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Augmentation in anterior cruciate ligament reconstruction—a histological and biomechanical study on goats

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Abstract We studied reconstruction of the anterior cruciate ligament (ACL) in skeletally mature goats. In one group, the autogenous tissue was augmented with polydioxanone (PDS), the other group had no augmentation. Histological complete incorporation and remodeling of the transplant was found in both groups. The newly formed connective tissues gradually assumed the microscopic properties of the normal ligament. The augmented group showed a delay in remodeling and maturation of the fiber bundles. Mechanically, the PDS-augmented transplants were stronger than the nonaugmented transplants immediately after surgery. During the first 6 weeks, a rapid decrease in strength of the augmented transplants was found, whereas the strength of the nonaugmented group gradually increased. The results of our experiment do not favor augmentation of autografts in reconstruction of the ACL.

Résumé Nous avons étudié la reconstruction du ligament croisé antérieur chez la chèvre à squelette mature. Dans un groupe le tissu autogène a été renforcé avec du polydioxanone (PDS), l'autre groupe n'avait aucun renforcement. Une incorporation histologique complète et un remodelage du transplant a été constaté. Les tissus conjonctifs récemment formés ont pris progressivement les propriétés microscopiques du ligament normal. Le groupe renforcé a montré un délai dans le remodelage et la maturation des faisceaux de fibres. Mécaniquement les greffes renforcées au PDS étaient plus fortes que les greffes non renforcées immédiatement après la chirurgie. Pendant les six premières semaines une baisse rapide de la force des greffes renforcées a été constatée, alors que la force du groupe non—renforcé a augmenté progressivement. Les résultats de notre expérience ne sont pas en faveur d'un renforcement des autogreffes dans la reconstruction du ligament croisé antérieur.

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Introduction

Surgical reconstruction of the anterior cruciate ligament (ACL) with autogenous tendons is a well-accepted treatment [20]. During the incorporation and remodeling of the transplant, a mechanical weakening occurs [1, 17, 21], and to avoid this, synthetic ligaments are used as augmentation. The initial favorable short-term results were, however, followed by a high failure rate [7, 12].

Augmentation with a bioresorbable material might be an attractive alternative. Polydioxanone (PDS), which is produced by polymerizing the monomer paradioxanon, can be processed into monofilaments of all kinds [22], and a PDS braid has been suggested as augmentation in ACL reconstruction [4, 6, 15, 17]. The present animal study was performed to correlate the histological transformation during revitalization of the augmented autograft with the mechanical characteristics.

Materials and methods

The final study was performed on 60 skeletally mature Dutch milk goats (Capra Hircus Sana) of about 2 years old and weighing 47.5±6.7 kg. The animal welfare committee of the university approved the study. Two groups of 16 goats each were operated with reconstruction of ACL. In one group, augmentation with PDS was used; the other group had no augmentation. Six goats were used in a pilot test of the surgical procedures. The animals were operated on both knees. The minimum period between the surgical procedures was 8 weeks.

The goats were all operated on using standard procedures. After a medial parapatellar incision, the infrapatellar fat pad was released

from its insertion to the anterior part of the intercondylar notch and the entire ACL resected. The periosteum of the anterior part of the proximal tibia and of the lateral side of the femur was elevated. Tunnels of 8 mm in diameter were drilled through the insertions of the ACL with a cannulated drill. The bone pegs obtained were kept in physiological saline. The location of the tunnels appeared critical for achieving a transplant that remained tensed throughout the normal range of motion. A strip of patellar tendon and medial parapatellar retinaculum was dissected, together with a part the fascia lata measuring 15 cm × 1–1.5 cm to serve as transplant.

The flat PDS braid used for augmentation was 7.5 mm wide and 30 cm long. It was folded twice—longitudinally and transversally. The interarticular part of the PDS braid was enveloped by the transplant using interrupted absorbable sutures. The augmented transplants were pulled through the tunnels and the PDS braid was first attached to the medial part of the tibia with a cortical screw and washer. With the knee in 30° flexion, the transplant was tensed and attached to the anterolateral part of the femur. The knee was moved through the full range of motion and, if satisfactory, the bone pegs were driven in the tunnels from outside in, resulting in a good fixation of the transplant and a good contact with the cortical wall [20]. The PDS braid was fixed with sutures after redundant parts were excised. After careful hemostasis, the fat pad was reattached to the intercondylar notch. Ampicillin 500 mg was administered as antibiotic prophylaxis. Postoperative radiographs were taken, and the goats were allowed load bearing the next day.

