A comparative evaluation of the tissue responses associated with polymeric implants in the rat and mouse

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Abstract: End product application is an important consideration when evaluating a material in an *in vivo* setting (Didisheim, Cardiovasc Pathol 1993;2:1S–2S). Small animal models allow high through-put evaluation of biocompatability. Previous preclinical evaluations have often used a rat subcutaneous model for the characterization of material-tissue interaction. Recent advances in genetic manipulation have provided mouse models with selective expression of a wide range of critical proteins. The rat model does not have many of the resources (i.e., knockouts, SCID, nude) that are present in mouse strains. The availability of these mice provides a resource to delineate the mechanisms regulating the healing associated with implants. However, before the mouse models can be used, they must be validated with respect to their ability to accurately assess tissue reponses to materials. In this study the tissue responses after the implantation of expanded polytetrafluoroethylene (ePTFE) were compared between rat and mouse. Discs of ePTFE (30-μm internodal distance) were implanted in subcutaneous and epididymal fat tissue of rats (Sprague-Dawley) and mice (129-SVJ). After 5 weeks the samples were removed and evaluated for vascular density, inflammation, and fibrous encapsulation. No difference in the vessel density was observed within the peri-implant subcutaneous and adipose tissue or within the porous material. However, a significant difference was found in the number of activated macrophages and giant cells between these two species. Implants in the rat exhibited greater numbers of activated inflammatory cells in the peri-implant tissue. The data indicate that the mouse and rat provide a comparable model for evaluating angiogenesis and neovascularization associated with synthetic porous implants. © 2001 John Wiley & Sons, Inc. J Biomed Mater Res 59: 682–689, 2002

Key words: tissue responses; polymeric implants; angiogenesis; neovascularization

INTRODUCTION

The National Institutes of Health currently estimate that 8–10% of the American population has a permanent medical implant. During the preclinical evaluation of these devices, animal models were often used to evaluate tissue-material interactions and device performance.[1] For example, animals ranging in size from dogs, sheep, and swine to small rodents have all been used and continue to provide critical information during the development of novel polymeric implants. The rat subcutaneous tissue implant site has proven to be a high-through-put, relatively low-cost screening technique for testing the initial tissue response to new materials.[2–4] This model has also permitted the evaluation of site-specific tissue responses to material structural alterations, changes in polymeric composition, and material surface modifications.[5,6] Recent advances in molecular biology and the development of genetic models of disease can greatly augment our knowledge of healing responses associated with polymeric implants.[7,8] The importance of a single gene can be evaluated through its absence or its abundance, as exemplified in knock-out and transgenic animals, respectively. Conversely, the interplay of thousands of genes or gene products can be determined in one assay, as exemplified by the use of genomic and proteomic techniques.[9–11] Yet, most of these new technologies are currently available for use solely in mice. Therefore, in order to take advantage of these new technologies, it is essential to understand the healing responses associated with polymeric implants in the mouse, and compare these with the more commonly used preclinical rodent species, the rat. The current study evaluated the angiogenic, inflammatory and fibrous encapsulation responses associated with poly-

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meric implants in the rat subcutaneous and adipose tissue and compared these responses to the mouse at a 5-week time point. The polymer used for these studies was 30 micron internodal distance, standard wall ePTFE.

MATERIALS AND METHODS

Study design

All procedures were approved by the University of Arizona Health Sciences Center Animal Care and Use Committee, following guidelines established by the National Institutes of Health. Anesthesia was induced in six Sprague-Dawley rats and six 129-SVJ mice by intraperitoneal injection of 50 mg/kg sodium pentobarbital or 2 mg/0.01 kg avertin, respectively. Discs of material (6-mm diameter for rats; 4-mm diameter for mice) were prepared from commercially available tubular ePTFE, 30-μm internodal distance, standard wall with a 4-mm internal diameter (ePTFE Vascular Grafts, Impra Inc., Tempe, AZ). Preparation of these discs from commercial, tubular vascular graft imparts a polarity to the discs. Throughout this evaluation the term ablumen is used to describe the outer curve of the material disc and lumen is used to describe the inner curve of the material disc. Each animal received a total of four discs, implanted into the right and left epididymal fat pads and the right and left rear haunch subcutaneous tissue. Five weeks after implantation, the samples were removed and placed in Histochoice fixative (Amresco, Solon, OH).

