
Tissue response to single-polymer fibers of varying diameters: Evaluation of fibrous encapsulation and macrophage density

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Abstract: An *in vivo* study was conducted to assess the sensitivity of fibrous capsule thickness and macrophage density to polymer fiber diameter. Single polypropylene fibers of diameters ranging from 2.1 to 26.7 μm were implanted in the subcutaneous dorsum of Sprague–Dawley rats. Results at 5 weeks demonstrated reduced fibrous capsule thickness for small fibers. Capsule thickness was 0.6 (± 1.8) μm , 11.7 (± 12.0) μm , 20.3 (± 11.6) μm , and 25.5 (± 10.0) μm for fibers in the ranges of 2.1 to 5.9, 6.5 to 10.6, 11.1 to 15.8, and 16.7 to 26.7 μm , respectively. Fibers very near to blood vessels had smaller capsules than did those with local vasculature further away. The macrophage density in tissue with fiber diameters 2.1 to 5.9 μm ($23.03 \pm 8.67\%$) was comparable to that of unoperated contralateral control skin ($18.72 \pm 10.06\%$). For fibers with diameters in the ranges of

6.5 to 10.6, 11.1 to 15.8, and 16.7 to 26.7 μm , macrophage densities were $33.90 \pm 13.08\%$, $34.40 \pm 15.77\%$, and $41.68 \pm 13.98\%$, respectively, all of which were significantly larger ($p < 0.002$) than that for the control. The reduced fibrous capsule thickness and macrophage density for small fibers ($< 6 \mu\text{m}$) compared with large fibers could be due to the reduced cell–material contact surface area or to a curvature threshold effect that triggers cell signaling. A next step will be to extend the analysis to meshes to evaluate fiber-spacing effects on small-fiber biomaterials. © 2000 John Wiley & Sons, Inc. *J Biomed Mater Res*, 52, 231–237, 2000.

Key words: microfibers; fibro-porous biomaterials; fibrous encapsulation; macrophage; polypropylene

INTRODUCTION

Soft tissue response to porous and fibro-porous biomaterials is influenced, in part, by the microarchitecture of the implant. For porous materials, effects of changes in the sizes of the pores have been studied. Results suggest that pore dimensions of at least 10 μm are needed to allow connective tissue ingrowth and to avoid global encapsulation.¹ However, for a solid implant with pores only on the surface, pore dimensions that facilitated attachment without signs of inflammation were 1 to 3 μm .² Fibrous capsule thickness was greater for materials with 0.22- μm pores compared to those with 8- μm pores.³ For percutaneous implants that go across the skin, epithelial migration was significantly less for materials with pore sizes 3 to 8 μm

than for those with pore sizes between 0.025 and 3 μm .⁴ A pore size range of 0.8 to 8.0 μm was shown to facilitate neo-vascularization 80- to 100-fold compared to samples with pores 0.1 and 0.8 μm or between 8 and 15 μm .⁵ Pore dimensions that encouraged migration of endothelial cells onto vascular grafts were in the 40 to 50 μm range.^{6,7} For corneal implants, stratification evaluated *in vitro* was improved when pores were between 0.1 and 0.8 μm compared with between 0.8 and 3.0 μm .^{8–10}

Davila¹¹ suggested that fiber dimensions also are important. Possibly a scissoring effect is induced on soft tissue trapped within acute angles between adjacent filaments. Stresses on the tissue are high for large fibers because they are relatively inflexible and thus pinch the tissue. Stresses are reduced for fine monofilaments because the fibers bend around the tissue. Thus fine monofilament materials perform better than large fiber materials. Clark¹² evaluated polyester samples with internodal distances of 14.5 to 100 μm , an open area percentage ranging from 5 to 23%, and fiber diameters from 39 to 161 μm . He demonstrated a strong correlation between inflammatory tissue reaction and the ratio of the percentage of open area to fiber diameter. Jansen,¹³ using sintered metal fiber-

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web materials, found that fibrous capsule thickness was reduced for higher porosity implants. Materials with fiber diameters of 22 and 50 μm and porosities of 80 and 86% were used. It is relevant to note that in the above-described studies,¹¹⁻¹³ fiber diameters were greater than 14 μm . In only one study were smaller fibers considered. Bernatchez,¹⁴ using an *in vitro* macrophage cell culture model, noted reduced cell spreading on 12- μm single gold fibers compared with 25- μm fibers. Thin-fibered, nonwoven polybutylene/polypropylene (2 to 12 μm in diameter fibers) materials and nonwoven polyester (10 to 12 μm in diameter fibers) materials experienced minimal cell spreading compared with thick-fibered woven polyester (40 μm in diameter fibers) materials and woven nylon (38 μm in diameter fibers) materials. However, because design variables were not separated in sample populations, it is not clear if the fiber diameter, porosity, material chemistry, or a combination of variables induced the favorable effect.