After 0, 1, 2, 4, 6, 12, 24, and 48 weeks, two animals were killed in each group. Bone-tendon-bone specimens were fixed en block in 0.1 M phosphate buffered (pH 7.4) 4% paraformaldehyde, radiographed, and decalcified in 25% phosphate-buffered (pH 7.2) EDTA. The specimens were embedded in polymethylmethacrylate and sectioned (7 μm) at fixed intervals in longitudinal and transverse directions. Sections were stained with hematoxylin-eosin. Thirty-two operated knees and two nonoperated controls were available for histology.

In three goats sacrificed at 24 weeks, the vascularity of new tissues was studied using perfusion with India ink with gelatin at 37°C. The knees were embedded in plaster of Paris and sectioned into 3-mm slices, cleared, and photographed. Mechanical testing was performed within 5 h after sacrificing the animals. Sixty operated knees, and ten nonoperated knees that served as controls, were available for testing. Four of the ten control knees were stored deep frozen and thawed prior to testing. PDS and nonaugmented ACL reconstructions were tested at 0, 2, 6, 12, 24, and 48 weeks postoperatively. At each time point, two control knees, five augmented, and five nonaugmented ACL reconstructions were available. All soft tissues were dissected, except for the reconstructed ACL. The tibia and femur were mounted in clamps and fixed with PMMA cement (Fastacryl) at a flexion angle of 70°.

The transplant or the ACL was aligned with the actuator axis of the tensile tester. The tests were performed at room temperature (20°C). The displacement speed was 100 mm/min. The failure mechanism was assessed by macroscopical visual inspection. The strength was defined as the maximal force in the tensile test. The statistical analysis of the strength was performed by the two sample tests of Student's t test with a significance level of 5% (p=0.05).

Results

Two goats died postoperatively at day 3 and 9 from a pneumonia. Infections were not seen. One goat was replaced at the time of operation because of severe synovitis and cartilage destruction. In one goat, a subcutaneous hematoma was found at 6 weeks and in another a patella luxation at week 12. These animals were replaced. After the operations, the animals limped or partially unloaded the operated leg for the first few weeks. From the fourth till the sixth week, gradual loading was noticed, and after 6 weeks, most animals were walking normally.

There were no dislocations of the transplant. In one goat, a swollen hyperemic synovium as well as a slight hydrops and a thinner transplant was seen after 24 weeks. In one specimen, moderate degenerative changes were found in the patella femoral joint after 48 weeks, and the ACL was thinner compared to others.

