ANTERIOR CRUCIATE LIGAMENT REPLACEMENT

BIOCOMPATIBILITY AND BIOMECHANICS OF POLYESTER AND CARBON FIBRE IN RABBITS

A. A. AMIS, S. A. KEMPSON, J. R. CAMPBELL†, J. H. MILLER

From Imperial College, London; the Royal (Dick) Veterinary School, Edinburgh and Glasgow Royal Infirmary

The anterior cruciate ligament was replaced in rabbits, using implants of carbon or polyester filaments with known mechanical properties. The biocompatibility of the implants was assessed in detail using light microscopy, and scanning and transmission electron microscopy. Mechanical tests were made of stability, in compression with normal joints and controls after excision of the ligament.

Some carbon fibre implants broke down in vivo, allowing instability; the fragments caused chronic inflammation. Intact carbon implants did not induce the formation of neoligaments; they were covered by tissue, but there was no ingrowth. Polyester did not degrade mechanically and supported early collagenous ingrowth within the implant, even in the mid-joint space. It was concluded that there was no justification for the use of carbon fibres as anterior cruciate replacements; polyester appeared to be suitable.

In order to avoid the use of autogenous grafts to replace a chronically ruptured anterior cruciate ligament, or to augment such a graft, a variety of synthetic materials have been investigated in animals. These include Dacron (Hinko 1981); Marlex (Meyers, Grana and Lesker 1979); Teflon (Butler 1964); Nylon (Vaughan 1963) and carbon fibre (Jenkins 1978). Many of these have been used clinically despite a lack of data regarding their performance.

One crucial concern is the fate of implants in the mid-joint space, relatively remote from vascular tissues. Despite reports that implants are infiltrated by fibrous tissue (Jenkins 1978; Meyers et al. 1979; Hinko 1981), its constitution and rate of formation remain uncertain. Arthroscopic findings (Rushton, Dandy and Naylor 1983; Leyshon et al. 1984), suggest that carbon implants were only covered by tissue and did not provide a basis for the formation of new ligaments. Thus McKibbin (1983) anticipated failure, since the friable carbon filaments have to remain as a load-bearing structure. Amis, Campbell and Miller (1985) showed that it took six months for reconstructed calcaneal tendons to regain normal strength; it seemed probable that, within the knee, tissue augmentation would take longer, giving ample opportunity for degradation of a prosthetic ligament. Stability after replacement has been little investigated, though this is the primary objective of ligament repairs. Only Cabaud, Feagin and Rodkey (1982) appear to have measured anterior drawer stability after experimental ligament replacement.

We present experiments in rabbits which examined tissue ingrowth into polyester or carbon fibre ligament replacements and measured the stability of the reconstructed joints.

MATERIALS AND METHODS

Implants. Carbon implants were made from Courtauld's Grafil type XA-S, with 24,000 filaments of nine micron diameter; their breaking strength was approximately 800 N. Polyester implants were made from ICI Terylene 50 f 20 type 100, with 5,000 filaments of 15 micron diameter, giving a breaking strength of 430 N. Previous work (Amis 1985) had shown that the natural anterior cruciate ligament had a mean breaking strength of 415 N in the mature California-breed rabbits with a mean body weight of 4.5 kg chosen for this investigation. The polyester implants were a good match for stiffness as well as strength, while carbon implants were both stronger and stiffer. All implants had a simple parallel-fibred structure to allow tissue ingrowth; a braid, for example, would strangle delicate ingrowth when the implant was loaded.
Operative technique. Under anaesthesia, and through a lateral parapatellar incision, the left anterior cruciate ligament was excised from 26 rabbits. A 2 mm hole was drilled from the anteromedial tibial facet, to emerge at the cruciate ligament insertion. The looped end of the implant was passed through a transverse hole in the tibial crest, then its free end passed through the loop, giving secure anchorage. The free end was passed proximally through the drill hole, then through the intercondylar notch and “over the top” of the lateral femoral condyle. Two holes were then drilled across the femur and the implant divided into bundles, which were passed through the transverse holes and tied together so that the implant was taut, and anterior drawer instability was prevented. The knot was stabilised by a Nylon ligature, and the wound closed. A bandage protected the wound for a few days and antibiotics were administered during and after operation. The animals were kept in standard cages.