The purpose of this research was to conduct *in vivo* studies to determine if tissue response was sensitive to fiber diameter in the 1 to 15 μm range. Single fibers of the same material (polypropylene) were implanted in rat dorsum for five weeks and fibrous capsule thickness and macrophage density then evaluated.

MATERIALS AND METHODS

Single fibers

Polypropylene (melt index 32.9, Aldrich, Milwaukee, Wisconsin) was used in this study since its stiffness is comparable to that of collagen fibers,^{15,16} the most common fibrous protein in skin. Fibers of diameters 2.0 to 27.0 μm were prepared. To prepare the fibers, a vessel of polypropylene was heated to approximately 210°C and then single fibers were drawn through a nozzle, a process that results in smooth, cylindrically shaped fibers varying in diameter depending on the draw rate. For each of four diameter ranges (2.0–5.9; 6.0–10.9; 11.0–15.9; and 16.0–27.0 μm) (Table I), 54 fibers were created. Fiber segments 1.0 cm in length were

cut, placed on a microscope slide, and their diameters assessed using a light microscope.

Using a dissecting microscope, fibers of different diameters were positioned parallel to each other in random order on 18 × 14 × 1-mm polycarbonate frames. There were 18 fibers per frame. Fiber ends were glued to the frame edges with cyanoacrylate (Fig. 1) such that none of the fibers extended outside the frame edge. A spacing of approximately 668 μm between fibers was maintained, a distance demonstrated in preliminary studies not to induce tissue response from adjacent fibers. The frame was necessary to provide mechanical support during implantation, and later it facilitated identification of fiber locations in tissue sections. Because all the frames had rounded and filed corners and edges, inflammation due to stress concentrations was minimized. The frames and fibers were gas sterilized before implantation.

Cytotoxicity evaluation was done on multi-fiber specimens using an agar diffusion test (US Pharmacopoeia, Rockville, MD). Endotoxicity evaluation was performed using a commercial kit (Associates of Cape Cod Incorporated, Falmouth MA). Fetal foreskin fibroblasts were used.

Animal model and surgical procedure

Animal care committee approval (June, 1998) was obtained for all procedures. NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) were observed. One frame was implanted in the lateral superior region of the subcutaneous dorsum (side selected randomly) of each of 12 Sprague-Dawley rats (mass 400 g), approximately one-third the distance between the head and tail. This location was over soft tissue; thus the frame did not abrade on underlying bone, it did not interfere with animal locomotion, and it could not be accessed by the animal's teeth post-surgery.

The fibers in the frame were fragile and breaking fibers during implantation was a potential problem, so a specialized surgical procedure was used. A 22-gauge needle and a 10-cc air-filled syringe were used to inject an air pocket subcutaneously while the animal was under vapor anesthetic (2% isoflurane with oxygen). [The skin will "inflate" only if the needle is positioned just beneath the smooth muscle

Group Number	Number of Fibers	Fiber Diameter		Fibrous Capsule Thickness
		Range (μm)	Mean ± SD (μm)	Mean ± SD (μm)
1	15	2.1–5.9	3.8 ± 1.1	0.6 ± 1.8
2	12	6.5–10.6	8.5 ± 1.5	11.7 ± 12.0
3	20	11.1–15.8	13.2 ± 1.5	20.3 ± 11.6
4	11	16.7–26.7	19.8 ± 3.6	25.5 ± 10.0

