Compromised Production of Extracellular Matrix in Mice Lacking Secreted Protein, Acidic and Rich in Cysteine (SPARC) Leads to a Reduced Foreign Body Reaction to Implanted Biomaterials

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SPARC (secreted protein, acidic and rich in cysteine), a matricellular glycoprotein, modulates the interaction of cells with the extracellular matrix (ECM). Recently, accelerated cutaneous wound closure and altered deposition of collagen were reported in SPARC-null mice. Herein we asked whether SPARC might influence the foreign body reaction to biomaterial implants. Polydimethylsiloxane (silicone rubber) disks and cellulose Millipore filters were implanted into wild-type and SPARC-null mice. In wild-type animals, significant levels of SPARC were observed in the cells and the ECM comprising the capsules around the implants. After 4 weeks, SPARC-null mice exhibited a significant decrease in the thickness of the foreign body capsule, as compared to that observed in wild-type mice. A significant reduction in capsular vascular density was also associated with the silicone implants in the SPARC-null animals. Electron microscopy revealed that collagen fibers in the capsules produced by SPARC-null mice were smaller and more uniform in size than those in wild-type animals. Furthermore, staining with picrosirius-red showed that the collagen fibers were less mature in SPARC-null than in wild-type mice. The altered ECM resulting in decreased capsular thickness, indicative of an altered foreign body reaction in SPARC-null mice, implicates SPARC as an important modulator of the encapsulation of implanted biomaterials. (*Am J Pathol* 2003, 162:627–635)

Extracellular matrix (ECM) can regulate the migration, proliferation, and/or differentiation of cells. The ECM is composed of structural proteins, proteoglycans, growth factors, and matricellular proteins that include throm-

bospondins 1 and 2, osteopontin, tenascin-C, and SPARC (secreted protein, acidic and rich in cysteine). Matricellular proteins are known to modulate cell-matrix interactions and aspects of the cell cycle that control proliferation or apoptosis.^{1,2} A number of matricellular proteins show increased expression in response to injury.^{2–4} For example, thrombospondin 2-null mice display accelerated excisional wound healing and an altered foreign body reaction (FBR) to silicone implants.^{5,6}

SPARC, also known as BM-40 and osteonectin, is a calcium-binding glycoprotein secreted by many cells, eg, fibroblasts, endothelial cells, and platelets.^{7–10} As a matricellular protein, SPARC does not contribute structurally to ECM; rather, it regulates the production of several ECM proteins.² Two principal functions of SPARC are its modulation of cell shape and inhibition of cell-cycle progression.² *In vivo*, SPARC is expressed in remodeling tissues, such as healing cutaneous wounds and bowel anastomoses, and during bone formation, adipogenesis, and angiogenesis.^{1,11,12}

Targeted disruption of the SPARC gene in mice results in a complex phenotype characterized by early cataractogenesis, increased amounts of subcutaneous adipose tissue, and progressively severe osteopenia.² The curled tails of these mice are suggestive of altered collagen fibrillogenesis. Furthermore, a significant reduction in collagen content, and in the diameter of the collagen fibers are characteristic of the skin of SPARC-null mice.¹³ Recently, accelerated cutaneous wound healing and enhanced fibrovascular invasion of sponge implants have been reported in SPARC-null mice.^{13,14} Given this phenotype we asked whether the lack of SPARC might influence the FBR to implanted biomaterials.

Supported by the National Science Foundation–Engineering Research Center (program grant EEC-9529161), the National Institutes of Health (grants GM 40711, HL 59475, and grant RR01296 from the National Center for Research Resources), the Helsinki University Central Hospital Research Fund (to P. P.), and The Gilbertson Foundation to The Hope Heart Institute (to P. P.).

Accepted for publication November 20, 2002.

