Ovine anterior cruciate ligament reconstruction using a synthetic prosthesis and a collagen inductor

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The evaluation of composite anterior cruciate ligament prostheses, the union of an inductor of collagen and a synthetic fibre, are described. They were implanted in 10 sheep models for six months. None of the ligaments were broken, and the dynamic radiography was stable during this time. After harvesting them, a histologic study was performed on the intra-osseou and intra-articular portion, and on the synovial tissue. Biocompatibility was excellent. An osseous anchorage was found in 50% of cases and a fibrous ingrowth with well oriented fibres in each case. The results of this study show that the matrix has only partially played its role, because there was no improvement of fibrous ingrowth compared with other studies. However this fact corresponds to results at only 6 months and it is necessary to identify the type of collagen.

1. Introduction

Ruptures of the anterior cruciate ligament (ACL) are frequent and continue to pose numerous surgical problems such as failure of the plasty [1], and osteoarthritis in unstable knees [2].

Having concentrated on prosthetic ligaments over the last decade, it seems that the number of failed artificial plasties (40%) [3] have lead to a more frequent use of autografts. However, to realize the autograft procedure it is necessary to harvest part of the patellar ligament, and a significant morbidity of the donar site has been reported [4]. Furthermore, a new injury to this same knee is always possible and sometimes it is difficult to take a second graft from the same knee. For this reason, the concept of artificial ligaments remains attractive and justifies experimental work.

Much work has been done since the first research by Butler in 1957. Many synthetic fibres have been tested:

polyesters (Dacron® a well-known example)
polyarylamids
polytetrafluoro-ethylenes
polyethylenes
carbon.

The two main reproaches that have been made against them concern:

Tolerance: numerous cases of synovitis have been reported with Dacron®. They seem to be linked to the presence of residual particles of lubricant used at the time of manufacture. Reaction to foreign bodies has also been reported when these fibres, and particularly carbon, wear.

Mechanical value: even if the prosthetic fibres have a resistance superior, or equal, to that of the natural ACL, they do not reproduce the natural viscoelasticity of the ligaments. These prostheses are, therefore, too rigid and thus rupture during repeated efforts due to their fatigue strength.

So, augmentation of the autoplasty with a synthetic ligament was proposed [5, 6]. As an alternative, we developed a synthetic ligament coupled with a collagen inductor.

In the present animal study, we investigated a non-cytotoxic prosthetic ligament whose strength would be at least greater than that of a healthy ACL and whose prosthetic braids were impregnated with a matrix enhancing collagen growth. This device was implanted for 6 months in the sheep model. Clinical and radiographical status, histological tolerance, collagen ingrowth and histological quality of bone anchorage are discussed.

2. Material and methods

2.1. Material

Use was made of a terephtalate polyester fibre coupled with an extracellular matrix composed of collagen, glycosaminoglycanes and chitosan (polysaccharides

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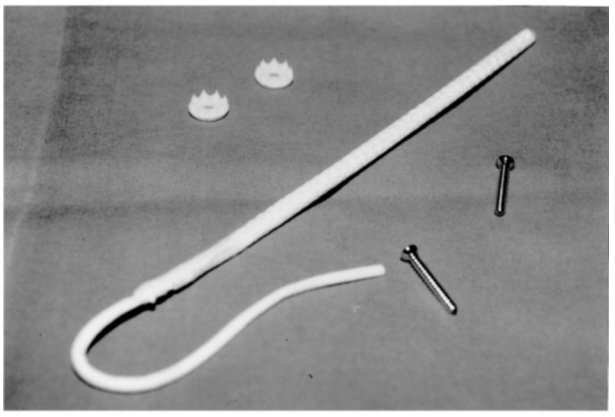

Figure 1 The prosthesis.

derived from Chitine), initially designed for the making of artificial skin.