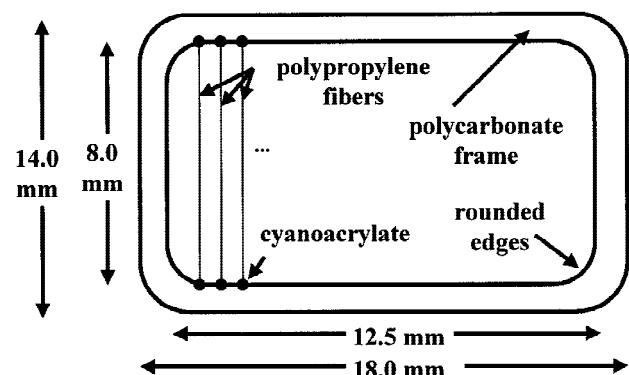


Figure 1. Schematic of polycarbonate frame strung with single fibers. There were eighteen fibers per frame.

layer (above the muscle fascia), thus insuring the pocket is created at a consistent depth.] A medial-lateral incision then was made at the superior edge of the air pocket, the skin elevated, and the frame inserted (Fig. 2). The incision was closed with staples, approximately 1 cm from the frame edge. All frames were left implanted for a 5-week period.

Tissue fixation and processing

The animals were euthanized in a carbon dioxide chamber, and the frame and surrounding tissue, as well as a comparably sized contralateral control sample, was excised. To avoid distorting the geometry of the tissue of interest, tissue was maintained at its *in vivo* dimensions by affixing the excised samples to wire meshes using staples at the boundaries. The samples were fixed in 4% paraformaldehyde for at least three days, dehydrated in serial solutions of 70, 95, and 100% ethanol, and then cleared in xylene.

After fixation, tissue within the central region of the frame was excised and embedded in paraffin under vacuum. Sections of 12 μm in thickness in planes perpendicular to the fiber axes were cut through a 0.14-mm distance. Histologic staining with hematoxylin and eosin was used to assess fibrous capsule thickness. Immunocytochemical labeling with ED1, using techniques similar to those described by Murry,¹⁷ was used to assess macrophage density.

Quantitative morphologic analysis

To assess fibrous capsule thickness, color images of fiber cross-sections were taken using a three-chip CCD camera (Optronics, Goleta, California) on a light microscope (Microphot-SA, Nikon, Melville, New York) with a computer data acquisition system. The system had a resolution of 2.5 to 8.7 pixel/ μm for the magnification range used. Identification of small fibers was difficult but facilitated through use of a polarizer on the microscope. For each fiber, contours of the

outer edge of the fibrous capsule and the fiber surface were traced using Image-Pro software (Media Cybernetics, Silver Springs, Maryland). The areas within the closed contours were calculated.

Tissue was considered part of the fibrous capsule if it met the following criteria: tissue was clearly disrupted; cell nuclei were deformed and formed around the fiber; and collagen fibers were formed around the fiber rather than in long strands parallel to the skin surface. Void regions of no tissue occasionally formed at the fiber-tissue boundary. Those regions were traced with another closed contour.

Fibrous capsule thickness was determined by first calculating the equivalent capsule radius, assuming a circular cross-section capsule

Equivalent capsule radius

$$= \sqrt{\frac{\text{Area within fibrous capsule contour} - \text{void area}}{\pi}}$$

and the fiber radius assuming a circular cross-section fiber,

$$\text{Fiber radius} = \sqrt{\frac{\text{Fiber area}}{\pi}}$$

Then, Fibrous capsule thickness = Equivalent capsule radius - Fiber radius.

Evaluation was conducted on one fiber from each group to assess variability in capsule thickness along a 0.14-mm length of fiber. Results indicated that there were no significant differences ($p < 0.01$) from one slice to the next for any group, thus indicating that any slice for each fiber could be selected for analysis. One-tailed *t* tests were performed to assess differences in fibrous capsule thickness between pairs of the four groups of fibers.

Macrophages labeled with ED1 appeared dark in tissue sections. Macrophage density was assessed using a point-counting method.¹⁸ A 117.6 $\mu\text{m} \times 81.8 \mu\text{m}$ sampling region, which included the totality of each fibrous capsule for all fibers, was used. Macrophage density results were expressed in units of μm^3 macrophages per 1 μm^3 volume of tissue. One-tailed *t* tests were performed to assess macrophage density differences between groups with different fiber diameters.

RESULTS

Cytotoxicity results demonstrated a score of less than 2 (mild reactivity) on the USP scale; thus the biomaterial samples were acceptable for implantation. The endotoxin level was 0.031 EU/mL, which is much less than the 0.500 EU/mL limit for medical devices.

