BASIC SCIENCE: GYNECOLOGY

Inflammatory cytokine and matrix metalloproteinase expression induced by collagen-coated and uncoated polypropylene meshes in a rat model

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OBJECTIVE: The objective of the study was to compare the influence of collagen-coated vs uncoated polypropylene meshes on the expression of genes critical for wound healing.

STUDY DESIGN: In 54 rats, abdominal wall defects were created, repaired by polypropylene sutures, and covered by an overlay of coated polypropylene (n = 20), uncoated polypropylene (n = 18), or no mesh (n = 16). Explants were harvested 7 or 90 days after repair and divided for histological, immunohistochemical, and messenger ribonucleic acid (mRNA) analyses. Real-time quantitative polymerase chain reaction arrays were used to profile the expression of 84 genes at the tissue-mesh interface.

RESULTS: One week after implantation, coated mesh elicited a slightly greater inflammatory response and increased mRNA expression of 4 proinflammatory cytokines compared with uncoated mesh. Both materials, however, induced a comparable expression of cytokines and matrix metalloproteinases relative to suture repair 90 days after implantation.

CONCLUSION: Collagen-coated polypropylene mesh induces elevated inflammatory cytokine expression compared with uncoated mesh early in the healing process, but the response to both meshes is similar 90 days after implantation.

Key words: coated polypropylene mesh, inflammatory cytokines, matrix metalloproteinases, pelvic organ prolapse, rat model

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The use of synthetic mesh for the transvaginal repair of pelvic organ prolapse has become increasingly popular as a mechanism to help improve the high failure rates observed with traditional procedures.[1-3] Polypropylene mesh is currently the most commonly used synthetic graft material in gynecologic surgery.[2] New variants are introduced frequently that are designed to reduce the inflammatory response and improve tissue ingrowth in an attempt to decrease local graft-related complications including erosion, infection, excessive scarring, and resulting dyspareunia.[2,4-7] No mesh, however, has demonstrated optimal biocompatibility and incorporation into host tissue, and limited long-term clinical data exist to determine which, if any, of the several variants is the best choice for vaginal surgery.[3]

The host response to implanted mesh follows a cascade of events involved in wound healing including coagulation, inflammation, angiogenesis, epithelialization, fibroplasia, matrix deposition, and contraction.[8,9] Biocompatibility is determined by the intensity of the foreign body reaction elicited by the mesh material and the host's ability to resolve the injury to the tissues during implantation.[10,11] Mesh characteristics such as pore size, chemical composition, filament structure, amount of implanted material, and biodegradability affect the processes of inflammation, angiogenesis, and tissue formation, and consequently may alter the wound healing process.[10-21]

Inflammatory cells recruited to the site of implantation produce signaling molecules that affect the tissue response to the biomaterial, but the molecular mechanisms directing this foreign body response are poorly understood.[9,22] Macrophages that adhere to the surface of implanted mesh become activated in an attempt to phagocytose the mesh fibers and fuse to form foreign body giant cells (FBGCs).[9] The subsequent secretion of cytokines, degradative enzymes, and reactive oxygen intermediates by these activated macrophages and FBGCs directs the inflammatory and wound-healing response to the material by influencing the behavior of other cell types including neutrophils (polymorphonuclear leukocytes [PMNs]), monocytes, lymphocytes, and fibroblasts.[9]

Our laboratory recently reported in vivo expression profiles for a wide array of genes involved in angiogenesis, wound healing, and extracellular matrix remodeling after the implantation of a variety of synthetic mesh materials used in inguinal and abdominal hernia repair including polypropylene, polyester, and polytetrafluoroethylene.[23] Evaluation of

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gene expression profiles and histologic specimens revealed that polypropylene and polyester induced a greater and more persistent inflammatory response than polytetrafluoroethylene, which elicited a response most similar to that induced by suture repair.

This study showed that different meshes induce the differential expression of inflammatory cytokines, matrix metalloproteinases, and growth factors, thereby influencing the extent of the foreign body reaction to these materials.[23] Furthermore, these results suggested that the particular mesh chosen for repair may affect the patient's wound healing response and clinical outcome. Increased understanding of the host response to implanted materials at the molecular level is needed to develop improved grafts with better wound-healing properties and fewer complications and to develop therapeutic targets intended to optimize the wound-healing response after implantation.