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Currently, a large number of biomaterial implants and devices are used clinically to replace or support inadequate or compromised tissues and organs. As drug-delivery systems and implants such as catheters, electrodes, and prostheses, these devices have been beneficial, if not entirely successful. However, the use of implants carries a risk of infection and impaired healing. Furthermore, an excessive FBR, starting with an acute inflammatory reaction followed by formation of a collagenous capsule, can impair the function of the implant, and is often associated with its subsequent failure.^{15,16} Ideal biomaterials are inert and induce minimal FBR, characterized in part by a thin capsule. Understanding the surface properties of biomaterials is desirable because they direct the type of macromolecular coat secreted and/or deposited around the implant by the cells of the injured tissue. Thus, identification of proteins that contribute to the FBR is expected to contribute to advances in the design of biocompatible materials and tissue engineering.

Silicone rubber, comprised of polydimethylsiloxane (PDMS), a silica filler and a crosslinking agent, is a commonly used biomaterial in percutaneous catheters, drug delivery devices, and breast prostheses.^{17–19} Its lack of porosity does not allow ingrowth of cells, ECM, or blood vessels. In contrast to PDMS, cellulose Millipore filters are porous.²⁰ In this study, we have used both types of biomaterials to test their FBR in mice. The phenotype of SPARC-null mice, which includes alterations in ECM assembly, led us to ask whether SPARC might be involved in one or more aspects of the FBR. Here we report a significant decrease in capsular thickness that is indicative of a reduced FBR in mice lacking SPARC.

Materials and Methods

Implant Materials and Experimental Model

Sheets of PDMS (Silastic NRV HP, lot HX 085147, 6 mm in diameter and 1.0 mm in width), cured by platinum-catalyzed hydrosilylation, were obtained from Dow-Corning (Midland, MI). The cellulose Millipore filters (type HA, mixed cellulose ester, pore size 0.45 μm , 6 mm in diameter, and 0.15 mm in width) were purchased from Millipore Corp. (Bedford, MA). The implants were prepared as described.⁶

C57Bl/6 $\times$ 129SvJ SPARC-null mice were generated as previously described.^{13,21} Genotypes were monitored by polymerase chain reaction using genomic-specific primers on purified tail DNA. Disks were implanted subcutaneously into 3- to 7-month-old SPARC-null [$n = 20$; $n = 11$ (PDMS), $n = 9$ (cellulose)] and wild-type (WT) [$n = 23$; $n = 12$ (PDMS), $n = 11$ (cellulose)] mice (two implants of the same material/animal) for 4 weeks. For surgery, the mice were anesthetized by isoflurane inhalation (Abbott Laboratories, North Chicago, IL), and their backs were shaved and cleaned. Incisions were made with a scalpel, and subcutaneous pockets adjacent to the incision site were created with blunt curved forceps. All instruments were rinsed in endotoxin-free water and were sterilized

before use. After implantation, the incisions were closed with staples (Autoclip; Clay Adams, Parsippany, NJ). SPARC-null and WT mice were handled and studies were performed according to the guidelines of the American Association for Accreditation of Laboratory Care and The Hope Heart Institute Animal Care and Use Committee. Preliminary studies with implantation periods of 2, 4, and 6 weeks were performed. Based on the results of these experiments, the animals were anesthetized after 4 weeks as above and were sacrificed. Implants were removed *en bloc* and were processed as described below.

Electron Spectroscopy for Surface Chemical Analysis (ESCA)

ESCA was performed on a surface science instrument (SSI; SSX100, Mountainview, CA) X-probe ESCA instrument with a monochromatized aluminum K_{α} X-ray source to determine the chemical composition of the implant surfaces. An electron flood gun was used to minimize surface charging. The photoelectron take-off angle for the analysis was 55°. The binding energy scale was referenced by setting the CH_x peak maximum in the C_{1s} spectrum to 285.0 eV. Surface elemental compositions were calculated from the peak areas. ESCA was performed on the materials before implantation and also after 4 weeks of implantation.

