PRE-MARKET APPLICATION

CFS™ FOR TREATMENT OF KNEE-LIGAMENT INJURIES

PLASTAFIL, INC.

VOLUME 3

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MECHANICAL TESTING OF THE CFS™ AND ITS FIXATION DEVICES

ABSTRACT

The bollard and toggle were loaded to failure in a manner that simulated their ordinary clinical use. The devices failed at average ultimate loads of 1495 newtons and 1154 newtons (at 0.5 cm/sec), respectively. The system consisting of the implant and fixation devices failed at an average ultimate load of 307 newtons. Comparable results for the fixation devices and the system were obtained at a rate of 0.1 cm/sec.

INTRODUCTION

The CFS™ and its fixation devices were mechanically tested to failure under conditions intended to reasonably simulate those occurring during clinical use of the CFS™.

METHODS

Carbon fibers, bollards, and toggles identical to those used in the clinical studies were used for mechanical testing. All tests were done in air at room temperature immediately after the implant had been removed from its sterile packaging.

Fresh human diaphyseal bone (tibia and femur) was used for the tests. The bones were cut into 2-inch specimens, and the marrow was removed by scraping and immersion of the specimen in acetone (1-4 hours) and ethyl alcohol (1 hour). For measurement of the ultimate mechanical strength of the bollard, a quarter-inch hole was drilled through the cortical faces of the specimen (approximately 0.5 inches along the bone axis, from one cut surface), and a brass screw was used to fix it to a metal plate that could conveniently be clamped in an Instron Testing Machine (testing machine). A bollard hole was drilled and radiused in line with and approximately 1 inch from the brass screw (approximately 0.5 inches from the specimen's other cut surface). The bollard was positioned in the bollard hole, and set and expanded with Kevlar fibers looped around the bollard shank.

For measurement of the ultimate mechanical strength of the toggle, a 4.8-mm hole was drilled through the cortical faces of the specimen (approximately 1.0 inches from either cut surface), and the hole was radiused on both cortical faces. A loop of Kevlar fibers was passed through the hole in each cortical face and looped around the toggle that was positioned across one of the holes. The bone specimen was clamped with its axis horizontal and the toggle was tested to failure by vertically loading the Kevlar fibers.

To test the ultimate mechanical strength of the system consisting of the implant and its fixation devices, the bollard and toggle were each attached to a bone specimen (as described above) and a carbon-

fiber bundle was used to transmit the load between the fixation points. The carbon-fiber bundle was looped around the toggle (lower bone specimen) according to normal clinical practice, and the carbon fibers were wrapped around the shank of the bollard one and one-half times after which the bollard was set and expanded. The tail of the carbon-fiber bundle was held to the bundle itself with three cerclage sutures (simulating normal clinical practice).

All tests were conducted to failure; they were performed at cross-head velocities of 0.1 and 0.5 cm/sec.

RESULTS

The ultimate mechanical strength of the bollard, toggle, and implant system are given in Tables 1-3. The tolerances given in the tables are standard deviations.

TABLE 1. Mechanical Characteristics of the Bollard (n, number of bollards tested).

RATE (cm/sec)	n	ULTIMATE LOAD (N)	STIFFNESS (kN/m)
0.1	6	1651 ± 334	204 ± 35
0.5	6	1495 ± 271	208 ± 42

TABLE 2. Mechanical Characteristics of the Toggle (n, number of toggles tested).

RATE (cm/sec)	n	ULTIMATE LOAD (N)	STIFFNESS (kN/m)
0.1	10	1419 ± 385	167 ± 71
0.5	10	1154 ± 345	187 ± 68

TABLE 3. Mechanical Strength of the Implant System (n, number of implants tested).

RATE (cm/sec)	n	ULTIMATE LOAD (N)	STIFFNESS (kN/m)
0.1	11	289 ± 67	92 ± 40
0.5	7	307 ± 71	67 ± 27

The bollards failed by shearing of the bollard head or by pulling out of the bone, with approximately equal frequency at both cross-head

velocities. The toggle failed by shearing at its midpoint. The system failed most often by breakage of the carbon fibers at the bollard; in 4 tests at 0.1 cm/sec and 1 test at 0.5 cm/sec the CFS failed at the toggle.

DISCUSSION

When the toggle and bollard were loaded under conditions relevant to their intended clinical use, they withstood loads beyond those which resulted in failure of the system consisting of the implant and the fixation devices. Consequently, neither the bollard nor toggle is likely to fail *in vivo* when used in the intended manner.

Under the conditions tested, the implant system was capable of sustaining ultimate loads of approximately 300 Newtons when loaded at 0.1-0.5 cm/sec. Thus the data indicates that, immediately following implantation, the CFS™ provides a maximum prosthetic fixation (on average) of about 300 N.

MICROBIOLOGICAL TESTING

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COMPARISON OF *IN VITRO* GROWTH OF *STAPHYLOCOCCUS EPIDERMIDIS* ON CARBON FIBERS AND POLYVINYL CHLORIDE

ABSTRACT

The propensity of carbon fibers to support colonization by *Staphylococcus epidermidis* was compared to that of polyvinyl chloride. Both materials were incubated in saturated bacterial cultures, and examined after 1-12 days of incubation. Throughout the time of observation, the carbon fibers were more resistant to colonization and glycocalyx formation by *S. epidermidis* than was polyvinyl chloride.

INTRODUCTION

Implanted biomaterials that become infected frequently exhibit an adherent biofilm known as a slime layer or glycocalyx (1). The glycocalyx facilitates bacterial attachment to biomaterials, and its composition and organization are important factors in promoting resistance to antibiotic therapy (2-4). Factors affecting the chemical composition and structure of the glycocalyx include the kind and strain of bacteria, and the physical and chemical nature of the implant surface (and perhaps its geometry).

Glycocalyx formation on an implant is an undesirable event because it may potentiate infection. It is therefore pertinent to determine the potential of carbon fibers in this regard, and to ascertain whether the material behaves differently from other implanted materials.

The purpose of this study was to observe the extent of glycocalyx formation on carbon fibers (CF) *in vitro*, compared with that exhibited by polyvinyl chloride (PVC) (a plastic polymer in common use for indwelling tubes). *Staphylococcus epidermidis* was chosen because it is known to be capable of colonizing PVC pediatric endotracheal tubes in 2-3 days.

METHODS

A bundle of 10,000 carbon fibers (each 8 μm in diameter) was cut into 1-cm sections, tied with sutures at each end to prevent separation of the fibers, and boiled to remove the glycerin/gelatin coating. PVC endotracheal tubes (3.5 mm ID, Sheridan, Portex, or Shiley) were cut into 1-cm sections, and the CF and PVC were placed in sterile tubes containing 20 ml of trypticase soy broth (Difco Laboratories). About 0.1 ml of coagulase-negative *S. epidermidis* (ATCC #12228) at a concentration of 10^5 CFU/ml was added to the experimental tubes; the control tubes were identical, except that bacteria were not added. The tubes were capped and incubated without shaking at 37°C for 12 days, and 2-4 samples of CF and PVC were removed from each of the experimental and control tubes after 1, 2, 4, 8, 10, and 12 days. The samples were fixed in 1% gluteraldehyde for 4 hrs, processed through graded alcohols, placed in hexamethyldisilazane for 5 minutes, allowed to dry at

room temperature, mounted on aluminum stubs, gold sputter-coated, and viewed with an AMR scanning electron microscope at an accelerating voltage of 25 kV.

RESULTS

The results are given in Figures 1-6. Bacterial colonization of PVC depicted in Figures 1-6 is representative, and depicts the extent of colonization at the indicated time of recovery; in contrast, the colonization depicted on the carbon fibers indicates the most dense colonization found at the indicated time of recovery. At 24 hrs after inoculation with S. epidermidis, clusters of 1-6 bacteria were seen on the PVC (Figure 1). Few bacteria, generally single or in pairs, were seen on the CF (Figure 1).

After 2 days, larger and more numerous colonies enclosed in a glycocalyx were observed on the PVC (Figure 2). Single bacteria or small colonies without a glycocalyx were seen among the larger colonies. Bacteria were infrequently seen on the CF (Figure 2).

After 4 days, the bacterial colonies on the PVC were larger and more densely distributed (Figure 3). Again, little bacterial growth and colony formation was seen on the CF (Figure 3).

After 8 days of incubation, the PVC colonies were still growing in size and density (Figure 4). Occasional small clusters of bacteria colonies without a glycocalyx were seen on the CF (Figure 4).

After 10 days, the glycocalyx-enclosed colonies on the PVC were larger but less dense per unit area (Figure 5). A few enclosed colonies were found on the CF (Figure 5). PVC and CF recovered after 12 days did not differ in appearance from the samples recovered after 10 days.

A relatively minor amount of broth aggregates and generalized debris was deposited on both the PVC and CF (Figure 6). The appearance and amount of the material deposited in the control cultures was essentially the same at 10 and at 12 days.

DISCUSSION

After 10 days' incubation, PVC was 40-50% covered with glycocalyx formations. A further increase at 12 days was not observed, possibly because the nutrients in the culture media had been exhausted thereby limiting further bacterial growth. In contrast, only scanty bacterial colonization and glycocalyx formation was observed on CF at all times of observation; the CF surface was more than 99% free of all material including bacteria, glycocalyx, and media debris. It is not clear whether the apparent relative resistance of CF to bacterial colonization is associated with the chemical nature of the substrate (pure carbon compared to a polymer chain), surface structure or hardness (CF

is significantly harder than PVC), or whether the geometry of the specimen (fibers compared to a sheet surface) are of fundamental importance in determining the observed difference in bacterial growth. It is clear, however, that CF was more resistant to colonization and glycocalyx formation by S. epidermidis than was PVC.

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(3) Gristina, A.G., Price, J.L., Hobgood, C.D., Webb, L.X. and Costerton, J.W.: Bacterial colonization of percutaneous sutures, Surgery 98:12-19, 1985.

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FIGURE 1. Representative areas of PVC (1 and 2) and CF (3 and 4) recovered after 1 day from cultures of S. epidermidis (* here and in succeeding Figures indicates the same area in paired photomicrographs).

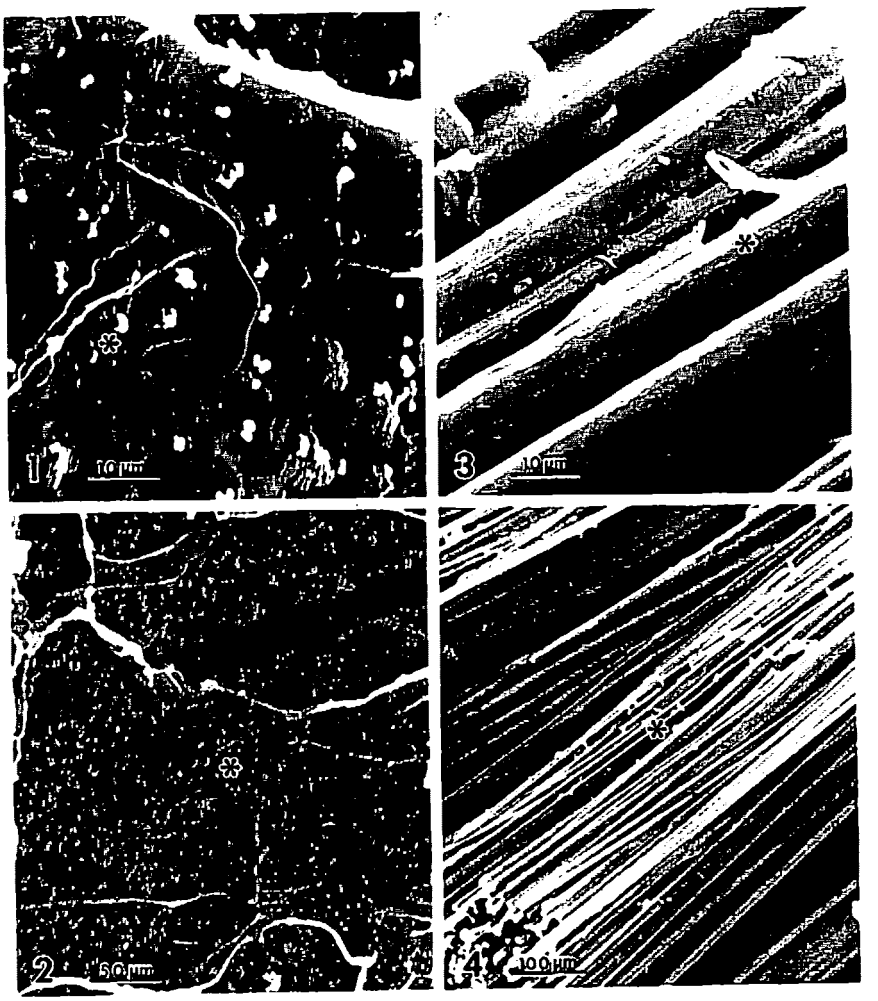


FIGURE 2. Representative areas of PVC (5 and 6) and CF (7 and 8) recovered after 2 days from cultures of S. epidermidis.

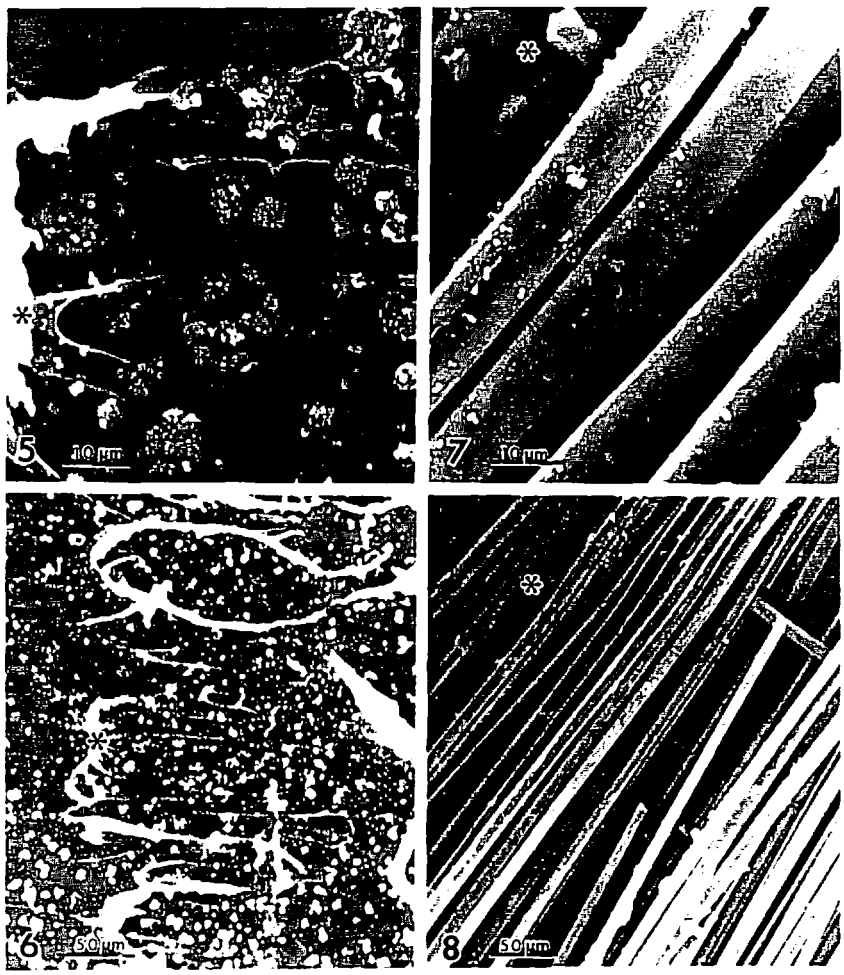


FIGURE 3. Representative areas of PVC (9 and 10) and CF (11 and 12) recovered after 4 days from cultures of S. epidermidis.

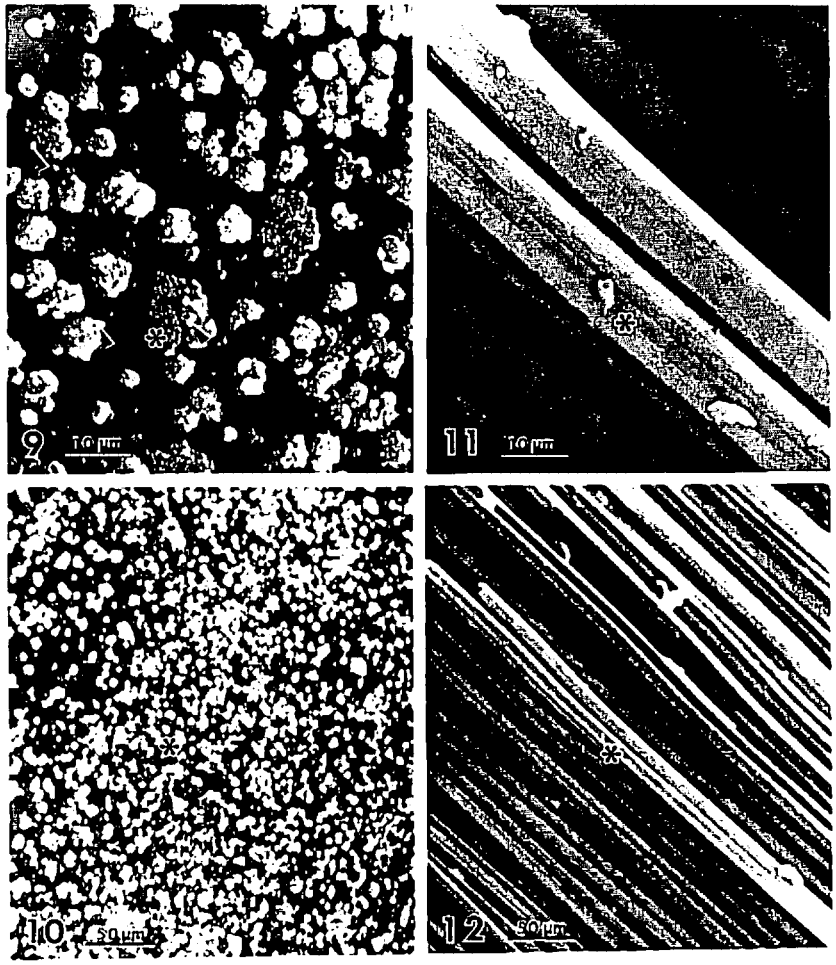


FIGURE 4. Representative areas of PVC (13 and 14) and CF (15 and 16) recovered after 8 days from cultures of S. epidermidis.

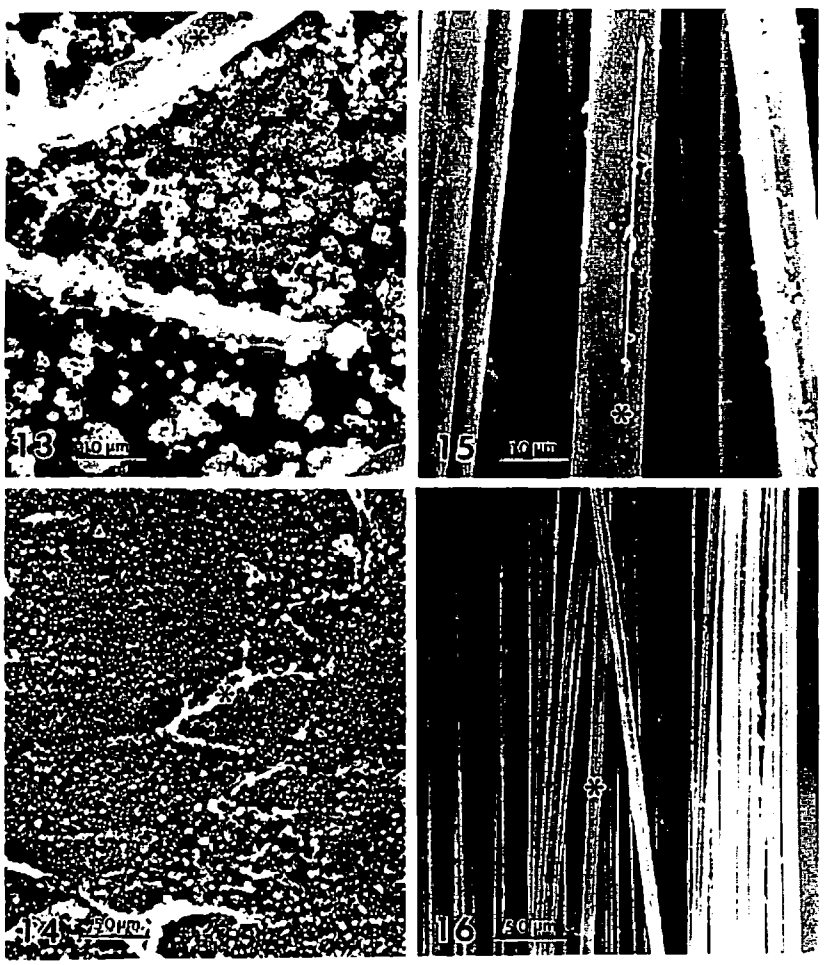


FIGURE 5. Representative areas of PVC (17 and 18) and CF (19 and 20) recovered after 10 days from cultures of S. epidermidis.

