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Tissue response to a braided poly-L-lactide implant in an experimental reconstruction of anterior cruciate ligament

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During follow-up periods of 6, 12, 24 and 48 weeks, the tissue response to a braided poly-L-lactide (PLLA) implant, 3.2 mm in diameter, was investigated in the reconstruction of experimental anterior cruciate ligament (ACL) ruptures in 32 sheep. In 16 sheep the cut ACL was removed and reconstructed with the fascia lata augmented with a PLLA implant. In 16 sheep the ACL was cut from its midportion, sutured, and thereafter augmented with a PLLA implant. The tissue reactions were typical of a scant non-specific-foreign-body reaction. The number of inflammatory and giant cells was greatest at six weeks, decreasing thereafter. Degradation of the PLLA was incomplete at 48 weeks. No signs of synovitis or changes in the cartilaginous surfaces were observed. The reconstructions in both groups were anchored to the bone by fibroconnective tissue, and remodelling of the bone was seen along the drill channels. After 48 weeks the maturation of the fibroconnective tissue and the orientation of the collagen fibres were higher ($p < 0.01$) in the fascia-lata-PLLA group than in the primary-suture-PLLA group. Histologically, the braided PLLA implants proved to be suitable for ACL repair in sheep. The augmentation of the fascia lata with the PLLA implant seemed to be preferable to that of the primary suture of the ACL.

1. Introduction

The repair methods of the anterior cruciate ligament (ACL) are controversial due to its complex functional anatomy [1]. Both autogenous and synthetic materials have been used in the treatment of ACL ruptures. Autogenous structures, including iliotibial band, patellar tendon, semitendinosus, and gracilis tendon, have the disadvantage of weakening due to necrosis and remodelling after implantation [2].

A synthetic replacement of the ACL may temporarily or permanently function as a scaffold (carbon fibre) [3, 4], as a stent or an augmentation device (polypropylene, Kennedy LAD) [5, 6] or as a permanent prosthesis (polytetrafluoroethylene, Gore-Tex) [7, 8]. Problems with non-absorbable synthetic implants are most often related to their stiffness and brittleness which can cause synovial-membrane reactions and even a failure of the implant [9]. Resorbable synthetic materials, such as polyglygolic acid (Dexon) and polydioxanone (PDS), have also gained attention in ACL reconstruction. They cause a mild tissue reaction which is a transient non-specific-foreign-body reaction but the rate of the degradation is, however, too rapid for ACL repair [10–13].

Poly-L-lactic acid (PLLA) is a synthetic, biodegradable alpha-hydroxy-acid polyester which belongs to the group of aliphatic polymers. *In vivo* it undergoes hydrolytic de-esterification into lactic acid which becomes incorporated in the tricarboxylic acid cycle and is subsequently excreted by the lungs as carbon dioxide [11, 14, 15]. The rate of degradation of PLLA has been reported to vary from 40 weeks to over three years depending on the molecular weight, size and shape of the implant [16, 17].

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Rods, screws and plates made of PLLA have been found suitable for experimental fixation of cancellous bone fractures and osteotomies of both non-weight-bearing [18–20] and weight-bearing bones [21]. Clinically, PLLA has, so far, been used in maxillofacial and mandibular surgery [22, 23] as well as in ankle fractures [24]. In ACL repair, polylactide has been previously used to coat carbon fibres to prevent their early migration and to control their degradation [4]. In this experimental study on sheep, a braided PLLA implant was used as an augmentation device with the fascia lata and primary suture of the ACL in an effort to protect the healing autogenous tissue. The aim was to evaluate the tissue response to the PLLA implant and to compare the two operation methods with each other.

2. Materials and methods

2.1. Materials

The raw material of PLLA was supplied by Boehringer Ingelheim (Germany), with an initial molecular weight of 250 000. The fibres for the braided implants were manufactured by melt-spinning at Tampere University of Technology. The fibres were drawn seven times their original length during the procedure, and the final diameter of the fibre was 0.35 mm. The tubular braids produced in the braiding machine (Herzog, Lohia Group, India) consisted of 28 parallel fibres twined together by six twisting fibres. The diameter of the braided implant was 3.2 mm and length 30 cm (Fig. 1). The implants were gamma-sterilized with a dosage of 3.5MRad.

2.2. Operative procedure

Thirty-two adult Finnish sheep, weighing 49 kg on average (range 30–91 kg), were operated on. Benzylpenicillin procaine (Prokain Penicillin, Novo, Denmark) 60.000 IU kg^{-1} was given intramuscularly (i.m.) as an infection prophylactic. The anaesthesia was carried out with the combination of medetomidine (Domitor, Farmos Group Ltd, Finland) 0.035 mg kg^{-1} and ketamine (Ketalar 50 mg ml^{-1}, Parke-Davis & Co., Spain) 1.5 mg kg^{-1} administered i.m. [25].

