COMPARISON OF THE STRUCTURE OF NEOTENDONS INDUCED BY IMPLANTATION OF CARBON OR POLYESTER FIBRES

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Carbon-fibre and polyester-fibre implants of comparable dimensions were used to replace the calcaneal tendon in 30 sheep. The neotendon produced in proximity to the polyester fibres was denser, more collagenous and more closely adherent than that in the carbon-based neotendon. Fragmentation of the carbon caused continuing cellular reaction which was associated with a poor collagen response.

Tendon repair has always provided problems for orthopaedic surgeons. Over the years many different types of prosthetic implant have been used in attempts to promote a good functional result (Henze and Mayer 1914; Grau 1958; Rogers 1970; Murray and Semple 1979). Jenkins et al. (1977) reported the use of carbon fibre to replace the calcaneal tendon in sheep and in rabbits. They claimed that carbon fibre induced the formation of new tendon and that in this respect it was fundamentally different from all other forms of tendon-replacement materials. Carbon fibre has since been used in other sites (Jenkins 1978) and in other species (Vaughan and Edwards 1978; Littlewood 1979; Goodship et al. 1980; Jenkins and McKibbin 1980; Weiss et al. 1981).

Carbon is said to have advantages over other implant materials because it is a basic constituent of tissues (Benson 1971), and is inert. Kenner, Williams and Eatherly (1973) implanted graphite rods intraosseoously for one year and found no change in the tissues, or in the mechanical properties of the carbon.

Polyester fibre is relatively inert in vivo: Levine (1968) showed that there was no change in its strength or its extensibility 17 months after implantation. Polyester is the most commonly used material for the construction of implants requiring tissue ingrowth, such as arterial grafts which have been commercially available since the mid 1950s (Hoffman 1977). Long-term studies (up to seven years) of such grafts have shown that tissue ingrowth takes place without adverse reaction (De Bakey et al. 1964).

Polyester has been used for several experimental tendon devices (King, Dunn and Bolstad 1975; Amstutz, Coulson and David 1976; King, McKenna and Statton 1977; Frazier 1981). The advantages of polyester implants over carbon in orthopaedic surgery are as follows: (i) its mechanical compatibility with the surrounding tissues can be ensured by designing the implant to have similar stiffness and energy-absorption characteristics to the structure being replaced; (ii) it is not liable to brittle fractures or disintegration during the implantation procedure as it has happened with carbon implants (Jenkins and McKibbin 1980); (iii) as polyester does not fragment, it improves the prospects of removing the implant in the event of infection.

The aim of the present study was to compare carbon fibres and polyester fibres (polyethylene terephthalate) when used to replace the calcaneal tendon in sheep.

MATERIALS AND METHODS

An initial trial on 10 sheep was carried out, followed by a second series of experiments on 20 sheep using a modified procedure.

The aim of the initial experiments was to reproduce exactly the work of Jenkins et al. (1977) who used carbon-fibre implants* consisting of three tows, each of 10 000 filaments nine micrometres in diameter, plaited together. The implants were uncoated (carbon fibres are normally supplied with a thin epoxy resin coating to promote bonding when used to reinforce plastics). They were washed in methylethyl ketone, as described by Jenkins et al. (1977). The plaited structure had an ultimate strength of 900 newtons at one per cent extension. Since the implants were doubled in use this gave sufficient strength to withstand the forces put on the calcaneal tendon when sheep canter (this was estimated to be 1.2

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* Grafil, type HM-S (high modulus or stiffness), supplied by Courtaulds, plc, UK.
kilonewtons at 26 per cent extension from work carried out by Jayes and Alexander, 1978).

The polyester implants* were constructed by plaiting together three bundles, each of 5000 filaments 15 micrometres in diameter. The polyester did not contain delustrant particles (which are normally added during production to give a matt-white colour) and was undyed. This implant had a strength of 1.3 kilonewtons at 26 per cent extension and had a similar bulk to that of the carbon implant. When doubled it was found that it had similar stiffness to the natural calcaneal tendon using tensile tests.