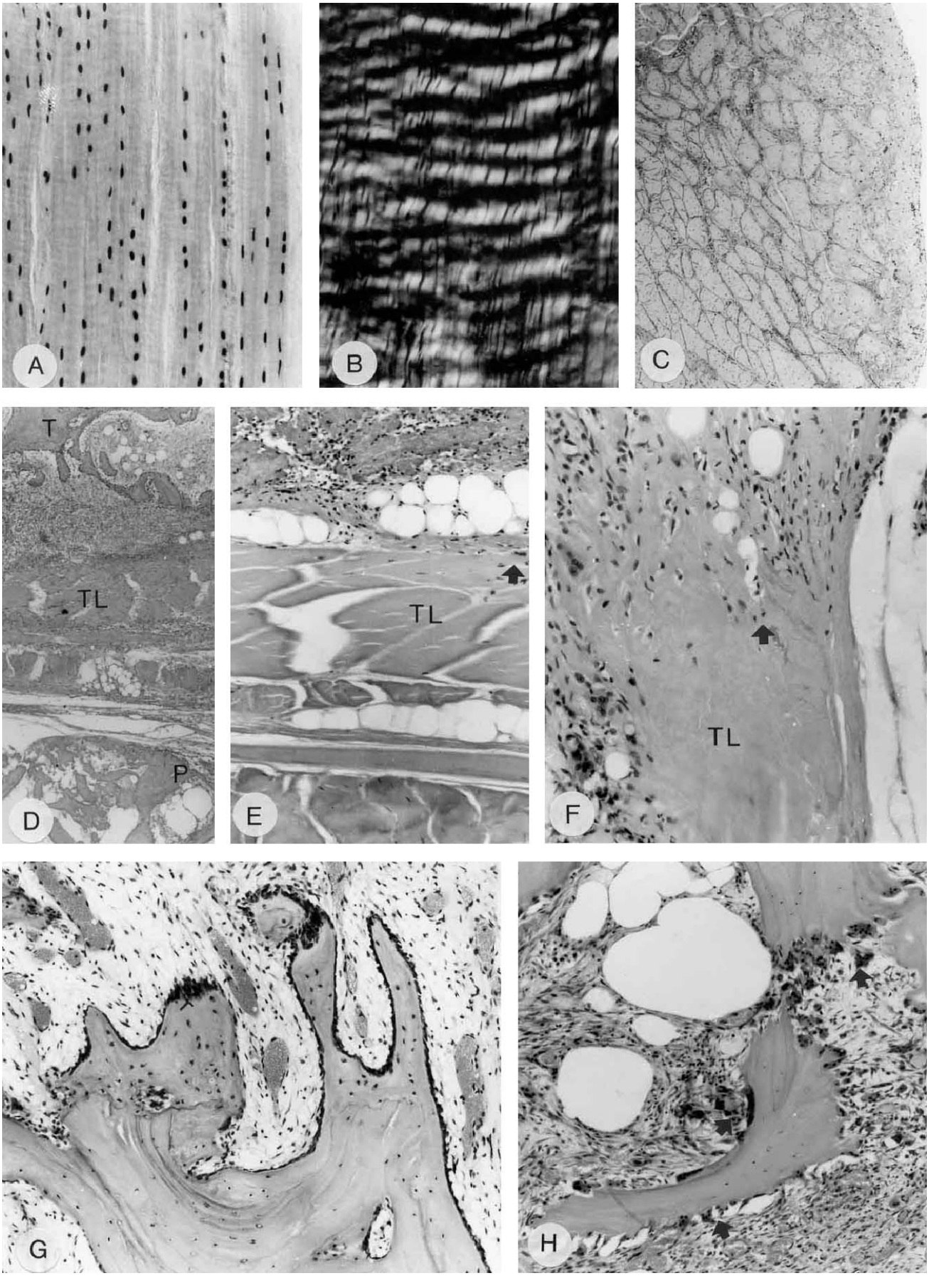
Histological analysis

In control samples, the ACL had the normal appearance of a ligament (Fig 1A–C). Directly postoperatively, the augmented transplant was thicker compared to the nonaugmented transplant. It had good contact with the infrapatellar fat pad. A good fixation of the bone pegs with the tunnel walls was achieved. There was also good contact between transplant and the PDS braid. Inside the joint, the transplant covered the major part of the PDS braid (Fig. 2A).

From week 1 and onwards, a progressive revascularization and fibrous tissue ingrowth (Fig. 1D–F) was seen whether the transplant was augmented or not. In this period, the transplant and the PDS fibers showed signs of disintegration. Giant cells engulfed PDS remnants, particularly seen after 6 and 12 weeks (Fig. 2A–C). After 12 weeks, the transplant was almost totally resorbed, and in the augmented group, more granulation tissue was present. The size of the PDS braid was considerably reduced. The newly formed collagen tissue bundles showed progressive orientation in the load-bearing direction in the joint as in the tunnels. After 24 and 48 weeks, the transplant assumed a synovial-like vascularised outer layer. In comparison to the control samples, the new ACL and the fibrous tissue of the ligamentous tissue situated in the tunnels of the transplanted knees was cell-rich and contained more vessels, but organization with bundles separated by connective tissue was lacking (Fig. 2F–H). After 48 weeks, the PDS braid was completely resorbed. In general the healing and remodeling process was somewhat advanced in the nonaugmented group.

