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Collagen scaffold: a treatment for simulated maternal birth injury in the rat model

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Abstract

Objective—We sought to determine the impact of a collagen scaffold on the healing response after simulated birth injury in a rodent model.

Study design—A total of 52 virgin animals were divided into the following groups: control (n = 18), injured untreated (n = 18), and injured treated with porcine small intestinal submucosa (SIS) (n = 16). Histopathology, immunofluorescence of collagens, and vaginal mechanical properties were used to assess the impact of injury and the subsequent healing response.

Results—Collagen I/V decreased by 44% after birth injury relative to the controls ($P = .001$). Birth injury resulted in inferior mechanical properties of the vagina with a decrease of 38% in the tangent modulus and 44% in the tensile strength. SIS improved the collagen I/V and I/III ratios by 28% and 46%, respectively, paralleling the trend in the mechanical properties.

Conclusion—Simulated birth injury negatively affected vaginal biochemical and biomechanical properties long term. SIS treatment mitigated the impact of birth injury by enhancing tissue quality.

Keywords

birth injury; collagen; collagen scaffold; rodent; vagina

Immediate trauma to the vagina and perineum are serious and common sequelae of vaginal childbirth, affecting roughly 75% of women and causing significant short- and long-term morbidity. Indeed, maternal birth injury is by far the greatest risk factor for the development of pelvic floor dysfunction later in life.^{1–8} The cost to society of pelvic floor dysfunctions is not trivial, estimated at >\$1 billion per year with approximately 338,000 patients requiring surgery in the United States annually for prolapse alone.^{9–11}

Despite the morbidity, damage to the vagina at the time of childbirth has received little attention.^{1,4,5} A major long-term motivation for understanding the tissue behavior following

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maternal birth injury is improvement of the healing response of the vagina, and ultimately tissue quality, when injury occurs.^{12–14} Enhancing recovery to the preinjured condition may in turn decrease susceptibility to pelvic floor disorders later in life. Current models of tissue behavior after maternal birth injury, which include the data acquired from magnetic resonance imaging before and after delivery,¹⁵ would be improved by the addition of biomechanical and biochemical (histomorphological) data characterizing the affected tissue. Such data are also necessary to evaluate the response to potential treatments that can be applied at the time of injury.

To explore possible treatment modalities for maternal birth injury, we turned to functional tissue engineering, which aims to enhance healing to improve the functional behavior of the injured tissue. Recently, extracellular matrix scaffolds have shown significant potential in a wide variety of applications including healing of the medial collateral ligament (MCL) and Achilles tendon after injuries.^{16,17} Small intestinal submucosa (SIS) is a naturally occurring acellular collagen matrix derived from porcine intestine that has also been used by gynecologists to augment prolapse repairs and as a tissue filler or replacement.¹⁶ In contrast to most graft materials, SIS is a bioinductive graft containing growth factors and cytokines that promote healing of damaged tissue.¹⁷ Some of the bioactive molecules that are contained within SIS include vascular endothelial cell growth factors, basic fibroblast growth factors, and transforming growth factor- β ,^{18–21} all of which induce angiogenesis and cell infiltration into the wound site.¹⁹ In addition, the application of SIS has been shown to recruit bone marrow–derived cells (possibly of a pluripotent nature) that remain at the injury site and aid in the healing response helping the injured tissues recover.²¹ In animal models, it has been shown that immune-mediated inflammatory reactions evoked by SIS are limited due to the chemical composition of the scaffold.¹⁷ Lastly the scaffold is completely degraded and replaced by native tissue over a period of 8–12 weeks.²² These factors are all thought to be the reason why scar formation is minimized with the use of this scaffold and the functional behavior of healing tissues is improved. Consequently, SIS can be utilized as a bioactive agent to augment healing toward a more regenerative pathway. SIS has been successfully used in this way to aid in the repair of tissues from the human vascular, urogenital (bladder and urethra), and musculoskeletal systems.^{23–26} When used to treat orthopedic injuries, SIS enhanced the mechanical properties of the healing tissue (a reflection of tissue quality) with an increase in tangent modulus and tensile strength.^{16,27}

The principal determinants of strength in the vagina and its supportive tissues are thought to be fibrillar collagens I, III, and V. Changes in ratios and architecture of these collagens likely contribute to altered vaginal tissue behavior.²⁸ Collagens III and V are increased in tissues following trauma and are associated with inferior mechanical properties: increased distensibility and lower tensile strength, compared to tissues with high concentration of collagen type I.^{29–32}