In the current study, we used a rat abdominal wall repair model to determine whether the foreign body response differs at the molecular level for 2 monofilament polypropylene meshes commonly used in vaginal surgery, including an uncoated mesh and a mesh that is coated with a hydrophilic, resorbable film of porcine collagen. The coating is intended to provide a theoretical protective effect by facilitating tissue ingrowth, decreasing the initial inflammatory response during healing, and thus decreasing the risk of mesh erosion, although evidence from clinical and animal studies to support this claim is lacking.[17,24-27]

de Tayrac et al[26] found that in the early postoperative period (1 week), coated polypropylene mesh implanted in the sheep vagina was less well integrated into the surrounding tissue and showed increased exudative and acute inflammation than the uncoated mesh. Boulanger et al[27] reported that the complete resorption of the collagen coating occurred at about 15 days, and they found that the coated mesh demonstrated delayed tissue integration compared with uncoated mesh 14 days after implantation in a rat abdominal model. Furthermore, Huffaker et al[17] found a statistically greater percentage of apoptotic cells at the interface of mesh and host tissue in rabbits vaginally implanted with coated mesh.

MATERIALS AND METHODS

Animals

A total of 54 adult male Sprague Dawley rats weighing approximately 300 g were used. Animals were obtained from Taconic (Germantown, NY) and housed in the Tripler Army Medical Center animal facility. Rats were assigned to abdominal wall repair using coated polypropylene (n = 20), uncoated polypropylene (n = 18), or suture repair with no mesh (n = 16) and were killed at either 7 or 90 days after repair. A power calculation prior to the commencement of the study determined that a sample size of 7 animals per group will allow detection of a 30% difference among means (assuming an SD of 15% of the mean) at an alpha level of 0.05 with 95% statistical power and a calculated effect size of 0.94 using a 1-way analysis of variance (ANOVA) design.

The study protocol was approved by the Institutional Animal Care and Use Committee at Tripler Army Medical Center. Investigators complied with the policies as prescribed in the US Department of Agriculture Animal Welfare Act and the National Research Council's Guide for the Care and Use of Laboratory Animals. Facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Mesh materials and study design

Full-thickness abdominal wall defects were repaired by polypropylene sutures to mimic native tissue repair and, in select cases, were covered by an overlay of monofilament polypropylene mesh coated with porcine collagen (Pelvitex; C. R. Bard, Inc, Covington, GA) or an uncoated monofilament polypropylene mesh (Prolene Soft; Ethicon, Inc, Somerville, NJ). Both macroporous meshes are similar in weight (38 and 41 g/m^2 for coated and uncoated polypropylene, respectively) and pore size (1.5 and 1.7 mm^2 for coated and uncoated polypropylene, respectively).[26] Meshes were cut into uniform strips at the time of surgery by using a precut plastic sterile template. Rats were killed 7 or 90 days after repair, and harvested explants (mesh with underlying abdominal wall) were divided for histological, immunohistochemical, and gene expression analyses.

Surgery and tissue collection

Surgery was performed as described.[23] Briefly, a 3.0 × 0.5 cm longitudinal full-thickness defect was created in the left lateral abdominal wall, which was subsequently closed with a continuous 4.0 polypropylene suture. In animals from the mesh repair groups, a 3.5 × 2.0 cm strip of mesh was fixed to the abdominal wall over the defect using 5.0 polypropylene sutures at its corners with a running 5.0 polypropylene suture (interrun distance 0.5 cm) along all four sides. The subcutaneous tissues and skin were closed with interrupted 3.0 nylon sutures. Rats were fitted with Elizabethan collars to prevent chewing on the incision site.

Animals were evaluated at least twice daily for the initial 48 hours after surgery and at least weekly thereafter for herniation or infection. On days 7 or 90, rats were killed, and the mesh with the underlying full-thickness abdominal wall (or similar region in suture repair animals) was removed. Length and width of the explanted mesh were measured to determine shrinkage (decrease in surface area) of the implant.