The left hind leg was shaved and scrubbed with an antimicrobial solvent. The exposure was through a lateral, parapatellar incision, and the patella was dislocated medially. The ACL was identified and cut in the midportion, and thereafter the anterior displacement of the tibia in regard to the femur was verified by the anterior drawer test. A channel, 4.5 mm in diameter, was drilled across the lateral femoral condyle starting from the point proximal to the insertion of the lateral collateral ligament and reaching the notch of the attachment of the ACL in the intercondylar fossa. A similar channel was drilled from the tibial insertion of the ACL extending to the anteromedial part of the crista tibiae. The edges of the drill channels were smoothened, and the channels were flushed copiously with saline.

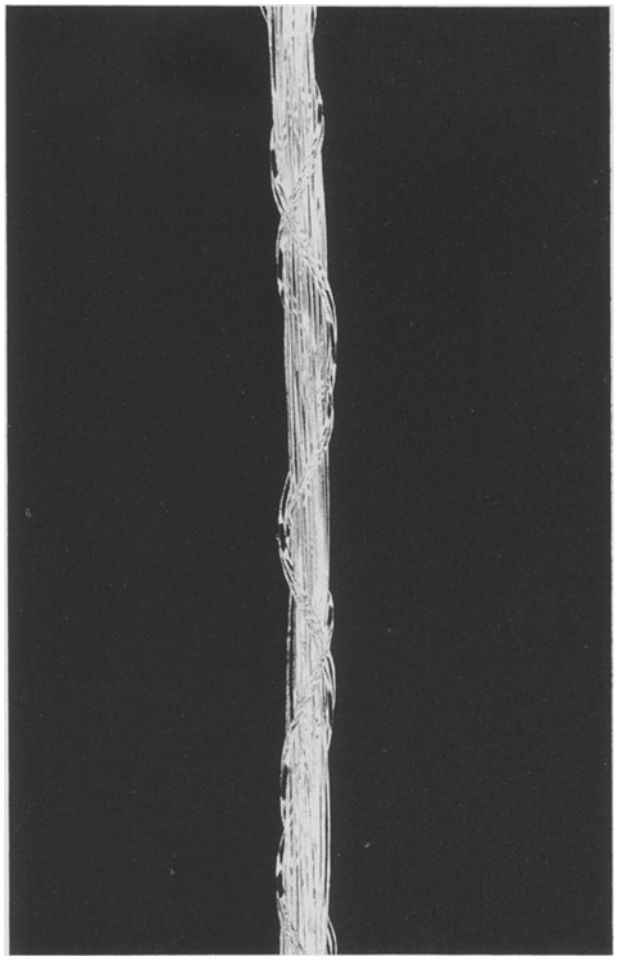

Figure 1 The braided PLLA implant 3.2 mm in diameter used in the augmentation of ACL rupture in sheep.

Two different operative procedures using the PLLA-fibre implant as an augmentation device were carried out. In the fascia-lata-PLLA group, consisting of 16 sheep, the cut ends of the ACL were excised, and the PLLA implant was covered with a pediculated fascia lata, 1 cm wide and 15 cm long, which was left attached distally to the lateral aspect of the femoral condyle. The PLLA implant covered with the fascia lata was then pulled through the femoral and tibial channels with a wire loop. Richards Fixation Staples type 12-8692 (Richards Medical Company, Memphis, USA) were used to fix the implant to the lateral condyle of the femur and to the medial aspect of the proximal tibia (Fig. 2). The fixation was carried out with the knee at 45° flexion, while the tension load of 40 N, measured with a tension isometer (MEDmetric Corporation, San Diego, USA), was applied to the implant.

In the primary-suture-PLLA group, consisting of 16 sheep, the cut ends of the ACL were sutured by two interrupted 3-0 polyglactin 910 sutures (Vicryl, Johnsson & Johnsson, Sweden). The PLLA implant, without coverage, was pulled through the drill tunnels and fixed as described above.

The anterior drawer test was used to show the adequate strain of the implant in the range of movement of the knee joint before closing the wound in layers. Postoperatively the sheep received phenylbutazone (Reumuzol 200 mg ml^{-1}, Farmos Group Ltd,