Immunohistochemistry

Immunolocalization of SPARC was performed as previously described.²² Samples were immersed in methyl Carnoy's fixative overnight at 4°C, embedded in paraffin, cut into 5- μm sections, and floated onto slides. The slides were heated on a slide warmer for 1 hour (60°C), deparaffinized, and rehydrated. Endogenous peroxidase activity was blocked by incubation in 1% H_2O_2 in MeOH for 20 minutes. Antigen retrieval was performed with Autolyse (Biomed, Foster City, CA), according to the manufacturer's instructions. The slides were subsequently washed in phosphate-buffered saline with 0.2% Tween 20 (PBST) and were blocked with 25% Sea Block (East Coast Biologics, North Berwick, ME) in PBST for 30 minutes at room temperature, after which they were incubated with an affinity-purified goat anti-SPARC polyclonal antibody (Gt 78, developed in our laboratory) at 10 $\mu\text{g}/\text{ml}$. The primary antibody was detected by incubation with a 1:500 dilution of peroxidase-labeled donkey anti-goat secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 hour at room temperature, and the immune complexes were localized with 3,3'-diaminobenzidine (Sigma, St. Louis, MO). Controls included sections treated only with secondary antibody as well as sections of testis from SPARC-null and WT mice.

Histological Analysis and Quantification of FBR

Five- μm sections were stained with hematoxylin and eosin (H&E); capsule thickness, vascular density of the

capsule, and number of foreign body giant cells lining the implants were analyzed with a Zeiss photomicroscope equipped with a calibrated ocular micrometer. To assess collagen deposition, we stained sections with picosirius red. Capsule thickness was determined at the 4-week time point on both sides of the capsule (one facing the skin and the other facing the body wall) at six to eight different locations. We measured capsules in 11 SPARC-null and in 12 WT mice with silicone implants (two implants/animal, four sections/animal), and in 9 SPARC-null and 11 WT mice with cellulose filter implants (two implants/animal, four sections/animal).

Vascular density was quantified by counting the number of erythrocyte-containing blood vessels and capillaries per high-power microscopic field ($\times 40$) within the collagenous capsule. In most cases, immunostaining with the endothelial marker, MECA (Developmental Studies Hybridoma Bank, Iowa City, IA), was used to confirm the identity of the vascular structures. Vascular density was measured in five to seven high-power fields of the capsule, on both sides of the capsule, and on the same number of samples used for the determination of capsule thickness. Values derived for vascular density were corrected for capsule thickness (vascularity index, expressed as number of vessels/ μm).

The number of foreign body giant cells was determined in all capsules. These cells were identified by their large size, location (lining the implants), and multiple nuclei.

Microscopic analysis of the FBR was performed independently by two investigators who were not aware of the identity of the samples.

Electron Microscopy (EM) and BrdU Immunostaining

Samples were immersed in Karnovsky's fixative and were processed for EM according to routine methods.²³ The diameters of the collagen fibrils in the foreign body capsule were measured from digitalized EM photographs (100 fibrils/animal, three animals/group) and are presented as frequency of fibrils per size category.

Six mice of each genotype were injected intraperitoneally (2 $\mu\text{g/g}$) on the day of sampling with 300 $\mu\text{g/ml}$ of 5-bromo-2'-deoxyuridine (BrdU) (Sigma) and were sacrificed after 8 hours. Samples were prepared for immunostaining as described,²⁴ and the number of BrdU-positive cells/capsule was determined by counting three to four microscopic fields per sample.

Isolation of Primary Dermal Fibroblasts and Cell Culture

Dermal fibroblasts were isolated from the skins of SPARC-null and WT animals.¹³ All experiments were performed with cells at early passage (P1 to P4), before senescence. Conditioned medium (CM) was sampled from wells containing the cells cultured on the implant material (disk) for 72 hours in Dulbecco's modified Ea-

