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Use of carbon fibers for repair of abdominal-wall defects in rats

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Carbon in the form of 8-gm fibers induces growth of connective tissue. 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 to 12 months postoperatively. The predominant tissues 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. Carbon fibers are potentially useful for reinforcing abdominal-wall defects. (SURGERY 1990;107:627-31.)

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WHEN AN ADEQUATE REPAIR of abdominal-wall defects cannot be achieved because the autologous tissue is either weak or missing, synthetic materials may be used to provide reinforcement for weak areas or replacement for missing tissue. Available synthetics have shortcomings, and the question arises whether implants made of newer materials would be an improvement.

Carbon, in the form of thin fibers (8 μm in diameter), acts as a scaffold for the ingrowth of connective tissue in tendons and ligaments,[1-4] and it might be useful in the repair of abdominal-wall defects.[5-7] When several bundles of 10,000 carbon fibers were braided and used as a side-to-side reef to repair large ventral incisional hernias in sheep, the results were superior to those obtained by use of polyester suture.[7]

Carbon fibers are hard and brittle, and methods of preparing histologic sections have only recently been developed. The purpose of this study was to measure and histologically characterize tissue ingrowth occurring in carbon fibers, compared with polypropylene mesh, 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.

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MATERIAL AND METHODS

Implanted material. A bundle of 3000 carbon fibers (each approximately 8 μm in diameter) was heated at 300° C in an inert atmosphere to liberate residual impurities, was wiped with a cellulose sponge to remove debris from broken fibers, and was coated with gelatin/glycerol to facilitate weaving. The implant was formed on a hand loom as a closed weave from a continuous bundle (Fig. 1). Individual carbon fibers are very strong,[3] but the mechanical strength of the implants used in this study was not directly measured. The average weight of the carbon-fiber implant was 35.2 mg/cm^2. Polypropylene mesh (Marlex; Bard Implants, Billerica, Mass.) was used as the control implant. It was an open-faced (about 33% open) knit of polypropylene fibers and weighed about 13.2 mg/cm^2.

Surgical procedure. Twenty-two female Sprague--Dawley rats (Charles River Laboratories, Needham, Mass.), 250 to 350 gm, were used in the study. After anesthesia (3 mg/kg xylazine hydrochloride Rompun] and 35 mg/kg ketamine, intramuscular) was induced, 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 with a 3.5 X 4.5 cm onlay of either carbon fibers or polypropylene mesh. The im-