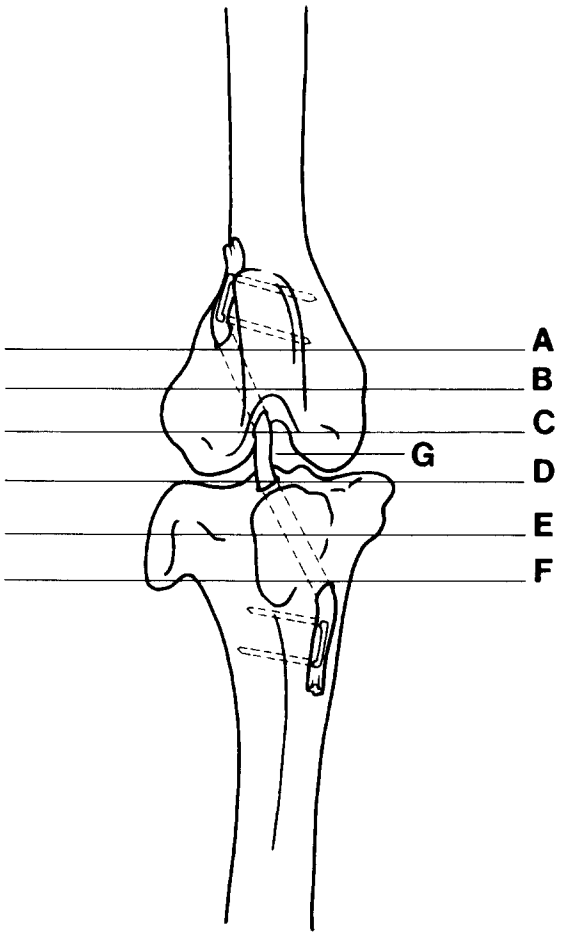

Figure 2 The sites of the specimen sections in the drill channels are presented in anteroposterior view. Specimens were taken from A to G in the operated-leg, and from A, C, D, F and G in the control leg.

Turku, Finland) 20 mg kg^{-1} and benzylpenicillin procaine 60.000 IU kg^{-1} i.m. daily for four days. The sheep were kept free in their stables without any external support or immobilization and they received a normal diet.

2.3. Follow-up studies

The follow-up periods were 6, 12, 24 and 48 weeks in both groups, four animals in each. Postoperatively, the clinical examinations were carried out daily for four days and thereafter weekly for three weeks. Special attention was directed to the general condition, wound healing and weight bearing of the operated leg. A scale from 0 to 4 was used (0 = no lameness, 1 = slight lameness, 2 = evident lameness, 3 = severe lameness and 4 = not using the leg at all).

At the end of the follow-up periods the sheep were sacrificed, and both knee joints were radiographed in anteroposterior (a.p.) and lateral projections (Siemens Polyphos 30 M, Kodak T-MAT G, film size 18 × 24 cm, intensifying screen Kodak Lanex medium, 48 kV and 5 mA s for the lateral and 50 kV and 8 mA s for the a.p. projections, with a 120 cm object distance). The unoperated right knee served as a control. The changes registered were osteophytes, bony spurs, at the proximal aspect of the trochlear groove and along the trochlear ridge in the femur as well as osteophytes at the proximal and distal end of the patella, the tibial plateau, and the margins of the femur in a.p. view. The changes were scaled from 0 (= no changes) to 3 (= advanced changes).

After the radiographical examination the knee joints were dissected. The reconstructed ACL was identified, cut from its insertions, and taken as a specimen. Transverse sections, about 1 cm thick, were cut in the femur and tibia in three planes in the operated leg (A–C and D–F in Fig. 2) and in two planes in the unoperated leg (A, C and D, F in Fig. 2). The specimens were fixed in ethanol, dehydrated, and then embedded in methyl methacrylate. For histological analysis, 5 μm thick sections were cut with a Jung Polycut S microtome and stained with Goldner–Masson and van Giesson. Polylactic acid was identified by its birefringence under polarized light. The specimens in the fascia-lata-PLLA and primary-suture-PLLA group were examined and assessed on a scale from – (= no findings) to + + + (= numerous findings).

Comparisons between the fascia-lata-PLLA and primary-suture-PLLA groups were analysed by using the Wilcoxon test. The histologic findings compared were the grade of PLLA degradation, maturation of the fibrous tissue, and the number of capillaries, giant and plasma cells, lymphocytes and osteoblasts, as well as the degree of bone formation in the drill channels. Value of p less than 0.05 were considered statistically significant.

3. Results

3.1. Clinical evaluation

The general condition of all sheep was good throughout the follow up, and no wound infections occurred. During the first postoperative days all sheep started to put weight on the operated leg, the degree of lameness being 2–3. The results of the clinical evaluation are presented in Table I. At the end of the follow-up period the sheep were moving normally. In the macroscopical post-mortem studies it was noticed that in the primary-suture-PLLA group two implants had ruptured after 6 weeks and one 48 weeks after the operation. In both groups one reconstruction was thin and loose after 6 weeks and one after 12 weeks in

TABLE I Clinical evaluation of lameness of the sheep one, two and three weeks postoperatively. A scale of 0 to 4 was used. (0 = no lameness, 1 = slight lameness, 2 = evident lameness, 3 = severe lameness, 4 = not using the leg at all)

Grade of lameness	Number of sheep fascia + PLLA/prim. suture + PLLA		
	Weeks postoperatively		
	1	2	3
0	–/–	6/2	12/11
1	7/8	7/11	4/3
2	8/6	3/2	–/2
3	1/2	–/1	–/–
4	–/–	–/–	–/–
Total	16/16	16/16	16/16

the primary-suture-PLLA group. The joint surfaces seemed fairly intact in most cases.