Histology

Specimens were fixed in formalin and embedded in paraffin, and serial sections (5 μm) were stained with hematoxylin-eosin, elastic/van Gieson, and Masson trichrome. Inflammation, neovascularization, and fibroblastic proliferation were scored on a scale of 0 to 4 (0, none; 1, minimal; 2, mild; 3, moderate; 4, severe) by a pathologist (J.R.M.) blinded to treatment as described.[17,18,23]

In addition, microscopic evaluation was performed to quantify the presence of FBGCs, PMNs, mononuclear cells (MN), newly formed vessels, and collagen deposition (amount and organization) using a semiquantitative scale analogous to that used by others[13,28] (Table 1). Five nonoverlapping fields per slide were scored at magnification ×400 by 2

TABLE 1
Scoring criteria during microscopic examination

	Score			
Category	**0**	**1**	**2**	**3**
FBGCs[a]	0	1–5	6–10	>10
PMNs[a]	0	1–5	6–10	>10
MNs[a]	0	1–5	6–10	>10
Vascularity[a]	0	1–3	4–10	>10
Collagen amount	None	Mild	Moderate	Abundant
Collagen organization	Totally disorganized	Slightly organized	Moderately organized	Well organized

FBGC, foreign body giant cell; *MN,* mononuclear cells; *PMN,* polymorphonuclear leukocytes.

[a] Number per high-power field (original magnification ×400).

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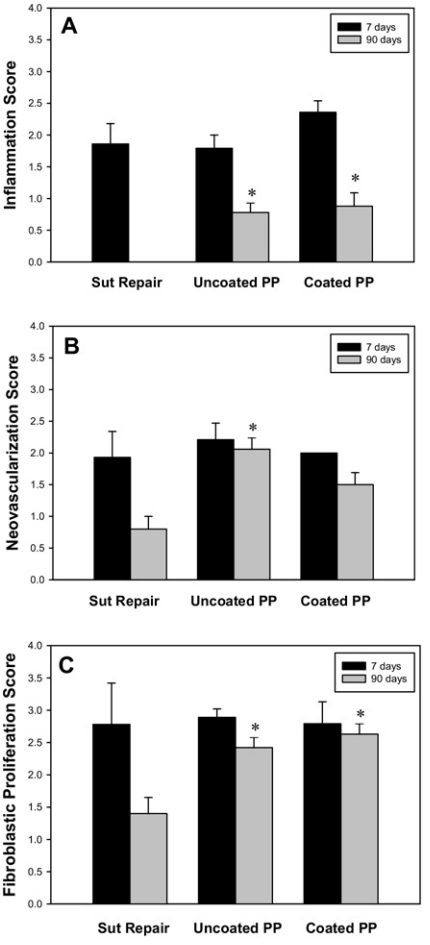
FIGURE 1
Scores after implantation of collagen-coated and uncoated polypropylene meshes or suture repair



Scores for **A,** inflammation, **B,** neovascularization, and **C,** fibroblastic proliferation after the implantation of collagen-coated and uncoated polypropylene meshes or suture repair. *Asterisk* indicates $P < .05$ relative to suture repair.

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blinded investigators (J.R.A. and L.MP.) at the tissue-mesh interface, and average scores were calculated. Digital images were captured using PictureFrame software (Optronics, Goleta, CA) and an Olympus IX71 microscope (Olympus America Inc, Center Valley, PA).

Immunohistochemistry

Immunohistochemistry was performed using matrix metalloproteinase (MMP) 9 (Millipore, Temecula, CA) and chemokine (C-X-C motif) ligand 2 (CXCL2) (BioVision Research Products, Mountain View, CA) rabbit polyclonal antibodies as described.[23] These markers were chosen for immunohistochemical analysis after demonstrating marked upregulation in the expression profiles from mesh groups relative to suture repair.[23] Degree of positive immunostaining was scored on a scale of 0 to 4 (0, none; 1, minimal; 2, mild; 3, moderate; 4, intense) by an investigator blinded to treatment (P.T.N.). Two sections per specimen per antibody were examined to confirm similar staining patterns within each section, and average scores were calculated.

Gene expression profiling

Samples were stored in RNALater (Ambion Inc, Austin, TX) at −20°C until ribonucleic acid (RNA) isolation was performed. Samples were homogenized using an Omni GLH homogenizer (Omni International, Kennesaw, GA), and total RNA was isolated using the Trizol method according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Extracted RNA was further purified using the RT^2 qPCR-grade RNA isolation kit (SABiosciences, Frederick, MD), quantified using a spectrophotometer, and stored at −70°C.

Complementary deoxyribonucleic acid was generated from 1 μg total RNA using the RT^2 first strand kit (SABiosciences) and analyzed using a rat RT^2 profiler PCR angiogenesis array (SABiosciences) by means of a Bio-Rad iCycler real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. This array is a set of optimized real-time PCR primer assays on 96-well plates for pathway-focused genes as well as appropriate RNA quality controls and internal control housekeeping genes to normalize the data for the amount of RNA added to each reverse transcription reaction.