Histology. Two rabbits with carbon and two with polyester implants were killed at each of 12, 18, 24 and 36 weeks postoperatively. Three animals with each implant type had their operated knee radiographed at six and 12 weeks. Immediately at death the knees were flushed with 6.3% glutaraldehyde in 1 M cacodylate buffer (Sabatini, Bensch and Barnett 1963) and two sections of the anterior cruciate ligament were taken from the mid-point of the joint space, for scanning electron microscopy (SEM), and transmission electron microscopy (TEM). All specimens were fixed further in 6.3% glutaraldehyde in cacodylate buffer, then post-fixed in 1% osmium tetroxide and dehydrated in acetone. The SEM specimens were critical point dried and gold coated. The TEM specimens were embedded in Araldite, thin sectioned and stained with uranyl acetate and Reynold’s lead citrate. The bones were decalcified in EDTA and processed for light microscopy.

Mechanical tests. Stability testing was performed on four rabbits with carbon implants, four with polyester, and two controls with no implant. All were killed 24 weeks postoperatively. Each intact knee was assessed by cyclic anteroposterior drawer testing at 90° flexion in an Instron 1122 materials testing machine (Fig. 1). The crosshead moved at 10 mm/min, its direction being reversed when a tibial displacing force of 20 N (approximately 45% body weight) was reached; a chart recorder drew load-displacement curves after three loading cycles. This was repeated twice: once after transection of all tissues crossing the joint except the cruciates and again after transection of the anterior cruciate, if one was present.

RESULTS

Gross appearances. All animals appeared to walk normally within one month of operation, but anteroposterior instability developed later in both the control rabbits with excised ligaments, and in seven of the 12 with carbon replacements. The five intact carbon implants and all 12 polyester implants had become covered by a thin sheath of pale pink tissue within the joint. There was no reconstitution of the ligament in the two excision cases, as expected. Large clumps of carbon were adherent to the joint lining after carbon implant failure.

Histology. The bone tunnels contained bundles of implant surrounded and infiltrated by fibrous tissue attached to bony trabeculae. There was no evidence of bone formation approaching the implants, nor of erosion where the implants emerged from the tibial plateau; this was confirmed by the radiographs.

Light microscopy revealed fibroblasts and fibrous tissue among the polyester filaments within the joint space; carbon implants were not examined in this way because of breakages, the intact implants being used for electron microscopy. The initial response to both types of implant appeared to be fibrous tissue covering. Subsequent ingrowth within the polyester implants started close to bone and advanced across the joint space within the fibrous sheath from both directions.

Scanning electron microscopy. There was a conspicuous
absence of tissue among the carbon filaments (Fig. 2), with only occasional clumps of material; no increase was seen in the tissue response up to 36 weeks. There was much more tissue within the polyester implants; it had penetrated into their centres even in the mid-joint space (Fig. 3).

Transmission electron microscopy. The intact carbon implants showed a thin fibrous sheath surrounding the carbon filaments. At 18 weeks this sheath contained numerous active fibroblasts with collagen fibres and carbon debris. By 36 weeks the outermost layer consisted of bundles of collagen fibres, the majority of which were parallel to the implant; a few fibroblasts remained. Isolated carbon filaments were seen within the deeper layers of the sheath at all times; by 36 weeks they were always surrounded by multi-nucleate giant cells which were enclosed by collagen fibres (Fig. 4). There was no evidence of tissue ingrowth in the centre of the carbon implants at 18 weeks; only small amounts of tissue were present by 36 weeks (Fig. 5).

The polyester implants had a well organised sheath, with bundles of collagen fibres aligned in the direction of loading, plus a few fibroblasts. As the polyester implants did not break up, there were no isolated filaments embedded in the tissue sheath. Strands of tissue ingrowth, containing groups of collagen fibres in their centres, were seen at the earliest time of observation at 12 weeks (Fig. 6). By 36 weeks there was good ingrowth into the centre of the implants, with fibroblasts and bundles of collagen fibres, most of them parallel to the polyester filaments (Fig. 7). Well defined pockets containing amorphous electron translucent material, thought to be synovial fluid, were associated with some of the polyester filaments (Figs 7 and 8). The tissue at the periphery of the polyester implants was densely packed and regularly organised collagen, with occasional fibroblasts (Fig. 8). The collagen fibres often adhered very closely to the polyester, with penetration and organisation between polyester filaments only 1.5 μm apart (Fig. 9). The collagen had formed bundles or layers of fibres, aligned with the direction of loading or wrapping concentrically around the implant filaments.