gle's medium containing 2% or 10% fetal calf serum. Control CM was collected from wells containing cells but no disks. CM was analyzed by immunoblotting and enzyme-linked immunosorbent assay for transforming growth factor (TGF)- $\beta 1$ (Promega, Madison, WI) and vascular endothelial growth factor (R&D Systems, Minneapolis, MN). Immunofluorescence staining for 4',6'-diamidino-2-phenylindole (DAPI), a nuclear stain, and phalloidin, which stains the actin cytoskeleton, was performed as previously described.⁶ In brief, after fixing, washing, and blocking, implants were incubated for 90 minutes in the dark with either DAPI (5 mg/ml, Sigma) or phalloidin (0.08 U/ml, Molecular Probes, Eugene, OR). The cell-covered disks were removed from the wells, mounted on slides, and examined by fluorescence microscopy. The proliferation and migration of dermal fibroblasts grown on the disks were evaluated by a wounding protocol *in vitro*,^{13,25} a collagen gel contraction assay,²⁶ and by a radial invasion of matrix by aggregated cells (RIMAC) assay.²⁷

Statistics

The Mann-Whitney *U*-test was used to assess statistical differences between groups. Values of $P < 0.05$ were considered to be statistically significant.

Results

Postoperative recovery of the mice was prompt and the wounds healed rapidly. Three implants in SPARC-null and two in the WT animals showed evidence of infection and were excluded from the analysis.

ESCA confirmed that implants were silicone (measured: C, 47.6%; O, 26.8%; Si, 25.6%; theory: C, 50%; O, 25%; Si, 25%) and mixed esters of cellulose (C, 40.3%; N, 11%; O, 48.7%), with no contamination. The implant materials were unchanged after 4 weeks of implantation. Increased carbon content and other changes in the surface of implants detected with ESCA reflected the development of adsorbed protein on the implants (data not shown).

Immunoreactivity for SPARC was observed in the skin and subcutaneous tissue of the WT animals. Figure 1A illustrates the model with the location of the FBR capsule around the silicone implant, as shown *in vivo* in Figure 1, B and C. The protein was deposited abundantly within and around the capsule. Areas of immunoreactivity included fibroblasts and endothelial cells, as well as the ECM of the capsules (Figure 1B). As expected, no staining was seen in the dermis or in the capsules surrounding the implants in SPARC-null mice (Figure 1C). There were no significant differences in the staining patterns of the capsules between silicone and cellulose implants. Figure 1D provides the histological orientation shown in Figure 1, E and F. That immunoreactivity was more prominent in capsules around the implants than in the corresponding uninjured dermis (Figure 1, E and F) indicates that SPARC was secreted in response to the biomaterials.

Figure 1. SPARC is expressed in foreign body capsules. **A:** A schematic indicating the location of the FBR capsule (gray) around the implant. The rectangle indicates the location of the microscopic fields of the implant and the capsule (shown in **B** and **C**). Immunohistochemical staining for SPARC (long arrows) in foreign body capsules (ca) around silicone implants from a WT (**B**) and a SPARC-null mouse (**C**) with silicone implants. SPARC was deposited within and in the vicinity of the capsule in WT mice (**B**) but not in SPARC-null mice (**C**). Areas of immunoreactivity include cells (fibroblasts, short arrows; endothelial cells, wide arrows) and ECM of the capsules. **D:** The histological orientation of the microscopic field shown in **E** (represented by the rectangle). Immunoreactivity for SPARC in a capsule of a cellulose implant in a WT mouse is shown in **E** and in **F**. Immunoreactivity appears more prominent in the capsule around the implants (long arrows) (**E**, **F**), in comparison to the uninjured dermis (U). Scale bars, 50 μm (**C** and **E**).

Analysis of FBR

Figure 2 shows histologically the substantial difference in capsular thickness that we observed between WT and SPARC-null mice. Implants (I) were removed 4 weeks after placement and sections were stained with H&E. The foreign body capsules (demarcated with arrows) consist of inflammatory cells, fibroblasts, and ECM. The foreign body capsules were thinner in SPARC-null as compared to WT mice. This finding was not dependent on the type of implant, as a similar reduction in capsular thickness was seen with both silicone (Figure 2, A and B) and cellulose implants (Figure 2, C and D).