Histology and immunohistochemistry

After fixation, the explanted discs were dehydrated, embedded in paraffin, sectioned at 6m and processed for hematoxylin and eosin (H&E) and immunocytochemical evaluation. Blood vessels of all diameters were identified using the lectin, *Griffonia simplicifolia* (peroxidase conjugated lectin-GS-1, 1:100; EY Laboratories). Additional sections were evaluated for the presence of activated macrophages and monocytes using ED1 antibody (monoclonal, mouse anti-rat ED1, 1:200; Serotec, Inc.) visualized with a peroxidase-conjugated secondary antibody. Nuclei were lightly counterstained with methyl green.

Vascular density

Vascular density was evaluated using the sections reacted with GS-1 viewed under a 40× water-immersion lens. The number of cross-sectional and longitudinal vessel profiles was counted in a total of 40 high-power fields (HPF = 54 × 54 μm^2). These HPF were randomly selected at the tissue-polymer interface, along the entire length of the polymer. The criteria for a positive vessel were as follows: 1) positive GS1 reaction, 2) an identifiable lumen, and 3) located within the designated HPF area. Vessel counts per HPF were assessed based on their location relative to the polymer. Four areas were defined as lumenal tissue (n = 10 HPF), lumenal polymer (n = 10 HPF), ablumenal tissue (n = 10 HPF), and ablumenal polymer (n = 10 HPF). Vascular density for each implant group was expressed as mean number of vessels/mm^2 ± standard error.

Inflammation

Using 54 × 54-μm^2 HPF, ED1-positive cells within the ablumenal (n = 10) and lumenal (n = 10) tissue associated with each material disc were counted. Inflammatory index for each implant group was expressed as mean number of inflammatory cells/mm^2 ± standard error.

Tissue capsule evaluation

Using a 20× objective, five random images were captured at the lumenal and ablumenal polymer surfaces from the H&E stained sections using a 20× objective and a Sony cats-eye camera. These images were categorized based on their position relative to the ePTFE disc (lumenal, ablumenal, edge or mid-graft) as well as capsule tissue type (fibrous or cellular capsule). Using a computer-based morphometric system (Metamorph Imaging Systems; Universal Imaging Corporation, West Chester, PA), three measurements of the capsule thickness were taken from each image, totaling 15 measurements per sample (five images per sample, three measurements per image). Values were expressed as mean thickness (μm) ± standard error.

Statistics

All statistical comparisons were completed using an ANOVA followed by a Bonferroni post hoc test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Vascularization

The vascular responses observed after implantation of porous ePTFE were evaluated and distinguished based on vessel location. An angiogenic response refers to the development of new vessels in the tissue surrounding the material, whereas neovascularization refers to the penetration of new vessels into the pores of the material implants. The neovascular and angiogenic values were not significantly different between

the two species (Fig. 1). Both species had patent vessels in the peri-implant tissue (i.e., angiogenesis) with identifiable lumens and resident red blood cells. However, few vessels were observed within the pores of the material (i.e., neovascularization; Fig. 2). Thus, the angiogenic response in the peri-implant tissue was always greater than the level of neovascularization. In addition, there was a trend toward greater vascularity in the adipose tissue relative to the subcutaneous tissue.

Inflammatory response

Unlike the vasculature, the inflammatory response differed not only between the species of animal, with rat greater than mouse, but also between the site of implantation, adipose greater than subcutaneous tissues.

Studies performed using adipose tissue implants illustrate that the rat exhibits a significantly greater inflammatory response, defined as number of ED-1-positive cells, compared with that of the mouse. Analysis of ED1 reactivity to different surfaces of ePTFE established no difference between the lumenal and ablumenal surfaces for the mouse implants. Of interest, a side-specific difference in the inflammatory response was observed in implants placed in the rat adipose tissue, where the lumenal surface exhibited significantly greater inflammation compared with the ablumen (Figs. 3 and 4). Similar to studies in the adipose tissue, samples implanted into the subcutaneous tissues resulted in a significantly greater inflammatory response in the rat compared with the mouse. No differences were observed for either species between lumenal and ablumenal surfaces (Fig. 3).