Directly after surgery, the gaps between transplant and tunnel wall were filled with fibrin and debris. From week 1, a pronounced cellular wound repair reaction started, and bone in the periphery of the tunnel wall started to proliferate. From week 2, the bone pegs showed progres-

Fig. 1A Control: Collagen fibers are arranged in parallel rows packed in small bundles (×180); **B** Control: same as A with polarized light (×50); **C** Control: cross section. Note small bundles of collagen fibers separated by connective tissue (×50); **D** 2 weeks: Low magnification of cross section of transplant in femoral condylar. *P* bone-peg, *T* tunnel wall, *TL* transplant (×40); **E** and **F** 2 weeks: transplant (*TL*) shows a varying degree of disintegration and ingrowth of fibroblasts (*arrows*) (×180); **G** 4 weeks: extensive remodeling of the bone of the tunnel-wall (×120); **H** 4 weeks: resorption by numerous osteoclasts (*arrows*) of the peripheral bone of the bone peg (×80)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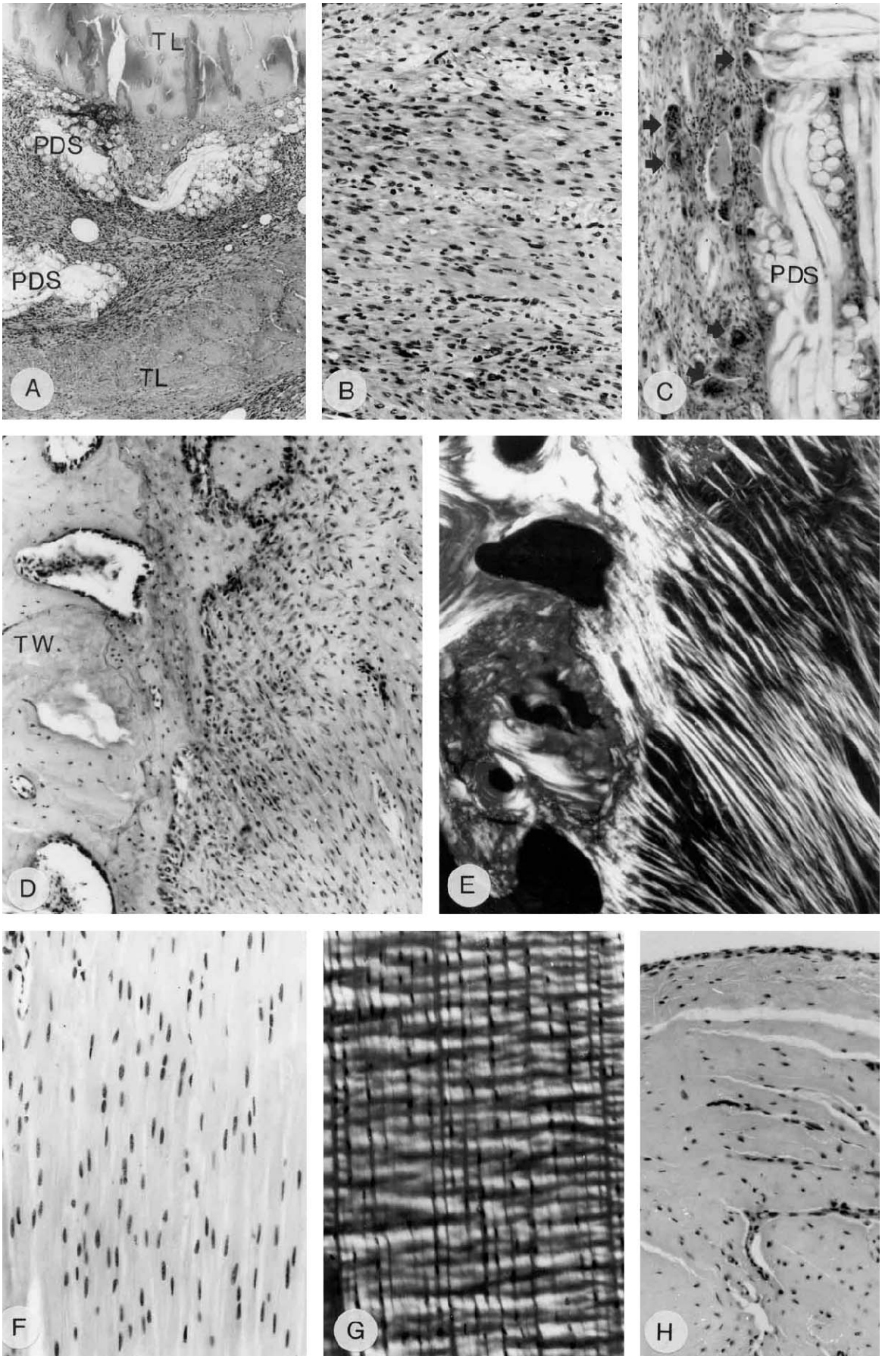
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Table 1 Mechanical strength (newton) of reconstructed anterior cruciate ligament (ACL) with and without augmentation. *SD* standard deviation