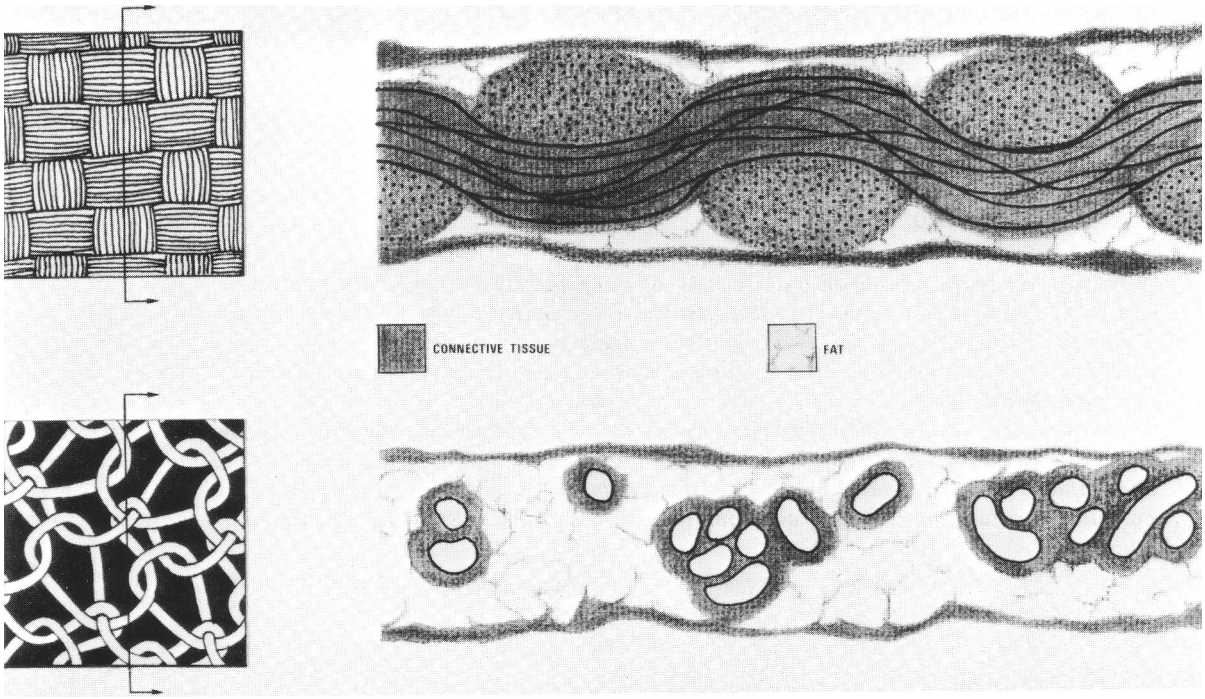

Fig. 1. Implant materials used and tissue response at 12 months. The carbon-fiber implant (top left) was a closed weave formed from a 3000-fiber bundle. The polypropylene implant (bottom left) (Marlex) was an open knit formed from a single strand of polymer. The tissue response to the implants at 12 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.

planted material was sutured around its perimeter to the abdominal wall with running stitches (6-0 Proline; Ethicon, Inc., Somerville, N.J.) 2 to 4 mm apart. The skin was closed with a running subcuticular stitch, and each animal was housed individually and was fed and given water ad libitum.

Histologic procedures. Animals were killed at 3, 6, and 12 months postoperatively (T-61 euthanasia solution; Gaylor Pharmaceutical, Decatur, Ill.), and the implant was excised along its margin, was separated from loosely adhering soft tissue, and was 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 from the central portion of the implant were dehydrated and embedded in epoxy. The blocks were cut either on an ultramicrotome (at 0.75 μm) or a diamond saw (at 125 μm), and the tissue sections were stained with a mixture of toluidine blue 0 and basic fuchsin. 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, were embedded in wax, were sectioned at 10 m, and were stained with hematoxylin and eosin. Every section of each node was mounted on glass slides and was coverslipped to permit an unambiguous assessment of the presence of debris in the nodes. All slides of the lymph nodes were examined blindly by one of us (R.P.M.).

Statistically comparisons were made with 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 more than 15% to 20% of the exposed surface of each implant. In most rats recovered 12 months postoperatively, sharp dissection was required to separate the im-

Table I. Comparison (carbon fibers versus polypropylene) of tissue-induced in abdominal-wall defects

	Tissue wet weight (mg/cm^2)			
Recovery time (mo)	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

NOTE: 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' for carbon fibers 13.2 mg/cm2 for polypropylene) from the specimen weight. n, Number of rats
*p <0.05.

plant from the liver and intestine (also more than 15% to 20%). Gross infection, seroma formation, enteric fistulas, 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 μm 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 I). 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 to 50 μm 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 (Fig. 1). Occasionally, pairs of bundles in the weave were locked tightly together, and these bundles contained only scanty amounts of tissue. The typical appearance of the carbon-fiber implant at 12 months is illustrated in Fig. 1. Tissue was found throughout the bundles (Fig. 2, *A*) except in occasional regions where the bundles were wedged tightly together. The tissue inside the carbon--fiber bundle was invariably dense connective tissue (Fig. 3, *A*); loose connective tissue and fat were not observed

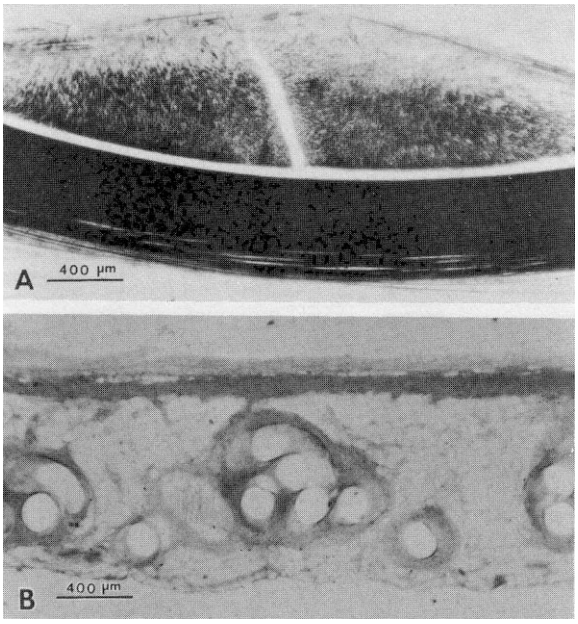

Fig. 2. Tissue ingrowth into carbon-fiber and polypropylene implants at 12 months postoperatively. The planes of the views correspond to those illustrated in Fig. 1. **A**, The view plane is perpendicular to the upper bundle, thereby permitting visualization of the tissue inside the bundle. Tissue cannot be visualized in the lower bundle in this view because of the thickness of the specimen (10 to 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.