The mean ($\pm$ SD) total thickness of the capsule in WT mice was 90 ($\pm$ 12) μm for the silicone implants and 79 ($\pm$ 32) μm for the cellulose filters. In SPARC-null mice the values were 44 ($\pm$ 8) μm and 27 ($\pm$ 5) μm , respectively. The difference in the total capsular thickness between SPARC-null and WT mice was statistically significant ($P < 0.01$) for both implants (Figure 3). On average, the capsule was thicker on the skin side compared to the body side, but this difference failed to reach statistical significance (Figure 3). Results from 20 SPARC-null and 23 WT mice consistently pointed to a marked diminution in capsular thickness in SPARC-null as compared to WT mice.

By our conservative counting of functional, erythrocyte-containing blood vessels, we noted a reduction in vascular density in the foreign body capsules associated with silicone implants in SPARC-null mice (Figure 4B), compared to WT mice (Figure 4A). The mean ($\pm$ SD) number of blood vessels per high-power field in the capsules are shown in Table 1. The values were corrected for

capsule thickness and have been presented as vascularity indices (vessels per μm capsular thickness). Although a similar trend in a diminished vascularity index was apparent in SPARC-null animals containing cellulose implants, this difference failed to reach significance (Table 1). There were no significant differences in the number of blood vessels between the skin and body side of the capsules.

There appeared to be more foreign body giant cells around the implants in WT mice. Their number per implant in WT mice with silicone and cellulose filter implants was 42 ± 22 and 27 ± 11 , respectively, with corresponding values in SPARC-null mice of 35 ± 14 and 17 ± 8 , respectively. However, these differences did not reach statistical significance.

Immunostaining with BrdU indicated that there were proliferating cells in the foreign body capsules. However, there were no significant differences in the number of BrdU-positive cells between SPARC-null mice and WT mice or between the different types of implants (data not shown).

Collagen Deposition in the Foreign Body Capsule

Histochemistry with picrosirius red indicated that there was a greater quantity of mature collagen fibers (stained red) in the foreign body capsules of WT compared to SPARC-null mice (Figure 5). The green-yellow color under polarized light indicated that, in SPARC-null mice (Figure 5, B and D), the collagen fibers were smaller

Figure 2. SPARC-null mice exhibit diminished encapsulation of silicone and cellulose implants. Implants (I) were removed 4 weeks after placement and sections were stained with H&E. Capsule thickness (between arrows) was significantly reduced in SPARC-null mice (B, D), in both silicone (A, B) and cellulose implant groups (C, D), in comparison to WT animals (A, C). Scale bar, 100 μ m.

(presumably with less crosslinking) and were less mature than those in WT mice (Figure 5, A and C).

The FBR capsules were also analyzed by EM, as shown in Figure 6A. The collagen fibrils of the capsule were found to be smaller and more uniform in size in

Figure 3. SPARC-null mice exhibit a significant decrease in the thickness of the foreign body capsule, relative to that in WT mice. Capsule thickness was measured with a micrometer at six to eight different locations on the capsule, on both sides of the capsule. Means ($\pm$ SD) are presented. **White columns** indicate the thickness of the capsule facing the skin, **gray columns** indicate the thickness of the capsule facing the body wall, and **black columns** indicate the total capsule thickness.

SPARC-null animals (31 ± 5 nm), compared to WT counterparts (43 ± 7 nm) (Figure 6B). These findings indicate altered collagen fibrillogenesis in the foreign body capsules in SPARC-null mice.

Characterization of Dermal Fibroblasts Cultured on Implants

Dermal fibroblasts isolated from SPARC-null and WT mice were cultured on silicone and cellulose disks. Immunofluorescence staining for nuclei with DAPI or actin fibers with phalloidin indicated that the cells attached and grew on the biomaterial disks. No significant differences in cell numbers as a function of the disk or the animal group were seen. CM (with 2% fetal calf serum) from fibroblasts grown on the biomaterials revealed 0.4 to 0.9 ng/ml of TGF- β 1 and 35 to 65 pg/ml of vascular endothelial growth factor, respectively. There were no significant differences in TGF- β 1 or vascular endothelial growth factor levels between SPARC-null and WT cells or between cultures grown on different biomaterials.