**Clinical procedure.** Carbon-fibre or polyester-fibre implants were used to replace the left calcaneal tendon of 10 adult sheep of various breeds in the manner described by Jenkins et al. (1977). The skin and paratendon were incised posterolaterally under general anaesthesia in a sterile field. The tendon was cut at the musculotendinous junction and at its calcaneal insertion to produce a defect approximately seven centimetres long. The implant was passed through the semitendinous zone of the muscle in an anteroposterior direction 2.5 centimetres proximal to the cut end (thus passing through the tendon sheets which are oriented in the frontal plane). The ends of the implant were threaded in opposite directions through a horizontal hole in the os calcis, and then tied together immediately proximal to the bone. In the first four sheep the load was spread within the muscle by three stout silk sutures which supported the implant; a soft bandage was applied after closure of the wound. The remaining six sheep underwent a similar procedure, except that the proximal loop of each implant was supported by an extensive sling of silk sutures in an attempt to spread the load widely across the tendinous material within the distal part of the muscles; after closure of the wound these limbs were supported by a plaster of Paris splint for four weeks.

The experiment was redesigned after initial trials to overcome the problems encountered. It was decided to splint the limbs for at least two months. Since the implants would be augmented by a considerable deposition of fibrous tissue by the time that load-bearing started, it was decided to reduce the bulk (and hence the strength) of the implants. The design was changed to a double-twist construction, using a total of 20,000 carbon filaments or 12,000 polyester filaments.

In a further group of 20 sheep the calcaneal tendons were excised between a point two centimetres distal to the musculotendinous junction and the attachment to the os calcis. The implant was folded in half, and the loop formed at the midpoint was passed distally through a vertical hole in the os calcis. The loop was brought proximally round the end of the bone so that it completely encircled the os calcis and the free ends of the implant were passed through the loop (Fig. 1). This gave a strong distal fixation without having to knot the implant. The two free ends of the implant were then fixed through the tendon stump and the musculotendinous junction by the Bunnell method, knotted together anteriorly (to avoid subcutaneous knots) and the knots secured by binding with a No. 1 silk suture. A plaster of Paris splint was applied and kept in place for two months. The sheep were killed at intervals of 1½, 2, 3, 4, 6, 9, 12 and 18 months.

![Fig. 1](https://example.com/fig1.png) Diagram to show how the implant loop was passed through a vertical hole in the os calcis to encircle the end of the bone completely.

**Histological examination.** Histological examinations of the tendons were carried out, the tissue being fixed, sectioned, mounted and stained by routine methods. In addition to light microscopy, scanning electron microscopy was used to examine sections from four sheep killed three months after operation. Transmission electron microscopy also was used in 14 cases. The popliteal and iliac lymph nodes from the left side were also fixed and examined by light microscopy and by transmission electron microscopy.

*Transmission electron microscopy.* Small pieces of tissue were taken from the central and peripheral regions of the neotendons for transmission electron microscopy. Tissue was also taken from the popliteal and iliac lymph nodes from the left side. The tissue blocks were fixed in 6.25 per cent glutaraldehyde in 0.2 molar cacodylate buffer (pH 7.4) (Sabatini, Bensch and Barnett 1963) and post-fixed in one per cent osmium tetroxide in 0.2 molar cacodylate buffer (pH 7.4) (Palade 1952). The specimens were dehydrated in acetone and embedded in Araldite. Ultra-thin sections were stained with uranyl acetate and Reynolds' lead citrate, and viewed in a Philips EM 400. *Scanning electron microscopy.* Specimens of neotendon were prepared for scanning electron microscopy by fixation in 6.25 per cent glutaraldehyde in 0.2 molar cacodylate buffer (pH 7.4) (Sabatini et al. 1963) and dehydrated in ethanol. The tissue blocks were dried to the critical point and sputter coated with gold.

* Terylene, 50F20 type 100, supplied by ICI.
RESULTS

Clinical examination. Initial series (10 sheep). The four sheep (two with carbon-fibre implants, and two with polyester-fibre implants) which had only soft-bandage support bore weight on the affected limb on the first or second day after operation, with no evidence of hyperflexion of the hock. By the fourth day, however, abnormal flexion of the joint commenced and became more obvious with time; up to 60 degrees of deformity occurred after removal of the support at 16 days.

The six sheep in which rigid support was applied for up to four weeks also showed evidence of excess flexion of the hock after removal of the splint, although never to such a severe degree.