Capsule formation

The thickness and composition of tissue capsules formed around all disc implants was assessed. The capsule formation differed between the rat and the mouse only in the adipose tissue. In the rat, the ablumenal surface stimulated a fibrous capsule whereas the capsule on the lumenal surface was more cellular in nature. The thickness of this capsule did not vary significantly between these two surfaces or with respect to the edge or mid position along the material disc (Table I).

In the mouse, the capsule that formed around the material disc in the adipose tissue consisted predominantly of an extracellular matrix capsule for both the ablumen and lumenal surfaces. This capsule was thin and uniform (Fig. 5). Although the capsule that formed in the rat was not significantly thicker at all positions along the disc, there was a clear trend in the rat adipose tissue for a much thicker capsule than the mouse in adipose tissue. Specifically, significant differences between the rat and mouse were noted at the ablumenal edge and lumenal mid positions along the material disc implant (Table I).

In the subcutaneous location, the rat and mouse produce the same type of capsule with respect to fibrous tissue formation and thicknesses. Both develop an extracellular matrix rich capsule around the entire surface of the disc implant. Additionally, there was a trend for the capsule thickness in the mid region on the lumenal surface to be thicker than the edge region (Fig. 5).

DISCUSSION

This study served as a comparative evaluation of the vascularization, the inflammatory response, and

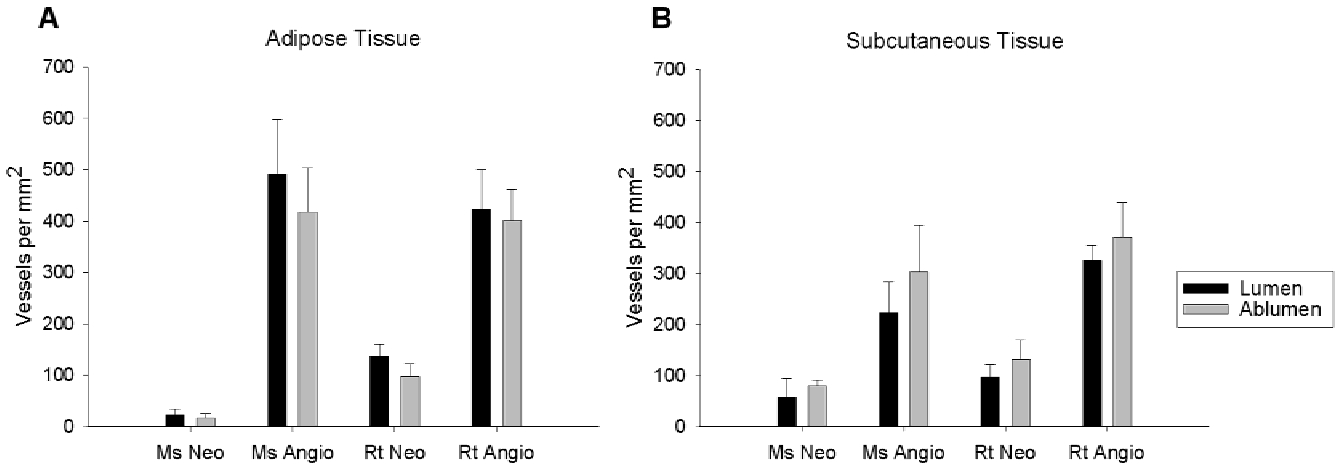

Figure 1. Adipose (A) and subcutaneous (B) vessel density associated with implanted ePTFE expressed as mean number of vessels per mm^2 ± SEM. GS-1 positively stained vessels were counted at the tissue/polymer interface. Neovascularization (Neo) refers to vessels within the material. Angiogenesis (Ang) refers to vessels in the tissue associated with the material.