From the implant studies a total of 58 fibers were found in the hematoxylin and eosin-stained sections. No fibers were found in four of the animals, presumably due to fiber damage during surgical implantation. There were at least eleven fibers in each of the four groups. Diameter ranges and means are shown in Table I.

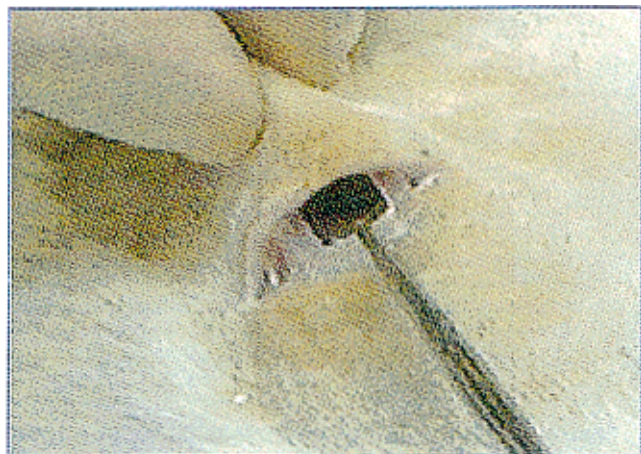


Figure 2. Frame implantation. A frame is being inserted into a subcutaneous tissue pocket in the lateral dorsum of a rat.

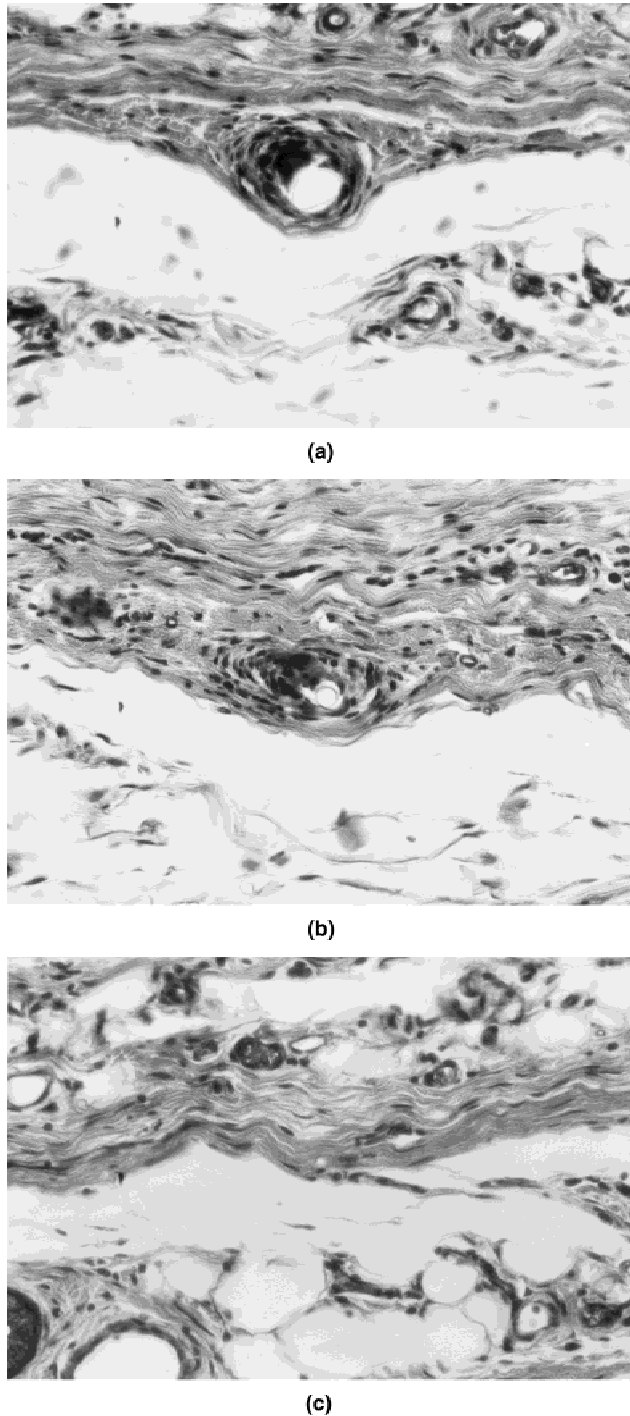


Figure 3. Tissue sections stained with hematoxylin and eosin. (Original magnification $\times 400$) Fiber diameters: (a) 26.4 μm ; (b) 11.9 μm ; and (c) 4.1 μm . A fibrous capsule is apparent in (a) and (b); no capsule is present in (c). The fiber is in the center of the image.