This matrix is not cytotoxic, either in its unmanufactured state, or at the time of its degradation (studies of cytotoxicity were conducted at the laboratory of Doctor Echinard, Lyon, in collaboration with the Pasteur Institute of Lyon).

The prosthesis came in the form of a tubular braid, 6 mm in diameter, and made up of fibres woven in such a way that the size of the pores, in the absence of traction, was 1.5 mm^2 (Fig. 1).

The polyester support was impregnated in order to obtain homogeneity and cohesion between the matrix and the synthetic fibres. Thus, there was a thin layer of matrix on the surface of the implantation. The prosthesis was tested in a tensometer, showing an ultimate tensile load of 700 N, and a maximum stretch of 40% at this load.

2.2. The animal model

The sheep was chosen as an animal model because its ACL is morphologically close to that of humans.

Ten mature sheep were operated on, all of them being of the same race and weighing an average of 65 kg.

One sheep was used as a control by cutting the ACL individually and submitting it to the same post-operative procedure.

2.3. Implantation

Implantation was done under general anesthesia using an over-the-top technique with a tibial tunnel of 3 m. Fixation was ensured by a screw on a spiked washer, after tensioning the prosthesis by hand to eliminate the anterior drawer created by the section of ACL.

2.4. Post-operative care

An antibiotherapy was administered for 7 days. Total weight-bearing was permitted and the animal returned to the meadow towards the 15th day, until euthanasia at 6 months.

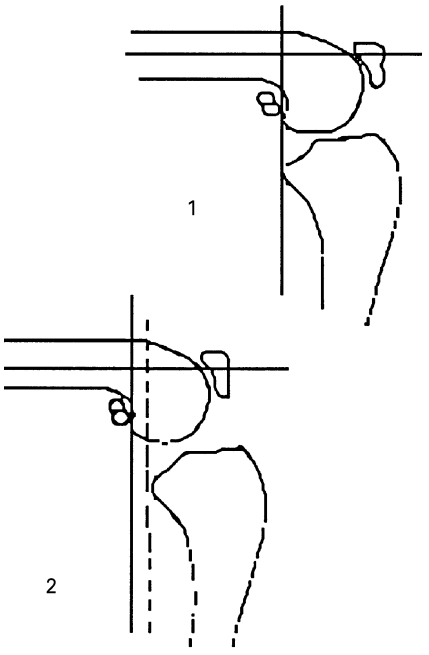

Figure 2 Measurement of the anterior drawer. (1) normal ACL = the posterior line of the tibia correspond to the posterior femoral condyle; (2) ACL = rupture, the posterior line of the tibia is before the posterior femoral condyle. The difference between 1 and 2 is the anterior drawer.

2.5. Post-operative radiography

In the post-operative days, radiographs of the operated knee were taken from the front and side in drawer at 90° flexion, in order to measure the radiographical anterior drawer (Fig. 2). A radiograph of the control knee was done in the same way.

2.6. Clinical and macroscopic results

Before the animal was sacrificed, an assessment of the knee's articular function was made, and afterwards an articular exploration was carried out: the aspect of the reconstructed ACL and of the cartilage was noted.

2.7. Radiographic evaluation

Comparative radiographies, with anterior drawer were done in the same way as after implantation.

2.8. Tolerance assessment

After arthrotomy, a sample of articular liquid was taken in order to evaluate the inflammatory reaction, as regards the tolerance of the prosthesis. The articular liquid sample was stained with May-Grunwald-Giemsa, enabling the calculation of a numeration-formula of the liquid (total number of cells of polynuclear neutrophiles, eosinophiles, lymphocytes and monocytes).

Samples of articular liquid were taken from six apparently healthy, unoperated sheep of the same race, which served as controls.

The results of the two groups were compared using the statistics software Statview®, by means of Mann–Whitney non-parametric tests.