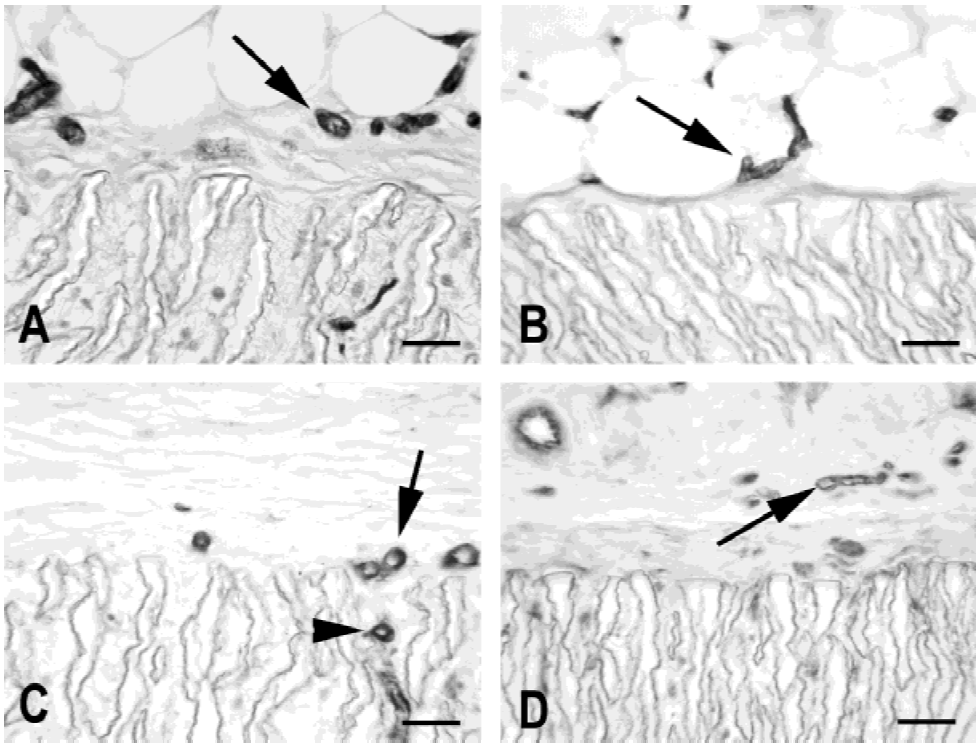

Figure 2. Light micrographs of GS-1-positive tissue cross sections. ePTFE cross section in rat adipose tissue (A). ePTFE cross section in mouse adipose tissue (B). ePTFE cross section in rat subcutaneous tissue (C). ePTFE cross section in mouse subcutaneous tissue (D). Vessels with lumens are observed in association with implanted polymers (arrows). Occasionally, vessels were seen in the pores of the ePTFE (arrowhead). Bar = 30 μm.

the tissue capsule formation associated with porous polymeric implants placed in rat and mouse subcutaneous and adipose tissues. These three parameters were chosen on the basis of their known influence on device function.[1,12–14] Furthermore, the mouse model was chosen because it represents an animal model that permits genetic manipulation of the key factors in the healing response associated with biomedical implants. The rat and mouse developed similar levels of vascularization in association with these discs, yet the mouse stimulated less of an inflammatory response toward implanted ePTFE. In addition, the mouse tended to form a thinner, fibrous capsule surrounding the disc implants, whereas the rat adipose capsule was thicker and more cellular.

Vascular response

The subcutaneous, adipose, and muscle sites of rats and rabbits have been common implant locations for