We hypothesized that untreated vaginal tissue heals incompletely following maternal birth injury, which is exhibited as inferior mechanical properties due to elevated levels of collagen types III and V relative to type I collagen when compared to the uninjured vagina. The application of SIS after a maternal birth injury would restore the vagina to its original mechanical behavior and biochemical composition. In this study, we therefore sought to characterize changes in vaginal collagen ratios and tissue microarchitecture as well as changes in the mechanical properties of the vagina that occur following simulated birth injury in the presence and absence of tissue augmentation using SIS in a well-established rat model.^{33–36}

MATERIALS AND METHODS

Animals

Approval for this study was received from the Institutional Animal Care and Use Committee from the University of Pittsburgh, PA. A total of 52 Long-Evans 3-month-old virgin rats were used in this study. Animals were divided into the following groups: control (n = 18), injured untreated (n = 18), and injured treated with SIS (n = 16). Injured rats underwent a simulated birth injury via balloon distension and recovered for 4 weeks. Sixteen animals underwent treatment with either a sheet (n = 8) or a gel suspension (n = 8) of SIS, placed over the site of vaginal injury at the time of the simulated birth injury. Control and injured virgin animals were in similar phases of the menstrual cycle (estrus and metestrus) as determined by vaginal smears. Variables such as total weight, genital hiatus (GH) (diameter of vaginal opening), and total vaginal length (TVL) were obtained. Measurements of the GH and TVL were recorded before and after injury. After sacrifice, the weights and diameters of the excised vaginas were measured.

Simulated birth injury

We utilized an established model of simulated birth trauma.^{37–39} A 16F Foley catheter was custom fit with a balloon. The tip of the catheter was trimmed so that it was flush with the end of the balloon. Experimental animals were anesthetized and the catheter-balloon construct was placed in the vagina. Following filling of the balloon, the animal was placed supine on the edge of a table and the Foley catheter was allowed to hang with 130-g weight attached to its free-hanging end. The catheter with inflated balloon stayed in place for 2 hours. The balloon was then deflated and the catheter removed. Gross examination of vaginas was performed to assess the extent of injury. Balloon distention with 5-mL volume produced full-thickness tears in all animals (Figure 1).

Application of the porcine SIS

Following balloon injury, the vaginal full-thickness tear was visualized. A strip of sheet SIS (Cook/Biotech Inc, Bloomington, IN), 10 × 3 × 0.2 mm, or the equivalent amount of the gel form of SIS was placed over at the site of vaginal injury underneath the vaginal epithelium. For the sheet form, the luminal side faced the tear. The gel form of the SIS was made following previously described technique.⁴⁰ The edges of vaginal epithelium were reapproximated over the collagen scaffold with 5-0 polyglactin suture secured to a microclamp (Ethicon Inc, Somerville, NJ), thereby covering the SIS. For the animals that went untreated after birth injury, analogous sutures were placed in the vagina to control for the effects of suture placement. All animals were allowed to recover for 4 weeks after which they were euthanized according to IACUC guidelines.

Histological analysis

Thirty rats were utilized for the histological and immunofluorescence portions of the study: control (n = 10), injured untreated (n = 10), and injured treated with SIS (n = 10). Following sacrifice, the vagina was dissected away from its attachments to the pelvic sidewall, pubic symphysis, levator ani muscles, and sacrum. The full-thickness midportions of the vaginas were excised, imbedded in Optimal Cutting Temperature media (Sakura Finetek Inc, Torrance, CA), cut into 5- to 7- μ m sections with a cryostat, and stained with Masson trichrome for examination of gross morphologic features. Histopathologic examination was carried out to assess the extent of tissue injury. Our histologic endpoints included vaginal thickness on a cross section (epithelium to inferior margin of the muscularis), presence of a cellular infiltrate (absent, present), and disruption of tissue architecture (yes/no). The

transverse diameter of the cross section of the midportion of the vagina was measured across from one to another antiluminal side of the epithelium.