The rat RT^2 profiler PCR angiogenesis array (SABiosciences) profiles the expression of 84 genes involved in modulating the biological processes of angiogenesis and wound healing. A complete list of genes contained in the array can be viewed on the following link: http://www.sabiosciences.com/rt_pcr_product/HTML/PARN-024A.html. Arrays were repeated with 3-5 different rats in each experimental group using total RNA isolated from a single rat per array.

Statistical analysis

Values were expressed as mean ± SEM. Mesh-induced changes in gene expression analyzed using the RT^2 profiler PCR array (SABiosciences) were calculated using software from SABiosciences. Differentially expressed genes were considered significant using a cutoff value greater than a 4-fold change and $P < .01$. ANOVA was used to to determine differences in histologic parameters and im-

TABLE 2
Microscopic examination scores 7 days after surgery presented as mean (SEM)

Implant material	FBGCs	PMNs	MNs	Vascularity	Collagen amount	Collagen organization
Suture repair (n = 7)	0.06 (0.03)	0.48 (0.17)	2.71 (0.22)	2.01 (0.20)	2.00 (0.19)	2.14 (0.26)
Uncoated polypropylene (n = 7)	0.31 (0.09)[a]	1.33 (0.30)	3.00 (0)	2.47 (0.09)	2.42 (0.16)	2.21 (0.10)
Coated polypropylene (n = 7)	0.07 (0.03)[b]	2.03 (0.34)[a]	2.88 (0.09)	1.73 (0.23)[b]	2.07 (0.22)	1.50 (0.18)[b]
P value[c]	0.01	0.004	0.35	0.04	0.28	0.03

ANOVA, analysis of variance; *FBGC,* foreign body giant cell; *MN,* mononuclear cells; *PMN,* polymorphonuclear leukocytes.

[a] $P < .05$ compared with suture repair; [b] $P < .05$ compared with uncoated polypropylene; [c] ANOVA followed by Tukey's test for pairwise multiple comparisons.

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munohistochemical staining among experimental groups, and the Tukey's test for pairwise multiple comparisons was used for post hoc analyses to identify specific differences. Comparisons were also performed using the Student *t* test. Corresponding nonparametric tests were used when indicated. Statistical analyses were performed using SigmaStat 3.5 software (Systat Software, Inc, Point Richmond, CA), with $P < .05$ considered significant.

RESULTS

Postoperative course

All animals had a normal postoperative recovery except 1 rat in the 90 day coated mesh group that was euthanized on postoperative day 10 for wound dehiscence and extrusion of mesh at the incision site. Ten rats without Elizabethan collars chewed their sutures within 48 hours after surgery, which required resuturing of the midline incision (coated mesh, n = 4; uncoated mesh, n = 2; suture repair, n = 4). These rats were among the first group of animals to undergo surgery as part of this study, during which time we had not yet begun fitting the rats with Elizabethan collars to prevent chewing on the incision site. Because an opened incision may impair healing at the mesh site, these rats were removed from analysis to eliminate this potential confounder. Therefore, we achieved sample sizes of n = 15 (coated mesh), n = 16 (uncoated mesh), and n = 12 (suture repair), with half in each group killed 7 days and half in each group killed 90 days after repair.

Postoperatively 2 rats implanted with coated mesh developed seromas appreciable on physical examination. These rats exhibited no change in feeding or activity indicative of infection. Clear, nonpurulent fluid was aspirated from 1 of these animals on postoperative day 14. Seromas resolved spontaneously by 90 days.

Macroscopic findings

Seven days after implantation, the coated polypropylene mesh was less integrated into the host tissue than the uncoated mesh, but both types were well incorporated at 90 days. The collagen coating was still visible at 7 days but was fully absorbed at 90 days. Minimal serosanguinous fluid between the mesh and the skin was observed in some animals sacrificed at 7 days (coated mesh, n = 5 of 7; uncoated mesh, n = 1 of 7; suture repair, n = 1 of 7). These rats had not exhibited any change in behavior indicative of infection. Because an onlay repair was used, adhesions were minimal in both groups and attached only to the suture repair line; no contact occurred between the mesh material and the intraperitoneal cavity. Both meshes demonstrated minimal shrinkage (approximately 4% mean decrease in surface area).