	ACL reconstruction mean (SD)	ACL reconstruction with augmentation mean (SD)
0 week	98±26 (N=5)	441±114 (N=5)
2 weeks	136±24 (N=5)	283±102 (N=5)
6 weeks	159±43 (N=5)	139±40 (N=5)
12 weeks	346±128 (N=5)	421±113 (N=5)
24 weeks	512±112 (N=5)	482±165 (N=5)
48 weeks	542±244 (N=5)	755±389 (N=5)

sive osteoclastic bone resorption resulting in complete disappearance after 12 weeks (Fig. 1G and H). After 6 weeks, the bone formation resulted in a dense trabecular bone with contact to the transplant by Sharpey fiber-like fibrous tissue (Fig. 2D–E) orientating from the tunnel wall into the transplant.

Two weeks after the operation, the synovium was swollen and hyperemic and the viscosity of joint fluid reduced. From week 4, these changes subsided. The perfusion studies showed many paraligamentous vessels in the control ligaments that originated from the infrapatellar fat pad and the posterior synovial fold, with small branches entering the ACL.

Twenty-four months after reconstruction, endosteal vessels from the femoral and tibial bone tunnels, besides the previous mentioned vessels from the synovial fold, contributed significantly to the vascular supply of the reconstructed ACL.

Mechanical analysis

The ACL in ten control samples failed in tension at 1,373 N (±349). At all time periods, the augmented and nonaugmented ACL reconstructions were weaker (Table 1). At the time of implantation, the PDS-augmented reconstruction failed at 441 N. This was higher than the failure load of the nonaugmented reconstructions (98 N), but considerably lower than the reported strength of the PDS braid (1,050 N). There was a statistically significant decrease in strength from 0 to 6 weeks postsurgery ($P<0.05$). After 6 weeks, the strength increased. A statistically significant difference in strength was only found between 6 weeks and 12, 24, and 48 weeks.

The strength of the nonaugmented reconstruction increased gradually from 0 to 12 weeks (Table 1). Strength at 12 weeks was significantly greater than at 0, 2, and 6 weeks ($p<0.05$). There were no significant differences between 12, 24, and 48 weeks. Only at weeks 0 and 2 was the PDS-augmented reconstruction stronger than the nonaugmented reconstruction ($P<0.05$).

Fig. 2A–H Cross section of PDS-augmented (*PDS*) transplants (*TL*) in tibial condylar (**A–C): A** 4 weeks: transplant (*TL*) adjacent to the bone peg (not shown, upper part) is avital but morphologically in tact; lower part of transplant (*TL*) is revitalized (×45); **B** 4 weeks: detail of area adjacent to tunnel wall showing replacement of the transplant by fibrous tissue (×180); **C** 4 weeks: detail of PDS braid (PDS) encapsulated by fibrous tissue and with giant cells on the surface of the braid (*arrows*) (×140); **D** 6 weeks: transplant without augmentation. Longitudinal section of tunnel wall (*TW*) in femoral condylar; **E** 6 weeks: same location as D but with polarized light (×180); **F** 24 weeks: non-augmented ACL reconstruction (×180); **G** 24 weeks: same as F with polarized light (×50); **H** 24 weeks: cross section. Note absence of clear organization of tendon in bundles of collagen fiber's connected by connective tissue septae (×180)