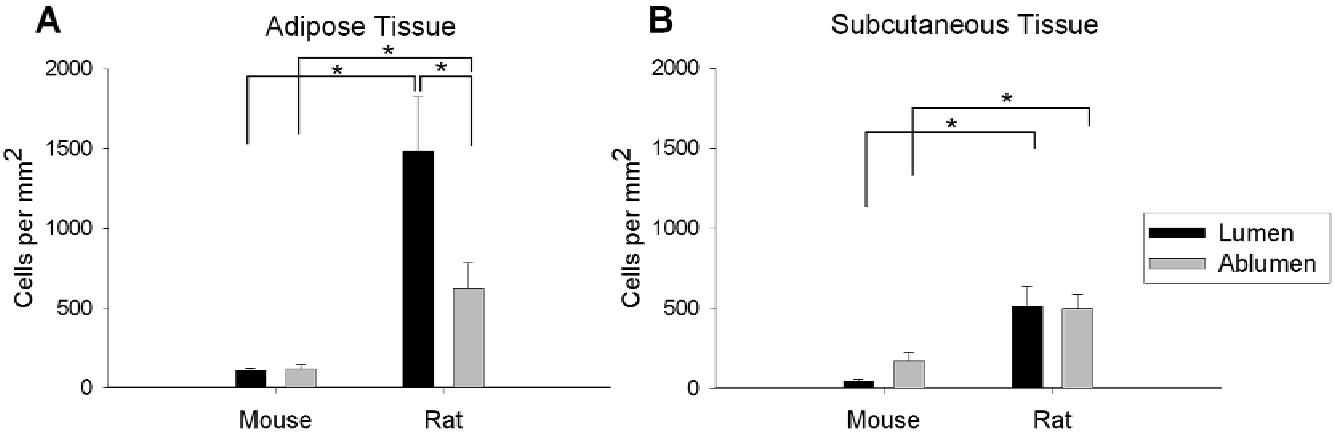

Figure 3. Adipose (A) and subcutaneous (B) ED1-positive cells associated with implanted ePTFE expressed as mean number cells per mm^2 ± SEM. ED1 positively staining cells were counted in the tissue associated with the implanted material. *Significant at $p < 0.05$.

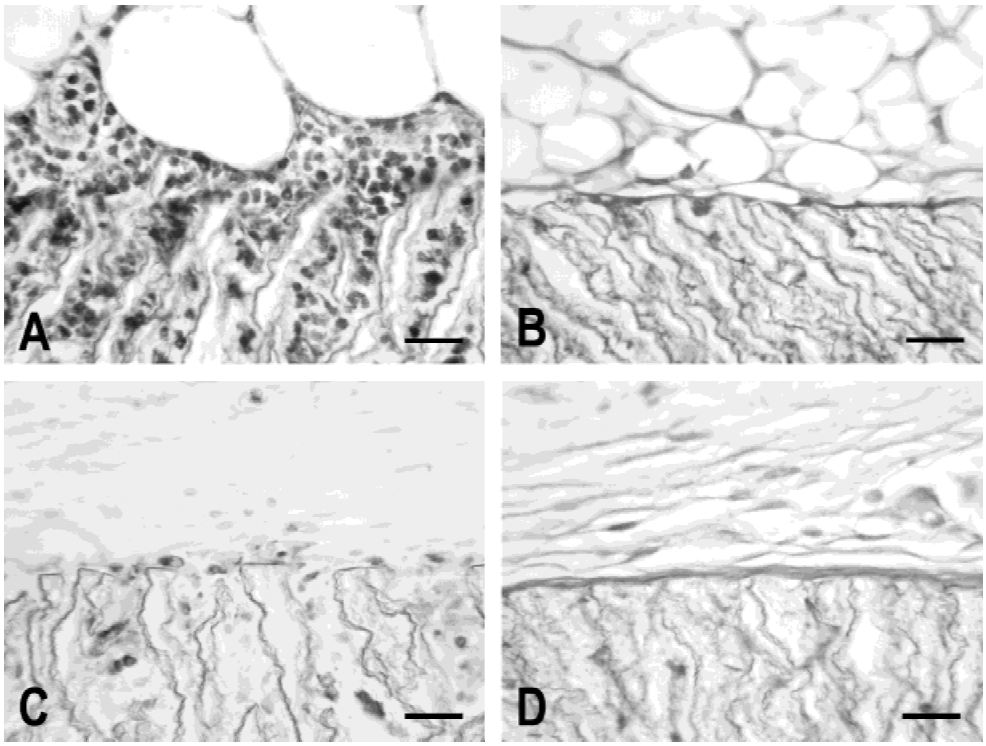

Figure 4. Light micrographs of ED1-positive tissue cross sections. ePTFE cross section in rat adipose tissue (A). ePTFE cross section in mouse adipose tissue (B). ePTFE cross section in rat subcutaneous tissue (C). ePTFE cross section in mouse subcutaneous tissue (D). Positively stained cells are identifiable by the dark color. Bar = 30 μm.

the evaluation of tissue reaction, in particular the vascular response to material implants.[4,15,16] These implant sites are often used during the initial determination of material safety and tissue responses. Additionally, they have been used for the evaluation of material modification for improved end-device function. Studies in this area have focused on the ability to improve integration of these materials into the body through a decreased capsule formation and an increased vascular response to the material.[17]