The aspect of the synovial tissue was noted and a sample, fixed in 10% formaldehyde at pH 7, was taken to look for inflammatory reaction, and the

presence of foreign body cells and prosthetic particles. The synovial tissue specimen, fixed in 10% formaldehyde at pH 7, was submitted to serial sectioning before being embedded in paraffin. These blocks were cut with a microtome in sections of thickness 4 μm which were then stained with Hematoxylin-Eosin-Saffran and afterwards studied under light microscopy.

2.9. Prosthetic study

The entire prosthesis was harvested, including the tibial tunnel fixation, and fixed in 10% formaldehyde at pH 7.

After dehydratation, the prosthesis was embedded in methyl-methacrylate, then cut into three blocks:

- an osseous tibial block comprising the tunnel and the prosthesis (block I);
- a block corresponding to the passage between the articular and the tibial osseous portion of the prosthesis (block II);
- a block comprising exclusively the intra-articular portion of the prosthesis (block III).

Blocks I and III were sectioned perpendicularly to their major axis to appreciate peripheral and central fibrous ingrowth. Block II was sectioned longitudinally to study the Sharpey's fibres. The sections were of 50 μm thickness.

Block I's sections, which are osseous tissues, were then stained with Solochrome-Cyanine, while those from block III (fibrous tissue) were stained with Hematoxylin-Eosin, and finally those from block II (osseous and fibrous tissues) were treated according to both techniques.

All of the sections were also subjected to microradiography. Observation was made under standard and polarized light microscopy.

A study was also carried out under a scanning electron microscope with a microanalysis to look for possible chemical abnormalities: 15 microanalyses of the zone where the synthetic fibres were completely embedded in the bone, were conducted in order to estimate the quality of the bone-prosthesis interface. The series of measurements taken of the prosthesis towards the osseous tissue enabled the precise dosing of carbon, phosphorus, magnesium, calcium, sulphur, and oxygen.

3. Results

3.1. Clinical and macroscopic results

For the control sheep, walking and running were normal. Of the others, only one showed lameness, on the operated side, in walking and running. There was no difference in the range of motion between the two knees of each sheep.

The scar was dry and non-inflammatory in all cases. There was an abundant intra-articular liquid in five cases; moderate or none in five cases. The synovial appeared inflammatory in one case (the same animal which had abundant intra-articular liquid and lameness).

All of the animals had femoro-patellar chondritis, two of which appeared advanced. A femoro-tibial arthritis was observed in one case. Furthermore, two large calcifications were observed in front of the tibial spine. Dissection showed their independence in relation to the osseous surfaces.

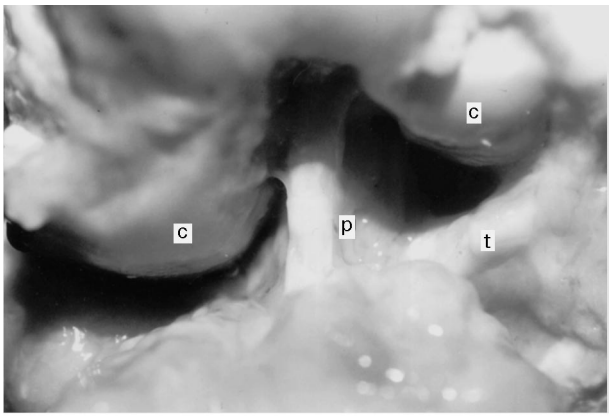

Figure 3 Macroscopic result.

No rupture of the prosthesis was evident. On the other hand, in two cases superficial erosion was observed. The braid was enveloped by a fibrous sheath in eight cases, with vessels on the surface of the ligament in two cases (Fig. 3).

3.2. Radiographic evaluation

The radiography analysis showed:

for the control sheep:

- hooked tibial spine
- peripheral tibial osteophytes
- no femoro-patellar arthritis.

for the others

- hooked tibial spine in two cases
- peripheral tibial osteophytes in four cases
- femoro-patellar arthritis in four cases
- calcification in front of the tibial spine in two cases confirming the macroscopic aspect.