Microscopic findings

Average scores for inflammation, neovascularization, and fibroblastic proliferation are presented in Figure 1. Scores for FBGCs, PMNs, MNs, vascularity, and collagen deposition at the mesh site are presented in Tables 2 and 3. At 7 days, coated mesh had reduced scores for FBGCs, vascularity, and collagen organization compared with uncoated mesh, but histologic parameters were similar between the 2 meshes at 90 days. Scores for inflammation were slightly higher for coated mesh (mild to moderate) than

TABLE 3
Microscopic examination scores 90 days after surgery presented as mean (SEM)

Implant material	FBGCs	PMNs	MNs	Vascularity	Collagen amount	Collagen organization
Suture repair (n = 5)	0.06 (0.06)	0.10 (0.06)	1.17 (0.17)	1.89 (0.13)	1.60 (0.25)	2.55 (0.20)
Uncoated polypropylene (n = 9)	1.27 (0.12)[a]	1.09 (0.24)[a]	2.94 (0.04)[a]	2.40 (0.07)[a]	2.63 (0.15)[a]	2.67 (0.11)
Coated polypropylene (n = 8)	1.15 (0.06)[a]	0.93 (0.25)	2.89 (0.06)[a]	2.28 (0.10)[a]	2.75 (0.10)[a]	2.63 (0.18)
P value[b]	.001	0.03	< .001	.008	< .001	.89

ANOVA, analysis of variance; *FBGC,* foreign body giant cell; *MN,* mononuclear cells; *PMN,* polymorphonuclear leukocytes.

[a] $P < .05$ compared with suture repair; [b] ANOVA followed by Tukey's test for pairwise multiple comparisons.

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uncoated mesh (minimal to mild) at 7 days, although it did not reach statistical significance ($P = .06$). Inflammation decreased and was minimal at 90 days for both mesh types but remained elevated relative to suture repair (Figure 1, A).

Molecular findings

Comparison of gene expression profiles 7 days after implantation revealed that 4 inflammatory cytokines were up-regulated in the collagen-coated mesh group compared with the uncoated mesh group (Table 4). Relative to suture repair at 7 days, coated polypropylene induced the differential expression (>4-fold difference and $P < .01$) of 6 genes, whereas uncoated polypropylene induced the differential expression of 2 genes. No differences in expression were detected between the mesh types at 90 days. However, the identical 7 genes encoding inflammatory cytokines, MMPs, and other matrix proteins were found to be differentially expressed in both the coated and uncoated meshes relative to suture repair 90 days after surgery (Figure 2 and Table 5).

Immunohistochemical findings

Because messenger ribonucleic acid (mRNA) expression may not reflect the functional level of cytokines and other matrix proteins because of potential transcriptional or translational regulations, immunohistochemistry was performed at the host-mesh interface to confirm, at the protein level, a subset of differences observed in the gene expression profiles using 2 markers that demonstrated the greatest increase in mesh groups relative to suture repair.[23]

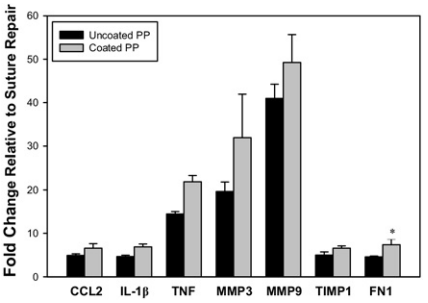
Scores for CXCL2 and MMP9 immunostaining are presented in Tables 6 and 7, respectively. Both mesh types tended to have elevated scores for CXCL2 and MMP9 relative to suture repair at 7 days and 90 days, with the coated mesh tending to have higher scores than the uncoated mesh for both markers, particularly at 7 days. CXCL2 and MMP9 were expressed in inflammatory cells, fibroblasts, and in the vasculature at 7 days in all groups, but by 90 days, staining was localized predominantly to macrophages and FBGCs surrounding mesh fibers (Figure 3).