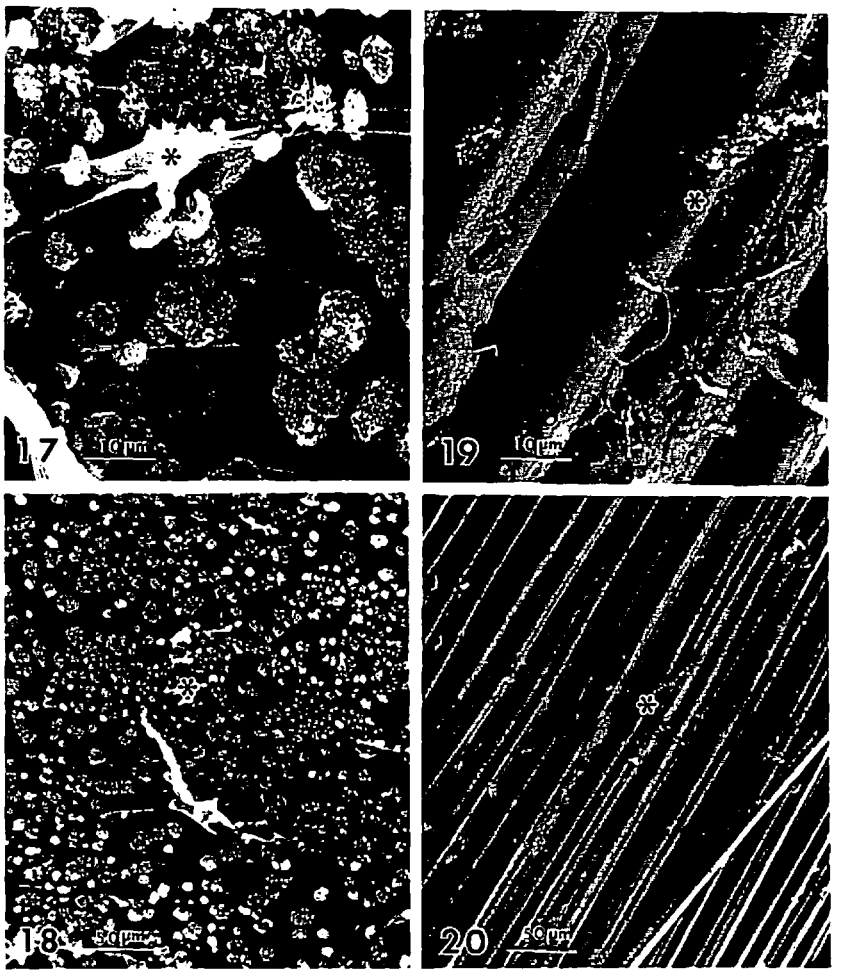
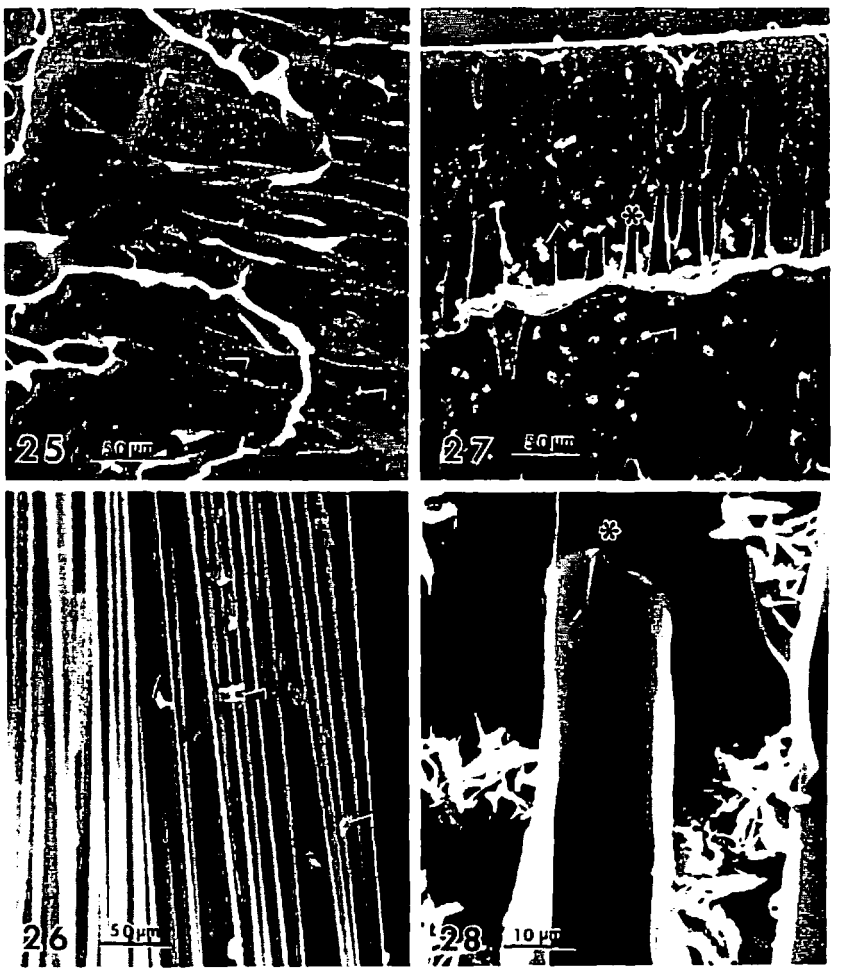


FIGURE 6. Representative areas of PVC (25, 27, 28) and CF (26) recovered after 4 (25,26) and 8 (27,28) days in broth (without bacteria).



ANIMAL TESTING

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TISSUE REACTIONS TO CARBON-FIBER IMPLANTS IN MICE

ABSTRACT

Carbon fibers, 8 microns in diameter, were implanted in mice either adjacent to sciatic nerve, in axillary fat, or in quadriceps muscle. The tissue reactions to both intact fibers and fiber debris were studied at 1 and 5 weeks postoperatively. The implants elicited a thin encapsulating granulation tissue and a mild giant-cell reaction. Orientation of fibroblasts and new fibrous tissue occurred along the axis of the intact carbon fibers, out to a lateral distance of about 5 cell thicknesses. No histomorphological changes attributable to the carbon were seen in any of the host tissues studied. Transport of carbon particles to the lymph nodes was not observed.

INTRODUCTION

Depending on the particular repair, carbon fibers may be routed through fat or near nerves or muscles, and it was therefore necessary to determine whether the fibers elicited any adverse reaction in these tissues. The primary aim of the present study was to examine these questions in mice for implant times up to 5 weeks. We also examined the regional lymph nodes to determine whether carbon fibers were transported via the lymphatic system.

METHODS

The carbon fibers employed in this study were identical to those used in the clinical study. An unsized bundle of 8-micrometer fibers, 95% minimum purity, was heated at 300°C for 20 minutes to remove residual organic contaminants. Carbon-fiber debris present in the bundle as a consequence of the original manufacturing process was removed by passing the bundle through a water bath and wiped with pure cellulose. The carbon-fiber bundle was then passed through a bath of gelatin and glycerine (and wiped again with cellulose) to facilitate handling of the carbon fibers during surgery.

White, male, DUB/ICR mice, 8-10 weeks of age, were used in this study. Carbon fibers were implanted in the form of 5-mm, 5,000-fiber bundles (Fibers), or as carbon-fiber debris (Debris) (1.0-300 microns) produced by grinding the carbon fibers with a mortar and pestle. Each Fiber and Debris implant weighed approximately 5 mg.

Under ether anesthesia, the Fibers were implanted either in a surgically-created pouch in the quadriceps muscle, immediately adjacent to the sciatic nerve, or in the axillary fat pad; they were held in place at the implant site using only the surrounding tissue. The Debris was implanted by suspending it in 0.2 ml of sterile saline, and injecting it into the same muscle or fat. Place-

ment of the Debris suspension adjacent to the nerve however, was done under full view after surgically exposing the implant site.

Six groups of mice, each containing 6 animals, were implanted with carbon. For each of the three tissues, one group of mice received Fibers and one group received Debris. In addition, 6 groups of sham-operated mice corresponding to the 6 treatment groups were included in the study. Each sham group received surgery (or injection) that was identical to that received by the mice in the treatment group with which they were paired except that carbon was not implanted.

Half of the mice in each treatment and control group were killed one week after being implanted, and the remaining animals were killed at 5 weeks post-implantation. Both the implant site and the regional lymph nodes were recovered, fixed in 10% buffered formalin, embedded in Paraplast, sectioned at 6-10 micrometers, and stained with hematoxylin and eosin or Wright's stain. The lymph nodes were recovered and examined by polarizing light microscopy to determine the presence of carbon particles. The histomorphological descriptions given here relate to the responses observed in the implanted animals that were distinct from the changes that occurred in the sham-operated animals.

RESULTS

Nerve

At 1 week post-implant, the Fibers were covered with granulation tissue 5-10 cells thick at the mid-shaft of the implant, increasing to approximately 30 cells thick over the ends of the implant. The majority of the cells of the granulation tissue were fibroblasts, with some polymorphonuclear leukocytes, and fewer lymphocytes. Epithelioid cells at various stages of development were present. Less mature fibroblasts were located towards the periphery of the granulation tissue, and exhibited no apparent orientation. Spindle-shaped fibroblasts located up to 5-8 cell thicknesses from the tissue-carbon interface were oriented in the direction of the Fibers. The connective tissue in this region was more dense and more organized than that present in the more peripheral granulation tissue. The most highly organized granulation tissue occurred in the region between the individual strands located in the outer portion of the implant. In this region, both the tissue and the cells were oriented along the fibers. No cells or tissue were seen in the deep portion of the implant.

At 1 week post-implant, the Debris was enclosed in fibrous tissue, 3-5 cells thick, that had no apparent organization or orientation; it was equally distributed between and outside the implant particles themselves. The cells were mostly fibroblasts and polymorphonuclear leukocytes, with occasional lymphocytes. Epithelioid cells were seen, but in no greater numbers than those seen in association with the Fibers. Occasional macrophages were seen.

At 5 weeks post-implant, the granulation tissue surrounding both the Fibers and the Debris was more dense, mature, and vascularized, and less inflammatory-cell infiltrate was seen. Individual strands in the periphery of the Fibers had become completely surrounded by orientated tissue (Figure 1) which appeared to penetrate deeper into the implant itself (compared to 1 week post-implant). In contrast to the reasonably well-defined capsule seen around the Fiber implants, a distinct capsule was not seen around the Debris. The histomorphological picture presented by the Debris at 5 weeks post-implant was one of a relatively unorganized granulation tissue underlying, and extending up to several cell thicknesses beyond the Debris itself (Figure 2). Giant cells rarely occurred in any of the implants (an average of fewer than 1 per high-power field). In the Fiber implants, the giant cells were usually found localized at the ends of the carbon strands (Figure 3).

No necrosis, fibrosis, inflammation, or demyelinization of nerve was seen at either 1 or 5 weeks post-implant (Figure 2). The mice exhibited no apparent functional neurological deficits.

Fat

At 1 week post-implant, the granulation tissue that formed around both the Fibers and Debris was greater than that observed in the muscle. Despite this apparent relative irritability of fat, only occasional macrophages or giant cells were seen. At 5 weeks post-implant, the granulation tissue that enveloped both the Fibers and Debris was similar in amount to that seen in the muscle (Figures 4 and 5). More giant cells were seen in the Fiber implants (usually located at the ends of the strands), as compared to the Debris implants, but the overall giant-cell reaction remained very low (fewer than 1 per high-power field).

No histopathological changes attributable to carbon were seen in the fat tissue itself.

Muscle

The reaction in muscle to both Fibers and Debris (Figure 5) was similar to that seen in the nerve implants. A reactive process in the muscle fibers themselves occurred at 1 week post-implant, manifested by an increased number of muscle-cell nuclei and a slight basophilia, but this effect also occurred in the sham-operated animals. In both groups, the zone of reactive muscle extended up to 12 cell thicknesses from the carbon, and it was not observed at 5 weeks post-implant.

None of the regional lymph nodes in any of the implanted mice contained carbon particles, or showed any other evidence of lymphatic transport of the carbon.

DISCUSSION

The general responses of muscle, nerve, and fat to the presence of carbon in the form of Fibers and Debris were similar. A granulation tissue layer formed around the implants thereby isolating them from the surrounding tissue. No histopathological changes attributable to carbon were seen in either nerve, fat, or muscle.

Giant cells rarely occurred in any of the three tissues, even at ~~5 weeks post-implant.~~ In the Fiber implants, the giant cells typically were found at the ends of the strands. A mild acute inflammatory response -- manifested principally by polymorphonuclear leukocytes and lymphocytes -- occurred at 1 week. The extent of the inflammatory reaction was significantly less at 5 weeks, and the ratio of the number of lymphocytes to the number of polymorphonuclear leukocytes was increased thereby indicating the onset of a chronic inflammatory reaction. The granulation tissue present at 5 weeks was less in extent compared to the tissue present 1 week post-implant, but the degree of vascularity was increased. Thus the overall trend seen in the development of the granulation tissue was a cessation of proliferation of the fibrous tissue itself (accompanied by a maturation of the existing fibrous tissue) and lessening of the inflammatory response, but an ongoing cellular activity as indicated by the presence of capillaries.

There was no evidence of lymphatic transport of the carbon. Occasionally, macrophages in the granulation tissue were seen with small dark granules apparently inside the cell, but it was not possible to confirm actual phagocytic activity or the presence of intracellular carbon. The main response, even to the Debris, was a walling-off reaction.

Little evidence was seen of an orientational effect in the granulation tissue, except within 2-5 cell thicknesses of the peripheral portion of the Fiber implants. At 5 weeks post-implant, the outer carbon fibers had been separated from the main bundle and encapsulated by a thin ring of collagenous tissue. It was only within this region that orientational effects (on both the collagen and the fibroblasts) were observed.

In summary, the carbon fibers were isolated from the surrounding tissue by a thin quiescent fibrous layer -- that was structurally organized only in the immediate vicinity of the Fibers -- and there occurred no morphological changes in the surrounding tissue.

FIGURE 1

Typical appearance of oriented granulation tissue immediately adjacent to the outer strands of the Fiber implant at five weeks post-implant. Hematoxylin and eosin stain. The scale indicates 5 micrometers.

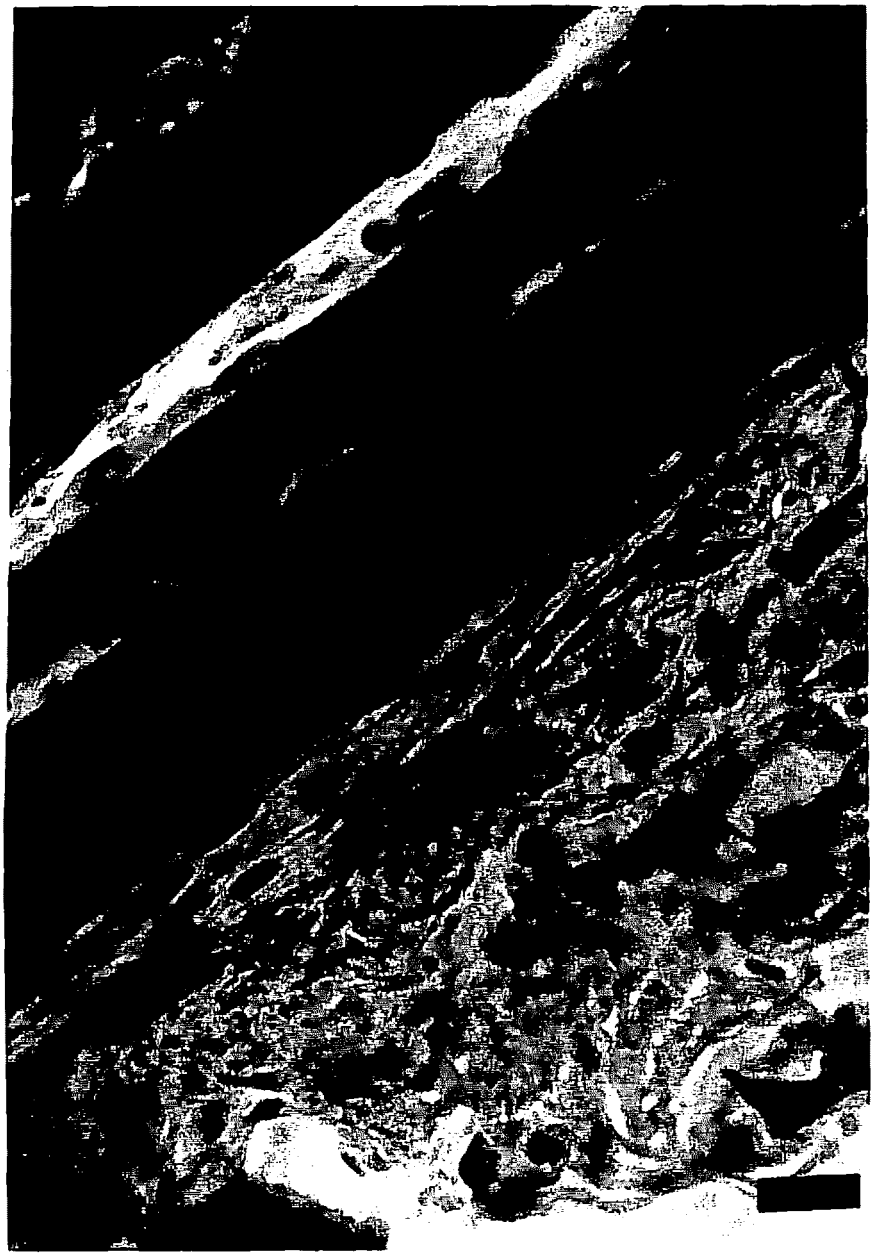

FIGURE 2

Debris embedded in granulation tissue between nerve (top) and muscle (bottom) at 5 weeks post-implant. Note minimal granulation-tissue reaction in the epineurium. The nerve fibers and the muscle appear normal. Hematoxylin and eosin stain. The scale indicates 20 micrometers.

FIGURE 3

The typical clustering of giant cells near the Fiber ends at five weeks post-implant. A portion of the relatively thick (about 30 cell thicknesses) end cap of granulation tissue can be seen. Hematoxylin and eosin stain. The scale indicates 20 micrometers.

FIGURE 4

Debris in fat 5 weeks postoperatively. It is embedded in randomly-organized connective tissue that extends several cell thicknesses beyond the Debris itself. Hematoxylin and eosin stain. The scale indicates 40 micrometers.

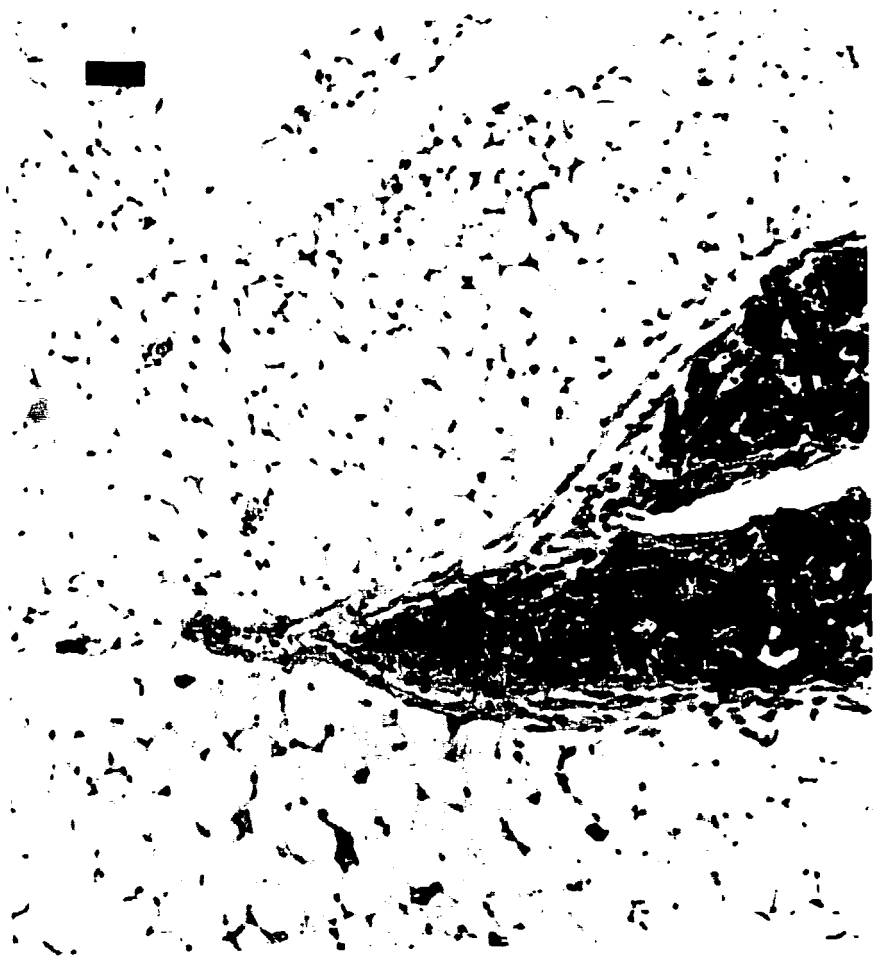

FIGURE 5

Carbon debris in muscle 5 weeks postoperatively. It is isolated from adjacent normal-appearing muscle by a thin layer of granulation tissue. Hematoxylin and eosin stain. The scale indicates 40 micrometers.