3.2. Radiographic assessment

No radiographic changes were seen during the first 12 weeks. At 24 weeks one sheep both in the fascia-lata-PLLA and primary-suture-PLLA group had a radiodensity indicating a small calcification laterally to the insertion of the ACL in the tibia and femur, respectively. At 48 weeks two sheep, both in the primary-suture-PLLA group, had grade 1–2 osteophyte formation in the patella (Fig. 3b). One sheep in the fascia-lata-PLLA group had grade 1 osteophyte formation in the trochlear groove of the femur and a calcification, 5 mm in diameter, in the patellar ligament. In addition, four sheep had a calcification, 2–9 mm in diameter, at the insertion of the ACL in the tibia (Fig. 3b).

3.3. Histologic results

3.3.1. Six weeks

Degradation of the PLLA fibres was seen under polarized light in all sections both in the fascia-lata-PLLA and the primary suture-PLLA group. Fibrocytes and giant cells surrounded the PLLA fibres and small capillaries were seen (Fig. 4). The connective tissue was immature, and no significant difference was found between the groups. Some plasma cells and lymphocytes were noticed (Table II). In two specimens in the primary-suture-PLLA group a clump of polymorphonuclear (PMN) cells was seen. In the drill channels there were a few osteoblasts and osteoclasts. Remodelling of the bone was obvious, and the trabeculae seemed to rearrange so as to surround the drill channel. The fascia lata covering the PLLA implant was visible in the specimens of the fascia-lata-PLLA group (Fig. 5a and b).

3.3.2. Twelve weeks

Birefringent polymeric material was seen in the PLLA fibres both in the fascia-lata-PLLA and primary-suture-PLLA group. The matured fibrocytes and collagen strands wrapped the individual PLLA fibres. The giant cells were located mainly around the PLLA fibres; only a few of them were seen elsewhere in the connective tissue. The number of lymphocytes remained almost unchanged in the fascia-lata-PLLA group but had diminished in the primary-suture-PLLA group (Table II). The number of osteoblasts ($p < 0.01$), as well as the degree of remodelling of the bone along the drill channels ($p < 0.001$), was considerably higher in the fascia-lata-PLLA group than in the primary-suture-PLLA group. The fascia lata was still seen as a sheath around the PLLA fibres in the fascia-lata-PLLA group.

3.3.3. Twenty-four weeks

The degradation of the PLLA surrounded by some giant cells had progressed similarly in both groups.

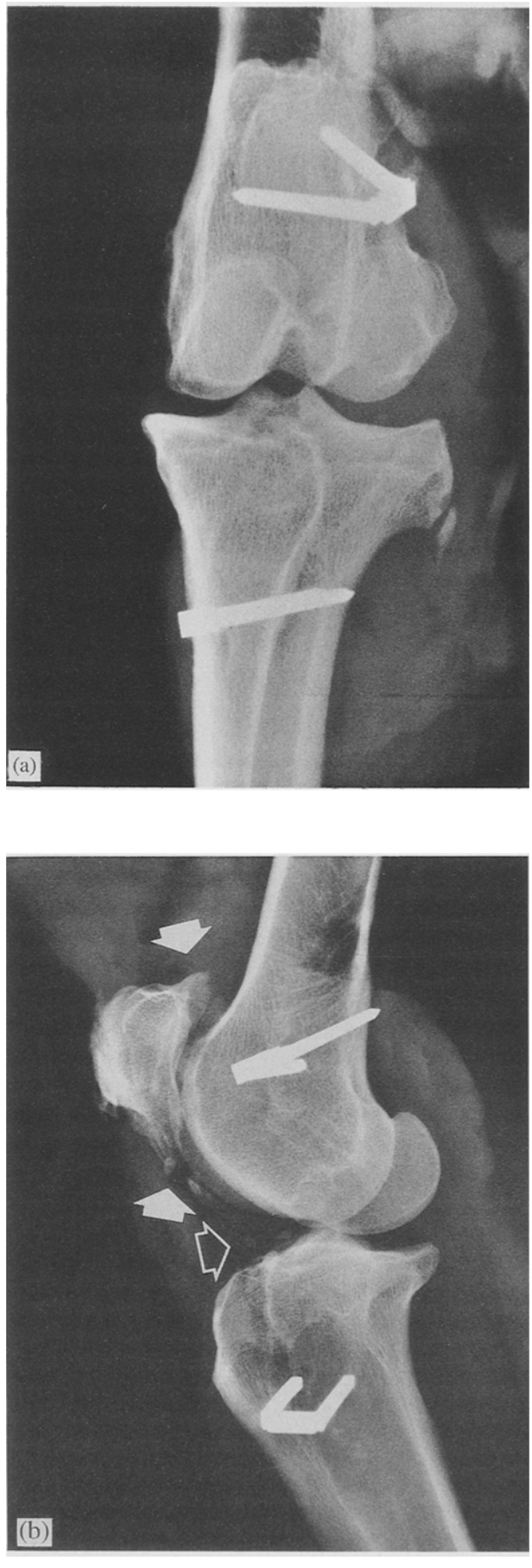

Figure 3 Radiographs in the (a) anteroposterior and (b) lateral view of the knee joint of a sheep in the primary-suture-PLLA group 48 weeks after the ACL reconstruction. Grade 2 osteophytes are seen in the lateral view in the patella (solid arrow) as well as small osteophytes near the tibial insertion of the ACL (open arrow). The fixation of the PLLA implant was carried out using Richards Fixation Staples.