The control sheep's radiographic anterior drawers had only advanced by 1 mm, as much compared with the healthy knee as compared with immediate postoperative measurement. For the others, the average differential between D-1 and D-180 on the prosthetic side was 1.1 mm (note that four sheep had a null differential) and the average differential on the prosthesis knee compared with the healthy side at 6 months, was 0 mm.

So, there is no significant difference between D-1 and D-180 and between D-180 and the control side (Table I)

3.3. Tolerance assessment

The synovial samples showed no sign of inflammatory reaction or foreign body reaction (giant cells).

Concerning the samples of articular liquid, there was no significant difference between the different elements of the different groups. It can be concluded that there was an absence of inflammatory reaction.

TABLE I Difference between anterior drawers

	Sheep number										
	1	2	3	4	5	6	7	8	9	10	Average
A: D-1/D-180	− 1	6	0	− 1	0	3	3	1	0	0	1,1
B: Op/No-Op	− 8	8	0	0	1	3	3	1	0	0	0

A: Difference between the anterior drawers on the operated side to the first day and to 6 months.
B: Difference between the anterior drawers on the operated and on the non-operated side at 6 months.

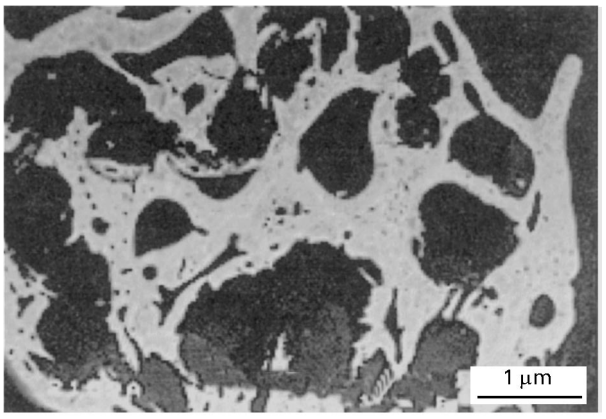

Figure 4 Micro-radiograph of the tibial tunnel; osseous ingrowth around and into the braid. (×20)

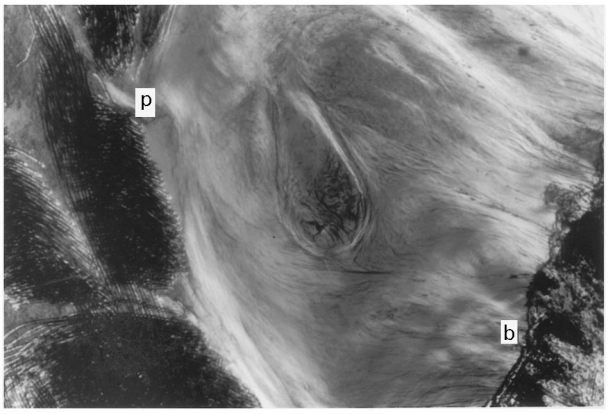

Figure 5 Junction of fibre attachment to bone: b, bone; p, prosthesis. (×48)

3.4. Prosthetic study

The study of the tibial portion showed an osseous ingrowth around the braid in five out of the ten cases. This concerned a well-differentiated bone in every case, and furthermore, the polarized light study showed the presence of oriented fibrous tissue between bone and prosthesis. In four cases, there was an osseous ingrowth in the centre of the prosthesis, and in one case, the prosthetic braids were entirely dissociated by the bone. There was no sign of resorption (Fig. 4).

At the junctional block, close contact between the marginal osseous tunnel and the prosthesis was found in seven cases. In nine cases, polarized light showed the existence of "Sharppey fibres", which appeared mineralized in six cases. The intra-articular peri-prosthetic's fibres were still oriented. These were abundant in five cases, and rare in another five cases (Fig. 5). Oriented fibres were found within the prosthesis. No cellular reaction to foreign bodies was found. There were numerous vessels, as much on the surface as in the depths of the prosthesis. No infiltration into the wall of the vessels was displayed. There was moderate cellular infiltration in the fibrous tissue, without any giant cells.