We also found minimal differences between WT and SPARC-null fibroblasts in their ability to contract collagen gels. Furthermore, culturing these cells on disks before the collagen gel contraction assay did not alter these results (data not shown). Consistent with previous data,¹³ cell culture wounding studies showed that dermal fibroblasts isolated from SPARC-null mice migrated faster than cells from WT mice. Culturing fibroblasts on disks before wounding the monolayer did not change the difference in cell migration (data not shown). The CM from SPARC-null and WT dermal fibroblasts cultured on the biomaterials were also tested in a RIMAC assay, which measures the invasion of collagen matrix by cells. Although there was no apparent effect of the fibroblast CM on the invasion of collagen by endothelial cells, the same CM was found to stimulate the migration of WT fibroblasts in the RIMAC assay (data not shown). In summary, studies with dermal fibroblasts cultured on silicone and cellulose disks revealed minimal differences among the groups and showed that both types of biomaterial were inert (ie, did not leach cytoreactive substances). We conclude that the difference in the capsule observed be-

Table 1. Reduced Vascular Density in Foreign Body Capsules of SPARC-Null Mice

Genotype	Implant	Vascularity index*
Wild-type	Silicone	0.90 ± 0.30
	Cellulose	0.70 ± 0.15
SPARC-null	Silicone	$0.50 \pm 0.15^\dagger$
	Cellulose	0.56 ± 0.13

*Vascular density (vessels/high-power field of the capsule) was quantified from hematoxylin and eosin-stained sections by counting of the number of erythrocyte-containing vessels per microscopic high-power field ($\times 40$) within the collagenous capsule. Analysis was performed on the same number of animals as were the measurements of capsule thickness. Values were corrected for capsule thickness and the derived vascularity index (vessels/ μ m) represents the means $\pm$ SD.

[†]The reduction in the number of blood vessels was statistically significant only in the silicone implant group ($P < 0.01$), but there was no significant difference in the number of blood vessels between the skin side and the body side of the capsules.

Figure 4. Reduced vascular density in foreign body capsules of SPARC-null mice. FBR in response to silicone implants (I) was associated with less vascularity in SPARC-null mice (B) in comparison to WT mice (A). **Arrows** denote capillaries in the capsule. Scale bar, 50 μ m.

tween WT and SPARC-null mice reflects an intrinsic defect in dermal fibroblasts that lack SPARC, ie, compromised production and assembly of fibrillar (primarily type I) collagen.

Discussion

SPARC-null mice are characterized by early cataractogenesis,^{21,28,29} increased amounts of subcutaneous adipose tissue,¹³ progressively severe osteopenia,³⁰ and altered cutaneous wound healing.^{13,31} We now report a reduced FBR as a novel part of the phenotype of SPARC-null mice. The mechanism responsible for most if not all of these phenotypes appears to be aberrant deposition and/or assembly of ECM.^{13,14,28} Findings of altered FBR are consistent with known functions of SPARC that modulate the interactions of cells with the ECM.

Tissue injury results in a series of responses that eventually lead to normal healing, with ECM formation and remodeling, minimal scarring, and resumed function. However, with implanted biomaterials the normal repair process is compromised. The healing is abnormal, slow, and results in a FBR. Excessive formation of a collagenous capsule and the foreign body giant cell response can even-

tually lead to impaired function of implanted devices, with subsequent failure. The repair process of the implant area in mice lacking SPARC approaches that of normal healing of soft tissue after injury, and indicates that blocking of SPARC might counteract an undesirable FBR.