Mature connective-tissue ingrowth was seen between the PLLA-fibre implants both in the fascia-lata-PLLA and primary-suture-PLLA group, though the collagen fibres were not well oriented. The number of lymphocytes had decreased in both groups (Table II). The formation of bone trabeculae around the drill channels was clearly seen in both groups. In some specimens there was hypertrophy of the synocial cells but there was no inflammatory cells in the synoviae. The cartilaginous surface of the joints was intact.

3.3.4. Forty-eight weeks

The round spaces of the original PLLA fibres were still present and PLLA was demonstrable under polarized light in the sections of both groups (Fig. 6). The maturation of the fibroconnective tissue and the orientation of the collagen fibres were significantly higher ($p < 0.01$) in the fascia-lata-PLLA group than in the primary-suture-PLLA group (Fig. 7a, b and c). Some foreign-body-type giant cells were still seen around the PLLA fibres. The amount of inflammatory reaction (lymphocytes and plasma cells) in the primary-suture-PLLA group was superior ($p < 0.05$) to that in the fascia-lata-PLLA group (Fig. 8). The drill channels were surrounded by a solid tube of mature bone with quiescent lining cells at the margin and no osteoid rim visible. No bone ingrowth into the PLLA implant was noticed (Fig. 9a and b). The fascia lata was still visible as a fairly intact sheath of tissue.

TABLE II The histologic results assessed on a scale from – (no findings) to + + + (numerous findings) are presented by summing up the number of the findings at the femoral and tibial drill channels, the entry points of the drill channels in the femur and tibia, and the intra-articular substitute of the ACL; statistical evaluation was made between the fascia-lata-PLLA (F + P) and primary-suture-PLLA (P) group. Values of p less than 0.05 were considered statistically significant

Time scale	Maturation of connective tissue		Number of giant cells		Number of plasma cells and lymphocytes	
	F + P	P	F + P	P	F + P	P
6 weeks						
–	0	0	11	10	36	31
+	5	12	10	11	16	22
+ +	19	12	5	7	0	2
+ + +	2	4	0	0	0	0
12 weeks						
–	0	0	21	16	38	47
+	2	3	3	7	12	4
+ +	15	15	2	4	2	4
+ + +	9	10	0	1	0	1
24 weeks						
–	0	0	15	20	47	51
+	0	1	9	5	6	3
+ +	10	8	3	1	1	0
+ + +	17	18	0	1	0	0
48 weeks	$p < 0.01$				$p < 0.05$	
–	0	0	16	22	47	41
+	0	2	9	5	5	10
+ +	3	9	1	0	0	3
+ + +	23	16	0	0	0	0

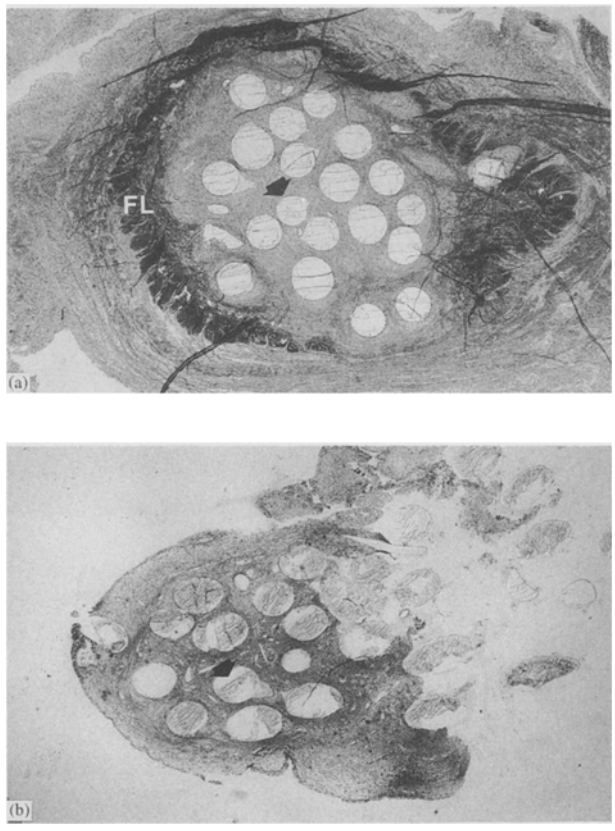

Figure 5 Histologic section of the reconstructed ACL 6 weeks after the operation. PLLA fibres (arrow) are presented both in (a) the fascia-lata-PLLA group, and (b) the primary-suture-PLLA group. Fascia lata (FL) is clearly visible. (Goldner–Masson, magnification × 12.)