The intra-articular ligamentory portion contained no calcification. It was made up of a peripheral fibrous envelope, thin in six cases, and thick in three (Fig. 6). The fibres were oriented eight out of ten times. The fibrous ingrowth within the prosthesis was abundant in three cases, constituted of oriented fibres in seven cases. Numerous vessels without inflammatory reaction were present (Fig. 7).

In the electronic microscope study, with the exception of calcium and phosphorus, traces of all of other elements were found. The study of the calcium/phosphorus ratio, which reflects the degree of osseous

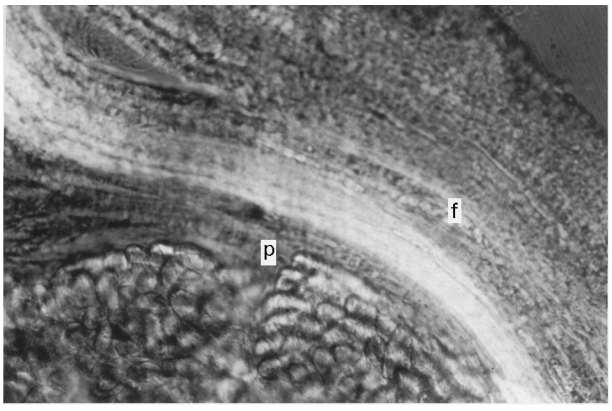

Figure 6 Intra-articular part; peripheral fibrous envelope: (×240) p, prosthesis; f, fibrous.

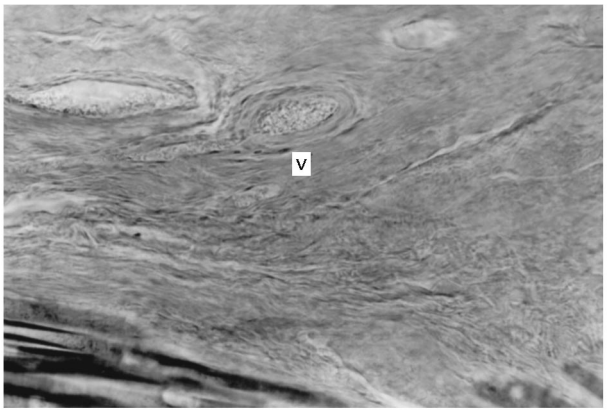

Figure 7 Vessel. (×240)

mineralization was conducted systematically. Of the 15 measurements taken, a change of the Ca/P ratio was discovered five times and was around 3. This value was measured at the zone of close contact

between synthetic fibre and osseous tissue. The increase in the ratio corresponded four out of five times, to a decrease in the amount of phosphorus. The amount of calcium remained unchanged.

The microanalyses carried out on the ligamentary portions of the samples found only organic substance.

4. Discussion

The sheep seems to be a valuable animal model because its anatomy and the size of its articulations are quite close to those of humans. The size and diameters of the sheep's ACL are 50 to 66% those of humans [4]. In the dog model, the ACL is smaller. The femorotibial angle is more open than in the canine, where the knee works permanently in more important knee flexion.

The kinematics of the appearance of arthritis after the ACL tear's seems to be different from the canine model, which in 6 months reproduces the natural evolution of the unstable knee towards arthritis. Concerning sheep, the same evolution could not be found, perhaps due to an insufficient number of control sheep in this study. Furthermore, AMIS [7] describes a degenerative change that only appeared after 24 months. At six months, the knee was normal. The frequency of radiographic femoro-patellar arthritis was very high. In comparing our results with those of other studies, we notice that all of the authors emphasize the frequency of femoro-patellar arthritis in sheep when they constitute the animal model [7–10]. The causes are, perhaps, the physiological position of the ovine knee in flexion and the necessity of patella luxation for placement of the prosthesis.

