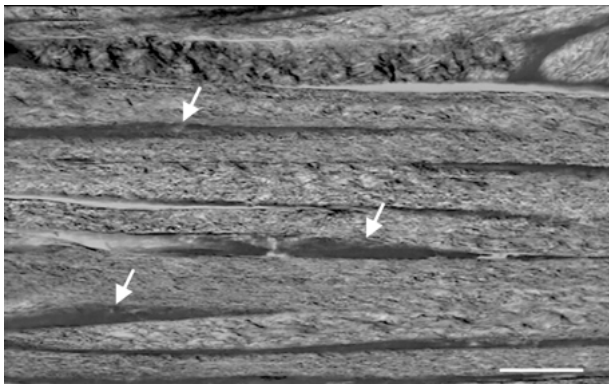
In several parts of the sections, a close contact between the PUUR fibers and the newly formed bone was observed. In the core of the band, the majority of the PUUR fibers seen in the ground sections were intact, and fibrous connective tissue was discernible between the PUUR fibers (Fig. 10(b)). The connective tissue had a dense, oriented, ligament-like structure, with only few cells between the parallel oriented collagenous fibers (Fig. 10(b)).

In both ACL and control sections, the articular cartilage showed a normal morphology without signs of pathology.

Discussion

In the present study, we demonstrate the mechanical properties and the first *in vitro* and *in vivo* results of a degradable PUUR fiber with the intended use in ACL reconstruction. The combination of the chemical composition, fiber spinning and textile manufacturing generates the final properties of the band [38]. Earlier augmentation devices, degradable or non-degradable, have a rigid texture and thus not the mechanical properties of a normal ACL or a tendon graft [24]. The rigidity directs most of the loading to the augmentation device, with failure due to stress-shielding or fatigue of the device as a consequence [23]. The novel PUUR band has a similar elasto-mechanical loading profile as a human ACL tested post mortem [24], and after cyclic loading in 50 repetitive cycles, no relaxation or fatigue was observed. To our knowledge, the newly developed





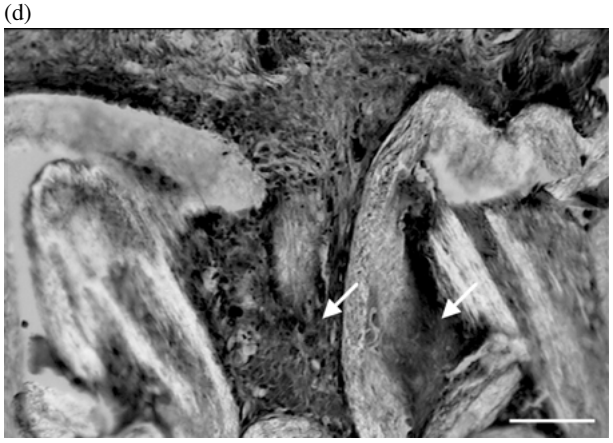


Figure 7 LM Micrograph of histological section of rabbit knee with PUUR band. 6 months observation time (a) polarized light, (b) Htx-Van Giseon staining. Bar = 400 μm. (c) Enlarged area from Fig. 7(b), illustrating tissue ingrowth between PUUR fibers. Bar = 100 μm. (d) Enlarged area from Fig. 7(b) demonstrating ingrowth of bone between fibers. Bar = 100 μm.

PUUR band is the first synthetic ligament device with such a loading profile [39, 40].

Among the most commonly used autografts in ACL reconstruction are free patellar tendon grafts. A critical period of time occurs 3 to 6 months after grafting, during revascularization and reorganization of the free patellar tendon graft. At this time, the graft displays reduced mechanical strength with a risk for residual elongation, and consequently instability of the knee [41]. Accordingly, a degradable augmentation device could be used in conjunction with the tendon graft to provide

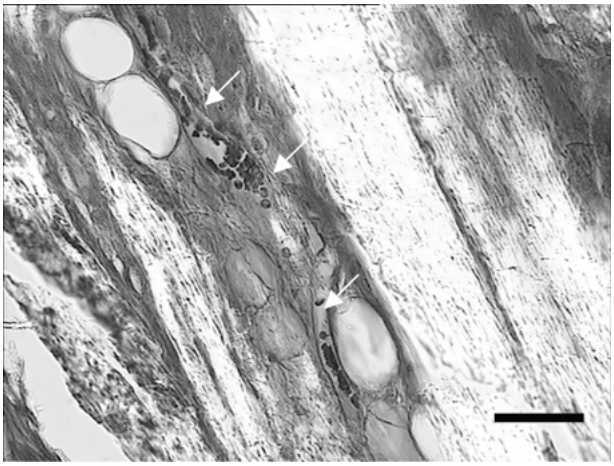
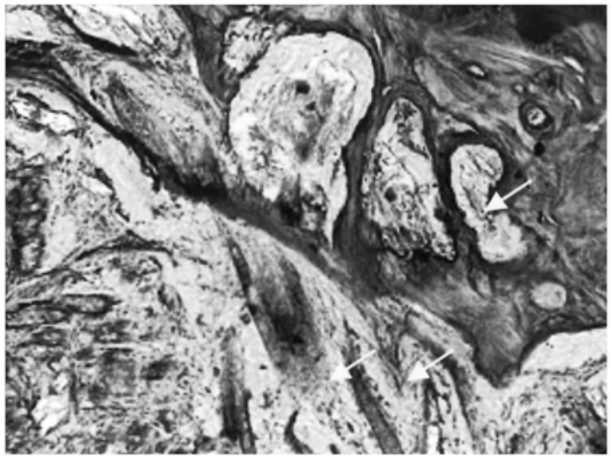