SPARC is a component of the capsule formed in response to biomaterial implants (Figure 1). Levels of SPARC were maximal in the capsule and were reduced progressively with increasing distance from the implant; low levels are normally seen in uninjured skin.¹¹ This spatial and temporal expression is coincident with the FBR and capsule formation and provides strong evidence that SPARC is involved in the response to biomaterials. The main finding of our study further supports this view. The foreign body capsules were significantly thinner in SPARC-null compared to WT mice. Because the reduced capsule thickness was detected in animals with both nonporous silicone and porous cellulose filter implants, the surface properties of these implants do not seem to affect the quality of the capsule in the absence of SPARC. Furthermore, based on experiments of 2 and 6 weeks, differences in the capsular thickness appeared not to be a function of time. In a related study, the foreign body capsules of bovine pericardial implants, sampled

Figure 5. Picrosirius-red staining indicates that implant capsules (ca) from SPARC-null mice exhibit less mature collagen fibers in comparison to those from WT mice. **A:** Representative foreign body capsule in a WT mouse with silicone implant. **B:** Representative foreign body capsule in a SPARC-null mouse with silicone implant. **C:** Representative foreign body capsule in a WT mouse with cellulose filter implant. **D:** Representative foreign body capsule in a SPARC-null mouse with cellulose filter implant. **Long arrows** indicate mature collagen (red), and **short arrows** denote immature collagen fibrils (yellow-green). Scale bar, 50 μ m.

Figure 6. EM of collagenous capsules around implants. **A:** The collagen fibrils (white arrows) were smaller and more uniform in size in SPARC-null mice, in comparison to WT mice (original magnification, $\times 20,000$). **B:** The diameters of the collagen fibrils in the foreign body capsule were measured on digitalized EM photographs (100 fibrils/animal, three animals/group). Values are presented as frequency of fibrils per size category. The collagen fibrils are smaller in SPARC-null mice (black columns) in comparison to those of WT (white columns).

as late as 3 months after placement, were found to be thinner in SPARC-null compared to WT mice (unpublished results). Thus, in neither case does time appear to be the deciding factor in the determination of capsule thickness. We have also found no evidence for degradation of FBR capsules in SPARC-null mice.

Because collagen I is the main constituent of the FBR capsule, the finding of reduced capsule thickness is supported by our previous data showing that the amount of collagen in the skin of SPARC-null mice is reduced to half that of WT mice at 6 months of age.¹³ Picrosirius red-staining and EM indicated altered collagen deposition in the capsules of SPARC-null mice; collagen fibrils were smaller and more uniform in size in the absence of SPARC. We propose that the mechanism of reduced FBR in SPARC-null mice is the decreased collagen content, immature collagen fibrils, and altered ECM in the capsules produced by these animals. An explanation for the accelerated wound healing in SPARC-null mice is their production of an ECM that is more readily contracted by dermal fibroblasts present in the granulation tissue.¹³ In the context of FBR, we would predict a decreased capsule thickness around implants, as reported here. The biochemical mechanism by which SPARC influences col-

lagen fibrillogenesis and deposition is not known; however, SPARC might facilitate the crosslinking of collagen into insoluble matrix (AB Bradshaw, in preparation), an effect that could account for the reduced foreign body capsule thickness in SPARC-null mice. SPARC binds directly to collagen I fibrils and could promote aggregation of fibrils to generate larger fibrils and fibers. For fibril maturation, it is probable that thinner fibrils initially align laterally and subsequently become crosslinked. SPARC could also be involved in an event that occurs earlier than crosslinking, that is, in the alignment of the adjacent fibrils themselves. We have recently shown that the lens capsule (a thick basement membrane surrounding the avascular lens) is severely compromised in SPARC-null mice. The aberrant distribution/assembly of collagen IV and laminin 1 results in an increased permeability that potentially contributes to cataract formation.²⁸