USE OF CARBON FIBERS FOR REPAIR OF GASTROCNEMIUS TENDONS IN RABBITS

ABSTRACT

The gastrocnemius and plantaris tendons of rabbits were removed and replaced with carbon fibers or nylon. The rabbits were sacrificed at various post-operative times up to 3 years, and the tissue associated with the implants was studied histologically. The regional lymph nodes were examined for the presence of carbon-fiber debris. Injury-induced fibrosis occurred in the carbon-fiber and nylon reconstructed rabbits; it did not differ between the groups in histological character or amount. Foreign-body fibrosis also occurred in both groups; it was histologically indistinguishable between the groups, but it differed markedly in amount -- significantly more foreign-body induced tissue occurred in the carbon-fiber implanted rabbits. In both groups, observations made after 1 year of implantation regarding histological character and amount of tissue were identical with those made 2 and 3 years after implantation. Transport of carbon particles to the iliac, inguinal, or popliteal nodes was not observed.

INTRODUCTION

The main purpose of this study was to use an appropriate animal model to determine the nature of the tissue reaction to carbon fibers, and the time within which the reaction becomes stabilized. A further goal was to determine whether the tissue formed in association with carbon fibers was unique, or was histologically identical to tissue that forms under other conditions. A third purpose was to determine whether carbon-fiber debris occurred, and could be transported by the lymphatic system to the lymph nodes.

We addressed these questions in the context of a functional repair, so that the data would be pertinent to the clinical study. A functional repair of the rabbit gastrocnemius tendons using carbon fibers was reported (1), but the procedure described did not result in a functional repair in our hands (2). We developed an alternative surgical procedure, showed that it resulted in a functional repair (2), and employed it in this study.

METHODS

Carbon Fibers

The carbon fibers employed in this study were identical to those used in the clinical study. An unsized bundle of 10,000 8-micrometer fibers, 95% minimum purity, was heated at 300°C for 20 minutes to remove residual organic contaminants. Carbon-fiber debris present in the bundle as a consequence of the original manufacturing process was removed by passing the bundle through a water bath and wiping with pure cellulose. The carbon-fiber

bundle was then passed through a bath of gelatin and glycerine (and wiped again with cellulose) to facilitate handling of the carbon fibers during surgery.

Animal Surgery

White, female, New Zealand rabbits (3-5 kg) were used in all studies. After induction of anesthesia (intravenous pentobarbital, 30 mg/kg), the gastrocnemius/plantaris tendon complex of one leg was exposed through a longitudinal incision and the tendon sheath was opened in line with the incision. The plantaris muscle was transsected proximally at the musculotendinous junction and distally at the calcaneus and excised, exposing the two gastrocnemius tendons. A 1-cm segment of each tendon was removed.

In 30 rabbits, the carbon fibers were passed through a transverse drill hole in the calcaneus, then crossed through the distal end of the tendons immediately proximal to the calcaneus and passed longitudinally through the 1-cm gap into the proximal ends of the tendons. A Bunnell-type weave was used proximally, and the fibers exited from the tendons immediately distal to the musculotendinous junction. The ends of the carbon fibers were twisted together and then glued with bone cement (Howmedica). Both gastrocnemius tendons were incorporated in the repair as a single tendon. A long-leg cast (20° flexion at the knee joint and 10° plantar flexion at the ankle joint) was applied for three weeks.

Thirty additional rabbits received the same operative and post-operative treatment except that monofilament nylon (ethylon 2-0) was used instead of carbon fibers. After weaving the nylon through the proximal tendon, the ends were secured with knots.

Twenty rabbits in each group were killed at various times 1-12 months post-operatively and 10 rabbits were killed at 1, 2, and 3 years post-operatively (n=3 or 4 in each group). The implant site was recovered and immediately fixed in formalin.

The popliteal lymph node was examined grossly, and representative specimens were fixed in formalin for histologic evaluation. In 3 carbon-fiber rabbits (recovered 3 years post-operatively), the entire popliteal node was prepared for histologic study. The inguinal and iliac nodes were identified by injecting areas that they drain with Trypan blue, 2-4 hours prior to sacrifice. The nodes were subsequently recovered and prepared for histologic study.

Microscopy

The specimens containing carbon fibers were passed through a graded series of alcohols up to absolute ethanol, propylene oxide (PO), 1:1 mixture of PO and Spurr embedding media, 1:2 mixture of PO and Spurr, and then a pure mixture of Spurr under vacuum. Each

sample was then embedded in a mold and heated at 60°C (±5°C) for 18-24 hours. The blocks were trimmed and cut (0.75 micrometers) on an ultramicrotome (LKB Model 5) using a diamond knife. Other blocks were cut on a diamond saw (South Bay Technology, Model 650) and polished using a graded series of abrasives to a final thickness of approximately 125 micrometers. The tissue sections were stained with Spurlock (Toludine O/basic fuchsin) and coverslipped.

For scanning electron microscopy (SEM), the central portion of the specimen was dehydrated through a graded series of alcohols up to 100% absolute alcohol. It was then frozen using liquid nitrogen and cut traversely using a razor-blade and hammer. The cleaved surface -- which consisted of the implant and adjoining tissue in cross-section -- was subject to critical-point drying (Balzers CPD020), coated with gold (Denton Vacuum Desk-II) and examined in an SEM (AMR 1200) at an accelerating voltage of 25 kV.

The lymph nodes were fixed in formalin, dehydrated, embedded in wax, sectioned at 10 micrometers and stained with hematoxylin and eosin.

RESULTS

Removal of the gastrocnemius and plantaris tendons and stabilization of the gap using carbon fibers or nylon was followed by proliferation of reparative tissue that bridged the tendon gap in about 4 weeks. Most carbon fibers remained associated in a well-defined bundle which became enveloped by a fibrous sheath. During 1-4 months post-operatively, the peripheral fibers in the bundle became encircled by tissue. During 4-12 months post-operatively, the process of tissue ingrowth into the bundle continued, resulting in a carbon-fiber bundle whose average diameter increased continuously.

A fibrous sheath also formed around the nylon suture, and was observed in every specimen recovered after about 1 month post-operatively.

At 1 year post-operatively, the surgical site in the rabbits reconstructed with nylon consisted mainly of an intrasynovial tube of tissue surrounding the nylon sutures (Figure 1). The tissue was the normal injury response in the rabbit following removal of the gastrocnemius and plantaris tendons (injury fibrotic reaction); microscopically, it resembled normal tendon. In addition to injury fibrosis, a further fibrotic reaction occurred due to the presence of the nylon suture (Figure 2). Each nylon suture was enveloped and isolated from the injury fibrosis by a relatively more cellular and less dense form of connective tissue, 10-15 micrometers thick, that formed in intimate association with the nylon. This foreign-body response occurred together with the injury response, but produced a histologically distinct form of connective tissue.

At 1 year post-operatively, the surgical site in the carbon-fiber rabbits consisted of injury fibrosis that was histologically indistinguishable from that seen in the rabbits reconstructed using nylon, together with tissue that formed in intimate association with the carbon fibers (Figure 3). Most carbon fibers remained together as an identifiable bundle, but some fibers flared from the main bundle and appeared (via SEM) to be directly enveloped by the injury fibrosis (Figure 3). Each flared carbon fiber, however, was itself surrounded by a concentric ring of foreign-body fibrosis (Figure 4). The carbon-fiber bundles were permeated with tissue that was histologically identical to that which encircled the nylon (Figure 5).

No macroscopic or microscopic changes were seen in either the nylon or carbon-fiber reconstructed rabbits at 2 years or 3 years post-operatively, compared to the respective observations made after 1 year. With regard to each material, the histological appearance of both the injury-induced and foreign-body-induced fibrous tissue in the 2- and 3-year rabbits was indistinguishable from that seen after 1 year.

The amount of tissue ingrowth (per unit length of the carbon-fiber bundle) was estimated from calculations of the cross-sectional area occupied by the carbon-fiber bundles (Table 1).

TABLE 1. Average Cross-sectional Area of Carbon-Fiber Bundles

POST-OPERATIVE TIME (years)	N	AREA (mm^2)
1	4	6.1 ± 1.1
2	3	6.3 ± 1.9
3	4	6.2 ± 1.7

Carbon-fiber debris was not observed grossly or microscopically in any lymph node or biopsy. The lymph nodes in the carbon-fiber and nylon reconstructed rabbits were indistinguishable.

DISCUSSION

Excision of the gastrocnemius and plantaris tendons in the rabbit followed by stabilization of the resulting gap using nylon sutures resulted in the proliferation of relatively dense connective tissue that bridged the defect. In addition, the nylon suture itself was recognized as a foreign body, and it elicited a fibrous reaction typical of that engendered by a non-toxic implanted material. The reaction consisted of an apparent attempt to wall off the nylon by formation of a relatively less dense and more

cellular species of connective tissue, about 10-15 micrometers thick. At 1 year post-operatively, the histological appearances of the two forms of connective tissue were clearly distinguishable. No change in the amount or histological character of either species of connective tissue occurred in the animals recovered at 2 or 3 years post-operatively. Qualitatively similar reactions occurred in the carbon-fiber rabbits: They exhibited injury-induced and foreign body-induced fibrotic reactions that were clearly distinct at 1 year post-operatively, and which exhibited no observable changes at 2 or 3 years post-operatively. Both species of connective tissue were histologically identical to the corresponding tissue seen in the nylon reconstructed rabbits; the only salient difference between the two groups was in the amount and organization of the foreign body-induced fibrotic tissue.

The foreign-body reaction induced by nylon produced 10-15 micrometers of connective tissue which was observable beginning 1-3 months post-operatively, and thereafter underwent no further qualitative or quantitative changes. Similar observations were made in the vicinity of individual carbon fibers that flared from the main carbon-fiber bundle. The carbon fibers within the bundle, however, exhibited a range in amount of associated tissue: Some bundle fibers were intimately associated with 10-15 micrometers of connective tissue, while other carbon fibers were devoid of induced tissue.

The average thickness of tissue induced by the carbon fibers can be estimated from the data in Table 1. In a tendon or ligament containing 20,000 8-micron carbon fibers, the average thickness, t, of the fibrous layer surrounding each carbon fiber is $t = (14.14r - 8)/2$ (r in mm, t in micrometers) where r is the effective cross-sectional radius of the carbon-fiber bundles*. An area of 6.2 mm^2 corresponds to a radius of 1.4 mm, which yields $t \cong 6$ micrometers.

The foreign-body tissue induced by nylon was about 10-15 micrometers thick, and the question arises why the average long-term tissue response to the carbon fibers was less than that observed for nylon. One possibility is that the chemical or physical properties of the nylon surface were recognized differently (or to a different extent) than was the carbon surface, resulting in more foreign-body connective tissue. The specific structural or chemical properties of surfaces that govern or determine the nature of the fibrotic response are unknown, and consequently it is difficult to evaluate this possibility. A more likely explanation is that accessibility to the carbon-fiber interstices by collagen-producing cells is sometimes restricted by mechanical factors that tend to bind carbon fibers together (by effectively generating radially-directed forces). Clearly, if physical factors constrict some carbon fibers, tissue ingrowth cannot occur because cells

* "Use of Carbon Fibers for the Repair of the Anterior Cruciate Ligament in Goats", Equation (3) (contained in this PMA).

Prior to 1 year post-operatively, the collagen-producing cells were gaining increasing access to the interstices of the carbon fibers, with the result that the average thickness of foreign-body tissue was increasing. After 1 year, however, average bundle diameter (hence, average thickness of tissue per fiber) was no longer a function of time, indicating that the fibrotic reaction had become stable.

It seems likely that the marked difference in the amount of foreign-body fibrosis between nylon and carbon fibers is due to the large difference in effective surface area exposed to the physiological environment (the carbon fibers have an effective surface area more than 200 times greater than that of the nylon). Although it is clear that the larger surface area of the carbon fibers is associated with significantly more fibrotic tissue, the effect on the structural organization of the induced tissue is more difficult to evaluate. The roughly parallel alignment of the carbon fibers appears to partially constrain the growth of connective tissue, resulting in a relatively organized pattern in which the collagen tends to align along the direction of the carbon fibers. This structuring phenomenon is less conspicuous in the case of the nylon. The precise contribution to the integrity of the reconstruction brought about by the structuring or ordering effect of the induced tissue is unknown.

There are three lymphatic drainage systems in the hind limb of the rabbit (3). The popliteal node drains the lower part of the hind limb, and its efferent channel continues to the iliac nodes. The medial subcutaneous region of the middle and upper leg drain mainly into the inguinal node. A third lymphatic bed consists of vessels that drain the muscles of the lower limb. This lymphatic channel drains the efferent lymphatic from the popliteal node (without entering the node itself) and continues to the iliac nodes.

Phagocytization of carbon was not observed in any connective tissue specimen, and carbon was not observed in any lymph node biopsy. There was apparently no mechanism by which the carbon fibers could be degraded sufficiently to produce particles that could be phagocytized. When India ink was injected intravenously, the carbon was phagocytized by cells in the spleen, liver, bone marrow, and by Kupffer cells (4): When it was injected intraperitoneally, intramuscularly, or subcutaneously, the carbon was phagocytized by cells in the regional lymph nodes (4). But India ink is a colloidal suspension of 30-nm carbon particles (about 10^6 atoms). Such small debris was not implanted (as a contaminant)

* Bundles of carbon fibers (5,000-10,000) that were tied together with loops of nylon suture exhibited essentially no fibrotic ingrowth when recovered from their implant site (pouches in rat muscle) after 1 year.

initially in our study, and the body apparently lacks a mechanism to produce such debris; the absence of carbon-fiber debris in the regional lymph nodes is therefore plausible.

CONCLUSION

When rabbit gastrocnemius tendons are replaced with carbon fibers or nylon, two histologically distinct forms of connective tissue are observed. One tissue is a result of the body's attempt to heal the injury, and the second tissue is a reaction to the presence of a foreign body. At 1 year post-operatively, the histologic appearance and extent of the injury-induced fibrosis in the nylon and carbon reconstructed rabbits were indistinguishable, and this absence of difference persisted at 2 and 3 years post-operatively. After 1 year, the foreign-body induced fibrosis in the carbon and nylon reconstructed rabbits was identical, and consisted of a more cellular and less dense (compared to the injury-induced fibrosis) species of connective tissue. The only salient difference between the two groups was the extent of the foreign-body tissue reaction: The nylon was enveloped by 10-15 micrometers of tissue but each carbon fiber was enveloped (on average) by 6 micrometers of induced tissue. Because there were two nylon sutures but 20,000 carbon fibers, the total amount of induced tissue associated with the carbon reconstructed rabbits was significantly greater. The carbon used in this study was not degraded, phagocytized, or transported to regional lymph nodes.

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(4) Oghiso, Y.: Distribution of colloidal carbon in lymph nodes of mice injected by different routes, Jap. J. Exp. Med. 49:223-234, 1979.

FIGURE 1. SEM cross-sectional view of nylon-suture reconstruction following removal of gastrocnemius and plantaris tendons (1 year post-operatively). The nylon filaments are about 200 micrometers in diameter.

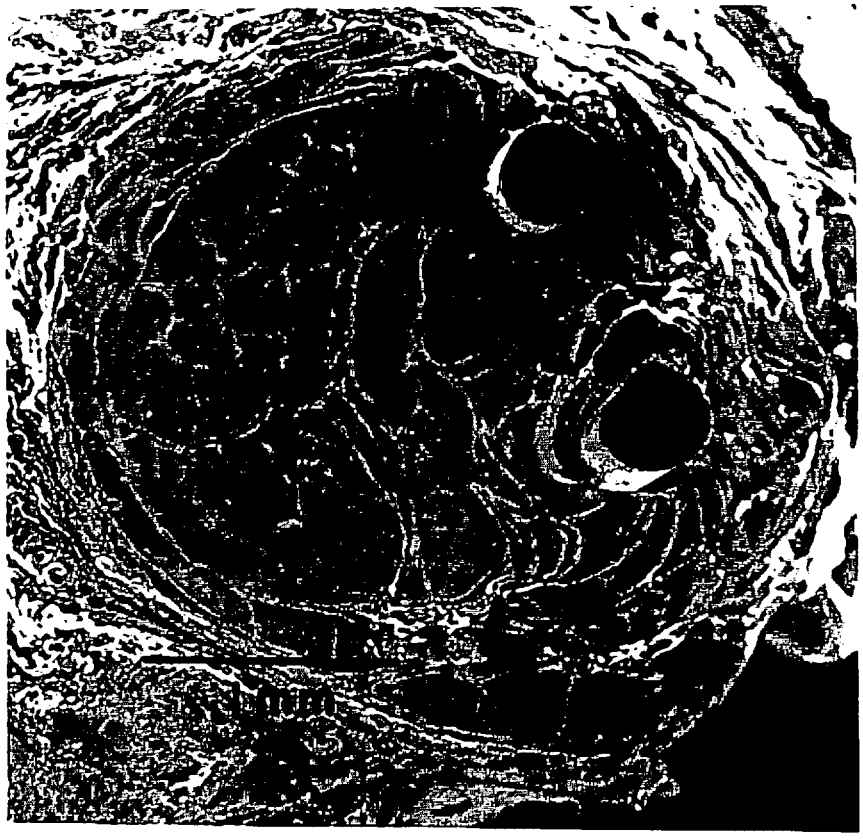


FIGURE 2. Foreign-body induced fibrous tissue occurring in the vicinity of a nylon suture in a rabbit reconstructed using nylon sutures, following removal of gastrocnemius and plantaris tendons. The featureless structure on the left is the nylon suture, adjacent to which is the foreign-body induced fibrosis. The injury-induced fibrosis is located on the right. Specimen cut at 0.2 micrometers, stained with Toludine blue. 100X objective.

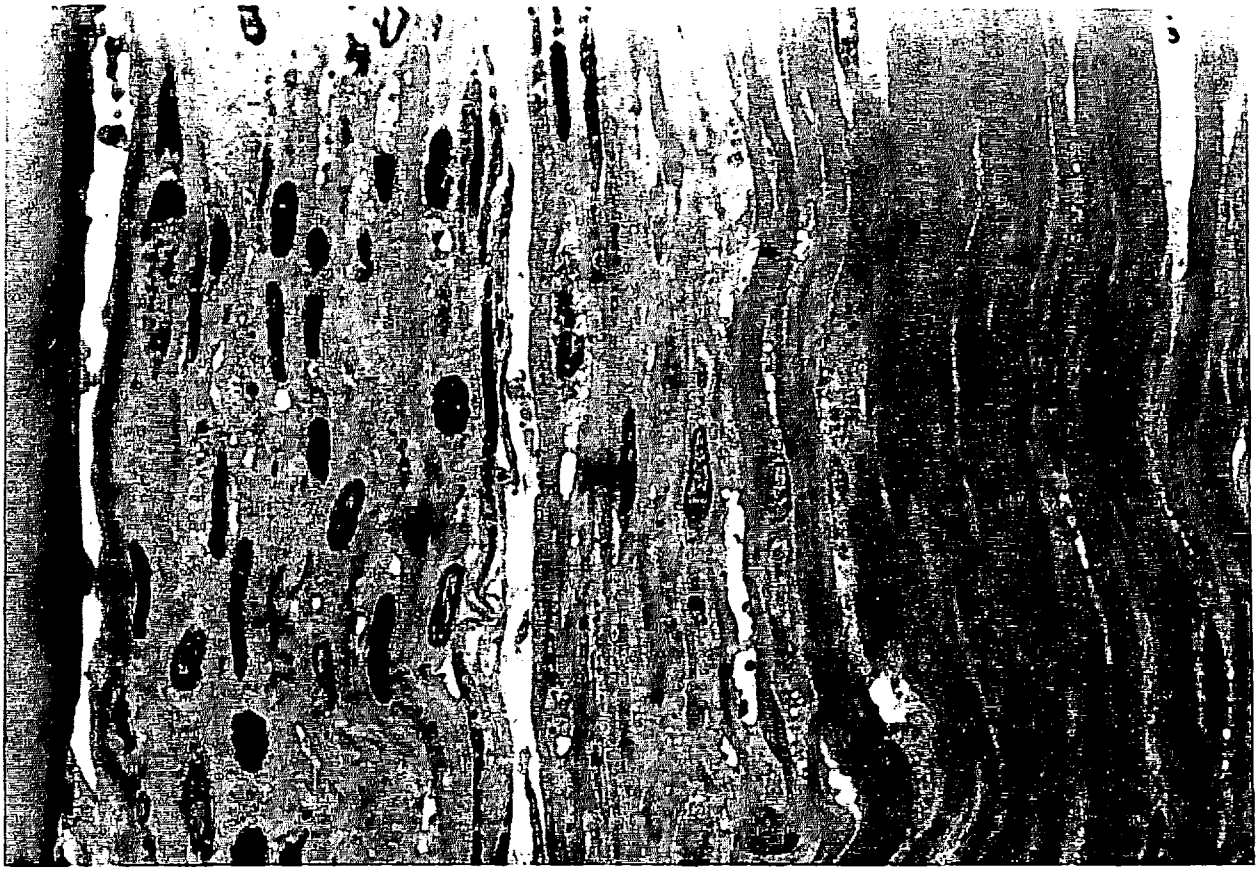


FIGURE 3. SEM cross-sectional view of carbon-fiber reconstruction following removal of gastrocnemius and plantaris tendons (1 year post-operatively).