Six of the 10 sheep (three with carbon implants, and three with polyester implants) showed clinical evidence of sepsis, and in three of them (two with carbon implants and one with a polyester implant) skin erosions were present near the attachment of the prosthesis to the os calcis, adjacent to the knots in the implant. These animals bore little weight on the affected limb. Histologically, the fibres of the implants were surrounded by necrotic debris or masses of inflammatory cells. No organised tissue was present in the vicinity of the prosthetic fibres, although a thick tubular rim of "neotendon" had formed around the infected core. The tissues from these sheep with infection have not been included in the histological descriptions of tendon structure.

Second series (20 sheep). All the sheep in the second experimental group (and four of those in the initial series) developed neotendons that were thicker than normal tendons. The maintenance of rigid support for two months prevented the development of severe flexion deformities. In the long term, all the sheep so treated regained normal function and locomotion, with no evidence of infection, regardless of the type of implant they had received. There was no discernible difference in the gross appearance resulting from the choice of implant material. The treated limbs were identifiable by their thickened tendons.

Histological examination. Light microscopy. Separation of fibres occurred in both the polyester and carbon implants due to the ingrowth of cells and of collagen in a fashion similar to that reported by Forster et al. (1978). A thick layer of neotendon was formed on the surface of the implants. The more densely packed the carbon or polyester fibres, the poorer the collagen response. Towards the edges of the implant, its fibres were often widely separated and therefore surrounded by more mature, more collagenous fibrous tissue than was seen in the densely-packed core of the implants. The neotendon rim also showed dense collagenous material.

Giant cells and macrophages were found in association with both types of implant material. In some sections the fibres of the implant appeared to be surrounded by the cytoplasmic processes of megakaryocytes. Eosinophils and plasma cells were also present. As the interval after operation increased more fibrous tissue was seen—even towards the centre of the implant where the collagen fibres were more widely spaced. Thicker bands of fibrous tissue occurred along the course of ingrowing vessels. The fibres of the implant were often bunched into groups and surrounded by thicker zones of fibrous tissue but with each filament separated from its neighbours by thinner, more delicate fibrous tissue.

Throughout the experimental period the cellular response in the sections of carbon-fibre neotendons was always more obvious than that in the polyester neotendons. Fragments of carbon fibre were scattered throughout the sections and were often seen to be engulfed by phagocytes. The fibrous tissue response to the carbon-fibre implant remained fairly delicate and, although there were many dilated blood vessels present, the perivascular collagen response was poor. The amount of collagen present in the carbon neotendon after six months was comparable to that of the polyester neotendon at three months after operation.

In the sections of polyester neotendon taken soon after operation the fibrous-tissue response was also very delicate. However, by three months more collagen material was present, with evidence of thick bands around blood vessels and polyester fibres; there was a progressive increase in fibrous material thereafter. During the same three-month period the cellular response diminished.

Three of the neotendons (one carbon, two polyester) showed evidence of low grade localised infection even though no clinical evidence had been noted. However, except in the regions very close to such foci, where many polymorphonuclear leucocytes were present, the cellular and fibrous response was as described above.

The lymph nodes showed a non-specific post-operative reaction to both types of implant. Occasional fragments of carbon filaments were found in the lymph nodes several months after operation.

Transmission electron microscopy. The typical picture of the carbon neotendon six weeks after operation showed carbon fibres surrounded by large active phagocytes (Fig. 2). The phagocytes were closely associated with the surface of the carbon fibres and showed many cytoplasmic processes; there were no collagen fibres in the vicinity of the carbon fibres (Fig. 2). In contrast, the polyester neotendon at six weeks showed an abundance of collagen fibres and numerous active fibroblasts (Fig. 3). The collagen fibres were densely packed around the polyester fibres and showed early signs of organised orientation. Phagocytes were also present, but were neither as numerous as in the carbon neotendon nor were they associated with the polyester fibres (Fig. 3). There was a conspicuous absence in the polyester neotendon of spaces filled with oedematous fluid which were seen in the carbon neotendon (Figs 2 and 3).