Fibrous capsule thickness

If a fibrous capsule formed around the fiber, it tended to be elliptically shaped with its long axis parallel to the skin surface. Nuclei of activated macrophages and foreign-body giant cells tended to align

concentric with the fiber. Small void spaces occasionally present between a fiber and its capsule were expected as a result of tissue-processing procedures.

Qualitatively, small fibers had smaller capsules than did large fibers [Fig. 3(a–c)]. For the mid- to large-diameter fiber range, some of the collagen within the capsule appeared pinker than surrounding tissue, which might be interpreted as new collagen formation.

Quantitatively, fibers of diameters smaller than 6.0 μm had much smaller capsules than did those with diameters larger than 6.0 μm (Table I). *P* values for comparison of Group 1 fibrous capsule thickness with those from Groups 2, 3, and 4 were 0.0044, 0.0000, and 0.0000, respectively (Table II). The percentage of fibers with no capsule in a group decreased from 87 to 33 to 5 to 0% for Groups 1 to 4, respectively. There was much scatter in the capsule thickness data for diameters larger than 6.0 μm (Fig. 4). No rat consistently showed larger or smaller capsule thickness than another rat. The R^2 value for a linear fit to capsule thickness versus fiber diameter for all data was 0.42.

An interesting observation was noted for fibers close to vascular structures. Five fibers in the study had nearby vessels. The results demonstrate that fibers with vessels within 13.0 μm of the fiber surface had much smaller capsules than those with the vessels further away, irrespective of the fiber diameter (Table III). The mean capsule thickness for fibers less than 13.0 μm from vascular structures was 6.9 μm while for those greater than 13.0 μm away the mean capsule thickness was 26.4 μm .

Macrophage density

Unactivated macrophages, apparent as small rounded cells in ED1-labeled sections, were distributed throughout all experimental and control tissue sections. Sections with mid- to large-diameter fibers had more unactivated and activated macrophages than did sections with small-diameter fibers [Fig. 5(a–c)]. Sections with fibers smaller than 6.0 μm had no large macrophages. Activated macrophages tended to encircle the fiber within the fibrous capsule.

Quantitatively, fibers of diameters smaller than 6.0 μm had much lower macrophage densities than those with diameters larger than 6.0 μm (Fig. 6). *P*-value comparisons of Group 1 data with Groups 2, 3, and 4

TABLE II
***P* Values: Capsule Thickness Comparisons Between Groups**

	2	3	4
1	0.0044	0.0000	0.0000
2		0.0291	0.0034
3			0.1053

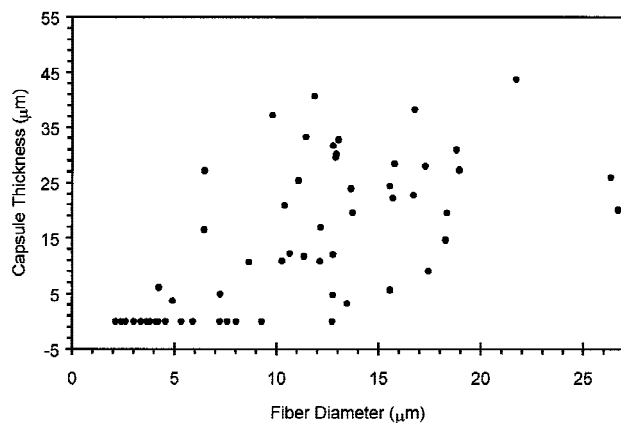


Figure 4. Capsule thickness for all fibers. Fibers of diameters smaller than 6.0 μm demonstrate minimal to no fibrous capsule compared with those larger than 6.0 μm in diameter.

data were 0.0171, 0.0083, and 0.0013, respectively (Table IV). Differences in means between groups 2 and 3, 3 and 4, and 2 and 4 were minimal, indicating minimal dependence on fiber diameter for the range of 6.5 to 26.7 μm . Group 1 macrophage densities were comparable to those for control tissue ($p = 0.0645$).