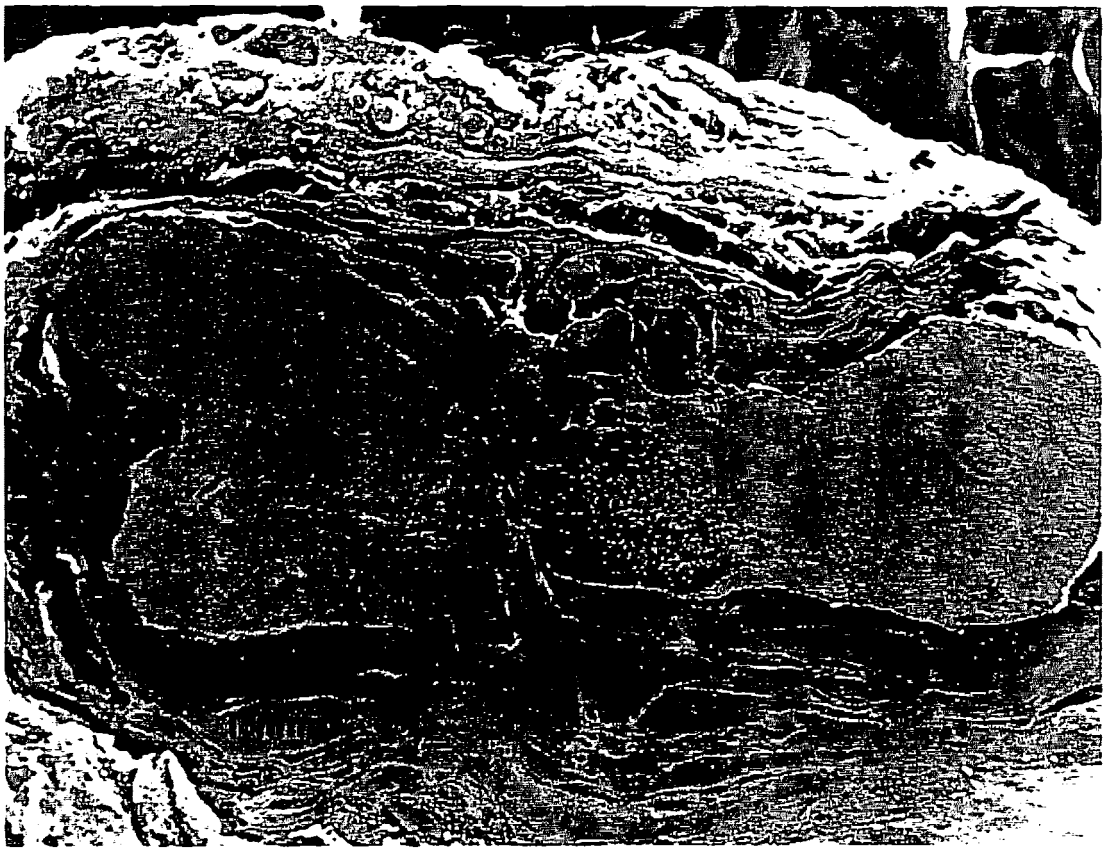
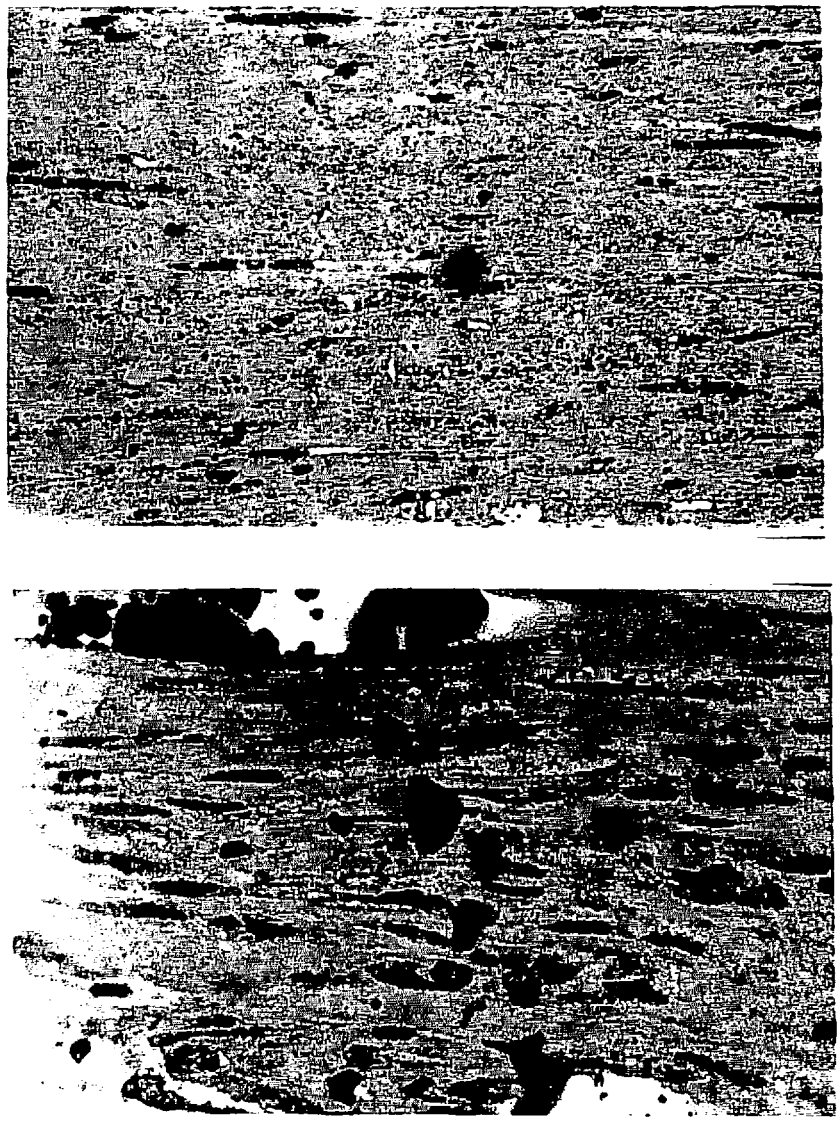


FIGURE 4. Cross-sectional view from a carbon-fiber reconstructed rabbit. The foreign-body induced tissue surrounding the individual flared carbon fibers can be seen. Specimen prepared at 125 micrometers and stained with Toludine blue. The black band is injury fibrosis that formed around the carbon-fiber bundles (the upper portion of one bundle can be seen in the lower portion of the photomicrograph). About 20 carbon fibers flared from the main bundle and were enveloped by injury fibrosis. These fibers induced a concentric ring of tissue that can be identified because it stains more lightly than injury fibrosis. 10X objective.

FIGURE 5. Histological appearance of the foreign-body induced fibrous tissue occurring in the middle of a carbon-fiber bundle in a rabbit recovered after 1 year. 10X, 100X objective for upper and lower, respectively.



USE OF CARBON FIBERS FOR REPAIR OF THE ANTERIOR CRUCIATE LIGAMENT IN GOATS

ABSTRACT

The anterior (cranial) cruciate ligament in goats was removed and replaced with carbon fibers; the implantation procedures and methods of fixation were similar to those used in patients. The carbon fibers were covered in the joint with either fascia lata or fat pad. The operated limb was not immobilized. The goats were sacrificed at 1.5, 3, and 18 months post-operatively, and the recovered tissues were examined grossly, and subjected to biomechanical and histological study. The implant broke near the bollard during the immediate post-operative period, but remained in place in the joint. Gross examination of each joint revealed no carbon-related changes in the articular cartilage, and no carbon-fiber debris in the local lymph nodes. The ultimate tensile load sustained by the implant at 18 months was (on average) 519 newtons; this value was significantly greater than the ultimate tensile load sustained at 1.5 or 3 months post-operatively. Progressive ingrowth of connective tissue into the intra-articular implant was observed, culminating in an induced ligament at 18 months having (on average) a diameter of about 5 mm.

INTRODUCTION

The rationale for the intra-articular use of carbon fibers is that they induce tissue that ultimately strengthens the implant site. The main purpose of this study was to determine the nature and extent of intra-articular tissue induction in an appropriate animal model. We chose the goat because its stifle joint was sufficiently large to permit a surgical procedure reasonably similar to that used in patients.

Immediately after surgery, the ultimate tensile strength of the carbon-fiber system (the carbon fibers together with their attachments to bone) is probably about 300 newtons, which is about 15% of the strength of a normal goat anterior cruciate ligament. Since it is not practical to stabilize a repair site in a goat or other large animal in a manner that reasonably simulates the controlled loading that occurs in patients following surgery, it was likely that the repairs would be disrupted 1-2 days following surgery (when the goats were turned out to pasture). This likely result in the goat paralleled the worst-case development that might occur with patients -- rupture of the implant in the immediate post-operative period -- and consequently the absence of immobilization in the goat permits estimation of the effect on knee-joint tissue and mechanical stability of an acutely ruptured implant in patients.

METHODS

Implant

The carbon fibers employed in this study were identical to those used in the clinical study. An unsized bundle of 10,000 8-micrometer fibers, 95% minimum purity, was heated at 300°C for 20 minutes to remove any organic contaminants. Carbon-fiber debris present in the bundle as a consequence of the original manufacturing process was removed by passing the bundle through a water bath. The carbon-fiber bundle was then passed through a bath of gelatin and glycerine and wiped with pure cellulose to remove any residual debris and facilitate handling of the carbon fibers during surgery.

The implant consisted of a bundle of 10,000 carbon fibers, each 8 micrometers in diameter, which was doubled back on itself and loosely twisted to form a loop at one end (20,000 fibers in cross-section). The implant was fixed on the tibia using a toggle (13 goats) or a stainless-steel rod (10 mm long, 1 mm in diameter) (2 goats), and on the femur using a bollard (13 goats) or by suturing to soft tissue (2 goats).

Surgery and Recovery

Mature random-bred goats of both sexes were used. The anatomical characteristics of the goat stifle (knee joint) necessitated several changes in the implant procedure (compared to that followed for patients) (Figure 1). The joint was opened and the anterior (cranial) cruciate ligament was cut at its attachments and removed. A drill hole was made in the tibial crest, and a second drill hole was made which exited near the tibial insertion of the ligament (Figure 1). The implant was passed through the holes and over the top of the lateral femoral condyle; the portion in the joint was covered with fat pad or fascia lata. The implants were placed on the right side (the left side was unoperated), and were recovered at 6 weeks (N = 6), 3 months (N = 4), and 18 months (N = 5) post-operatively. Following sacrifice the regional lymph nodes were examined grossly, and the stifles were removed and frozen (-20°C) until studied.

Gross Examination and Mechanical Testing

The joint was opened, and the synovium and articular surfaces were examined for the presence of carbon-fiber debris. Local lymph nodes were dissected and examined grossly for the presence of carbon fibers. The posterior cruciate ligament was then cut and removed, leaving the implant as the only structure connecting the tibia and the femur. The bones were mounted in a mechanical testing machine (Instron) with the long axis of the bones at 45°, and the implant was subjected to a tensile load at a cross-heads velocity of 1 cm/sec. One of the stifles recovered at 18 months was

examined microscopically without being subjected to prior mechanical testing.

Microscopy Preparation

The intra-articular portion of the implant and adjoining soft tissue were removed and fixed in 10% buffered formalin. The specimens were transversely bisected, and the proximal and distal portions were used for scanning electron and light microscopy, respectively.

For scanning electron microscopy (SEM), the proximal portion of the specimen was dehydrated through a graded series of alcohols up to 100% absolute alcohol. It was then frozen using liquid nitrogen and cut transversely using a razor-blade and hammer. The cleaved surface -- which consisted of the implant and adjoining tissue in cross-section -- was subjected to critical-point drying (Balzers CPD020), coated with gold (Denton Vacuum Desk-II) and examined in an SEM (AMR 1200) at an accelerating voltage of 25 kV.

For light microscopy, 2-3 mm thick transverse sections were cut from the proximal end of the distal half of the specimen. The sections were cut into 1.5-mm cubes and passed through a graded series of alcohols up to absolute alcohol, propylene oxide (PO), 1:1 mixture of PO and Spurr embedding media, 1:2 mixture of PO and Spurr, and then a pure mixture of Spurr under vacuum. Each sample was then embedded in either a capsule or a mold and heated at 60°C (± 5°C) for 18-24 hours. The blocks were trimmed and cut (0.2-0.3 micrometers) on an ultramicrotome (LKB Model 5) using a diamond knife. The tissue sections were floated on glass slides, dried on a hot plate, stained using a modified Spurlock (Toludine O/Basic Fuchsin) and cover-slipped.

Plainimetric Determination of Tissue Ingrowth

The cross-sectional area of the carbon-fiber bundle was determined plainimetrically (Gelman Instrument Co.) from 8x10-inch photographs of SEM views of the specimen cross-section. Let A_m be the average of three plainimetric measurements of the cross-sectional area (in mm^2). The radius, r, of the circular cross-section having an equivalent area (equivalent radius) is

$$r = \sqrt{A_m/\pi}/M$$

where M is the magnification on the photograph. The equivalent surface area is A ($A = \pi r^2$). Let A_c be the cross-sectional area occupied by the 20,000 8-micrometer carbon fibers ($A_c = (20{,}000)(\pi)(0.004)^2 = 1\ mm^2$). Let A_t be the area of new tissue per carbon fiber. Then,

$$A_t = (A-A_c)/2x10^4. \qquad (1)$$

Assume that the new tissue forms around each carbon fiber such that

D is the diameter of the composite. Then,

$$A_t = \pi(D/2)^2 - \pi r_c^2 \qquad (2)$$

where r_c is the radius of a carbon fiber. Eliminating A_t from Equations (1) and (2), we obtain D (in micrometers).

$$D = 20\sqrt{A/2\pi} = 10\sqrt{2}\, r. \qquad (r \text{ in mm}) \qquad (3)$$

Equation (3) relates the equivalent radius of the implant (derived directly from the plainimetric measurements) to the diameter of the structural unit formed by the growth of tissue surrounding each carbon fiber. For each goat, both r and D were calculated from the plainimetric measurements.

Statistics

Statistical comparisons were made using Student's t test at a chosen level of significance of $P < 0.05$.

RESULTS

Gross Observations and Mechanical Testing

All goats walked normally without limping at the time of sacrifice.

At 6 weeks postoperatively, implant fixation was lost in each animal, either by loosening of suture fixation or rupture of the implant 1-4 cm distal to the bollard. By 18 weeks, thinning of the fascia lata used to cover the carbon fibers had occurred, and the implant was visible in the joint where it remained covered by a thin translucent sheet of connective tissue. At 18 months, the implant had a larger diameter (compared to 3 months) and was covered with a thicker (but still translucent) fibrous sheath. In each case, the carbon fibers had broken near the bollard prior to recovery of the specimen. There were no loose carbon fibers present in the joint in any goat, and there was no evidence of either mechanical deterioration of articular surfaces, or the presence of carbon fibers in local lymph nodes. The ultimate tensile strength of the carbon-fiber reconstructions is listed in Table 1. The average ultimate tensile load of the intact goat anterior (cranial) cruciate ligament was 2044 newtons.

TABLE 1. Ultimate Tensile Strength (in Newtons) of Carbon-Fiber Reconstructed Anterior (Cranial) Cruciate Ligaments in Goats. The increase in strength between 3-18 months was statistically significant.

POST-OP TIME (Months)	N	ULTIMATE TENSILE LOAD (newtons)	
18	5	*519 ± 119	
3	4	280 ± 25	
1.5	7	272 ± 86	($P < 0.05$)

SEM Observations

The SEM cross-sectional appearance of implants recovered at 18 months is shown in Figures 2-4. In each instance, a relatively uniform pattern of tissue invagination of the carbon fibers is seen throughout the carbon-fiber bundles. Figures 5 and 6 are typical examples of the extent of fibrous ingrowth that occurred after 3 months; little tissue was seen in the interior of the implant.

Histological Evaluation

The tissue induced by carbon fibers is shown in longitudinal section in Figure 7. Tissue ingrowth formed an interstitial matrix between individual carbon fibers which was composed of collagen, cellular elements, and proteinaceous staining material. Cellular components included elongated spindle fibrocytes, and macrophages. Macrophages were present in small numbers in all specimens, and they occasionally formed multinucleated giant cells. Polymorphonuclear inflammatory cells (neutrophils and eosinophils) were not seen.

In goats examined 1.5 months after implantation, the interstitial matrix closely adhered to the carbon fibers and was amorphous, vacuolated and globular. Cellular components were minor consisting of only occasional fibroblasts and macrophages. Three-month implants exhibited a marked increase in cellularity, primarily of fibroblastic type cells with elongated nuclei oriented longitudinally along the carbon fibers (Figure 7). Fewer macrophages were present and were found singularly or in clusters of 3-6 cells, with occasionally multinucleated giant cells. Carbon was not seen inside cells. Eighteen-month implants were similar to 3-month specimens but contained more fibroblasts including plump active cells and dormant cells with dark elongated nuclei and very little cytoplasm. Macrophage type cells were present singularly and in clumps of 10-12 cells. The non-cellular matrix had a fibrillar collagenous appearance oriented longitudinally between widely separated carbon fibers (Figure 7).

Plainimetric Data

The average equivalent radius of the 18-month specimens was 2.4 mm (Table 2): the corresponding values at 3 and 1.5 months were 1.7 and 1.4 mm, respectively.

TABLE 2. Plainimetric Determination of Tissue Ingrowth. A, r, D, are implant cross-sectional area, equivalent radius, and diameter of basic structural unit, respectively. N, number of goats. The increase and equivalent radius between 1.5-3 months and 18 months were statistically significant.

POST-OP TIME (months)	N	A (mm^2)	r(mm)	D(μm)
18	5	*17.4±6.5	*2.4±0.6	34±9
3	4	9.4±1.0	1.7±0.1	
1.5	6	5.9±0.9	1.4±0.1	

*P < 0.05

DISCUSSION

The main purpose of the study was to determine whether tissue ingrowth into carbon fibers occurred in the knee joint. The operated limb was not immobilized; consequently, although the implants remained in position in the joint, they probably broke within a few days following surgery.

The intra-articular tissue reaction to carbon fibers was essentially the same as that observed following implantation at extra-articular sites. A thin fibrous sheath formed around the carbon-fiber bundle, and by 3 months postoperatively the peripheral carbon fibers in the bundle had become invaginated by newly induced connective tissue. Radially-directed tissue growth continued during the next 15 months. By 18 months postoperatively each carbon fiber was surrounded by a tube of newly induced connective tissue, 34 micrometers in diameter (on the average). The average cross-sectional area was 17.4 mm^2, of which only about 1 mm^2 was occupied by the carbon fibers themselves. Thus, the presence of the carbon fibers induced the growth of new tissue having a cross-sectional area about 17 times that of the implanted material. The elicited connective tissue was oriented in the direction of the carbon fibers and occurred, essentially, in the absence of a chronic inflammatory response. Despite the absence of immobilization (and the consequent early rupture of the implant) the mechanical data (Table 1) indicates that the induced connective tissue was capable of sustaining modest mechanical loads.

Results of histopathology studies indicated the carbon-fiber implants were essentially pathologically inert. The absence of neutrophils indicated that the fibers were not chemotactic and that the site remained sterile. The lack of lymphocytic and plasma cells indicated that the fibers did not elicit an immune response. Likewise, an allergic reaction to the fibers was not indicated because eosinophils were not observed.

FIGURE 1. Carbon-fiber reconstruction of the goat anterior (cranial) cruciate ligament.

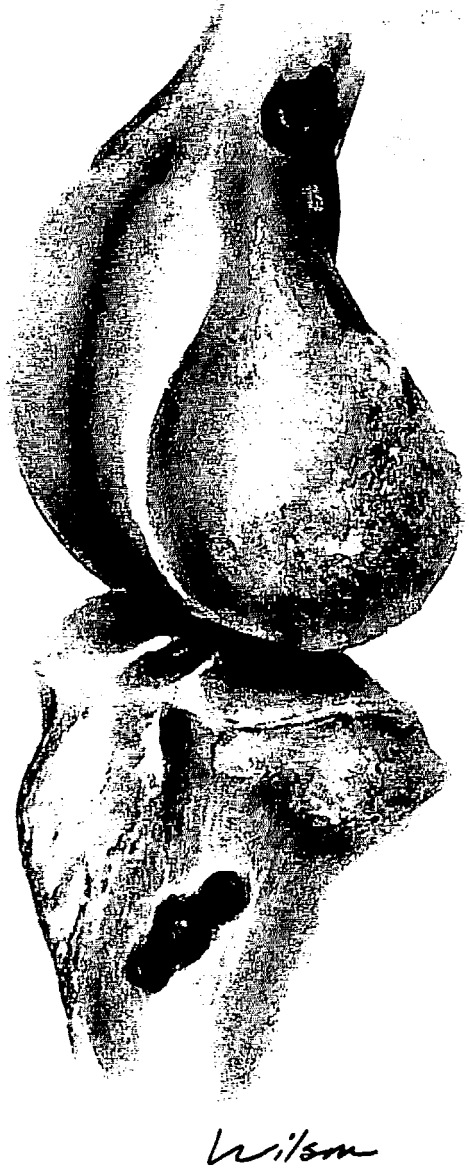


FIGURE 2. SEM view of cross-section of implant recovered after 18 months. Figures B and C are higher power views from areas indicated on Figure A.