Discussion

At the end of the observation period, ACL reconstructions with or without PDS augmentation appeared to be incorporated into a macroscopically rather normal looking ligament. There is no agreement as to the ultimate fate of the transplant. Whether the cells of the transplant remain viable after transplantation [2], or the cells die and the matrix is repopulated by fibroblasts from the host, a process that is called "ligamentization" is still debated [1]. As with our study, several studies have shown initial necrosis of the transplant followed by revitalization in which most of the matrix is replaced by new, irregular-orientated collagen bundles that slowly assume a final new organization morphologically resembling that of the original ligament. The main difference between the reconstructed transplant and the normal ligament is the changed microarchitecture of the incorporated transplant. Furthermore, the transition between ligament and bone shows only Sharpey fibers instead of normal fibrocartilage, as reported in the literature [20].

In the reconstructed ACL, the contribution of vessels originating from bone tunnels contribute to microvascularity. In the normal ACL, a few vessels enter via this route, and most vessels are coming from the ligamentous branches of the middle genicular artery and from some terminal branches of the inferior genicular arteries [5]. We assume that the microvascular difference to the control ACL is a remnant of changed microvascularity of the transplant during incorporation and that in time this will be further remodeled into a normal ligament.

Immediately after surgery, the augmented transplants failed at the screw fixation, while later, after incorporation in the bone tunnels, failure occurred in the substance of the ligament. The reduction in strength of the PDS braid occurred much faster than was expected [16]. The rapid degradation was accelerated by the good revascularization potential in this animal model.

During the first weeks failure occurred in the relatively week fixation in the bone tunnels. This makes a good estimation of the strength of the PDS-augmented reconstruction difficult. The values are, however, in the same order as found by other groups [21]. They found that after

12 weeks, the remnants of the PDS braid had a tensile strength of 12% of the original value. At 48 weeks, the PDS-augmented transplants in our study showed a nonsignificant increase in mechanical properties compared to the nonaugmented transplants. This final result is in agreement with other experimental studies in which no differences were found in the ultimate strength of the reconstruction [3, 9].

In view of rapid histological and mechanical degradation of the PDS braid, we assume that the stress-shielding effect of the braid is mainly restricted to the first 2–6 weeks. Stress-shielding effects have also been seen in other studies [18]. This might be caused by the lack of load on fibroblast stimulation and remodeling. The relation between load and a vital fibroblast population is well documented in the periodontal ligament [11] and in in vitro systems [10]. In contrast to studies in which the transplants were combined with nonresorbable materials, the stress shielding was only a temporary effect [13]. It may be assumed that the type of synthetic material and the speed of degradation determine the time course of this effect.

Clinically, ACL reconstruction with augmentation allows for early mobilization with continuous passive motion in order to prevent muscle atrophy [14], and the augmentation procedure improves the initial stability of the reconstructed ligament [8, 19]. Particularly the clinical results of acute proximal ACL ruptures may benefit from augmentation [19]. Stability tests [8] have shown that after a follow-up of two years, 77% of the knees had normal stability if tested with a physiological (89 N anterior-posterior traction) stress. But 57% of the knees failed if tested under maximal anterior-posterior stress.

In conclusion, we have demonstrated that the initial effect of PDS augmentation on the strength of a reconstructed ACL has no long-term effect. Clinically, the benefits of such an augmentation seem doubtful.