By eight weeks after operation there were more collagen fibres present in the carbon neotendon than at
six weeks (Fig. 4). The collagen fibres were never seen in
close association with the carbon fibres, but were present
in the areas between carbon fibres. Most of the collagen
fibres were oriented parallel to the long axis of the carbon
implant; the others ran in an oblique direction (Fig. 4).
The most conspicuous feature of the carbon neotendon
at eight weeks was the presence of numerous multinu-
cleate giant cells which encircled the carbon fibres (Fig.
4). Fragments of carbon-like material were seen in
vesicles within the cells (Fig. 4).

At nine weeks after operation the polyester neo-
tendon contained densely packed collagen fibres and
numerous fibroblasts. The collagen fibres had a more
definite orientation, with the majority of the fibres
parallel to the long axes of the polyester fibres. The other
collagen fibres encircled the implant filaments, and thus
were perpendicular to the long axes of the polyester
fibres. The polyester neotendon had a similar appearance
at 12 weeks.

The most conspicuous feature of the carbon implants
at 12 weeks was the presence of small fragments of
carbon fibre scattered throughout the neotendon (Fig. 5).
Giant cells were seen closely juxtaposed to the carbon
fibres and eosinophils were conspicuous (Fig. 6); collagen
fibres were randomly scattered amongst the phagocytes
and oedematous debris.

The maximal density of collagen fibres in the carbon
neotendon was established by six months (Fig. 7) and a
large fibroblast population was seen at four months; at
neither stage were collagen fibres observed juxtaposed to
the carbon fibres. Multinucleate giant cells were still
conspicuous at six months. The overall density of the
carbon neotendon was at its maximum at six months,
after which there was a general decrease in the number
of collagen fibres and a change in their distribution. By
12 months the collagen fibres were confined to small
isolated bundles associated with fibroblasts. Large areas
of the central core of the carbon neotendon showed “clear
regions” at 12 and 18 months (Fig. 8) (these regions
would have been filled by oedematous fluid in vivo).

The polyester neotendon at six months had a dense
well-ordered structure (Fig. 9). The majority of the
collagen fibres were parallel to the long axes of the
polyester fibres. During the observations it became
obvious that the polyester fibres always retained the same
orientation relative to other polyester fibres, but that the
carbon fibres became increasingly disorganised.

One year after the insertion of the polyester implant
there was some localised cellular reaction. Polymorpho-
nuclear leucocytes were observed closely associated with
the polyester fibres, but the collagen fibres were still
present in well organised dense bundles. Polyester
neotendons examined 18 months after insertion had
collagen fibres densely packed and mostly oriented
parallel to the long axes of the polyester fibres. The cells
present 18 months after implantation were fibroblasts.

Scanning electron microscopy. Figures 10 and 11 show
samples of polyester and carbon neotendons at three
months after operation. The surfaces of the polyester
fibres (Fig. 10) were smooth, while the carbon fibres (Fig.
11) had numerous uneven ridges running along their
length.

The tissue surrounding the polyester fibres was very
dense and well organised (Fig. 10). A concentrically-
layered arrangement of fibres was discernible adjacent to
the polyester. It was conspicuous throughout the obser-
Transmission electron micrographs of the carbon neotendon. Figure 4—At eight weeks fibroblasts (f) were occasionally seen and were associated with the collagen fibres (cf). The carbon fibre (c) was surrounded by large, multinucleate giant cells (g). (×5000.) Figure 5—At 12 weeks fragments of carbon fibre (f) were scattered throughout the neotendon. The collagen fibres (cf) were randomly oriented and loosely packed. (×11 000.) Figure 6—Eosinophils (e) were more conspicuous at 12 weeks although giant cells were still in evidence juxtaposed to the carbon fibre (c). Cell debris was scattered throughout the oedematous areas. (×6000.) Figure 7—The maximal density of active fibroblasts (f) and collagen fibres was established at six months (×5000). Figure 8—By 12 months the collagen fibres (cf) were confined to isolated bundles. There were large oedematous areas surrounding the carbon fibres (c). (×5000.)
vations that the polyester neotendon showed a consistent picture with a predominance of solid collagenous material, with only occasional cavities up to 50 micrometres in diameter.

