GYNECOLOGY

Impact of prolapse meshes on the metabolism of vaginal extracellular matrix in rhesus macaque

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OBJECTIVE: The impact of polypropylene mesh implantation on vaginal collagen and elastin metabolism was analyzed using a nonhuman primate model to further delineate the mechanism of mesh induced complications.

STUDY DESIGN: Forty-nine middle-aged parous rhesus macaques underwent surgical implantation of 3 synthetic meshes via sacrocolpopexy. Gynemesh PS (n = 12) (Ethicon, Somerville, NJ) and 2 lower-weight, higher-porosity, lower-stiffness meshes (UltraPro [n = 19] [Ethicon] and Restorelle [n = 8] [Coloplast, Minneapolis, MN]) were implanted, in which UltraPro was implanted with its blue orientation lines perpendicular (low stiffness direction, n = 11) and parallel (high stiffness direction, n = 8) to the longitudinal axis of the vagina. Sham-operated animals were used as controls (n = 10). Twelve weeks after surgery, the mesh-tissue complex was excised and analyzed.

RESULTS: Relative to sham, Gynemesh PS had a negative impact on the metabolism of both collagen and elastin—favoring catabolic reactions, whereas UltraPro induced an increase only in elastin degradation. Restorelle had the least impact. As compared with sham, the degradation of collagen and elastin in the vagina implanted with Gynemesh PS was increased with a simultaneous increase in active matrix metalloproteinase (MMP)-1, -8, -13, and total MMP-2 and -9 (all $P < .05$). The degradation of elastin (tropoelastin and mature elastin) was increased in the UltraPro-implanted vagina with a concomitant increase of MMP-2, and -9 (all $P < .05$). Collagen subtype ratio III/I was increased in Gynemesh PS and UltraPro perpendicular groups ($P < .05$).

CONCLUSION: Following implantation with the heavier, less porous, and stiffer mesh, Gynemesh PS, the degradation of vaginal collagen and elastin exceeded synthesis, most likely as a result of increased activity of MMPs, resulting in a structurally compromised tissue.

Key words: collagen and elastin, matrix metalloproteinases, rhesus macaque, synthetic mesh, vagina

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Lightweight polypropylene mesh has been widely used in the surgical repair of pelvic organ prolapse to improve anatomical outcomes.[1,2] However, mesh-related complications including mesh exposure through the vaginal wall and erosion into adjacent structures, pain, and infection have raised concerns, prompting the Food and Drug Administration to issue 2 public health notifications warning of complications related to prolapse mesh and calling for mechanistic studies.[3-5]

To date, the impact of mesh on the vagina has not yet been clearly defined, and the mechanism by which mesh complications occur remains unknown. In a well-controlled nonhuman primate sacrocolpopexy model, heavier weight meshes with lower porosity, and higher stiffness were shown to have a profoundly negative impact on the vagina including a decrease in the amount of collagen, elastin, and smooth muscle.[6]

The resulting thinner and biomechanically inferior vagina seemed a perfect scenario for the development of mesh exposure, a process in which mesh becomes visible through the vaginal epithelium. Because collagen and elastin are key structural proteins that maintain the mechanical and structural integrity of the vagina, their content and stability are likely critical factors in the pathogenesis of mesh exposures.

In this study, we aimed to define alterations in collagen and elastin metabolism following the implantation of synthetic meshes varying by weight, porosity, and stiffness. We hypothesized that heavier, less porous, and stiffer meshes would be associated with increased collagen and elastin degradation characterized by

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increased matrix metalloproteinases (MMPs) and an increased ratio of collagen subtypes III/I.

To test this hypothesis, we compared the impact of 3 distinct polypropylene meshes with varying textile and structural properties: the heavier, less porous and stiffer prolapse mesh (Gynemesh PS; Ethicon, Somerville, NJ) vs 2 lighter, more porous, and less stiff meshes with (Ultrapro; Ethicon) and without (Restorelle; Coloplast, Minneapolis, MN) an absorbable component: poliglecaprone 25. Meshes were implanted via sacrocolpopexy in the rhesus macaque. Because UltraPro is highly anisotropic,[7,8] it was implanted with its blue orientation lines perpendicular (low stiffness direction) and parallel (high stiffness direction) to the longitudinal axis of the vagina. The production and degradation of collagen and elastin, the collagen subtype III/I ratio, and the levels of MMP-1, MMP-2, MMP-8, MMP-9, and MMP-13 were examined.