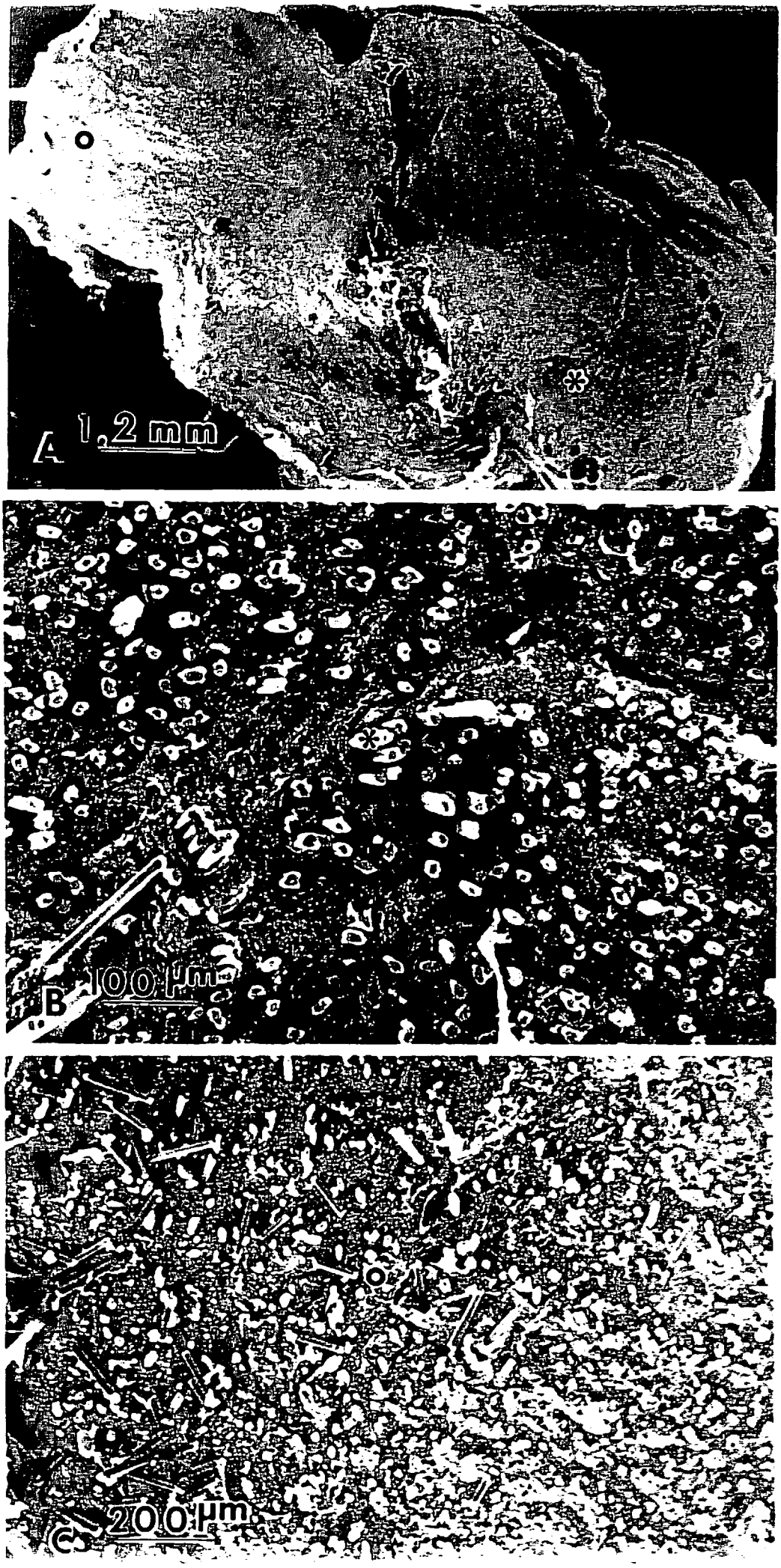
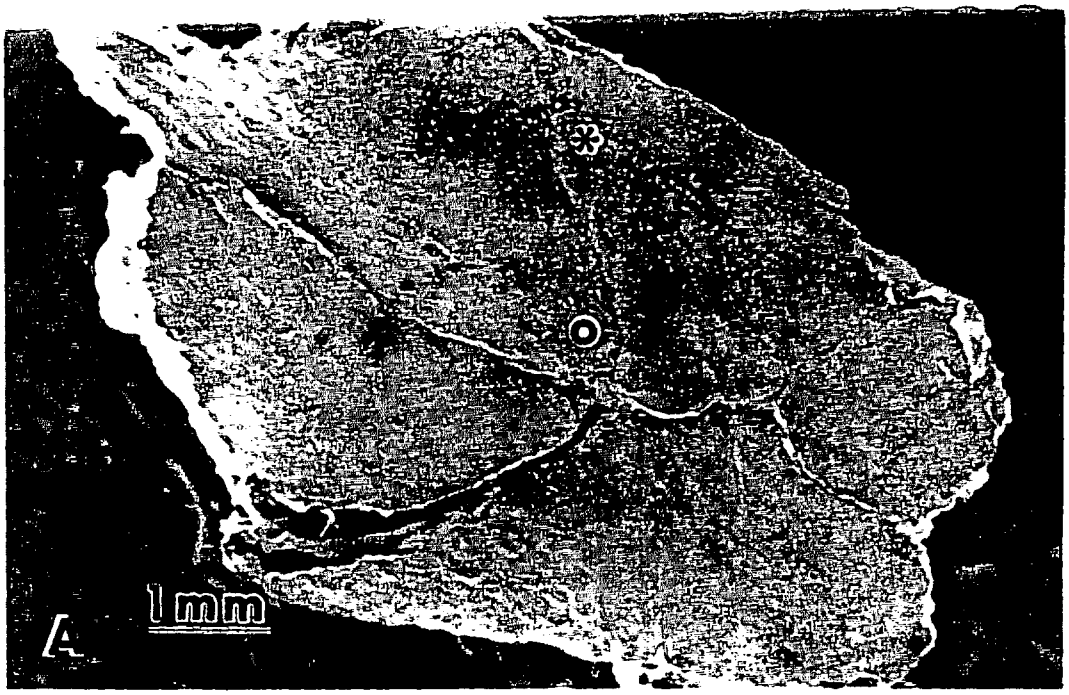
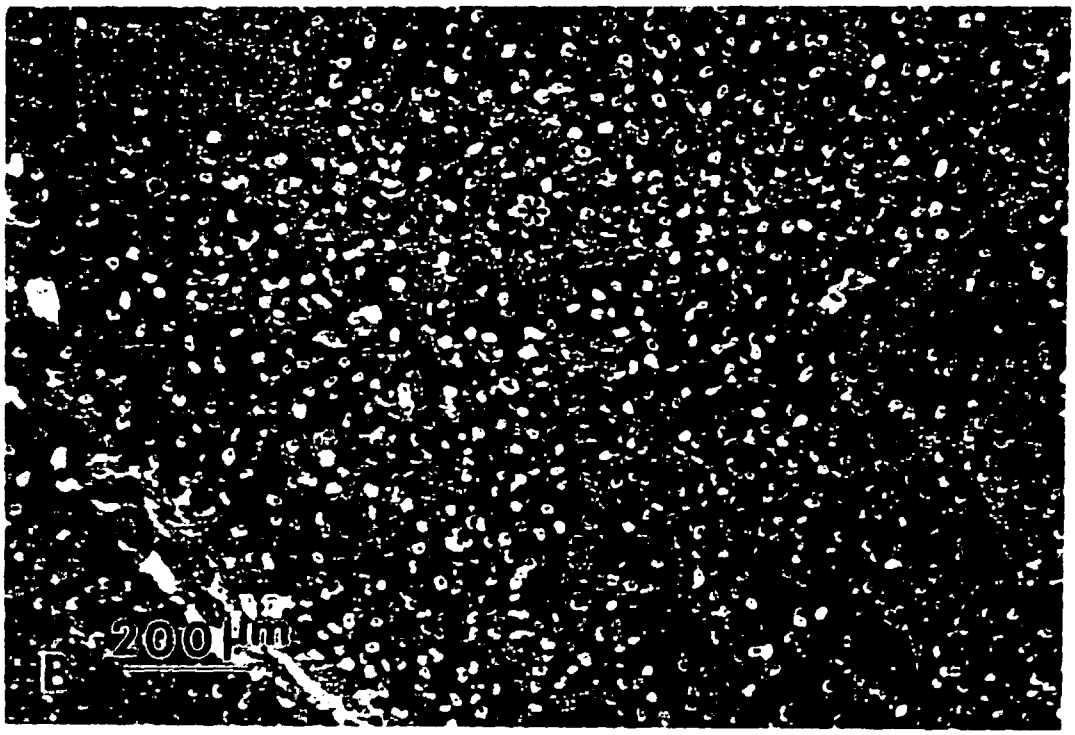

A
1,2 mm
B
100 µm
C
200 µm

FIGURE 3. SEM view of cross-section of implant recovered after 18 months. Figures B and C are higher power views from areas indicated on Figure A.


1mm
A


200 µm
B


200 µm
C

FIGURE 4. SEM view of cross-section of implant recovered after 18 months. Figure B is a higher power view from the area indicated on Figure A.

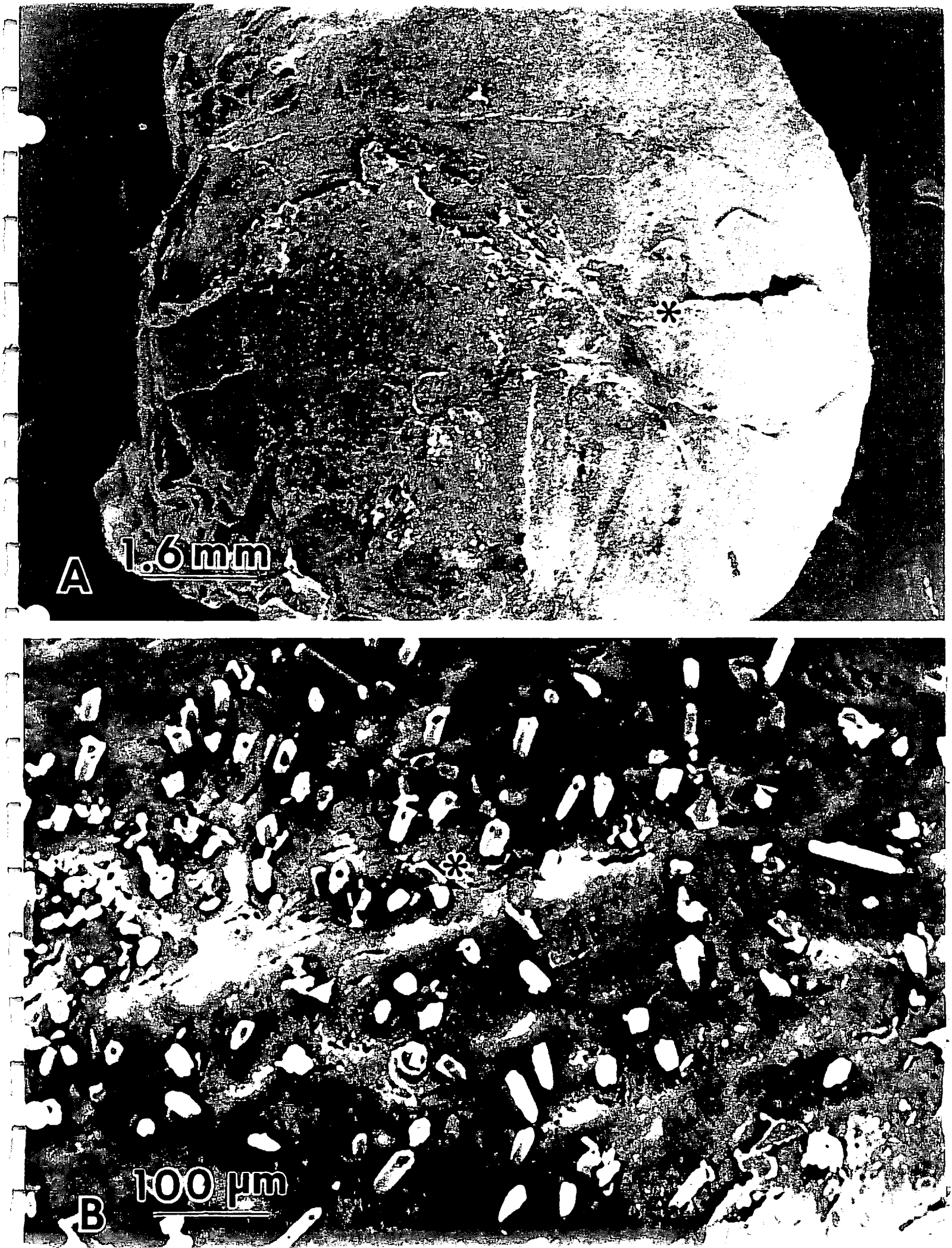

A
1.6 mm
B
100 µm

FIGURE 5. SEM view of cross-section of implant recovered after 3 months. Figure B is a higher power view from the area indicated on Figure A.

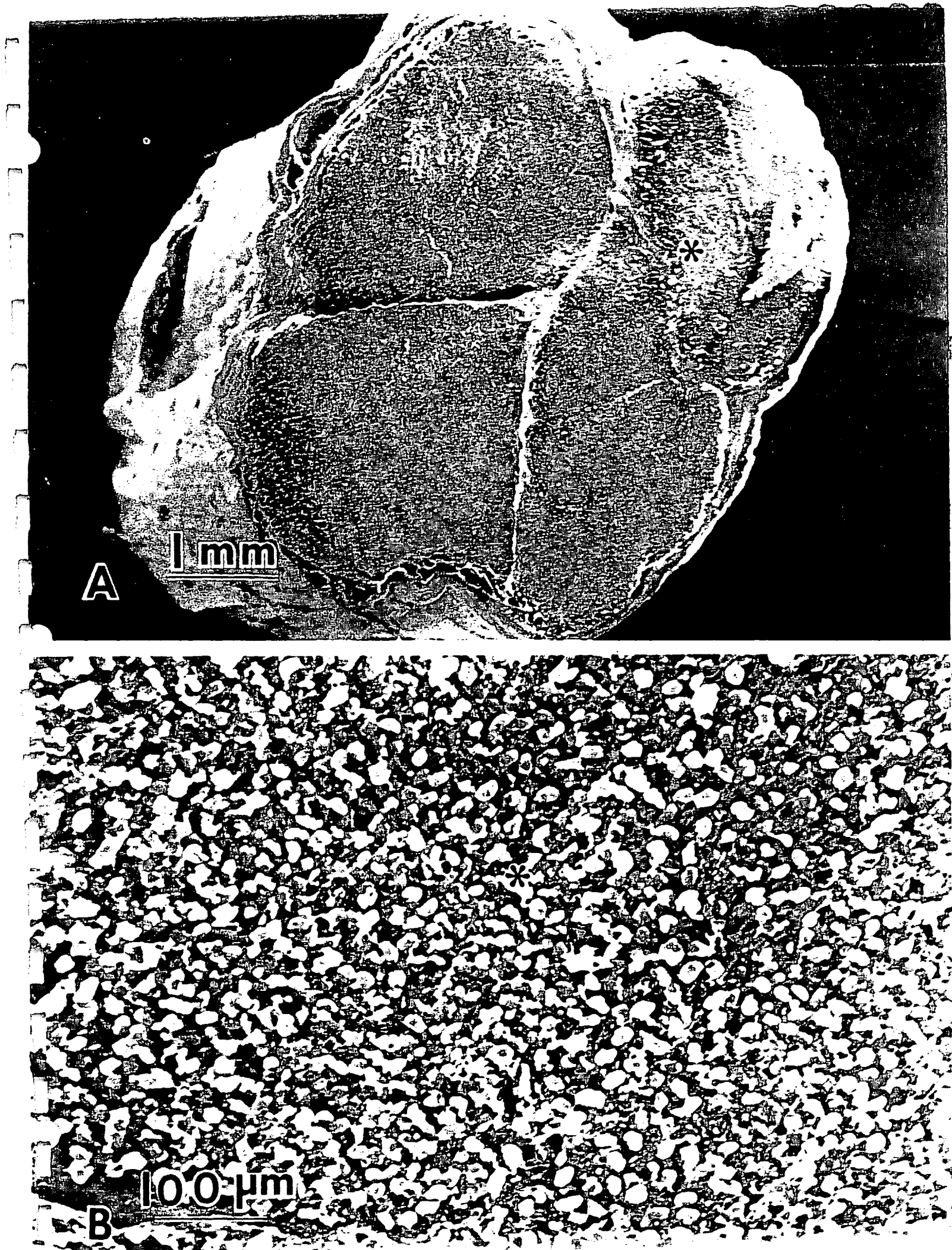
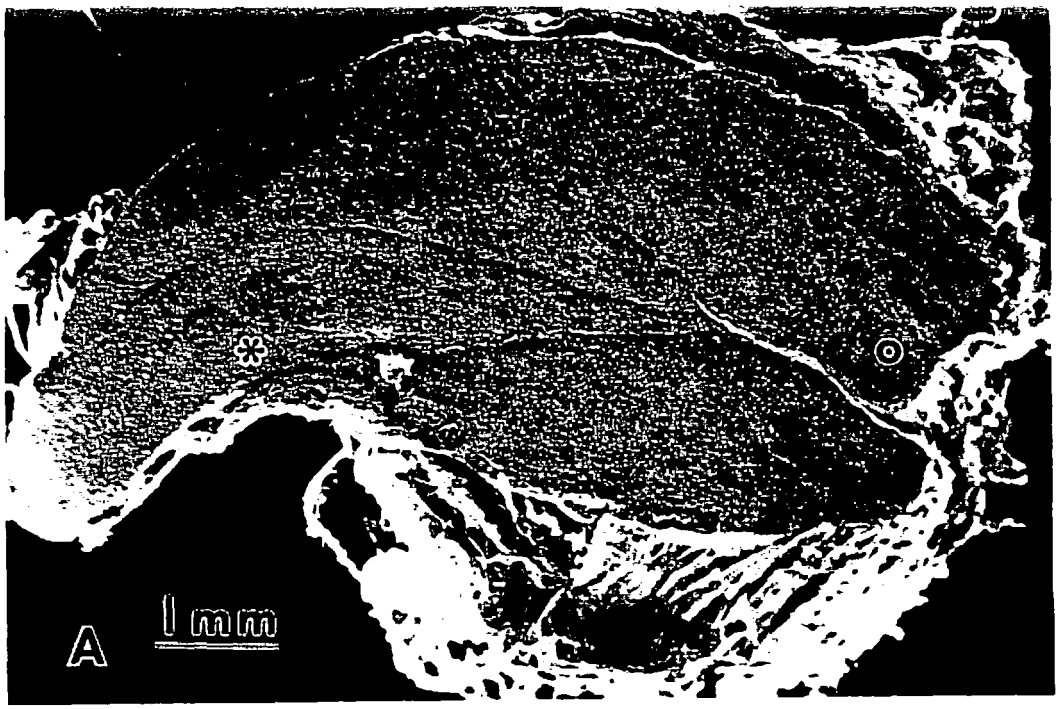
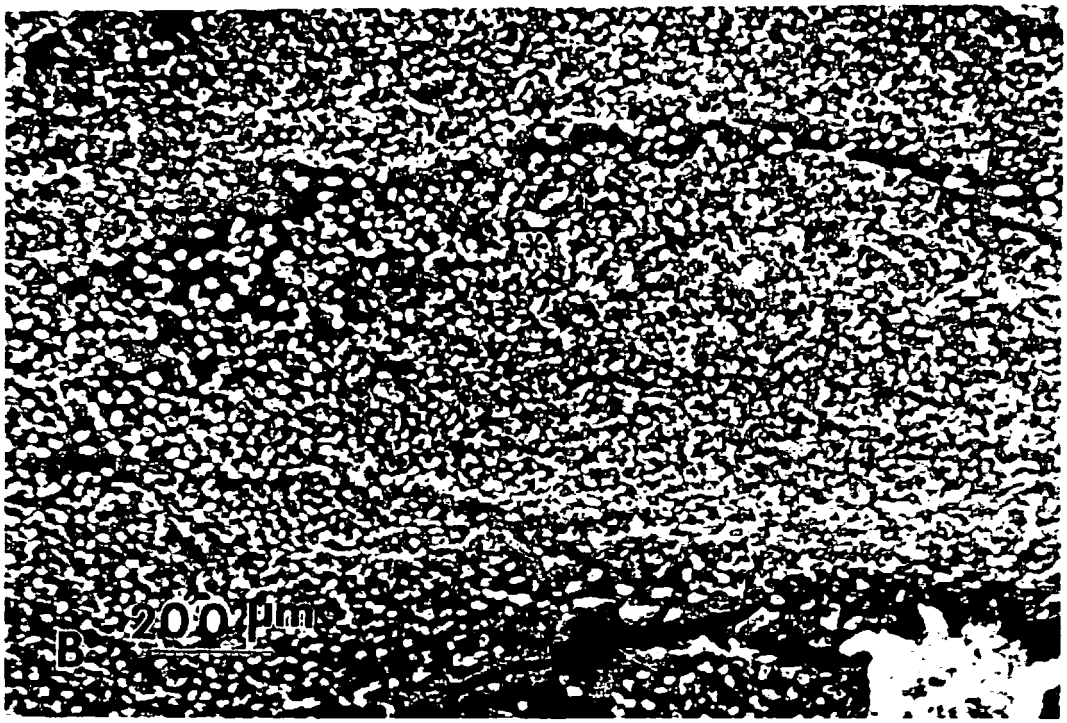