References

1. Amiel D, Kleiner JB, Akeson WH (1986) The natural history of the anterior cruciate ligament autograft of patellar tendon origin. Am J Sports Med 14:449-462
2. Arnoczky SP (1983) Anatomy of the anterior cruciate ligament. Clin Orthop 172:19–25
3. Asahina S, Yamamoto H, Muneta T, Ishibashi T, Furuya K (1995) Evaluation of anterior cruciate reconstruction reinforced by the Kennedy ligament augmentation device. An arthroscopic and histological study. Int Orthop 19:229–233
4. Blauth W, Hassenpflug J (1985) Reconstruction of the cruciate ligament with special reference to synthetic substitute materials. Unfallchirurg 88:118-125
5. Bolton CW, Bruchman WC (1985) The GORE-TEX expanded polytetrafluoroethylene prosthetic ligament. An in vitro and in vivo evaluation. Clin Orthop 196:202–213
6. Bonnaire F, Berwarth H, Munst P, Eichinger S, Kuner EH (1995) Kann das Ergebnis nach Kreuzbandoperationen arthrometrisch erfasst werden? Ein Vergleich der subjektiven Einschatzung, des Lysholm-Scores, der klinischen Stabilitatseinstufung und der Stabilitatsmessung mit dem KT 1000 nach komplexen Kniebandverletzungen. Unfallchirurgie 21:83–91
7. Claes L, Durselen L, Kiefer H, Mohr W (1987) The combined anterior cruciate and medial collateral ligament replacement by various materials: a comparative animal study. J Biomed Mater Res 21 [Suppl 3] 319–343
8. Hehl G, Strecker W, Richter M, Kiefer H, Wissmeyer T (1999) Clinical experience with PDS-II augmentation for operative of acute proximal ACL ruptures—2-year follow-up. Knee Surg Sports Traumatol Arthrosc 7:102–106
9. Holzmuller W, Wehmeyer M, Rehm KE, Perren SM (1994) Histologische Untersuchungen zum Ersatz des vorderen Kreuzbands mit PDS-augmentierten Patellarsehnentransplantaten. Unfallchirurg 97:144–150
10. Huang D, Chang TR, Aggarwal A, Lee RC, Ehrlich HP (1993) Mechanisms and dynamics of mechanical strengthening in ligament equivalent fibroblast populated collagen matrices. Ann Biomed Eng 21:289–305
11. Lekic P, McCulloch CA (1996) Periodontal ligament cell population: the central role of fibroblasts in creating a unique tissue. Anat Rec 245:327–341
12. Margevicius KJ, Claes LE, Durselen L, Hanselmann K (1996) Identification and distribution of synthetic ligament wear particles in sheep. J Biomed Mater Res 31:319–328
13. McPherson GK, Mendenhall HV, Gibbons DF, Plenk H, Rottmann W, Sanford JB, Kennedy JC, Roth JH (1985) Experimental mechanical and histologic evaluation of the Kennedy ligament augmentation device. Clin Orthop 196:186–195
14. Oberbillig C, Kirschner P (1989) Postoperative kontinuierliche passive Bewegung (CPM) nach Augmentation von vorderen Kreuzbandrupturen mit Polydioxanon-Bandern. Fruhergebnisse einer prospektiven Studie. Unfallchirurgie 15:145–151
15. Puddu G, Cipolla M, Cerullo G, Franco V, Gianni E (1993) Anterior cruciate ligament reconstruction and augmentation with PDS graft. Clin Sports Med 12:13–24
16. Ray JA, Doddi N, Regula D, Williams JA, Melveger A (1981) Polydioxanone (PDS), a novel monofilament synthetic absorbable suture. Surg Gynecol Obstet 153:497–507
17. Rehm KE, Schultheis KH (1985) Ligament replacement with polydioxanone (PDS) Unfallchirurgie 11:264–273
18. Tohyama H, Yasuka K (1998) Significance of graft tension in anterior cruciate ligament reconstruction. Basic background and clinical outcome. Knee Surg Sports Traumatol Arthrosc 6 [Suppl 1] 30–37
19. Trager D, Pohle, K, Tschirner W (1995) Anterior cruciate ligament suture in comparison with plasty. A 5-year follow-up study. Arch Orthop Trauma Surg 114:278–280
20. van Rens TJ (1977) Fixation of ligament structures in the knee. In: Injuries of ligaments and their repair, Chapchal G (ed), George Thieme, Stuttgart
21. van Rens TJ, van den Berg AF, Huiskes R, Kuypers W (1986) Substitution of the anterior cruciate ligament: a long-term histologic and biomechanical study with autogenous pedicled grafts of the iliotibial band in dogs. Arthroscopy 2:139–154
22. Wissing J, van Vroonhoven TJ, Schattenkerk ME, Veen HF, Ponsen RJ, Jeekel J (1987) Fascia closure after midline laparotomy: results of a randomized trial. Br J Surg 74:738–741