TABLE
Demographics of nonhuman primates in the study

Groups	Age, y[a]	Parity[b]	Weight, kg[a]	POP-Q stage[b]
Sham	13.3 ± 2.6	3.5 (2, 6)	7.5 ± 1.3	0 (0, 1)
Gynemesh	12.3 ± 2.4	4 (2, 5)	7.9 ± 1.6	0 (0, 0)
UltraPro Per	12.0 ± 2.5	2 (1.5, 4.5)	7.4 ± 1.3	0 (0, 1)
UltraPro Par	12.9 ± 1.0	4 (4, 5.5)	8.4 ± 1.3	0 (0, 0)
Restorelle	13.8 ± 1.7	5 (3, 5.3)	10.0 ± 2.8	1 (0, 1)
P value[c]	.43	.66	.02	.30

Gynemesh PS; Ethicon, Somerville, NJ. UltraPro; Ethicon. Restorelle; Coloplast, Minneapolis, MN.

POP-Q, Pelvic Organ Prolapse Quantification; *UltraPro Par*, UltraPro Parallel (high stiffness); *UltraPro Per*, UltraPro Perpendicular (low stiffness).

[a] Mean ± SD; [b] Median (first quartile, third quartile); [c] Comparison of overall *P* value among the groups.

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Materials and Methods

Mesh

Sterile samples of Gynemesh PS, UltraPro, and Restorelle were obtained. Their structural properties were described previously.[6,9]

Animals

Animal groups in the current study were the same as those in a previous study[6] except that a new animal was added to the UltraPro Perpendicular group. Parous middle-aged, nonhuman primates (rhesus macaques) were maintained and treated according to protocols approved by the Institutional Animal Care Use Committee of the University of Pittsburgh (no. 1008675) and in adherence to the National Institutes of Health Guidelines (Washington, DC) for the use of laboratory animals.

Surgical procedures

Two animals were excluded from the study at the time of surgery, the first because of a large mass in her right leg and enlarged pelvic lymph nodes and a second with stage IV endometriosis. In the end, a total of 49 animals were used. Thirty-nine animals were implanted with mesh via sacrocolpopexy after hysterectomy[6]: Gynemesh PS (n = 12), UltraPro Perpendicular (n = 11), UltraPro Parallel (n = 8), and Restorelle (n = 8). Ten animals underwent the identical surgery (sham) without insertion of mesh (n = 10). Twelve weeks later, the mesh-tissue complex was harvested en toto, and the epithelium was carefully removed prior to biochemical analyses.

Western blot: precursors of collagen I, and III

Following extraction using a high salt buffer (pH 7.5), the total protein concentration was determined in duplicate (DC protein assay; Bio-Rad Laboratories, Hercules, CA). Proteins at 10 μg/well were separated on 8% polyacrylamide gels and examined by standard procedures of Western blot. Precision plus Protein WesternC standards (Bio-Rad Laboratories) were used to indicate the molecular weight.

Primary antibodies included COL1A1 1:400 (L-19, goat polyclonal; Santa Cruz Biotechnology Inc, Santa Cruz, CA) and COL3A1 1:200 (C-15, goat polyclonal; Santa Cruz Biotechnology). Signal intensity of bands was quantitated via UN-SCAN-IT (version 4.3; Silk Scientific Co, Orem, UT). The blotted membranes were stained with Coomassie Blue, and the protein bands were quantified to represent the loading control for each well.

Protein amounts were expressed as arbitrary units, relative to the loading control and an internal positive control (protein extracts from a human prolapsed vagina) that was loaded in duplicate on each gel.

Western blot: tropoelastin and tropoelastin degradation

Tropoelastin monoclonal antibody at 1:200 (BA-4, mouse; Abcam, Cambridge, MA) and polyclonal antibody at 1:400 (ab21605, rabbit; Abcam) were used to detect tropoelastin at approximately 60 kDa (monoclonal) and tropoelastin degradation products (series of bands <50 kDa), respectively. To reduce the amount of nonspecific binding by the polyclonal antibody, we did the following: (1) established optimal binding conditions utilizing a progressive series of dilutions of the primary antibody and (2) confirmed the absence of nonspecific binding by the secondary antibody by performing parallel blots in which the primary antibody was eliminated.

Western blot: MMP-1, MMP-8, and MMP-13

The primary antibodies including MMP-1 at 1:200 (41-1E5, mouse monoclonal, recognizing both latent and active forms; EMD Millipore, Temecula, CA), MMP-8 at 1:400 (115-13D2, mouse monoclonal, recognizing both latent and active forms, EMD Millipore), and MMP-13 at 1:200 (VIIIA2, mouse monoclonal recognizing