SPARC could also affect the localization of other ECM proteins. In type I collagen-deficient *mov-13* mice, Iruela-Arispe and colleagues³² have shown that fibroblasts that do not express collagen I do not incorporate SPARC into ECM synthesized *in vitro*, nor is SPARC evident in type I collagen-containing tissues *in vivo*. SPARC is known to regulate TGF- β 1 expression, at least in mesangial cells. Francki and colleagues³³ showed significantly decreased levels of TGF- β 1 in SPARC-null mesangial cells that were associated with diminished expression of collagen I. Our finding of reduced ECM production in the FBR capsules is consistent with these data. Thus, part of the ECM regulation in the FBR could be mediated by TGF- β 1. Although we did not find differences in TGF- β 1 levels in our studies *in vitro*, we anticipate that cultured cells do not necessarily reflect the subcutaneous environment.

The formation of blood vessels and the expansion of the vascular network are regulated by numerous factors. Angiogenesis involves the coordinated activity of stimulated endothelial cells in the environment of the ECM. As described for many of the matricellular proteins, SPARC interacts with growth factors, such as platelet-derived growth factor and vascular endothelial growth factor.³⁴ The secretion of SPARC by endothelial and smooth muscle cells/pericytes into the ECM and its increased expression in remodeling/angiogenic tissue render it well-placed to regulate vascular sprouting and growth. The activities associated with SPARC and its proteolytic fragments are thought to facilitate different steps in the formation of new vessels.³⁴ In our previous studies, SPARC-null mice displayed enhanced fibrovascular invasion of subcutaneous polyvinyl alcohol sponges, in comparison to the response in WT mice.¹⁴ Therefore, it was at first surprising to find reduced vascularity in the silicone-associated foreign body capsules in SPARC-null mice. However, these attenuated capsules might not require extensive vascularization. In fact, vascularization is likely to be a concomitant or secondary response that is regulated in part by ECM, and especially, by collagen (AD Bradshaw and colleagues, in preparation).³⁵ A similar relationship between capsule thickness and its vascularity was recently reported by Kyriakides and colleagues,⁵ who found that thrombospondin-2-null mice exhibited in-

creased capsule thickness as well as increased vascularity around silicone implants.

BrdU staining revealed no significant differences between SPARC-null and WT mice in the percentage of proliferating cells in foreign body capsules. This finding is in accord with other studies in which no proliferative differences were found in cutaneous wound healing or in association with fibrovascular invasion of subcutaneous sponges.¹⁴ Thus, changes in rates of cell proliferation do not seem to be responsible for the altered capsules in SPARC-null mice, despite the observation that SPARC inhibits cell-cycle progression *in vitro*. BrdU analysis, on the other hand, was performed only 30 days after implantation. Therefore, we cannot exclude the possibility that alterations in cell proliferation have an effect during early capsule formation.

SPARC-null dermal fibroblasts have been shown to display accelerated migration, relative to WT fibroblasts, in a wound healing assay *in vitro*.¹³ This finding was confirmed in the present study. Contact between the biomaterials and fibroblasts before wounding did not alter the cellular rates of migration. The time frame for wounding studies *in vitro* is usually 12 to 72 hours, whereas the duration of our implant studies was 30 days. It is therefore unlikely that differences in the rate of migration of the fibroblasts would explain the altered foreign body capsules in SPARC-null mice.

In summary, an altered ECM that results in reduced capsule thickness is a manifestation of an altered FBR to both nonporous silicone and porous cellulose filter implants in SPARC-null mice. These data indicate that SPARC is an important mediator of foreign body encapsulation. Local gene therapy to reduce SPARC should therefore be considered as an adjunct to implanted biomaterials as a means of reducing the FBR and improving biocompatibility.

Acknowledgments

We thank P. Stephans, P. Stayton, and S. Gollidge at the National Electron Spectroscopy for Surface Chemical Analysis and Surface Analysis Center for Biomedical Problems (NESAC/BIO) at the University of Washington for surface analysis; the Developmental Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health and Human Development and maintained by The University of Iowa, Department of Biological Sciences, for MECA-32 antibody; A. Francki and R. Vernon for collaboration; and S. Lara and J. Carbon for technical support.

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