In contrast, the carbon neotendon showed a very variable picture: some zones contained no collagen fibres while others did contain solid collagenous material. The tissue surrounding the carbon filaments was mostly porous and with no apparent organisation (Fig. 11). Numerous large cavities (up to 200 micrometres in diameter) were seen in the tissue matrix of the neotendon (Fig. 11). Cellular material (ranging from cellular processes to large whole cells) was observed adhering to the surface of the carbon filaments (Fig. 11).

DISCUSSION

The results of these experiments showed that, given a suitable operative technique and postoperative care, both carbon and polyester implants may be used to bridge defects of calcaneal tendons. In this situation a large cyclic load is imposed on the implants and their anchorages. The strength of the implants was ensured by appropriate mechanical testing and design, but the anchorages to the host tissues presented problems initially. In the first series, subcutaneous knots were too close to the os calcis and caused skin erosions with subsequent infection. Similar problems have been noted with implants at both the ankle and the knee in humans (Jenkins and McKibbin 1980); these workers overcame the problem by using a technique which avoided subcutaneous implant masses. There were also difficulties in securing the implants proximally into the musculotendinous junction. The implants migrated despite efforts to spread the load widely into the muscles using an extensive sling of sutures. The migration of the implants was responsible for flexion deformities. This was overcome by applying external support for eight weeks.

Valdez, Clark and Hanselka (1980) found that horses with implants could bear weight at 30 days, but recommended support and rest for 90 days. Jenkins and McKibbin (1980) immobilised human patients for six weeks. It was found that filamentous implants must be protected from bearing weight initially to allow adequate ingrowth of fibrous tissue. In time the fibrous tissue infiltrates the anchorages and strengthens them.

The light, transmission electron, and scanning electron microscopic studies all showed that the polyester-based neotendon developed collagenous tissue very similar in appearance to normal tendon. From the earliest stages the collagen fibres were able to form a close relationship to the polyester fibres; this led to the development of a well-organised collagenous tissue within the implant which became increasingly dense with time. In contrast, the carbon fibres created a massive cellular reaction in the early stages and a close bond never developed between the collagen and carbon fibres. The maximal development of collagen occurred at six months in the carbon-based neotendon. After six months the tissue reaction continued, leading to a steady decrease in the network of collagen fibres and producing large oedematous areas. These observations conflict with those of Jenkins et al. (1977) who reported no signs of foreign-body reaction. The presence of macrophages, lymphocytes and multinucleate cells have also been reported by Forster et al. (1978), Valdez et al. (1980), and Ráliš and Forster (1981).

The significance of the fragmentation of the carbon fibres has stimulated two opposing interpretations. The early interpretations suggested that the gradual mechanical weakening of the carbon implant due to fragmentation was responsible for the production of new tendon (Forster et al. 1978). Jenkins and McKibbin (1980) proposed that the carbon induces a new structure in a unique way and that the neotendon grows stronger rather than weaker with age. However, our present observations suggest that fragmentation of the carbon fibre stimulates a constant inflammatory response; the macrophages and multinucleate giant cells appeared to be continuously trying to remove the carbon-fibre debris. The presence of carbon particles within macrophages has also been reported by Valdez et al. (1980). It is thought that the presence of large numbers of phagocytes would inhibit rather than stimulate fibroblast activity and subsequent formation of collagen. Indeed, by 12 months the phagocytes were still present, closely associated with fragments of carbon fibre, and the proportion of collagen fibres had decreased leaving large oedematous areas. Further evidence of the inflammatory response to the fragments of carbon fibre was the presence of carbon particles in the lymph nodes. This lymphatic response
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Fig. 10

Scanning electron micrographs. Figure 10—The polyester fibres (p) at three months had smooth surfaces. The surrounding tissue was densely packed and appeared to be organised into concentric layers around the polyester fibres. (× 1700.) Figure 11—The carbon fibres (c) at three months had ridged surfaces. Large irregular cell masses (t) were seen adhering to the surface of the carbon fibres; the surrounding tissue was loosely organised. (× 4000.)
was also noted by Jenkins (1978). In contrast there was no long-term inflammatory reaction in the polyester neotendon which strongly indicated that polyester fibres were sufficiently inert in vivo.