1 mm
A
100 µm
B

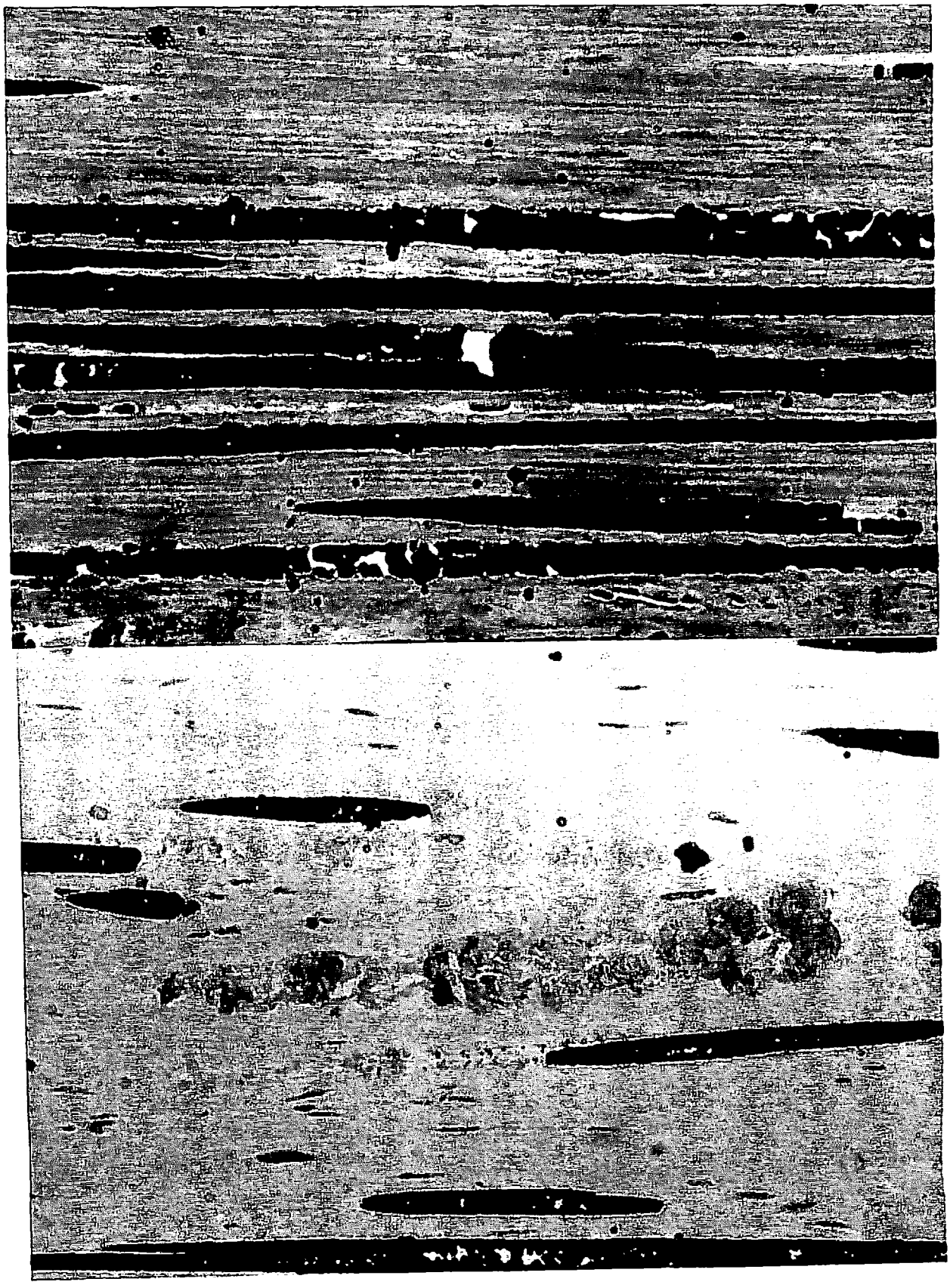
FIGURE 6. SEM view of cross-section of implant recovered after 3 months. Figures B and C are higher power views from areas indicated on Figure A.


A
1 mm


B
200 µm


C
200 µm

FIGURE 7. Light-microscope view of implant sectioned at 0.2 μm and stained with a modified Spurlock. Upper photograph, 3 months; lower, 18 months. The diameter (width) of the fibers is about 8 micrometers. The debris is an artifact produced when the diamond knife shattered the carbon fibers.



USE OF CARBON FIBERS FOR REPAIR OF ABDOMINAL-WALL DEFECTS IN RATS

ABSTRACT

The purpose of this study was to measure and histologically characterize tissue ingrowth occurring in carbon fibers implanted for up to 12 months in abdominal-wall defects in rats, compared with polypropylene mesh. Carbon fibers induced significantly more tissue ingrowth than polypropylene mesh at 6-12 months post-operatively. The predominant tissue associated with carbon fibers and polypropylene mesh were dense connective tissue and fat, respectively. Fragmentation of the implants did not occur, and implant debris was not found in the regional lymph nodes.

INTRODUCTION

The purpose of this study was to measure and histologically characterize tissue ingrowth in carbon fibers implanted for up to 12 months in abdominal-wall defects in rats. We also examined the organ compatibility of carbon fibers, and the question of lymphatic transport of carbon-fiber debris.

MATERIALS AND METHODS

Implanted Materials

The carbon fibers employed in this study were identical to those used in the clinical study. An unsized bundle of 3,000 8-micrometer fibers, 95% minimum purity, was heated at 300°C for 20 minutes to remove residual organic contaminants. Carbon-fiber debris present in the bundle as a consequence of the original manufacturing process was removed by passing the bundle through a water bath and wiping with pure cellulose. The carbon-fiber bundle was then passed through a bath of gelatin and glycerine (and wiped again with cellulose) to facilitate handling of the carbon fibers during surgery.

Surgical Procedure

Twenty-two female Sprague-Dawley rats, 250-350 gm, were used in the study. Following induction of anesthesia (3 mg/kg Rompun and 35 mg/kg Ketamine, IM), the abdomen was shaved and incised along the midline and a 3 x 4-cm segment of the anterior abdominal wall including fascia, muscle, and peritoneum was removed. The defect was repaired using a 3.5 x 4.5-cm onlay of either carbon fibers or polypropylene mesh. The implanted material was sutured around its perimeter to the abdominal wall using running stitches, 2-4 mm apart (6-0 Proline). The skin was closed using a running subcuticular stitch, and each animal was housed individually, and fed and watered ad libitum.

Histological Procedures

The animals were sacrificed at 3, 6 and 12 months postoperatively (T-61 euthanasia solution), and the implant was excised along its margin, separated from loosely adhering soft tissue, and fixed in buffered formalin. The inguinal lymph nodes were also recovered and fixed in formalin. The central 1 cm^2 of the implant was used for determination of wet weight. The removed portion was cut into 5-mm squares which were weighed individually to determine the positional variation of tissue ingrowth.

Samples were taken from the central portion of the implant and passed through a graded series of alcohols up to absolute alcohol, propylene oxide (PO), 1:1 mixture of PO and Spurr embedding media, 1:2 mixture of PO and Spurr, and then a pure mixture of Spurr under vacuum. Each sample was then embedded in a mold and heated at 60°C (±5°C) for 18-24 hr. Some of the blocks were trimmed and cut (0.75 micrometers) on an ultramicrotome (LKB Model 5) using a diamond knife. Other blocks were cut on a diamond saw (South Bay Technology, Model 650) and polished using a graded series of abrasives to a final thickness of approximately 125 micrometers. The tissue sections were stained using Spurlock (Toludine O/basic fuchsin) and cover-slipped. These techniques resulted in the preparation of true representative sections, and permitted a determination of the relationship between the section and the gross tissue from which it was prepared.

The lymph nodes were dehydrated, embedded in wax, sectioned at 10 micrometers and stained with hematoxylin and eosin. Every section of each node was mounted on glass slides and cover-slipped to permit an unambiguous assessment of the presence of debris in the nodes.

Statistical comparisons were made using Student's t test, with a chosen level of significance of $P < 0.05$.

RESULTS

Gross Observations

Polypropylene mesh and carbon fibers exhibited comparable propensities for adhesion formation. At 3 and 6 months postoperatively, adhesions that could be freed by blunt dissection were observed on over 15-20% of the exposed surface of each implant. In most rats recovered 12 months postoperatively, sharp dissection was required to separate the implant from the liver and intestine (also over 15-20%). Gross infection, seroma formation, enteric fistulae, and implant fragmentation were not observed.

At 3 and 6 months postoperatively, the carbon fibers were covered on both surfaces by fibrous tissue. Gross fibrous tissue associated with the polypropylene typically was minimal, but regions as thick as 50 micrometers were occasionally seen on the

visceral surface of the implant. At 12 months the outer fibrous table of the carbon fibers was less prominent, but the appearance of the polypropylene implants was unchanged. The average weight of the tissue ingrowth in carbon-fiber specimens was greater at both 6 and 12 months compared to polypropylene mesh (Table 1). When each 1-cm section was quartered and weighed separately, the average percent standard deviation in tissue weight for both materials was about 30% at each recovery time.

Microscopic Observations

At 3 months, the carbon-fiber implant was encapsulated by a 30-50 micrometer layer of connective tissue. The region between the bundles and the outer fibrous layers contained granulation tissue and fat. Tissue was not seen inside the carbon-fiber bundles, except for connective tissue surrounding the most peripheral fibers in each bundle. At 6 months, tissue ingrowth into the carbon-fiber bundles was observed. The growth extended into the bundles from the surfaces opposite the surfaces where the bundles overlapped (see Figure 1). Occasionally, pairs of bundles in the weave were locked tightly together, and such bundles contained only scanty amounts of tissue. The typical appearance of the carbon-fiber implant at 12 months is depicted in Figure 1. Tissue was found throughout the bundles (Figure 2A) except in occasional regions where the bundles were wedged tightly together. The tissue inside the carbon-fiber bundle was invariably dense connective tissue (Figure 3A); loose connective tissue and fat was not observed inside the bundles. The fibroblast-like cells seen in the dense connective tissue exhibited a preferential orientation in which their fusiform nuclei were aligned along the carbon-fiber direction (Figure 4A). The fibroblast-like cells appeared to form a sheath around individual carbon fibers (Figure 5).

In the polypropylene implants, at 3 months the space between the fibers was filled with blood vessels, loose connective tissue, and adipose tissue. During 6-12 months the connective tissue became mostly restricted to the region surrounding the fibers, and adipose tissue filled the remaining space (Figures 2 and 3B).

The lymph nodes of rats implanted with carbon fibers and polypropylene contained hemosiderin and unidentified particles. Neither the amount of debris, nor the occurrence of reactive cellular changes differed between the two groups, or between either group and a group of rats (N = 3) that had not received an implant.

DISCUSSION

The carbon fibers induced proliferation of fibrous tissue in full-thickness abdominal-wall defects in rats after 12 months (Figures 2A, 3A, 4 and 5). The growth of new tissue into the carbon fibers occurred along with a thinning of the outer fibrous layer. As a net result of these changes, the tissue weight per unit area

remained unchanged during 3-12 months (Table 1). Tissue growth was not uniform throughout the implant as evidenced by the observed 30% standard deviation in the weight of the induced tissue in the center of the implant (a phenomenon also exhibited by polypropylene mesh).

The carbon fibers induced a negligible chronic inflammatory reaction. We did not find evidence of lymphatic migration. Either carbon-fiber debris does not enter the lymph nodes in the model used, or it is histomorphologically indistinguishable from other debris which are normally found in the inguinal lymph nodes of rats. Lymph nodes from the three groups could not be separated by histologic examination by a blinded examiner.

The mass of carbon fibers used to form the implant was approximately the same as that implanted in a patient undergoing an anterior cruciate ligament repair (about 0.6 grams). As a consequence, the amount of carbon fibers per unit body weight implanted in the rat was greater than the corresponding value for the clinical cases by an amount equal to the ratio of the body weight of a human being to that of a rat (the ratio is about 250, assuming a patient body weight of 175 pounds). It is reasonable to expect that, if phagocytization and lymphatic transport of carbon fibers are physiological processes that can occur at an observable level, then carbon-fiber debris would have been seen in the regional lymph nodes of the rats. The absence of such observations suggests that the processes did not occur (to a material extent).

Polypropylene elicited a minimal inflammatory response and scanty fibrous tissue formation that did not change significantly in nature or extent after 3 months. The most prominent tissue present in the interstices of the polypropylene mesh was adipose tissue (Figures 2B and 3B).

Polyproplyne mesh apparently provides some clinical benefit in the short term (1-2 years) (1-3), but there have not been long-term studies of either its safety or efficacy in hernia repair. It is recommended by the manufacturer for use as an overlay or a cuff. As an overlay, polypropylene mesh is intended to stabilize a wound or suture line until healing takes place via scar formation. Since minimal fibrous tissue is actually induced by polypropylene mesh (Figures 2B and 3B), the implant must function as a frank prosthesis if it is to confer a long-term clinical benefit. Soft-tissue attachment of the mesh cannot realistically be expected to maintain mechanical integrity over long periods. If the mesh remains intact mechanical relaxation will probably occur at the suture line by cutting of the stitches through the soft tissue.

As a cuff surrounding weak tissue, polypropylene mesh facilitates (initially) a mechanically secure anastomosis between two tissue planes. Healing actually takes place via growth of connective tissue that bridges and binds the two tissues. The presence of any cuff material reduces the cross-sectional area through which healing tissue can grow by an amount proportional to the openness

of the material's weave or knit. The masking effect of polypropylene mesh is significant because it is about 67% plastic polymer and 33% open space. The problem is compounded when polypropylene mesh is used as a cuff on both edges of the wound.

Many plastics are carcinogenic in animal models (4,5). The absence of long-term follow-up involving human use of polypropylene raises the question of its long-term safety. In contrast, there have not been any reports indicating that carbon in any form is a mutagen or carcinogen.

In summary, in the animal model employed, carbon fibers can be fabricated into a mechanically sufficient implant that induces more high-quality tissue ingrowth than that obtained with polypropylene mesh. This stimulation of tissue ingrowth may provide a rationale for the use of carbon fibers to replace lost tissue or reinforce weak tissue when repairing abdominal-wall defects.

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TABLE 1. Comparison (Carbon Fibers versus Polypropylene) of Tissue Induced in Abdominal-Wall Defects. The specimen wet weight consisted of the weights of the implant material and its associated tissue. The tissue weight was obtained by subtracting the implant weight (35.2 mg/cm^2 for carbon fibers, 13.2 mg/cm^2 for polypropylene) from the specimen weight. N, number of rats.

RECOVERY TIME (months)	TISSUE WET WEIGHT (mg/cm^2)			
	N	CARBON FIBERS	N	POLYPROPYLENE
3	2	177 ± 48	2	125 ± 24
6	4	*213 ± 40	5	63 ± 12
12	4	*169 ± 64	5	103 ± 36

*$P < 0.05$

FIGURE 1. Implant materials used, and tissue response at 12 months. The carbon-fiber implant (top left) was a closed weave formed from a 3000-fiber bundle. The polypropylene implant (bottom left) (Marlex) was an open knit formed from a single strand of polymer. The tissue response to the implants at 12 months postoperatively is depicted (right side) in cross-section. The carbon-fiber bundles were permeated with connective tissue, and contained only scanty fatty deposits in the interstices between the bundles (top). Fat was the predominant tissue associated with the polypropylene implants (bottom): Connective tissue occurred in regions adjacent to the polymer.

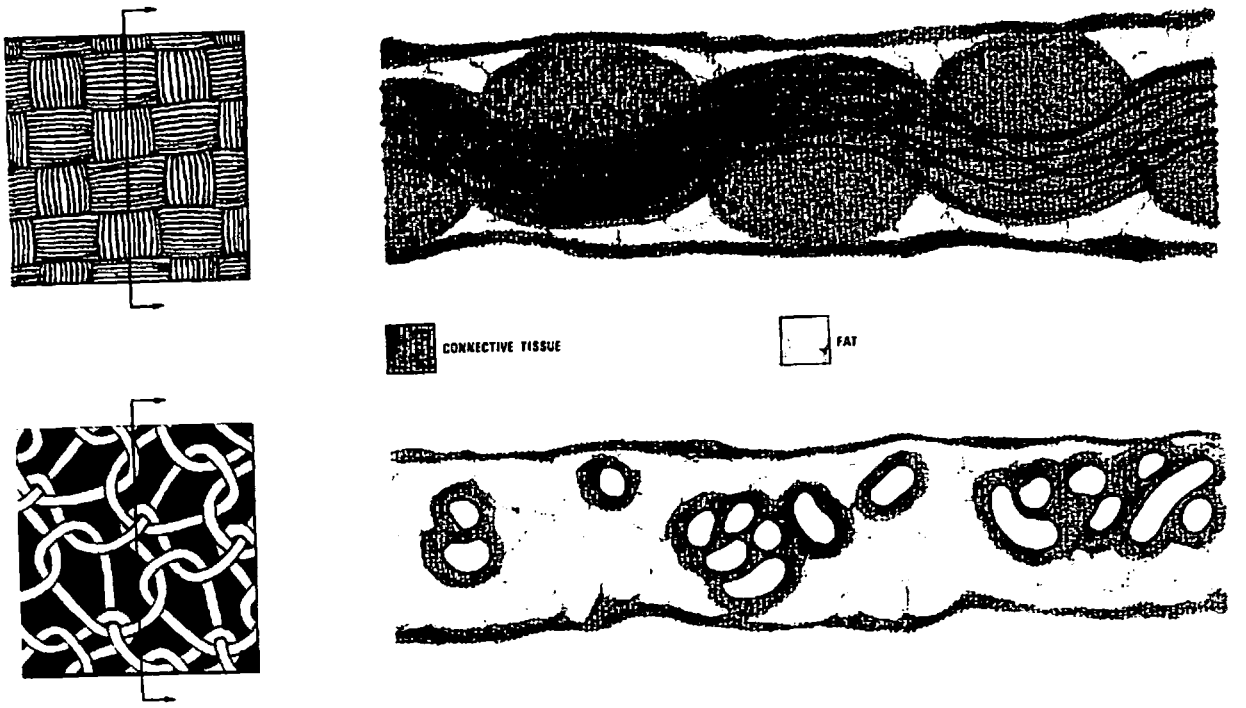

FIGURE 2. Tissue ingrowth into carbon-fiber and polypropylene implants at 12 months postoperatively. The planes of the views correspond to those illustrated in Figure 1. (A) The view plane is perpendicular to the upper bundle, thereby permitting visualization of the tissue inside the bundle. Tissue cannot be visualized in the lower bundle in this view because of the thickness of the specimen (10-15 carbon fibers). The separation in the upper bundle, and the gap between the two bundles are both artifacts of preparation. (B) The circular and oval structures are polypropylene fibers in tranverse and oblique section. The fibers are thinly covered by connective tissue, but fat is the predominant space-filling tissue.