4. Discussion

The success of the ACL repair using synthetic materials depends on the biocompatibility and the strength of the material as well as on the fixation of the reconstruction to the bone in order to ensure the stability of the knee joint.

The tissue reactions observed in this study were typical of a mild non-specific-foreign-body reaction which is considered a normal biological response to the degradation of the biodegradable implants [11]. In the literature, the accumulation of cells has shown to decrease with the degradation of the polymer [16, 26]. The number of inflammatory and giant cells in the present study was small, reaching its maximum at six weeks and decreasing thereafter. This can be ascribed to the tissue trauma after surgery combined with a foreign-body reaction. Plasma cells were very rare, and a moderate number of them was observed in only two specimens derived from one sheep in the primary-suture-PLLA group at 48 weeks, possibly indicative of an individual tissue response.

In the present study the degradation of the PLLA was incomplete at the end of the follow-up time, 48 weeks, and the original spaces of the fibres were seen although the interspaces as well as any defects caused by fragmentation or splitting of the PLLA were filled with fibroconnective tissue. Some fragmentation and ingestion of the polymeric material by giant cells was observed as early as six weeks. In previous studies, the

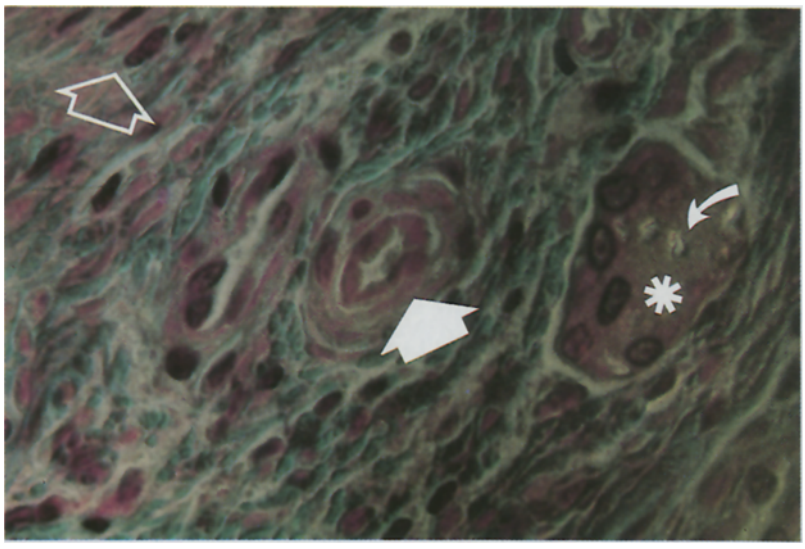

Figure 4 Photomicrograph from the intra-articular section at 6 weeks in the fascia-lata-PLLA group showing immature fibrocytes (solid arrow), a blood vessel (open arrow) and a giant cell (asterisk) with polymeric particles (curved arrow). (Goldner–Masson, magnification × 700.)

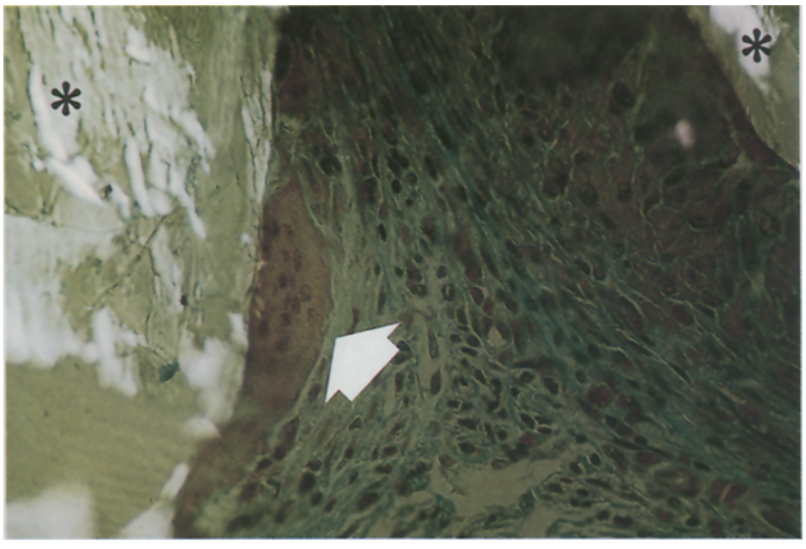

Figure 6 Photomicrograph under polarized light, showing partly fragmented PLLA fibres (asterisk) and a giant cell (arrow) in the primary-suture-PLLA group 48 weeks after the operation. (Goldner–Masson, magnification × 280.)