Comment

Understanding how the molecular mediators of wound healing are affected by various mesh materials is essential for the development of safe, biocompatible, and functional implants. This study revealed that collagen-coated polypropylene mesh induces elevated inflammatory cytokine expression compared with uncoated mesh during the early postoperative period. This response likely is associated with the collagen coating because of the following: (1) numerous inflammatory cells were observed to colonize the coating 1 week after implantation; (2) increased exudative and acute inflammation occurred at the coated mesh repair site; and (3) histologic and gene expression differences between the mesh types disappeared at 90 days, after which the coating was fully resorbed. These results are consistent with histologic studies in sheep and rabbit models for vaginal surgery[17,26] and in a rat abdominal model.[27]

Given that the collagen coating is intended by the manufacturer to elicit a reduced initial inflammatory reaction and thereby induce a better healing response with improved tissue integration and de-

FIGURE 2
Differentially expressed genes induced by meshes 90 days after implantation



Differentially expressed genes (>4-fold change and $P < .01$) induced by collagen-coated and uncoated polypropylene meshes 90 days after implantation. Data represent mean ± SEM mesh-induced fold change compared with suture repair with no mesh (n = 5 suture repair; n = 5 uncoated mesh; n = 4 coated mesh). *Asterisk* indicates $P < .05$ comparing the 2 mesh types.

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TABLE 4
Genes overexpressed in coated mesh group at 7 days

Symbol	Refseq	Description	Fold change mean (SEM)	*P* value[a]	Function
CXCL1 (CINC-1, Gro1)	NM_030845	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	9.4 (0.2)	.00004	Neutrophil chemoattractant
CXCL2 (Mip-2, Scyb2)	NM_053647	Chemokine (C-X-C motif) ligand 2 (macrophage inflammatory protein 2)	23.9 (4.2)	.03	Neutrophil chemoattractant
IL-1β (IL1B, Il1b)	NM_031512	Interleukin-1β	8.4 (1.2)	.0003	Inflammatory cytokine
IL-6 (ILg6, Ifnb2)	NM_012589	Interleukin-6	3.9 (0.5)	.03	Inflammatory cytokine

Refseq, reference sequence.

[a] Student *t* test.

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TABLE 5
Differentially expressed genes induced by meshes at 90 days[a]

Functional gene grouping	Symbol	Other gene names	Unigene	Refseq	Description	Function
Inflammatory cytokines	CCL2	MCP-1/Scya2	Rn.4772	NM_031530	Chemokine (C-C motif) ligand 2	Monocyte chemoattractant protein
Inflammatory cytokines	IL1B	Il1b	Rn.9869	NM_031512	Interleukin-1β	Inflammatory cytokine; elevated in chronic wounds
Inflammatory cytokines	TNF	RATTNF/TNF-alpha	Rn.2275	NM_012675	Tumor necrosis factor (TNF superfamily, member 2)	Cytokine; binds TNF receptors; plays a role in regulation of cell proliferation, induction of apoptosis, and inflammatory response
Proteases, inhibitors, matrix proteins	MMP3	Mmp3	Rn.32086	NM_133523	MMP3	Degrades extracellular matrix proteins; regulates chemokine signaling; required during wound contraction
Proteases, inhibitors, matrix proteins	MMP9	Mmp9	Rn.10209	NM_031055	MMP9	Degrades extracellular matrix proteins; regulates chemokine signaling; involved in matrix remodeling
Proteases, inhibitors, matrix proteins	TIMP1	TIMP-1/Timp	Rn.25754	NM_053819	TIMP metalloproteinase inhibitor 1	Acts as an inhibitor of metalloprotease activity; may play a role in vascular tissue remodeling
Proteases, inhibitors, matrix proteins	FN1	FIBNEC/fn-1	Rn.1604	NM_019143	Fibronectin 1	Extracellular matrix component; may play a role in fibrosis and tumor metastasis

Refseq, reference sequence; *TIMP*, tissue inhibitor of metalloproteinase.

[a] Gene descriptions located at http://www.sabiosciences.com /rt_pcr_product/HTML/PARN-024A.html. Accessed March 18, 2011.

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creased risk of mesh erosion, it is noteworthy that in this study, a higher percentage of rats implanted with the coated mesh (15%; 3 of 20 rats) had healing issues, including 1 with wound dehiscence and 2 with seromas, whereas none of the rats implanted with uncoated mesh displayed evidence of impaired healing.

In addition, the coated mesh was less integrated into the host tissue at 7 days than the uncoated mesh, and serosanguinous fluid was present in 5 of 7 (71%) rats implanted with coated mesh compared with 1 of 7 (14%) rats implanted with uncoated mesh or no mesh. Scores for inflammation were higher for collagen-coated mesh than uncoated mesh, although this difference did not reach statistical significance likely because of a small sample size. Taken together, these results suggest that the collagen coating may affect the early healing response in a detrimental manner.