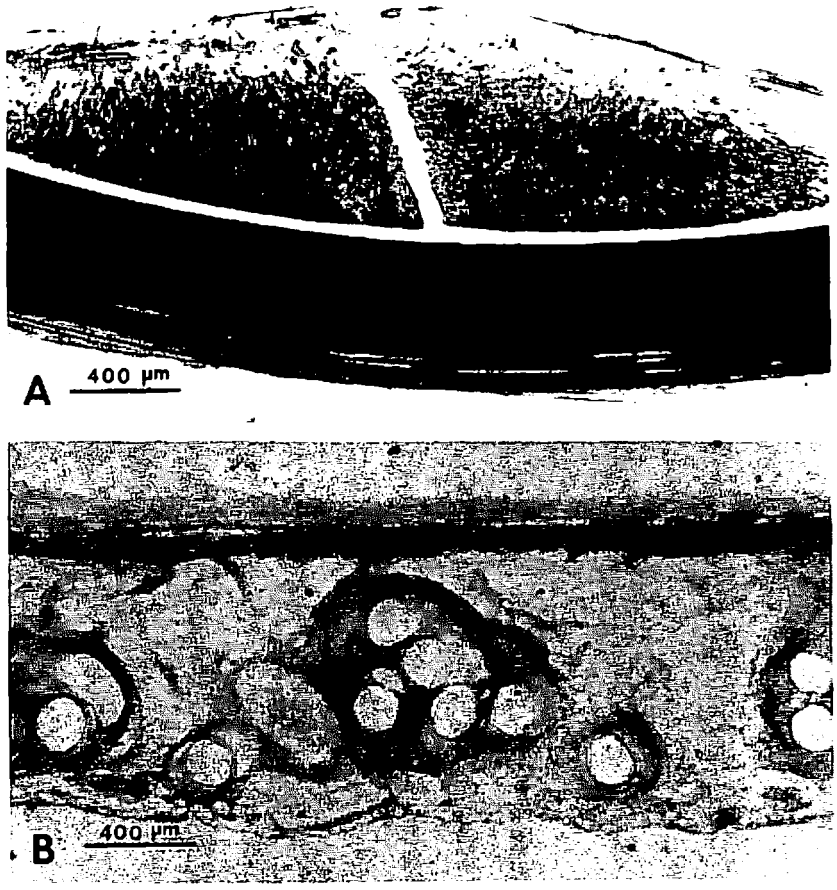

FIGURE 3. Representative tissue ingrowth into carbon fiber (A) and polypropylene (B) implants at 12 months. The specimens were cut at a thickness of 0.75 micrometers and stained with Spurlock. (A) The induced tissue is relatively homogeneous and consists of a fibrous matrix and numerous fibroblast-like cells. Occasional lymphocytes and giant cells were also observed. (B) Dense connective tissue typically occurs only within 20-30 micrometers of the polypropylene fiber. The remaining inter-fiber region contains loose connective tissue and fat.

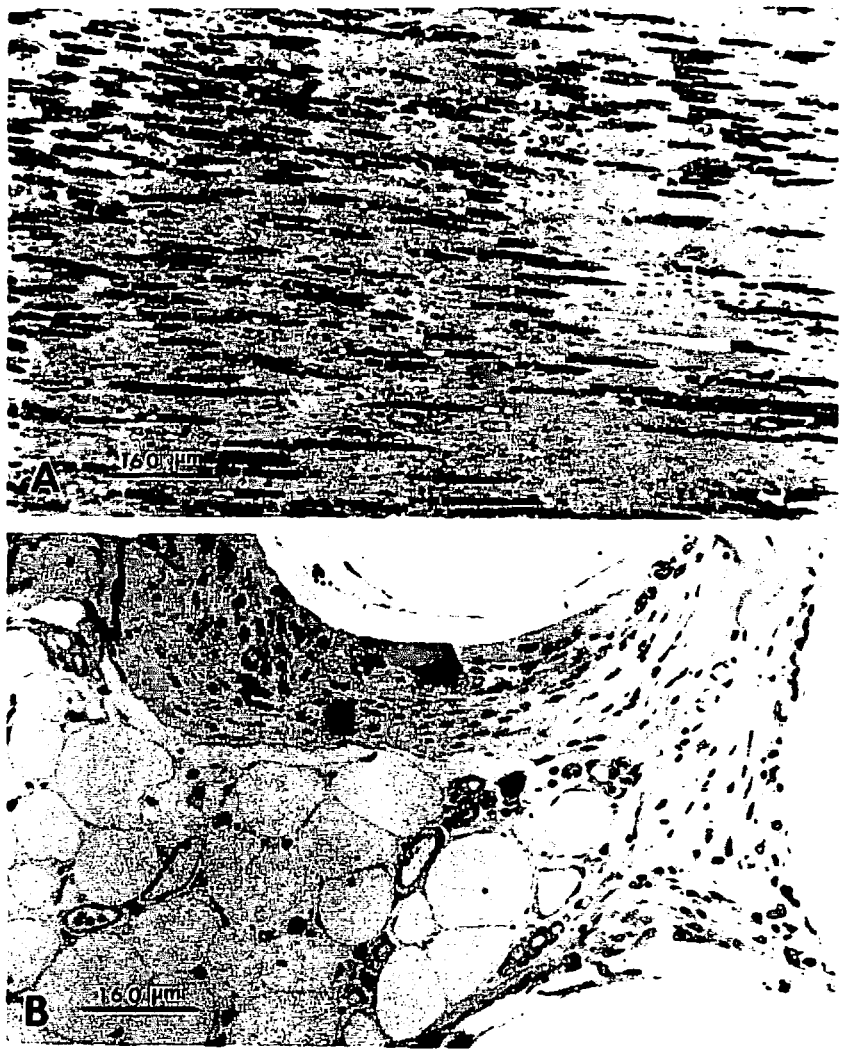

FIGURE 4. Representative induced tissue inside carbon-fiber bundles at 12 months. Both specimens were cut at 0.75 micrometers and stained with Spurlock. The carbon fibers were shattered by the diamond knife, and some of the debris was displaced from its original bed. (A) The angle between the carbon fibers and the specimen plane was 1-4°, and the preferential orientation of the nuclei of the fibroblast-like cells along the carbon-fiber direction can be seen. (B) Specimen cut at 19-23°. The nuclei are more round, as expected if the cells were oriented along the fibers.

FIGURE 5. Representative tissue adjacent to individual carbon fibers at 12 months. The specimen was cut at 0.75 micrometers and stained with Spurlock. The angle between the plane of the specimen and the upper carbon fiber permits direct visualization of the fibroblast-like cells immediately adjacent to the fiber. The fusiform cell nuclei are preferentially aligned along the fiber.

TREATMENT OF BOWED TENDON IN THOROUGHBRED RACEHORSES USING CARBON FIBERS

ABSTRACT

Bowed tendon is a common and debilitating form of tendinitis in Thoroughbred racehorses. The most frequently affected tendon is the superficial digital flexor tendon. Since antiquity, a variety of chemical and surgical procedures have been employed in an attempt to improve the generally poor prognosis for bowed tendon. In the present study, the use of carbon fibers for treating bowed tendon was studied in (1) horses that had failed standard therapy and (2) acutely injured horses. In the latter study, a historical control group was identified and used for purposes of evaluating the treatment with carbon fibers. Ability to return to the racetrack was the endpoint in both studies. In the first study, 65% of the horses that had suffered a single tendon injury and failed conventional therapy returned to the racetrack. Among horses that suffered injuries to 2-3 tendons and failed conventional therapy, 45% of the horses returned to the racetrack. In the second study (acutely treated horses) 74% of the horses treated with carbon fibers returned to the racetrack, compared to 23% of the horses that were treated conventionally. Each of the differences was statistically significant.

INTRODUCTION

Bowed Tendon and Its Treatment

In the horse, the superficial digital flexor muscle originates on the medial epicondyle of the humerus, and inserts on the first and second phalanges; it is joined by an accessory ligament which comes from its attachment on the posterior surface of the radius. The deep digital flexor originates on the medial epicondyle of the humerus, the medial surface of the olecranon, and the posterior surfaces of the radius and ulna, and inserts on the third phalanx. The action of both muscles is to flex the digit and carpus and to extend the elbow (Figure 1).

Tendinitis of the superficial digital flexor tendon (SDFT) is a typical disease of racehorses (1,2). It usually results from severe strain produced by excess physical stress, and can range from a slight subclinical injury to a complete rupture. Typically, it is accompanied by hemorrhage, edema, and swelling of the plantar/palmar surface (bowed tendon). The recommended treatment for bowed tendon is rest, anti-inflammatory agents, and blistering (use of a topical caustic agent) (1,2). If the subsequent physical demands on the injured tendon are moderate, this treatment can produce acceptable results. In the case of the Thoroughbred racehorse, however, significant physical forces are transmitted by the SDFT during racing, frequently resulting in re-injury. Since antiquity, attempts have been made to augment or improve the normal healing process sufficient to permit a horse to return to its

previous functional level. Thermocautery (firing) was perhaps the most common technique: it was performed in the hope that the resulting scar formation would strengthen the injured tendon. More recent approaches have included surgically-administered (non-thermal) injury, autologous-tissue grafting, and synthetic prostheses including carbon fibers; none of these procedures have demonstrated any improvement compared to other therapy (1,2).

Previous Studies Using Carbon Fibers

Carbon fibers have been used to treat transected or ruptured tendons using various weaves secured by knotting (3-5) (Figure 2); based on anecdotal observations, generally fair results were reported. The rationale apparently was that the carbon fibers would stabilize the lesion and that the induced tissue would strengthen the site. But knotted carbon fibers cannot sustain a mechanical load nearly as great as that which is normally transmitted by the tendon, and there is no evidence that carbon fibers form a mechanical bond with autologous tissue. Thus there is no evidence that weaving can stabilize a completely divided tendon.

In other studies, carbon fibers were implanted in sprained but intact tendons (6-8). Littlewood (6) hypothesized that the presence of carbon fibers in acutely injured (but intact) tendons, might alter the structural organization or the chemical nature of the resulting scar tissue, producing tissue that more closely resembled normal tendon. In 7 horses (5 that had failed previous treatment) bundles of 5,000 carbon fibers were passed through the affected region of the tendon using a cannula (Figure 3). Generally successful results were reported (3 horses returned to previous use, 4 horses in training). Goodship et al. (7) implanted 40,000 fibers in a surgically-created t-shaped bed in the superficial flexor tendon of 40 injured racehorses. Thirty-one of the horses were in training, 6 had returned to racing, and 3 had failed. No further follow-up was performed (9). Vaughan et al. (8) treated 34 horses (30 Thoroughbreds) with carbon fibers by passing a bundle of 10,000 fibers through the lesion using a curved needle (Figure 3), and reported a success rate of 21% (7 of 34).

Rationale for the Present Study

If carbon fibers were placed in acutely sprained tendons, a beneficial effect might be obtained by one of several mechanisms. Assuming that the carbon fibers were placed parallel to the tendon axis, the longitudinal orientation of the carbon fibers might serve to organize or orient the collagen tissue that occurs in response to the injury. That is, the presence of the carbon fibers in intimate association with the injury-induced collagen-producing cells might define a preferred orientation for the collagen. Another possibility is that the foreign-body induced tissue produced by carbon fibers -- the tissue that occurs irrespective of the presence of an injury -- could contribute mechanical strength by serv-

ing as an internal stent across the defect. A third possibility is that the strength contributed to the defect arises from the foreign-body induced tissue but that, in the context of an acute injury the foreign-body reaction proceeds more quickly (because of the availability of the mitotically active cell population and blood supply). In the case of a chronic injury, the hypothesis is that the foreign-body induced tissue is formed and serves as an internal stent.

In this study, our aim was to pass carbon fibers through a lesion in the tendon to permit the induced foreign-body tissue to join with normal tissue proximally and distally to the lesion (Figure 4). The hypothetical role of the carbon fibers was that they would serve as an inducer of new tissue (and possibly an organizer of proliferating scar tissue). Since it was not hypothesized that the carbon fibers would themselves carry a mechanical load, it was unnecessary to fix them within the tendon. This placement of the carbon fibers was accomplished using specially designed surgical instruments that permitted bridging of the lesion by the carbon fibers with minimal operative morbidity (Figure 5).

METHODS

Horses Studied

The use of carbon fibers was studied in two groups of Thoroughbred racehorses. Each horse in the first group had previously developed a bowed tendon and had been treated in a manner chosen by the horse's owner and trainer, and veterinarian. Most often, the treatment involved rest, anti-inflammatory agents, and blistering. Occasionally, other non-surgical (magnetic fields) and surgical (tendon-splitting) treatments were used. Each horse had failed its course of therapy and was unable to return to the intensive training needed to prepare for racing, as assessed at 4 months, or more, following the initial injury. That is, if the animal could not begin training after 4 months following its injury, it was regarded a treatment failure and considered as a candidate for this study. Horses were entered into the study provided that the injury was not within 2 cm of the annular ligament,* and the owner (1) agreed to the proposed plan for rehabilitation and (2) intended to carry out the rehabilitation plan to the point where the animal either failed treatment or successfully returned to the racetrack. Horses with unilateral or bilateral injuries to the SDFT or DDFT were accepted

* That is, the injury was at least 2 cm more proximal than the annular ligament, which is present at the level of the fetlock joint. The inability to place carbon fibers distal to the annular ligament (using the present operative technique) necessitated this exclusion criterion, in view of the hypothesis being tested (Figure 4). This criterion resulted in the exclusion of relatively few horses, because the most common site of injury is at mid-tendon.

into the study and stratified for the purposes of data analysis. All horses that met these criteria were offered entry into the study.

In a second study, Thoroughbred racehorses with unilateral injuries to the SDFT were treated acutely (generally within 30 days of injury) with carbon fibers (the other entry criteria were the same as those in the first study). The successful return of a Thoroughbred racehorse to the racetrack following a bowed tendon is relatively rare, and a prospective randomized study could therefore not be justified. To form a control group suitable for assessing the effects of the use of carbon fibers in acute injuries, we identified a continuous historical group of horses that had been treated for bowed tendon. Some of the horses were treated concurrently with the carbon-fiber horses (because the owner refused carbon-fiber treatment), but most were obtained from the period immediately preceding the use of carbon fibers as therapy for bowed tendons. A consecutive series was formed consisting of Thoroughbred racehorses having a single tendinous injury for which it could be confirmed that treatment was administered with the intention of attempting to return the animal to the racetrack.

Surgery and Post-Operative Treatment

The implant operation was performed under general anesthesia with the horse in lateral recumbancy, using specially designed instruments (Figure 5). The area between the carpus and the pastern joint (the joint distal to the fetlock) was shaved, and the limb was fixed in extension to obtain the correct angle for the introduction of the instruments. After the usual pre-surgical preparations, a stab wound was made through the skin and tendon sheath to the center of the superficial flexor tendon. In the case of the deep flexor tendon, the stab wound penetrated to its center through the superficial tendon. A trocar and cannula were inserted up the center of the tendon to the level of the carpo-metacarpal joint. The trocar was removed and the implant (a bundle of 40,000 fibers) was placed into the tendon through the cannula. The cannula was then removed, and the tendon sheath and skin were sutured. The entire procedure was completed in 5-10 minutes.

A support bandage was applied immediately after surgery and changed every 3-4 days as needed, and the horse was confined to a stall for 6 weeks. The horse was confined in a small paddock for the next 6 weeks and then pastured during the next 3-6 months where it was permitted unlimited exercise. At 6-9 months after surgery, the horse began a 3-6-month training period in which an attempt was made to slowly return it to its previous functional level.

Evaluation

Success of the treatment was determined based on the horse's ability to return to (or begin) competitive racing. If the horse

was able to complete its training program, return to the racetrack and complete at least one race, it was regarded as a success: All other outcomes (including failure during the 1st race) were regarded as a failure. Entry into both studies ended in November, 1987 and follow-up was continued until the fate of the last animal to receive carbon fibers could be determined (approximately January, 1989). The horses that were initially entered into the study that were treated successfully had the opportunity to enter races during several racing seasons.

Carbon Fibers

The carbon fibers employed in this study were identical to those used in the clinical study. An unsized bundle of 10,000 8-micrometer fibers was heated at 300°C for 20 minutes to remove residual organic contaminants. Carbon-fiber debris present in the bundle as a consequence of the original manufacturing process was removed by passing the bundle through a water bath. The carbon-fiber bundle was then passed through a bath of gelatin and glycerine and wiped with pure cellulose to remove any residual debris and facilitate handling of the carbon fibers during surgery. Four carbon-fiber bundles were lashed together at the ends using absorbable sutures, to form the actual implant.

Microscopy

Carbon fibers were placed in the uninjured superficial flexor tendon in 3 horses which were sacrificed at 6, 14, and 25 months after implantation. In 2 horses with chronic bowed tendons (more than 9 months) carbon fibers were implanted and recovered at 18 and 25 months after surgery, respectively. The regional lymph nodes were recovered in the 18-month animal.

The specimens containing carbon fibers were passed through a graded series of alcohols up to absolute ethanol, propylene oxide (PO), 1:1 mixture of PO and Spurr embedding media, 1:2 mixture of PO and Spurr, and then a pure mixture of Spurr under vacuum. Each sample was then embedded in a mold and heated at 60°C (±5°C) for 18-24 hours. The blocks were trimmed and cut (0.75 micrometers) on an ultramicrotome (LKB Model 5) using a diamond knife. Other blocks were cut on a diamond saw (South Bay Technology, Model 650) and polished using a graded series of abrasives to a final thickness of approximately 125 micrometers. The tissue sections were stained with Spurlock (Toludine O/basic fuchsin) and coverslipped.

The lymph nodes were embedded in wax, sectioned at 10 micrometers, and stained with hematoxylin and eosin.

Statistics

The data was evaluated using the 2x2 chi-square test, with the

Yates continuity correction. The chosen level of significance was $P < 0.05$.

RESULTS

The results for the horses that had failed conventional therapy are listed in Table 1. Numbers 1-17 each suffered an injury to one tendon (the superficial flexor tendon in all but one case), and failed their subsequent (listed) course of treatment. Horses 18-28 suffered injuries to 2 or 3 tendons, and subsequently failed treatment. Results for the horses that received carbon fibers for treatment of acute injuries are listed in Table 2; 16 horses received the implant within 30 days of injury, 2 within 60 days, and 1 within 90 days. Results obtained in the (largely) historical control group are listed in Table 3. Statistical evaluation of the data presented in Tables 1-3 is given in Table 4.

In the group that suffered a single tendon injury and failed conventional therapy, 65% returned to the racetrack. In the group that suffered 2 or more tendon injuries and failed conventional therapy, 45% of the horses returned to the racetrack. In the acutely treated group, about 74% of the horses returned to the racetrack, compared to about 23% of the horses that were treated conventionally. Each of the differences was statistically significant.

All carbon-fiber specimens exhibited similar histologic features consisting of tissue ingrowth between individual carbon fibers that dissected longitudinally. The tissue formed a matrix composed of collagen fibers, fibroblast/fibrocytic type cells, and small thin-walled blood vessels. Small numbers of macrophages were present in some perivascular locations but phagocytization of carbon-fiber particles was not observed in any specimen. Isolated neutrophils (but not eosinophils) were seen.

Between 6 and 14 months post-operatively what appeared to be a greater penetration of new tissue into the carbon-fiber bundle was observed but the specimens recovered 14-25 months post-operatively were indistinguishable with regard to the extent of tissue ingrowth. No carbon fibers were observed in the regional lymph nodes at 18 months post-operatively.

DISCUSSION

Eighteen horses that had an injury to one tendon and failed conventional therapy (Table 1) were implanted with carbon fibers at 9.3 ± 5.3 months following the initial injury, and 65% successfully returned to the racetrack. The question arises whether the observed significant improvement in outcome was due to the presence of the carbon fibers -- which would support the hypothesis that induced tissue forms a mechanically proficient internal stent -- or to the rest and post-operative care provided during the year between

implantation and return to the racetrack. Although it has not been satisfactorily measured previously, the success rate for a first treatment is generally regarded as low. It is therefore reasonable to assume that the success rate for a second treatment would be still lower. Consequently, it seems permissible to impute the 65% success rate to the presence of carbon fibers, and thus to interpret the results in support of the hypothesized mechanism of action of carbon fibers.

About 45% of the horses with multiple chronic injuries successfully returned to the racetrack. The 6 failures included both horses that had injuries to 3 tendons, and 4 horses that had bilateral injuries (only 2 of the 5 successfully treated horses were injured bilaterally). Taken as a whole, the results from this group suggests that the success rate is inversely proportional to the number of tendons initially injured; it was 65%, 45%, and 0% for 1, 2, and 3 injured tendons, respectively.

About 74% of the acutely injured horses returned to the racetrack compared to 23% in the control group. Although precise comparisons with previous reports are difficult it appears that a success rate of 23% for conventional therapy is comparable to the success rate reported by others (7). The higher success rate in the carbon-fiber group can reasonably be attributed to the presence of the implant because the horses in this group were treated the same as those in the control group but for the presence of the carbon fibers.

The results probably do not indicate that the acute use of carbon fibers produced a higher success rate than their use for treating chronic bowed tendon. The 65% success rate in the chronic group (1 injured tendon) can plausibly be compared with a very low (perhaps 0%) success rate in horses that failed one course of treatment and were then subjected to a second treatment. Thus, a 65% success rate can be attributed to the action of the carbn fibers themselves. Although 74% of the acutely injured horses returned to the racetrack, 23% could have been expected to return even in the absence of the carbon fibers. Thus, the present study provides no evidence that success rate is a function of time between injury and treatment (in horses that had an injury to only one tendon). We conclude, therefore, that the efficacy of the carbon fibers was associated with the stenting effect of the foreign-body induced tissue, and that any effect of carbon fibers on proliferating scar tissue in the context of an acute injury had no effect on outcome.

Histologic examination demonstrated that the carbon fibers were pathologically inert, and stimulated a tissue growth that stabilized the implants. The presence of only isolated neutrophils indicated that the fibers were not chemotactic, and the absence of lymphocytes also indicated that they do not induce an immune response. The lack of eosinophils demonstrated that the fibers did not elicit an allergic reaction.

Although the amount of tissue ingrowth into the carbon-fiber

bundle and the cross-sectional area of the tendon each varied from level to level, the carbon-fiber bundle generally occupied about 30% of the cross-sectional area of the implanted tendon. The actual strength that the induced tissue contributed to the tendon can be discussed by using a model that takes into account the injury lesion and the effect of the carbon fibers.