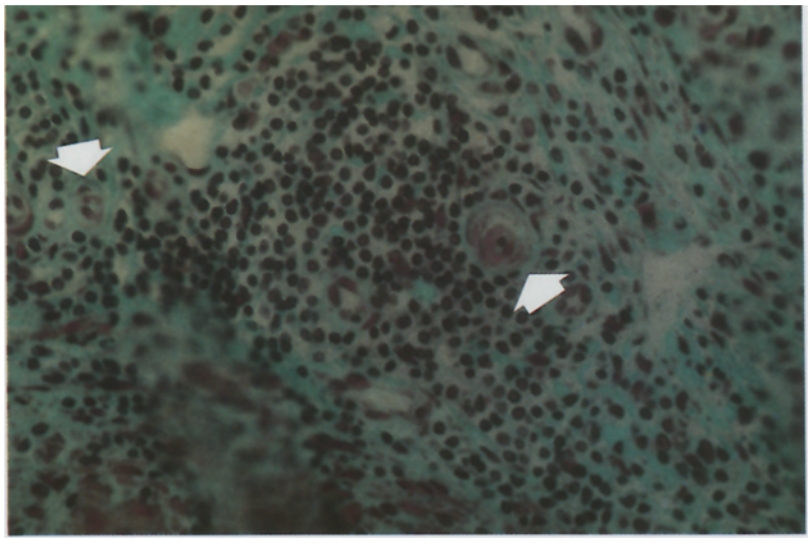

Figure 8 A clump of lymphocytes around blood vessels (arrow) in the primary-suture-PLLA group 48 weeks after the operation. (Goldner–Masson, magnification × 280.)

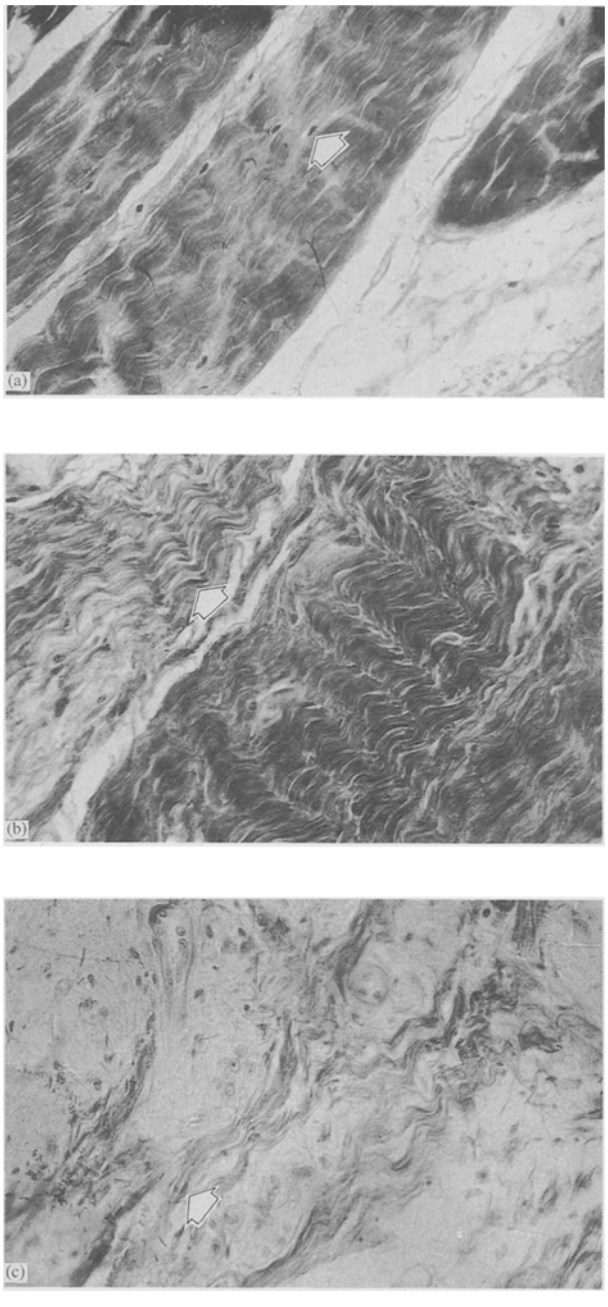

Figure 7 ACL specimens of the (a) the control, (b) fascia-lata-PLLA groups, and (c) primary-suture-PLLA groups 48 weeks after the operation. The maturation of the connective tissue and orientation of the collagen fibres were significantly higher ($p < 0.01$) in the fascia-lata-PLLA group than in the primary-suture-PLLA group. Fibrocytes are indicated with an arrow. (Goldner–Masson, magnification × 192.)

rods and screws made of PLLA have remained unaltered in the femur of rabbits without signs of PLLA degradation 48 weeks after the operation [21, 27]. This discrepancy in the resorption could be explained, besides by the different size and shape of the implants, by the greater mechanical stress on the reconstructed ACL caused by multiform motion of the knee joint than in the more rigid intramedullary placement of the rods and screws. Synovitis and arthritis of the knee joint caused by abrasive fragmentation of the implant have been reported to occur with several synthetic materials [28], particularly with carbon fibre [29]. PLLA seemed to provoke only a mild hypertrophy of the synovial cells without degenerative changes in the joint cartilage.