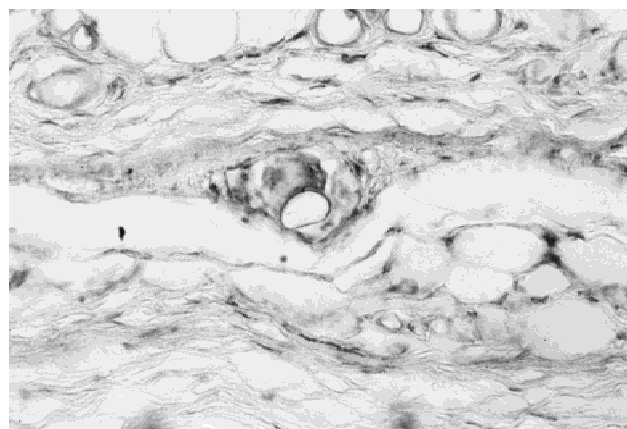
DISCUSSION

The result of minimal capsule thickness and normal macrophage density for Group I fibers (2.1–5.9 μm) compared with Group 2 (6.5–10.6 μm), 3 (11.1–15.8 μm), or 4 (16.7–26.7 μm) fibers demonstrates that the local environment at and around the fiber surface induces a lower level of activity by cells associated with an inflammatory response. Rather than attaching and spreading on the fiber surfaces, the cells remained their normal shapes and minimal fibrous encapsulation was induced.

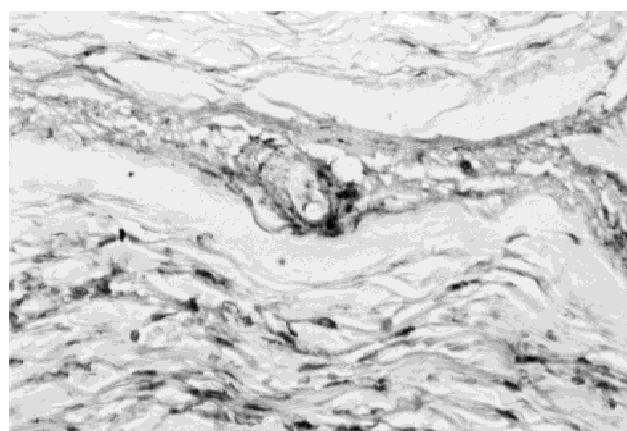
It is possible that a threshold surface area concept is at work here. This concept would suggest that below a certain threshold of cell–material contact surface area, there is an insufficient signal from the cytoskeleton and membrane to the nucleus to induce the unfavorable cell response. It has not been demonstrated, however, that such a threshold exists. It also is interesting to note here that the severity of response (fibrous capsule thickness, macrophage density) does

TABLE III
Capsule Thickness for Fibers Near Vessels

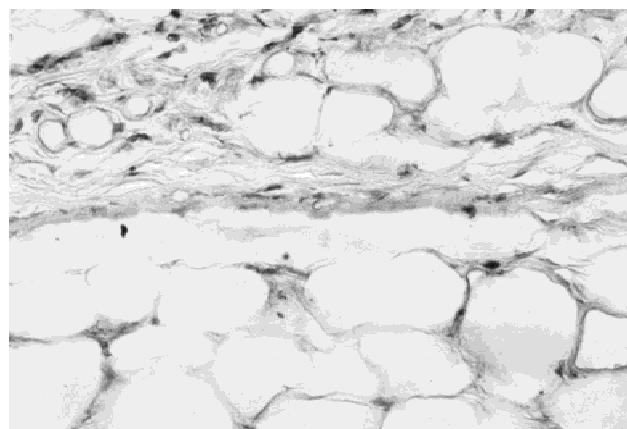
Distance from Fiber to Vessel (μm)	Fibrous Capsule Thickness (μm)	Fiber Diameter (μm)
8.2	5.6	15.6
10.5	3.2	13.5
12.9	12.0	12.8
62.5	33.2	11.4
67.3	19.6	13.7



(a)



(b)



(c)

Figure 5. Tissue sections stained with ED1. Fiber diameters: (a) 26.4 μm ; (b) 11.9 μm ; and (c) 4.1 μm . Many large activated macrophages are present around the mid-sized and large fibers (a and b) but not the small fiber (c). (Original magnification $\times 400$)

not show a strong linear correlation with surface area (fiber surface area is proportional to diameter).