There have been conflicting reports on the cause of tissue reaction to carbon fibres. Hench and Ethridge (1975) suggested that it is the surface texture of the carbon fibres that is responsible for tissue reaction. Ráliš and Forster (1981) noted a greater tissue reaction to pure carbon fibres than to those with an epoxy-resin coating. Other workers have reported that the carbon-fibre coatings are carcinogenic and responsible for adverse tissue reactions (Alexander et al. 1978). The extreme stiffness of carbon fibres causes mechanical incompatibility with soft tissues.

The surface chemistry of the carbon fibres may have an influence on the tissue response. The surface of the carbon fibres is affected by the oxidative treatment which is applied routinely during production and whose purpose is to aid bonding to the surroundings when these fibres are used to reinforce plastics. The surface chemistry of all types of treated carbon fibres (whether normal or high modulus) is likely to be similar, with their characteristic of biocompatibility dominated by the presence of reactive oxide side-groups (Johnson and Masters, personal communication 1981).

It is probable that differences in the purity of the solid material within the carbon filaments is of minor importance in comparison to their surface characteristics. The carbon fibres used for implantation do not have an epoxy-resin "size" on their surface, but may bear traces of lubricating chemicals used to aid handling during manufacture. Two methods have been used to remove the lubricants. The first method involved washing fibres in methyl ethylketone (MEK) (Jenkins et al. 1977; Valdez et al. 1980); however, Alexander et al. (1978) reported that this did not completely remove the surface coating. Valdez et al. (1980) claimed that washing with MEK reduced the granulomatous tissue reaction. The second method was to wash carbon fibres in acetone (Jenkins and McKibbin 1980).

Medical-grade polyester fibres have been used where tissue ingrowth is required, but not all polyester is suitable. The toxicity of polymeric implants can change with the presence of low molecular weight fractions, which may leak out in vivo or because of the presence of additives. The material used in the present study was undyed, unlike most polyester sutures, and did not contain delustrent. The surface of the filaments was contaminated by traces of a silicone oil lubricant used during manufacture, but this did not appear to compromise the biocompatibility of the implant. Such lubricants may be removed by using cold petroleum ether.

We feel that the smooth, well-oriented polyester fibres, with stiffness and extensibility similar to the calcaneal tendon, provided an ideal template for the development of a neotendon. Forster et al. (1978), however, said that the production of the new tendon in their experiments followed gradual mechanical weakening of the carbon implant due to fragmentation—a process which would allow an increasing load to fall on the newly formed collagen of the new tendon. The results of our present study do not support this hypothesis. The carbon fibre appeared unable to provide a suitable template because of fragmentation. It therefore seems likely that the carbon-based neotendons depend for their load carrying capacity on the collagenous sheaths, which quickly form as a surface covering for the implants.

Forster et al. (1978) had no success with implants of silk or nylon. This was thought to be due to two factors. First, both silk and nylon are extensively degraded in vivo (Levine 1968; Williams 1971; Hoffman 1977; Rostoker and Galante 1979); thus, there is a continuing reaction at the implant/tissue interface rather than the stable conditions allowed by a relatively inert implant material. Secondly, there is the effect of physical size: silk and nylon filaments are much coarser than carbon filaments, and the larger fibre-bundles evoke a different encapsulating response from the host. Larger implants cause the formation of sheets of collagenous connective tissue at the artificial interface which thicken in response to mechanical trauma (implant slippage) and gradually outstrip the blood supply (Davila, Lautsch and Palmer 1968). Fine fibres, however, break down the artificial interface to a microscopic level so that each is captured by a collagen envelope compatible (because of its small size) with the immediately adjacent blood supply (Davila et al. 1968). This allows the interstices of the implant to be invaded by well-vascularised tissue which has the potential to mature to a tendon-like structure. Bruck (1973) suggested that viable implants should have fibres with a diameter between one and five micrometres while Davila et al. (1968) suggested an upper size limit of 50 micrometres. These authors have suggested that a bundle of fine filaments of any inert material would induce the formation of a collagenous cord which could reorganise to form a tendon-like structure in response to repetitive stretching.

In this study a close bond between collagen and carbon fibres never developed. However, in the polyester neotendon close bonds between the collagen fibres and implanted fibres did develop and remained stable. The size (15 micrometres in diameter), the strength and the elasticity, along with the smooth surface and chemical inertness of the polyester fibres, all contributed to the successful formation of a load-bearing neotendon.

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