Vascular integration into porous materials (i.e., neovascularization) is important because blood vessels provide the exchange of nutrients, proteins, and molecules. It is believed that increased vascular integration to and within the material will facilitate proper device function.[14,17] Moreover, the process of endothelialization, reformation of an endothelial cell monolayer (in the case of vascular grafts), may also take place through the process of neovascularizantion within the pores of a polymer.[12,18] As stated above, there was no difference in porous polymer neovascularization between the rat and mouse for either tissue location. Similarly, no differences were observed in the sprouting of new blood vessels in tissue surrounding the implants (i.e., angiogenic response). These findings agree with the work of Khouw et al.,[19] who

TABLE I
Tissue Capsule Thickness[a]

Implant Site	Animal	Surface	Capsule Thickness (μm)		Capsule Classification
			Disc Edge	Mid-disc	
Adipose tissue	Rat	Ablumen	130 ± 20*	85 ± 19	Fibrous
		Lumen	108 ± 45	117 ± 46**	Cellular
	Mouse	Ablumen	12 ± 2	7 ± 1	Fibrous
		Lumen	14 ± 6	7 ± 1	Fibrous
Subcutaneous	Rat	Ablumen	56 ± 13	7 ± 1	Fibrous
		Lumen	77 ± 11	136 ± 12	Fibrous
	Mouse	Ablumen	73 ± 24	27 ± 6	Fibrous
		Lumen	64 ± 11	113 ± 12	Fibrous

[a]Values are means ± SEM.
*Significantly different from mouse ablumen disc edge.
**Significantly different from mouse lumen mid-disc; $p \leq 0.05$.