The acceptable fiber dimensions demonstrated here are in the range of collagen fiber dimensions, 0.5 to 3.0 μm .¹⁹ A biomimicry principle would suggest that cells prefer fiber dimensions comparable to the natural

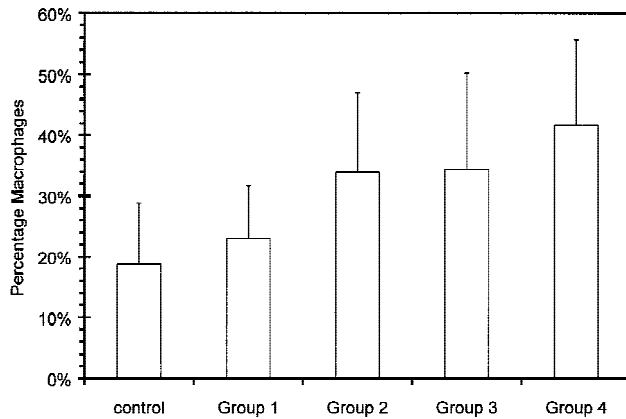


Figure 6. Macrophage densities for all implanted and control groups. Densities expressed as a percentage of tissue volume are comparable for control and Group 1 fibers while densities for Groups 2, 3, and 4 are much larger than the control values.

structures. It also is interesting to note, however, that the acceptable fiber diameter ($<6 \mu\text{m}$) overlaps with the range of pore dimensions acceptable for materials with porous surfaces (1 to $3 \mu\text{m}^2$; 0.8 to $8.0 \mu\text{m}^5$). Possibly surface curvature is a controlling parameter. If the cell membrane detects either convex or concave curvature in this range, it does not flatten out and proceed towards the classic encapsulation response but instead remains quiescent. Sensitivity of cell function to membrane surface curvature has been demonstrated for other signaling events.²⁰ It is relevant, however, that appropriate curvature does not appear to be the same for all applications, as indicated by different appropriate dimensions for endothelial cell migration^{6,7} and corneal applications.⁸⁻¹⁰

The acceptable fiber diameter range here ($<6 \mu\text{m}$) is consistent with Bernatchez's *in vitro* result that cell spreading is reduced for meshes with small-diameter fibers (2 to $12 \mu\text{m}$ compared with 38 to $40 \mu\text{m}$). Thus though it is possible in that study that porosity, material chemistry, or a combination of variables influenced response, the overlap of the acceptable fiber diameter ranges between that study and this one is relevant.

The reduced capsule thickness for fibers close to vascular structures is consistent with the expectation that tissue response is more favorable if nutrients are available locally. Fibro-porous meshes coated with ap-

propriate surface ligands to facilitate vascularization might be effectively used with thin-fiber materials to facilitate acceptance and to minimize encapsulation.

A next step will be to extend the analysis to the mesh level, that is, a network of fibers. It is expected that fiber spacings appropriate for allowing ingrowth and preventing detrimental interactions between fibers will be smaller than those described in the literature because of reduced capsule thickness around the fibers. There is more volume available for ingrowth. It will be particularly relevant to investigate behavior at the nodes to see if noncircular geometries alter the response.

CONCLUSIONS

Single polypropylene fibers implanted in the subcutaneous tissue of rat dorsum demonstrated high sensitivity to fiber diameter. Fibrous capsule thickness was minimal for fibers of diameters less than $6 \mu\text{m}$ compared with those of diameters greater than $6 \mu\text{m}$. Macrophage densities for fibers of diameters less than $6 \mu\text{m}$ were comparable to control macrophage densities while for fibers of diameters larger than $6 \mu\text{m}$, macrophage densities were substantially higher. Fibrous capsule thickness was reduced if vascular structures were near to the fibers. These results could have important application to the design of fibro-porous mesh implants.

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TABLE IV
P Values: Macrophage Density Comparisons
Between Groups

	1	2	3	4
Ctrl	0.0645	0.0013	0.0002	0.0002
1		0.0171	0.0083	0.0013
2			0.4629	0.1026
3				0.1086

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