Figure 8 LM Micrograph. Rabbit knee joint with PUUR band after 24 months. Arrows denoting blood vessels in the newly formed soft tissue between remaining PUUR fibers. Bar = 50 μm.

Figure 9 Micrograph. Section showing rabbit knee with PUUR device after 18 months. Connective tissue between PUUR fibers. Note the disruption of the fibers compared with the six months observation time (Fig. 7(c)). Bar = 100 μm.

resistance to the mechanical load during the time for revascularization and reorganization of the tissue.

It is imperative that a new degradable material is well tolerated and incorporated in the host tissue. As the synovial membrane is a richly vascularized tissue, it reacts promptly with an inflammatory reaction to any mechanical or chemical stimulus. To test for this, a piece of the band or bundle of fibers was sutured to the synovial membrane of the knee in order to detect any adverse reactions. Despite the close contact between the band and the membrane, no joint reaction or macroscopic inflammation was demonstrated. However, microscopic examination of the sections showed that in the area close to an implant, macrophages and FBMGCs were present. The mechanism for the focal accumulation of lymphocytes, occasionally seen at 13 and 18 months, is not known, nor their role at the site. One possibility may be friction between the band and the synovial membrane as the PUUR material only was sutured with two sutures, possibly allowing movement and friction. The presence of FBMGCs in association with the PUUR surface is not surprising, since it is likely that the slowly degrading PUUR present a large, unphagocytosable object. In addition, a rough surface topography may favor FBMGC formation and attachment to the surface, and it is likely



(a)

(b)

Figure 10 Light micrograph from ground section (minipig ACL study, 24 months). (a) Bone from site of osseous insertion surrounding fibers of the PUUR band. Bar = 300 μm. (b) Connective tissue (arrows) between the fibers of the PUUR band located in the central parts of the band. Intact parts of the PUUR band (denoted by arrows) Bar = 100 μm.

that the fiber surface topography will change during the process of degradation.

In the rabbit studies, where the PUUR bands were used as a full ACL prosthesis, the knee joint function was intact even after 24 months. The absence of osteoarthritis indicates knee joint stability, as instability of the joints is known to result in degenerative changes in the cartilage [42, 43]. At 24 months, some decay of the fibers was observed, indicating a possible degradation of the PUUR material, and the theoretical degradation time of the band *in vitro* implies that the remaining mechanical strength is negligible [38]. At this observation period, newly formed connective tissue was demonstrated at several locations bridging the extension of the natural ACL. This observation suggests that cells have migrated into the implanted PUUR band and formed new tissue at the time of degradation and, albeit speculatively, possibly carry parts of the biomechanical load.

Formation of new vessels between the fibers in the band was detected at several locations, without the presence of inflammatory cells, both at the periphery and in the center of the PUUR band. This implies that the fibers are tolerated by the host, and further, that angiogenesis is concomitant with stromal cell migration, tissue formation and differentiation. As in the rabbits, the macroscopically intact knee joints of the minipigs at time of euthanasia, indicated a stable knee joint function [42, 43]. In spite of the species differences, the minipig displayed a similar histological picture, with bone formation in the drilled tunnels and surrounding the PUUR band. In the central parts of the PUUR band, ligament-like connective tissue ingrowth was observed between the fibers.

After 24 months, signs of fiber degradation was seen in the rabbit ACL study. However, in the minipig ACL study, no clear signs of degradation of the fibers was apparent after 24 months. This inconsistency may be related to species differences in regard of the frequency of knee joint motion. Given that the loss of molecular weight occurs ahead of mass loss and volume degradation [44], a longer observation time is necessary to demonstrate mass degradation histologically. Accelerated hydrolysis experiments, to be presented in a forthcoming publication [38], have demonstrated a decrease in molecular weight and alterations of the mechanical properties of the PUUR material, which is consistent with the significant degradation of the material after 24 months *in vivo*.

In conclusion, a degradable PUUR band has been developed to serve in ACL reconstruction. In this study we have demonstrated mechanical stiffness and elastic function similar to normal human ACL, *in vivo* biocompatibility and tissue ingrowth, and a preserved joint function over 24 months in rabbits and pigs. Human clinical trials with ACL reconstruction using the PUUR band are in progress.

Acknowledgment

We are grateful to Professor Peter Thomsen, Institute of Anatomy & Cell Biology, Göteborg University, for his scientific expertise and advice related to biocompatibility.

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Received 28 November 2000
and accepted 19 July 2001