Mechanical testing. Virtually all the anteroposterior stability of the normal knees derived from the cruciates; transection of the anterior cruciate allowed gross tibial subluxation, with little secondary restraint. The rabbit thus seemed to depend on the anterior cruciate ligament to a much greater extent than man. Normal joints had no anteroposterior slackness; the anterior drawer test at 20 N (45% body weight) produced a mean displacement of only 0.64 mm (Fig. 10). Cutting the anterior cruciate did not change the posterior displacement curve significantly, but only 1 N produced 2 mm anterior displacement. Similar results were obtained knees after ligament excision (Fig. 10).

Three knees with broken carbon implants gave results similar to those of control excisions; one knee with an intact carbon implant showed 1.4 mm slackness and deflected anteriorly by 2.6 mm at 20 N. Two knees with polyester implants were not examined because of operative and preparation errors; the other two showed 0.6 and 1.5 mm slackness, and allowed tibial displacements of 1.7 and 3.2 mm at 20 N (Fig. 10).

DISCUSSION

Although Jenkins (1978) reported that carbon fibres were separated by the ingrowth of collagen, the arthroscopic findings of Rushton et al. (1983) and Leyshon et al. (1984) suggested that the appearance of "neoligament" was illusory; the carbon remained unincorporated within a sleeve of fibrous tissue, which in the majority of cases was not complete between one and four years after operation. Forster and Shuttleworth 630

Fig. 2

Scanning electron micrographs of the centre of the mid-joint section of implants. Figure 2 – Carbon filaments (arrowed) showing the occasional clumps of associated material (m) at 24 weeks postoperatively (x 250). Figure 3 – A polyester implant at 24 weeks postoperatively showing large irregular clumps of associated tissue (x 135).
Figure 4–In the inner part of the fibrous sheath, 36 weeks after operation, a carbon filament (cf) is surrounded by a multi-nucleate giant cell (g). Bundles of collagen fibres (C) surround the giant cell. This reaction was seen around all isolated carbon filaments at this stage (x 4500). Figure 5–At 36 weeks, in the centre of the carbon implant, there were only small areas of tissue (arrowed) among the filaments (x 4500).

Figure 6–The centre of the polyester implant at 12 weeks showed tissue ingrowth (arrowed) between the polyester filaments (P). Collagen fibres were also present (C) (x 2500). Figure 7–At 36 weeks, the centre of the polyester implant shows good tissue ingrowth, with numerous active fibroblasts (f) and bundles of collagen fibres (C) surrounding the polyester filaments (P). Some polyester filaments are surrounded by well defined pockets containing electron translucent material (arrowed), thought to be synovial fluid (x 2500).
(1984) found that the tissue response was extremely variable, while Valentin (1984) found no evidence of a new ligament and considered that synovial structures did not tolerate carbon fibre.

Our results show a consistent response to carbon fibres, which explains the differences between previous findings. Intra-articular carbon fibre does not provide a basis for tissue ingrowth or integration; the host response is to encapsulate it in fibrous tissue, thus isolating it. Fragmentation of the implant liberates carbon filaments, and smaller debris which can migrate, and provokes a multi-nucleate giant cell response within the deeper layers of the collagenous sheath (see Fig. 4); low magnification light microscopy allows this to be interpreted as carbon fibres being separated by ingrowth of collagen. The multi-nucleate giant cells indicate chronic inflammation; in normal healing, this is followed by repair of damaged tissues. However, if the irritation persists, tissue necrosis will ensue (Cheville 1983). Mendes et al. (1985) found active inflammation and necrotic foci within a carbon neoligament 18 months after implantation; the failure of cells to remove carbon fragments led to a failure in the healing mechanisms.