Interestingly, expression profiling revealed that the same 7 genes encoding inflammatory cytokines, MMPs, and other matrix proteins were up-regulated relative to suture repair in both mesh types 90 days after implantation. This finding suggests that coated and uncoated polypropylene meshes induce a similar long-term biologic response in excess of that elicited by suture repair. Although the chemical and physical properties of synthetic materials influence the foreign body reaction within the first 2-4 weeks after implantation, this reaction at the tissue-mesh interface is present for years or the lifetime of the implant.[9,14] Although a foreign body reaction is expected with all implanted materials and mild inflammation is necessary to induce angiogenic processes after implantation, chronic inflammation may adversely affect long-term incorporation of the material and may account for increased rates of mesh-related complications observed clinically.[4-7,12,14]

Elevated levels of tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β), which were increased in mesh repair groups at 90 days, are observed in chronic wounds.[22] These proinflammatory cytokines, produced predominantly by neutrophils and macrophages, are normally up-regulated during the in-

TABLE 6
Scores for CXCL2 immunohistochemical staining

Implant material	7 days mean (SEM)	n	90 days mean (SEM)	n
Suture repair	1.61 (0.39)	7	1.40 (0.37)	5
Uncoated polypropylene	2.64 (0.50)	7	2.47 (0.21)	9
Coated polypropylene	3.46 (0.19)[a]	7	2.38 (0.32)	8
P value[b]	.01		.05	

ANOVA, analysis of variance; *CXCL2*, chemokine (C-X-C motif) ligand 2.

[a] $P < .05$ compared with suture repair; [b] ANOVA followed by Tukey's test for pairwise multiple comparisons.

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flammatory phase of wound healing, but at high levels, and especially for prolonged periods of time, they may have a detrimental effect on healing.[22] Sustained expression of chemokine (C-C motif) ligand 2 (CCL2), a chemoattractant for monocytes/macrophages, enables a persistence of neutrophils and macrophages in chronic wounds and has been shown to contribute to a prolonged inflammatory response.[9,29] In addition, CCL2 is involved in the fusion of monocyte-derived macrophages on biomaterial surfaces to form FBGCs.[9]

TNF-α and IL-1β synergistically increase the synthesis of matrix metalloproteinases including MMP3 and MMP9, also shown in this study to be induced after the implantation of coated and uncoated polypropylene mesh.[22,30] MMP3 and MMP9 are members of a family of proteolytic enzymes that cleave components of the extracellular matrix in addition to a wide range of bioactive molecules, including cytokines and chemokines, growth factors, receptors, and adhesion molecules involved in wound healing.[30] They are important regulators of wound repair and are involved in the processes of reepithelialization, inflammation, and resolution of scar formation.[30-32]

A normal increase in MMP9 occurs in acute surgical wounds, which diminishes as healing occurs.[33] Likewise, a normal increase in MMP3 is required for wound contraction during the resolution phase.[30] In contrast, the elevated and prolonged production of MMP9 observed in many chronic wound types is thought to lead to delayed wound healing.[32] In this study, a 20- to 50-fold increase in MMP3 and MMP9 mRNA expression was observed 90 days after the implantation of coated and uncoated polypropylene mesh compared with suture repair, which demonstrated a return of these MMPs to physiologic levels at 90 days. It is tempting to speculate that the mesh-induced up-regulation of MMPs and proinflammatory cytokines contributes to persistent extracellular matrix remodeling and inflammation at the mesh-host interface, which may lead to erosion and other mesh-related complications.[4-7,12,14]

TABLE 7
Scores for MMP9 immunohistochemical staining

Implant material	7 days mean (SEM)	n	90 days mean (SEM)	n
Suture repair	1.86 (0.40)	7	1.25 (0.27)	5
Uncoated polypropylene	2.82 (0.34)	7	2.11 (0.34)	9
Coated polypropylene	3.43 (0.23)[a]	7	2.63 (0.14)[a]	8
P value[b]	.01		.02	

ANOVA, analysis of variance.

[a] $P < .05$ compared with suture repair; [b] ANOVA followed by Tukey's test for pairwise multiple comparisons.