Let A_t, A_i, A_c, be the cross-sectional areas of an intact tendon, the portion of the cross-sectional area of the tendon that is injured following a bowed tendon and subsequently heals, and the cross-sectional area of the carbon-fiber bundle, respectively. Following the acute injury stage, the bowed tendon resumes (approximately) its normal cross-section, but the connective tissue that formed to bridge the defect (scar) is mechanically weaker than the original (uninjured) tissue; it is the likely site of a rebow. Thus, the ultimate tensile load (UTL) that the tendon can sustain has been reduced because normal tissue has been replaced by (inferior) scar tissue at the level of the lesion.

Prior to injury, the UTL of the tendon was proportional to A_t. After injury, the UTL is proportional to $A_t - jA_s$, where j is a scale factor that accounts for the reduced mechanical strength of scar compared to normal tendon (j = 0 if scar is assumed equal to tendon, j = 1 if scar is assumed to contribute no mechanical strength). After reconstruction, the UTL is proportional to $A_t - jA_s + kA_c$, where k is defined for carbon-fiber induced tissue in a manner similar to the definition of j. The effect of carbon fibers (compared to conservative treatment) is given by the ratio $(A_t - jA_s + kA_c)/(A_t - jA_s)$.

$$(A_t - jA_s + kA_c)/(A_t - jA_s) = 1 + kA_c/(A_t - jA_s)$$

$$= 1 + (kA_c/A_t)/(1 - jA_s/A_t)$$

If $A_s/A_t = 0.5$, $A_c/A_t = 0.3$, $j = k = 0.5$, then the ratio above is 120%. Thus, under these assumptions, the effect of implanting carbon fibers is to increase the mechanical strength of the tendon at the level of the lesion by approximately 20%. Since it is at the level of the lesion that a previously bowed tendon is likely to fail, this has the effect of increasing the UTL of the tendon by 20%. This precise value depends, of course, on the assumed contributions to the mechanical strength due to the scar and induced tissue. But all reasonable assumptions lead to the conclusion that a significant increase in strength is conferred on the tendon by the presence of the carbon fibers because its contribution occurs in addition to the normal healing response.

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TABLE 1. Use of Carbon Fibers in Thoroughbred Racehorses that Failed Conventional Therapy. Degree of injury: 1, obvious but mild; 2, bulging and filling; 3, bowed out; 4, severe bow. S, superficial digital flexor tendon. D, deep digital flexor tendon. L, left forelimb; R, right forelimb. The number in parentheses is the number of races successfully completed at the time of the last follow-up.

NAME	AGE (years)	DEGREE OF INJURY	INJURED TENDON	INJURED LIMB	PRIOR TREATMENT			RESULT WITH CARBON FIBERS
					Type	Duration (months)	Result	
1. Mission in Orbit	5	3	S	L	Rest	19	Failure	Success (19)
2. Now and Then	6	3	D	L	Rest	14	Failure	Success (32)
3. Seward Junction	4	2	S	L	Rest Blistering	6	Failure	Success (2)
4. Nale the Crow	3	2	S	L	Rest Blistering	7	Failure	Failure
5. Satan's Crown	5	2	S	L	Rest	6	Failure	Failure
6. Rare Market	3	3	S	R	Rest Laser	9	Failure	Failure
7. Bold Jigger	6	2	S	L	Rest Blistering	7	Failure	Failure
8. Basic White	4	2	S	L	Rest	5	Failure	Success (1)
9. Oakland Bay	5	2	S	R	Rest	4	Failure	Success (17)
10. Eastern Saga	4	3	S	L	Rest Blistering	24	Failure	Success (7)
11. Anjuli	3	2	S	R	Rest Blistering	6	Failure	Success (5)
12. Regal Line	3	4	S	R	Rest Blistering	9	Failure	Success (1)
13. Cannock	4	3	S	L	Rest	6	Failure	Success (4)
14. Live In Lover	3	2	S	R	Rest	9	Failure	Failure
15. Royal Saint	4	2	S	L	Rest	9	Failure	Success (8)

Table 1, continued

NAME	AGE (years)	DEGREE OF INJURY	INJURED TENDON	INJURED LIMB	PRIOR TREATMENT Type	PRIOR TREATMENT Duration (months)	PRIOR TREATMENT Result	RESULT WITH CARBON FIBERS
16. Wellgroomed	6	2	S	R	Rest	12	Failure	Success (3)
17. Pettit Prince	5	2	S	L	Rest	6	Failure	Failure
18. Doctor T	3	3 	S D	R R	Rest Blistering	24	Failure	Success (5)
19. Timmy's Charro	3	2 	S S	L R	Rest Blistering	20	Failure	Failure
20. Houston Oaks	5	3 	S S	L R	Rest Blistering	19	Failure	Failure
21. Majestic Jabot	6	3 2 3	S S D	L R L	Rest	24	Failure	Failure (retired)
22. Prince Diplomat	4	3 	S S	L R	Rest	6	Failure	Success (6)
23. Lt. Farcliff	4	4 	S D	R R	Rest Blistering	13	Failure	Failure
24. Strawberry Drone	2	1 	S S	L R	None	4	Failure	Success (4)
25. Big Bad Blade	4	3 	S D	R R	Rest	11	Failure	Failure
26. Duncan's Mill	3	2 	S D	R R	Rest	6	Failure	Success (2)
27. Song Dancer	4	4 3 	S S D	L R L	Rest	6	Failure	Failure
28. Noble Notion	5	3 	S D	L L	Rest, Laser	18	Failure	Success (2)

TABLE 2. Use of Carbon Fibers in Acute Injuries. Degree of injury: 1, obvious but mild; 2, bulging and filling; 3, bowed out; 4, severe bow. S, superficial digital flexor tendon. D, deep digital flexor tendon. L, left forelimb; R, right forelimb. The number in parentheses is the number of races successfully completed at the time of the last follow-up.

NAME	AGE (years)	DEGREE OF INJURY	INJURED TENDON	INJURED LIMB	RESULT
1. Nil's Boy	2	2	S	L	Success (11)
2. Joy's Colt	3	1	S	R	Failure
3. Alydarmer	3	2	S	R	Success (6)
4. L'Enjolie Mache	3	2	D	L	Success (2)
5. No Illusions	3	2	S	R	Success (2)
6. One Iron	3	4	S	L	Failure
7. Hilarious Beau	3	2	S	L	Success (8)
8. Copeland Rode	2	2	S	R	Success (1)
9. B.J.'s Dawn	5	3	S	L	Success (7)
10. King Titch	2	2	S	R	Failure
11. Kwiktan	3	4	S	L	Success (14)
12. Gift Horse	3	3	S	L	Success (11)
13. Wild Wood	3	2	S	R	Failure
14. Garam Shah	3	3	S	L	Success (5)
15. Pet Mac	6	2	S	L	Success (4)
16. Only a Pound	5	1	S	L	Success (6)
17. Lucky Loot	4	2	S	L	Failure
18. Snowbank	4	2	S	R	Success (2)
19. Regal Shield	2	4	S	L	Success (2)

TABLE 3. Control Horses for Acute Implantation of Carbon Fibers. Degree of injury: 1, obvious but mild; 2, bulging and filling; 3, bowed out; 4, severe bow. S, superficial digital flexor tendon. D, deep digital flexor tendon. L, left forelimb; R, right forelimb. The number in parentheses is the number of races successfully completed at the time of the last follow-up.

NAME	AGE (years)	DEGREE OF INJURY	INJURED TENDON	TREATMENT	RESULT
1. Gambler's Debt	3	2	S	Rest Blistering	Success (3)
2. Terson	8	3	S	Rest Blistering	Success (9)
3. Majestic Coastline	3	3	S	Rest Blistering	Failure
4. Perdition's Son	3	2	S	Rest Blistering	Success (1)
5. Safecracker	5	3	S	Rest Blistering	Failure
6. Midnight Desire	3	3	S	Rest Blistering	Failure
7. Beaubridge	3	2	S	Rest Blistering	Success (2)
8. Happy Snookie	5	3	S	Rest	Failure
9. Once a Dancer	4	2	S	Rest	Failure
10. Tuff One To Follow	3	4		Rest	Failure
11. Enlightenment	3	3	S	Rest	Failure
12. Boldansexy	4	3	S	Tendon splitting	Failure
13. Explosive Wagon	5	2	S	Rest	Failure
14. Charming Jake	4	3	S	Rest	Failure
15. Dustin Power	3		S	Rest	Failure
16. Peg Em Out	4	3	S	Rest	Failure
17. Osnola Native	4	4	S	Rest	Failure
18. Dese Days	4	3	S	Rest	Success (5)

Table 3, continued

NAME	AGE (years)	DEGREE OF INJURY	INJURED TENDON	TREATMENT	RESULT
19. Tick	4	3	S	Rest	Failure
20. Round River	3	1	S	Rest	Failure
21. Shelay	8	2	S	Rest	Failure
22. Double Anniversary	6	1	S	Rest	Success (2)
23. Pick Up The Tab	4	1	S	Rest Laser	Failure
24. Sovereign Dignity	4	2	S	Rest Blistering	Failure
25. Scott's Thirst	5	3	S	Rest	Failure
26. Special Trip	3	2	S	Rest Magnetic therapy	Failure

TABLE 4. Statistical Analysis (Chi-Square Test) of Data from Horses that Received Carbon Fibers

HORSES THAT FAILED CONVENTIONAL THERAPY -- ONE INJURED TENDON

	Success	Failure
Conventional	0	17
Carbon Fibers	11*	6

* $P < 0.05$ ($X^2 = 13.44$)

HORSES THAT FAILED CONVENTIONAL THERAPY -- MULTIPLE TENDON INJURIES

	Success	Failure
Conventional	0	11
Carbon Fibers	5*	6

* $P < 0.05$ ($X^2 = 4.14$)

HISTORICALLY CONTROLLED STUDY OF CARBON FIBERS IN ACUTE INJURIES

	Success	Failure
Conventional	6	20
Carbon Fibers	14*	5

* $P < 0.05$ ($X^2 = 9.43$)

FIGURE 1. Anatomy of the forelimb of the horse. The suspensory ligament arises from the carpal bones, bifurcates, and extends to the proximal sesamoid bones. Near the carpus the SDFT is enveloped by a synovial sheath in common with the DDFT. The SDFT becomes flattened and broader, and at the fetlock it widens greatly forming a ring through which the DDFT passes.

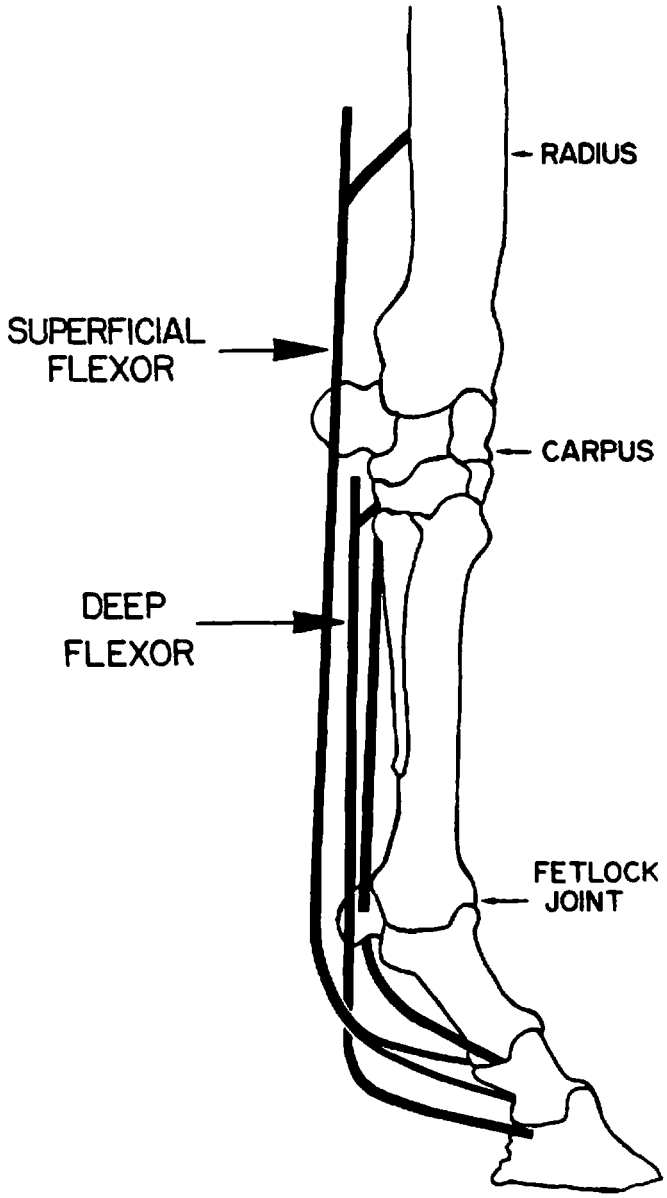

FIGURE 2. Surgical technique used to repair transected or ruptured tendons. Left, Valdez (3); Middle, Nixon et al. (5); Right, Brown and Poul (4).

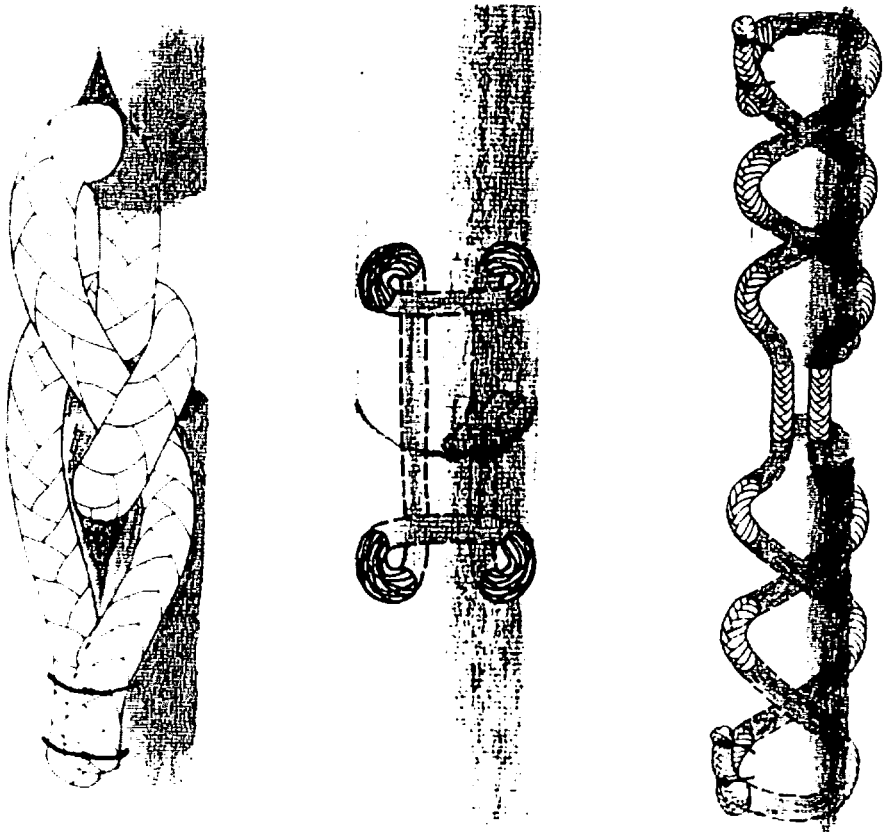

FIGURE 3. Surgical technique used to repair sprained tendons. Left, Goodship et al. (7); Middle, Littlewood (6); Right, Vaughan et al. (8).

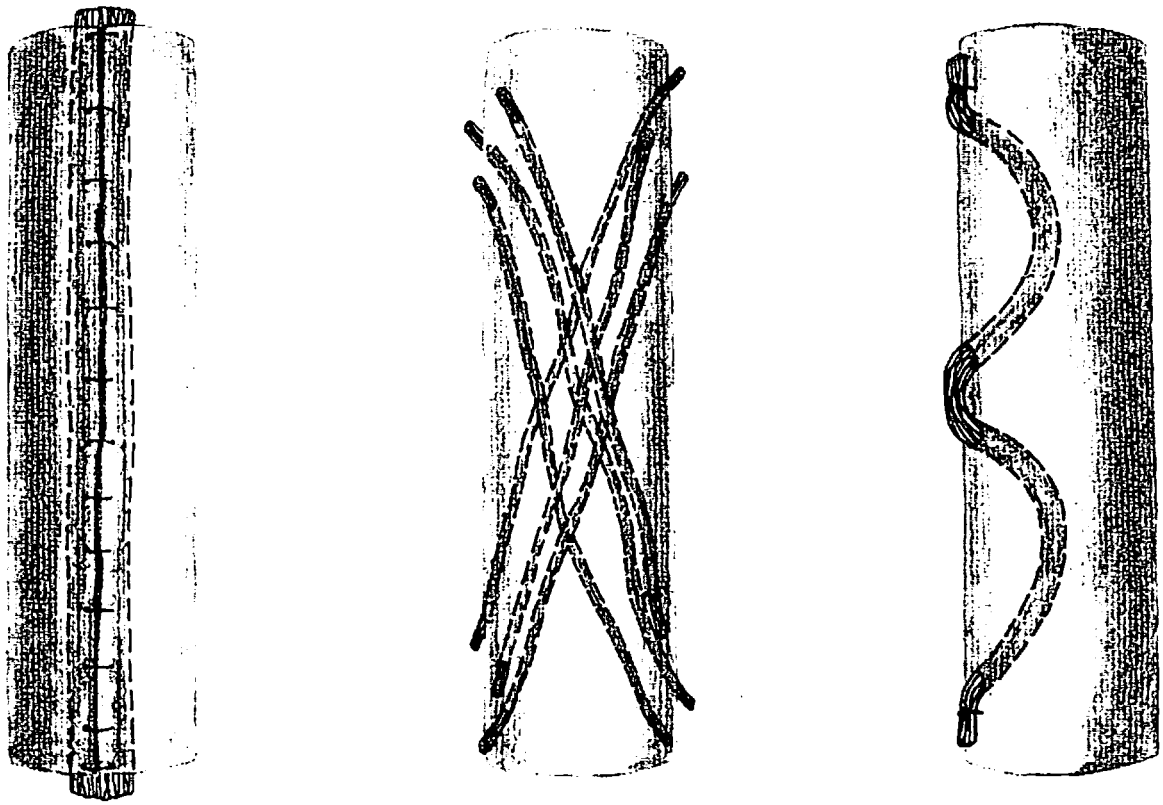

FIGURE 4. Left, Hypothesis for the mechanism of action of carbon fibers in sprained tendon. The induced foreign-body tissue forms an internal stent across the injury site. Right, Surgical technique used to repair sprained tendon.

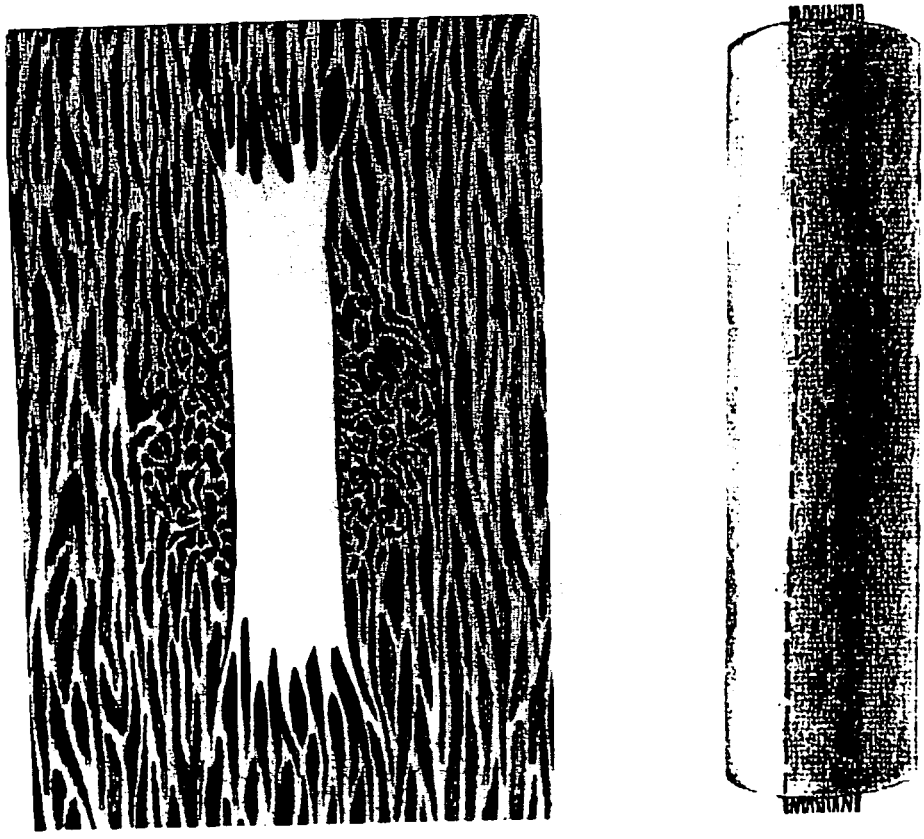

FIGURE 5. Surgical instruments used to place carbon fibers in the superficial and deep flexor tendons of Thoroughbred racehorses.