The suggestion that carbon fragments are removed by macrophages (Forster et al. 1978) is not supported by our observations. To be ingested the particles must be of the order of 0.5 μm; the carbon filaments are 8 μm in diameter and normally much longer, hence leading to the formation of multi-nucleate giant cells. Wolter (1983) has reported that 1 μm particles were phagocytosed, but that filaments provoked a multi-nucleate giant cell response and subsequent scarring.

Since our findings are pessimistic in comparison to those of others, the difference needs explanation. One possibility is implant variation, but the new XA-S fibres used in this study have 90% less impurities than the A-S fibres available commercially as implants. We believe that the sole reason for our negative findings is that we have looked more closely. Carbon is not amenable to thin sectioning and a new unpublished technique was developed to allow examination of the centres of the implants. Without this, Figure 5 could not have been produced and the micrographs, as in previous studies, would have been confined to the much better-looking periphery, where implant fragments were embedded in the fibrous sheath. In addition, electron microscopy allowed the tissue response to be examined in great detail.

Figure 8 – At 36 weeks the periphery of the polyester implant shows well organised bundles of collagen fibres (C) packing the space between the polyester filaments (P). Some filaments were surrounded by a thin layer of amorphous material (arrowed) (x 4250). Figure 9 – In another region of the same implant there is no layer of amorphous material surrounding the polyester filaments (P). Collagen fibres (arrowed) are seen next to the polyester; the collagen is well organised through a gap of less than 1.5 μm between the filaments (x 9500).
The initial biological response to polyester was similar to that with carbon, an encapsulation in fibrous tissue, which is a common reaction to a relatively inert implant (Davila, Lautsch and Palmer 1968). A similar reaction to polyester has been noted in the calcaneal tendon of sheep (Amis et al. 1984) and in arterial grafts (Tilney and Boor 1975). The functional orientation of collagen fibres associated with the polyester filaments is thought to indicate load bearing by the neoligament. This contrasts with the random array found by Arnoczky, Warren and Minei (1986), but their velour material was more extensible than the canine cruciate ligament, so it would not have carried load in use; this highlights the importance of using a properly-engineered implant, with suitable strength and stiffness, if the development of a neoligament is desired.

Tissue ingrowth was slow. Figure 7 shows that collagen proliferation was not complete in the middle of the polyester implant at the middle of the joint, even after nine months. The human cruciate ligament is three times longer than that of the rabbit, so maturation of collagen across a human knee may take two years.

The polyester implants contained well defined spaces which were thought to contain synovial fluid; this might aid movement between fibres (Mendes et al. 1985). Synovial fluid can provide sufficient nutrition by diffusion to sustain healing within the knee joint without vascular perfusion (Lundborg and Rank 1978); this could occur in an artificial ligament.

Without ingrowth, carbon implants may have to act as load-bearing prostheses for life, so failure might be anticipated (although not at first apparent clinically) as fragmentation progresses. Structures which do not accept tissue ingrowth are subject to fatigue, abrasion of the carbon implants showed that large forces were imposed, since the implants were initially stronger than the natural ligament. Thomas, Turner and Jones (1987) found carbon implant failures in every case, but started with weaker implants and inserted them through a drill hole in the femoral condyle. Lengthy immobilisation is not practicable in rabbits, so damage probably occurs before the tissue is encapsulated; polyester implants do not suffer this loss of strength. The use of the “over the top” route avoided fraying at a femoral drill hole, the rabbit knee being too small for the chamfering of sharp edges recommended by Strover (1985). The rabbit proved convenient for examining biocompatibility, but long-term implantation in larger animals is necessary to provide realistic mechanical data.

The report by King and Bulstrode (1985) that carbon debris from extra-articular reconstruction migrated into the joint throws doubt on the wisdom of using it even in the “retro-synovial” placement described by Strover (1985). Also, since King and Bulstrode used

![Diagram](image-url)
polyactic acid-coated carbon fibres, their finding throws doubt on the efficacy of the degradable coatings intended to avoid the migration of carbon fragments (Alexander, Weiss and Parsons 1983). Our study, in conjunction with clinical reports, suggests that carbon fibres should not be implanted within joints. In contrast, the benign integration of polyester filaments should prompt further investigation.

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REFERENCES