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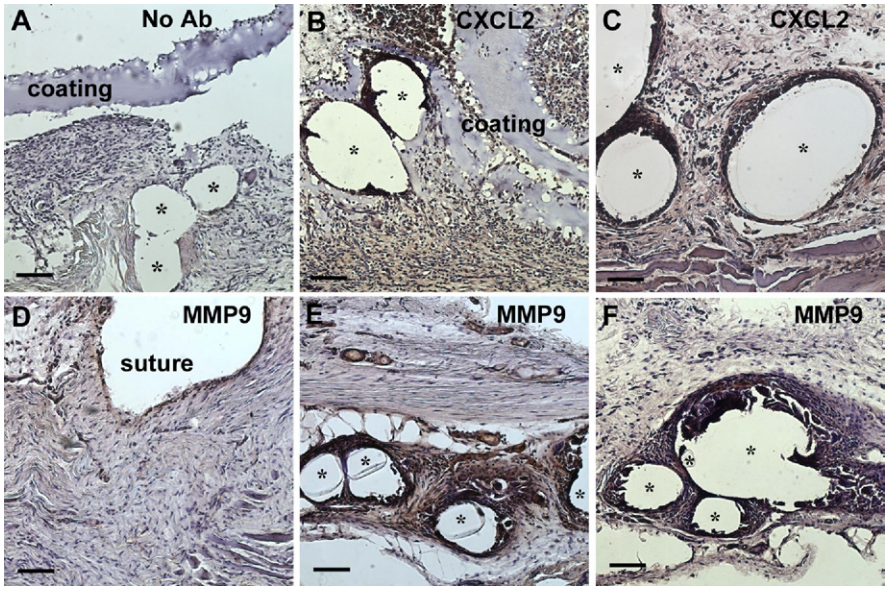
Interestingly, the mRNA expression of fibronectin was also up-regulated in mesh repair groups at 90 days. After implantation, biomaterials immediately acquire a layer of host proteins that interact with adhesion receptors present on host inflammatory cells.[9] These adsorbed proteins such as fibronectin, albumin, complement, fibrinogen, vitronectin, and others may be critical determinants of the foreign body reaction to implanted materials.[9] Because adsorbed proteins modulate inflammatory cell interactions and adhesion, their presence influences the inflammatory and wound-healing responses to the implanted materials.[9]

Limitations of this study exist. Gene expression profiles were evaluated to gain increased understanding of the molecular response to implanted materials, but it is possible that mRNA expression

FIGURE 3
CXCL2 and MMP9 immunostaining in mesh explants



CXCL2 (*panels* B and C) and MMP9 (*panels* D, E, and F) immunostaining in collagen-coated and uncoated polypropylene mesh explants. **A,** Coated mesh 7 days after implantation; negative control using no primary antibody (Ab). **B,** Coated mesh 7 days after implantation. **C,** Uncoated mesh 7 days after implantation. **D,** Suture repair with no mesh 90 days after surgery. **E,** Coated mesh 90 days after implantation. **F,** Uncoated mesh 90 days after implantation. Note absence of collagen coating at 90 days (*panel* E). *Asterisk* indicates mesh fibers. Original magnification ×200. Bar, 100 μm.

Pierce. Inflammatory cytokine and matrix metalloproteinase expression induced by coated and uncoated mesh. Am J Obstet Gynecol 2011.

may not reflect the functional level of the marker of interest because of potential transcriptional, translational, or post-translational regulations.[9] Future studies using proteomic analysis to investigate host responses are warranted.

In addition, this study involves the use of animals, and the responses observed may not be identical to those in humans. Data were obtained from a rodent abdominal model using a relatively small sample size, and this study did not examine effects in the vagina. However, histologic evaluation of synthetic meshes removed from human patients revealed similarities in the host response between rats and humans.[14,21]

Another weakness of the study is that the 2 mesh types used in this study were obtained from 2 different companies and are not identical with respect to mesh characteristics, although both macroporous meshes are reportedly similar in weight (38 and 41 g/m^2 for coated and uncoated polypropylene, respectively) and pore size (1.5 and 1.7 mm^2 for coated and uncoated polypropylene, respectively.[26]

This study was initiated as a first step to delineate the molecular mechanisms that direct the foreign body response to implanted meshes used in the transvaginal repair of pelvic organ prolapse. Findings from this study suggest that the porcine collagen coating provides no benefit over uncoated polypropylene mesh when used for vaginal surgery and may actually induce harm. For example, it is possible that the collagen coating may be associated with mesh erosions that are early and related to impaired healing of the vaginal incision. Additional clinical studies are warranted.

The ideal implant material is one that induces optimal strength of connective tissue, rapid wound healing, and minimal chronic inflammation, but the search for the ideal mesh continues. Increased understanding of the host response to graft materials ultimately will enable the development of improved meshes with better wound-healing properties and fewer complications. ■

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