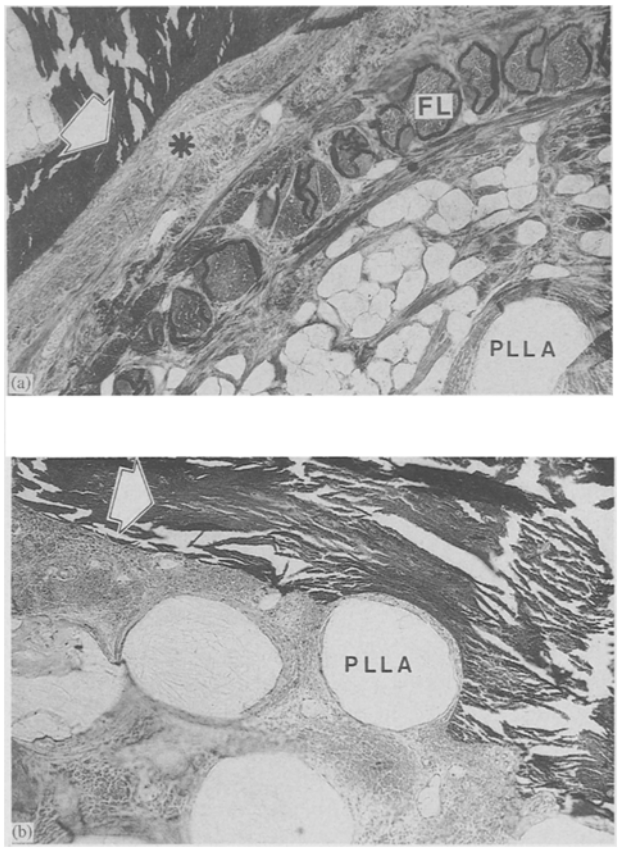

Figure 9 Photomicrograph showing the anchor of the ACL reconstruction 48 weeks after the operation in: (a) the fascia-lata-PLLA group, magnification × 48, and (b) the primary-suture-PLLA group, magnification × 12. A solid tube of bone (arrow) and fibrous tissue formation (asterisk) in the fascia-lata-PLLA group are presented around the drill channel. Fascia lata (FL) is still demonstrable. (Goldner–Masson.)

The ingrowth of the fibroconnective tissue around the PLLA fibres occurred similarly in both groups. The fairly loose design of the PLLA implants seemed to promote and support tissue growth in contrast to, for example, carbon fibre and Dacron which induce fibrous-tissue formation only to the outer sheet of the implant [6, 28]. The fibres of the connective tissue were more oriented in the fascia-lata-PLLA group than in the primary-suture-PLLA group after 48 weeks, resembling, however, more of a scar tissue compared with the control ACL. The orientation and maturation of the fibrocytes can have an effect on the mechanical properties by increasing the tensile force of the reconstruction. The difference of the connective-tissue maturation could be due to the effect of the fascia lata acting as a source of fibroblasts to the graft thus enhancing fibrous-tissue formation [30]. No necrosis of the fascia lata was observed after 48 weeks; the nutrition of the fascia lata probably arose from the surrounding connective tissue. Fascia lata is often used with synthetic materials to protect them from abrasion [31]. This effect was also noticed in the present study; the three failures of the reconstruction were all in the primary-suture-PLLA group.

The strength of the fixation of the implant at either end of the drill channel is important for long-term success, since the interface between a synthetic material and a living tissue is a region of high stress [32]. In

normal conditions most ligaments insert by gradual transition through layers of fibrocartilage and mineralized cartilage into the bone [33]. In the present study remodelling of the bone was already seen along the drill channels after six weeks, and the PLLA implants seemed to anchor to the bone by connective-tissue formation. In only a few samples bone formation was visible around the PLLA fibres at the periphery of the drill channel. In studies with carbon fibre, Neugebauer and Claes [34] reported the occurrence of bone ingrowth in the drill channel in sheep after one year. Arnoczky *et al.* [35] have studied the biologic attachment of bovine xenograft, Gore-Tex, Kennedy LAD and Dacron to canine bone. LAD exhibited a connective-tissue interface between the prosthesis and the trabecular bone, whereas all the other materials had a direct bony attachment. They draw the conclusion that the bone ingrowth is related to the surface characteristics of the prosthesis.

Biocompatibility of a material has been defined as the ability to perform with an appropriate host response in a specific application [36]. The findings of the present study, in our opinion, are in accord with previous knowledge about the good biocompatibility of polylactide. Histologically, the braided PLLA implants proved to be suitable for ACL repair in sheep. The firm fibroconnective-tissue attachment to the bone, as well as the earlier maturation of the fibrous tissue, suggest that the augmentation of the fascia lata with the braided PLLA implant is a preferable method to that of the primary suture of the ACL. The clinical success will depend on the mechanical properties of the PLLA implant which are under investigation. Future areas of research include the evaluation of long-term biocompatibility and tissue replacement of PLLA-fibre implants.

Acknowledgements

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