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 (Fig. 4, A). The fibroblast--like cells appeared to form a sheath around individual carbon fibers.

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 to 12 months the connective tissue became mostly restricted to the region surrounding the fibers, and adipose tissue filled the remaining space (Figs. 2 and 3, *B*).

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.

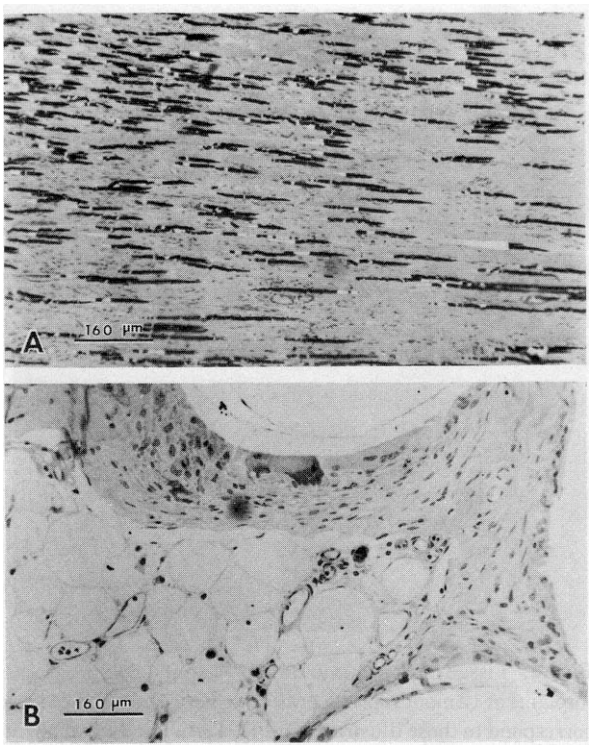

Fig. 3. Representative tissue ingrowth into carbon fiber **(A)** and polypropylene **(B)** implants at 12 months. The specimens were cut at a thickness of 0.75 μm and stained with a mixture of toluidine blue 0 and basic fuchsin. **A**, The induced tissue is relatively homogeneous and consists of a fibrous matrix and numerous fibroblast-like cells. Occasional lymphocytes and giant cells were also observed. **B**, Dense connective tissue typically occurs only within 20 to 30 μm of the polypropylene fiber. The remaining interfiber region contains loose connective tissue and fat.

Fig. 4. Representative induced tissue inside carbon-fiber bundles at 12 months. Both specimens were cut at 0.75 gm and stained with a mixture of toluidine blue 0 and basic fuchsin. 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 ito 4 degrees, 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 to 23 degrees. The nuclei are more round, as expected if the cells were oriented along the fibers.

DISCUSSION

The ability of carbon fibers to induce proliferation of fibrous tissue has previously been reported in tendons and ligaments,[1-4] and we found that it also occurred in full-thickness abdominal-wall defects in rats after 12 months (Figs. 2, *A*, 3, *A*, and 4). 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 to 12 months (Table I). 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, confirming similar observations at other implant sites.[2-4,8] 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 that is 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.

Propylene 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 (Figs. 2, *B,* and 3, *B).*

Polypropylene mesh apparently provides some clinical benefit in the short term (1 to 2 years),[9-11] 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 as a cuff. As an

overlay, polypropylene mesh is intended to stabilize a wound or a suture line until healing takes place via scar formation. Since minimal fibrous tissue is actually induced by polypropylene mesh (Figs. 2, *B,* and 3, *B*), 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 by 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.[12,13] The absence of long-term follow-up involving human use of polypropylene raises the question of its long-term safety. In contrast, there have been no reports indicating that carbon in any form is a mutagen or carcinogen. Attempts to induce tumors with carbon implants in animal models have failed.[8]

In summary, in the animal model used, 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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