Therefore, the implantation of this prosthesis on this model enabled a clinical and histologic evaluation in conditions closely resembling those of an artificial ligament's regular use in humans.

The operative technique has been known and used for a long time and does not present any particular difficulty, if it were not for the problem of the implant's fixation. The fragility of the ovine bone was a hindrance concerning the placement of the means of fixation. Only screwing seems feasible, even if it makes tensioning the prosthesis more difficult.

The rapid return to the meadow allowed testing knee use on a normal scale. This appears determinant and differentiates this study from one where the animal remains in the stall until sacrificed, as with the rabbit model [11]. This mechanical environment provided more reliable clinical conditions than the strength test performed *in vitro*, which was far from physiological conditions.

The prosthetic replacement seems to have restored the knee function, permitting normal activity. Only one animal showed lameness.

The stability of the radiographic drawers during this time confirmed the mechanical strength of the prosthesis, a strength already evoked clinically. This stability may be a consequence of the preloading of the prosthesis. Thus, preloading may eliminate secondary slackening of the prosthesis, but it can diminish the prosthetic pores' diameters and so the fibrous ingrowth. As reported in the literature, the main materials did not resist and broke. This is notably the case with isolated Kevlar [12], whereas this was not so in association with Fascia-Lata [5]. This was also the case with polypropylene, used as a substitution ligament [13].

DACRON® seemed to produce a more satisfactory performance from a mechanical point of view [14].

The biocompatibility proved to be satisfactory, since no inflammatory reaction or foreign bodies could be found. Concerning tolerance, little was mentioned by other authors about intra-articular inflammatory reaction. On the other hand, particles of Kevlar and carbon have been found further away, in the lymphatic system [12, 15].

Laboureau *et al.* [16] reported episodes of synovial inflammation when the prosthesis eroded due to friction.

While the preceding results were encouraging, the histological study tempers somewhat this optimism.

Prosthetic anchorage in the tunnel proved to be entirely osseous in only five out of ten cases. In the other five cases, there was a large amount of fibrous infiltration and this despite placement, with rough friction, of the prosthesis. This was performed to ensure close contact with the bone and therefore a secondary osseous anchorage. Probably, this fibrous interface was the consequence of micro-movements which opposed osseous colonization of the prosthesis.

In the case of the fibrous anchorage, the bundles of fibres reproduced the aspect of Sharpey fibres. The orientation of the fibres may signify the transmission of stress between the prosthesis and the wall of the tibial tunnel and so an efficient anchorage was realized.

In the literature, osseous ingrowth into the tunnels was rarely found and most authors [10] reported a fibrous-type anchorage. This was especially the case of Kennedy L.A.D.®, which did not generate any osseous growth, but on the contrary, resorption zones [8, 9].

Bolton *et al.* [17], with Gore-Tex®, and Macmaster [18], with xenografts, found close contact between the bone and the prosthesis. At 18 months for Paavolainen *et al.* [6] with GORE-TEX and at 24 months for Amis *et al.* [7] with polyester fibres total osseous anchorage was observed.

As far as the intra-articular-ligamentary portion was concerned, peripheral colonization of the prosthesis seemed to be relatively satisfactory. This was not the case for fibrous penetration within the braid. This could be one of the consequences of preloading the prosthesis at the time of its implantation, reducing the diameter of the braid's mesh and therefore limiting penetration of the collagen fibres.

On the other hand, in polarized light, superficial and deep fibres appeared oriented nine times out of ten. This indicated the placement in constraint of the fibrous tissues by forces in the same direction. There may be a reduction of the constraints, notably in shearing, for the synthetic fibres and therefore a probable increase in fatigue failure resistance.

Furthermore, the development of a large vascular network, as superficial as it was deep at the

neo-ligament and without any sign of inflammation, allows one to predict that, with time, an improvement in the fibrous quality of the ligament will occur.