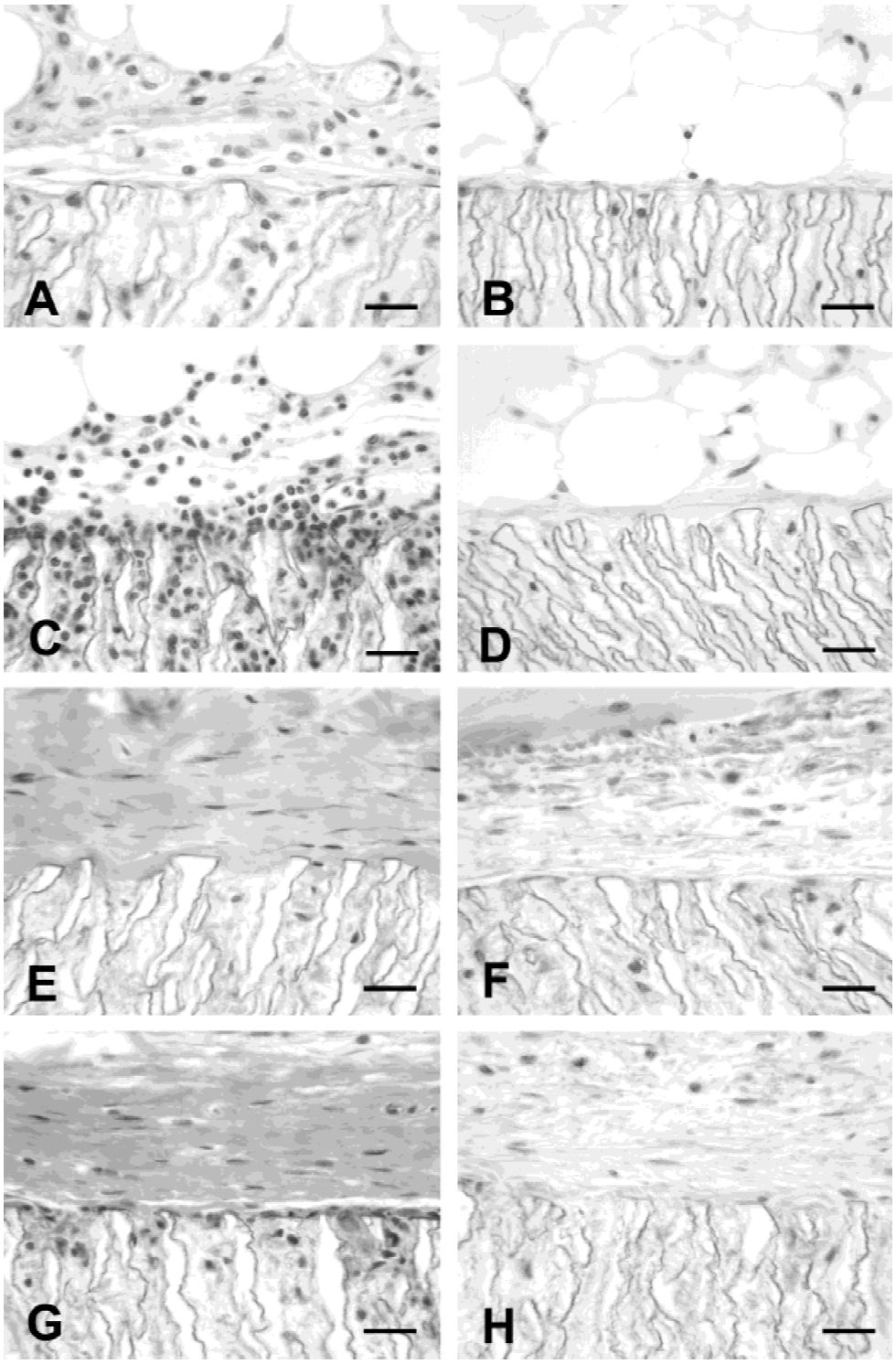

Figure 5. Light micrographs of hematoxylin and eosin-stained tissue sections. Images are grouped as adipose tissue (A–D) and subcutaneous tissue (E–H). The rat (A, C, E, G) and mouse (B, D, F, H) samples are displayed side by side for comparative purposes. Differences in the capsule thickness and composition between the mouse and rat are clearly visible in the adipose samples.

observed no difference in the peri-implant (angiogenic) vascular response between the rat and the mouse in response to implanted biodegradable materials. Thus, these two models are comparable with respect to vascularization of polymeric materials.

Inflammation

Excessive and chronic inflammation is a known factor contributing to implant failure.[13] As such, it is an important variable to be considered in an evaluation

of tissue response to biomaterial implants. In the present study a significantly lower inflammatory response was found in association with implanted material in the mouse when compared with the rat. This finding is in agreement with the study performed by Khouw et al.[19] In this study, the authors compared the foreign body reaction to biodegradable materials not only between the rat and mouse species, but also within distinct strains of each species. They found the foreign body reaction to be clearly different between the mouse and the rat, but only slightly different between the species strains.

Inflammatory cells are known to produce chemotactic and angiogenic factors, both of which can affect long-term implant function.[1,20,21] Variations in the degree of the inflammatory response could result in differing vascular responses to polymeric implants.[6] Similarly, inflammation could serve to impede device integration into the body. It is arguable that although the level of the vascular responses is the same, the mechanism behind each species response may be different. Alternatively, the inflammatory response in the mouse could have resolved before the 5-week time period.

Capsule formation

The presence of a fibrous capsule has also been shown to interfere with device function. This has been best documented in the field of in-dwelling sensor development, but expands into other applications as well.[3,5] In the case of glucose sensors, the formation of a nonvascular capsule inhibits the flux of glucose across a porous membrane for detection. Consequently, it is of interest to study this aspect of healing and examine how it is related to the vascularization of porous materials. In the current study, the mouse formed a thinner capsule in the adipose tissue, possibly allowing for vessel recruitment. Consequently, a lesser inflammatory response could more efficiently recruit vessels in the mouse because of the differences in capsule formation. However, the differences in the capsule thickness was limited to the adipose tissue alone, therefore, this is likely not the only explanation.

SUMMARY

The rat subcutaneous and adipose tissue implant sites have previously been described as testing sites for tissue responses associated with biomedical implants. In this study, the mouse (129- SVJ) model was compared with the rat (Sprague-Dawely) and focused on the tissue response to 30-μm internodal distance ePTFE at the 5-week time point. The rat and mouse implant models provide comparable data with regard to angiogenic and neovascular evaluations. The rat model, with its more extensive inflammatory response, may provide a more appropriate model of inflammatory cell recruitment and differentiation. The future use of genetic mouse models will contribute to our understanding and manipulation of the healing associated with biomaterial implants.

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