Concerning the intra-articular portion, most authors found a fibrous encapsulation [19]. Penetration into the prosthesis was more problematical. For Park *et al.* [20], Dacron® seemed to be better colonized by collagen fibres when the implant was coated with Fascia-Lata. MacPherson *et al.* [9], with Kennedy L.A.D.® found above all, a fibrous reaction of the encapsulation type to foreign bodies.

It was for this reason that Marcacci *et al.* [21] marked the collagen tissue with specific antibodies in order to determine the fibres' type (type I collagen after 18 months).

Dunn *et al.* [11] evaluated a composite collagenous prosthesis without synthetic scaffolding and reported total degradation of the prosthesis when replaced with a fibrous repair tissue. But in this study there was no collagen identification.

The micro-analysis in our study with electronic microscopy of the intra-articular-ligamentary portion did not find any measurable elements. Passuti *et al.* [12] has proven the presence of sulphur with Kevlar, which probably corresponded to impurities added during manufacturing. The anomaly of mineralization at the tibial tunnel, found in 30% of the measurements, remains unexplained.

Concerning the method used during the present study, the microradiographies and the polarized light study furnished most of the information concerning the intra-osseous portion. Solochrome-Cyanine and, even more so, Toluidine Blue only confirmed data from the preceding techniques.

With regard to the ligamentary portion, polarized light enable us to find the abundance and quality of the fibrous tissue. Use of Hematoxylin-Eosin was probably adapted for the study of vascularization and cellular reaction. However, its ideal use requires embedding the ligamentary portions of the sample differently, i.e. in paraffin, compared with those comprising the bone, i.e. in methacrylate.

5. Conclusion

Globally speaking, we tested an artificial ligament over a period of 6 months, which was satisfactory in terms of its functioning and tolerance. Polyester proved satisfactory for its solidity, since no ruptures were found. The histological results, however, show a slightly insufficient result as to the osseous anchorage. Reasons for this were, perhaps, the relative failure of the anchorage device or insufficient size of the interfibre pores when the braid was under tension. With regard to ingrowth, while the braid's sheath was satisfactory, penetration into the prosthesis and between the fibres was less so. The matrix, therefore, only partially played its role. Compared with other studies concerning prosthetic braids not treated by collagen inductors, the matrix does not appear to improve the results. This does not take into account the potential type of collagen, which was not established here. The characterization of the fibrous tissue sheathing the prosthesis will be done by using "markers" coupled with antibodies in another study.

This consideration is counterbalanced by the fact that the animals were sacrificed at 6 months. The time period was shorter than the American series [6, 7, 19].

Central ingrowth occurs perhaps, in a centripetal, and very progressive way. The presence of abundant neo-vascularization in the absence of any signs of inflammation seems a good prognosis.

For Arnoczky *et al.* [14], however, in his study of Dacron® in the canine, the only difference between 6 months and 1 year was the orientation of the fibres. The waiting duration will not increase the quantity. However, his Dacron® were in the form of strips and not hollow braids and therefore, there could be no central invasion there. That was different for Amis *et al.* [7] who showed an increase of prosthesis diameter with time.

But if this prosthesis satisfies a part of the schedule of conditions of artificial ligaments, there are still improvements to be made.

Thus one can foresee in the future a ligament which is not only composite but also comprises different parts according to their position in the knee:

- A part destined to be in the osseous site; while respecting the principle of Arnoczky *et al.* [22] on osseous anchorage, it could also benefit from a surface treatment promoting osseous ingrowth.
- An intra-articular part that supports the regeneration of collagen tissue. It would be composite with one part constituting the scaffolding and one part which would be an inductor for collagenic synthesis. The support should have a mesh of sufficient size to promote fibrous penetration. In other respects, this weave should reabsorb itself when the collagen regeneration has been accomplished, in order to progressively inload the neo-ligament.

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Received 6 February
and accepted 14 March 1996