Immunofluorescence

Frozen embedded tissues were cut into serial sections of 5–7 μm and stored at 20°C until they were ready for use. The sections were incubated with either collagen I and III or collagen I and V primary antibodies at room temperature for 1 hour. The primary antibody to collagen I is rabbit anticollagen I (1:100) (Abcam, Cambridge, MA), collagen III is mouse anticollagen III (1:1000) (Sigma, St Louis, MO), and collagen V is mouse anticollagen V (1:1000) (Chemicon, Billerica, MA). Optimal dilutions of primary antibody were determined by a series of previous titration experiments. The secondary antibodies for collagens I, III, and V are goat antimouse Cy3 (Jackson, West Grove, PA), goat antirabbit Alexa 488 (Molecular Probes, Eugene, OR), and goat antimouse Cy5 (Jackson). Smooth muscle F-actin was labeled with Alexa 647 Phalloidin (1:500) (Molecular Probes). Sections were incubated with secondary antibody for 60 minutes, followed by 5 washes of bovine serum albumin and 5 washes of phosphate-buffered saline. Slides were stained for 30 seconds with Hoechst stain, followed by 5 washes of phosphate-buffered saline. Sections were then mounted with gelvatol and a coverslip and dried overnight at 4°C in the dark. Samples simultaneously labeled with 3 different primary antibodies (collagens I, III, and smooth muscle actin or collagens I, V, and smooth muscle actin) were scanned with an Olympus Fluoview BX61 confocal scanning laser microscope with a $\times 60$ objective. One of the authors (M.A.), blinded to the identity of the slides, performed all the analyses. Each specimen was analyzed at 10 random sites of the subepithelial and muscularis layers of the vagina. Fluorescence microscopy was interfaced to a quantitative computer program (Matlab). Results are represented as mean pixel intensity ratios per square area. The ratio of collagen I/III or I/V was used as an indicator of remodeling and the quality of the healing response. Regions of interest were drawn around vessels, which were identified by their morphology and positive staining of vascular smooth muscle F-actin, labeled with Alexa 647 Phalloidin (1:500) (Molecular Probes). Initial studies showed no significant difference in mean pixel intensity ratios of collagens I/III and I/V with and without regions of interest containing vascular structures. Therefore, vascular structures were not excluded in the final analysis.

Biomechanics

To characterize the biomechanical properties of vaginal tissue a uniaxial tensile test in the longitudinal direction was performed. In all, 22 rats were utilized for this portion of the study: control ($n = 8$), injured untreated ($n = 8$), and injured treated with SIS ($n = 6$). SIS-treated animals utilized for biomechanical testing included 3 rats treated with the gel form of SIS and 3 treated with the sheet form. After sacrifice, the vaginas were carefully dissected from the surrounding connective tissue, wrapped in saline-soaked gauze, placed in a plastic bag, and immediately stored at -20°C .^{41,42} On the day of testing, the tissue was thawed immediately before performance of the uniaxial tensile test. Custom-designed soft-tissue clamps were utilized to grip each vaginal sample at the proximal and distal ends, thus forming the clamp-specimen-clamp complex. To provide a uniform stress and strain distribution within the midsubstance of the vaginal sample, for each uniaxial tensile test an aspect ratio (length/width) of the sample was insured to be minimum of 5.43.⁴⁴ The cross-sectional area of each sample was measured using a laser micrometer system in 3 areas along the length of the sample to calculate an average cross-sectional area for the tissue, as previously described.^{45,46} Throughout the experimental protocol the sample was kept moist using 0.9% saline. Black contrast markers were placed on the tissue near the midline of the sample for strain measurements. These markers were captured and tracked using a camera system (Keyence CV-2600) and motion analysis software (Spicatek Inc, Maui, HI) to

calculate the strain in the midsubstance of the tissue. The clamp-specimen-clamp complex was placed into a 37°C saline bath and attached to a uniaxial tensile testing machine (Instron 5565). The proximal vagina was attached to a 50-lb load cell (Honeywell model 31, resolution 0.1 N), and the distal end was secured to the base of the material testing machine. The specimen was aligned with the loading axis of the machine and allowed to equilibrate in the saline bath for 30 minutes prior to testing. A small preload (0.1 N) was applied to the tissue and 10 cycles of preconditioning to 7% of the clamp-to-clamp distance was performed at a rate of 10 mm/min. A load to failure test was performed immediately after the preconditioning regimen, as previously described.⁴¹⁻⁴³ The load (force, N) and elongation (distension, mm) of the tissue were recorded and used to generate a load-elongation curves, which were then converted to a stress-strain relationship to calculate the mechanical properties of the vagina in the longitudinal direction. Here, stress is defined as the load (a measure of the force applied to the tissue) divided by cross-sectional area (measured by the laser micrometer), and strain was defined as the change in the marker distance divided by the original distance between the markers ($\Delta l/l_0$ or change in length of the specimen relative to its initial length). The slope of the linear region of the stress-strain curve was defined as the tangent modulus (a measurement of stiffness), while the tensile strength and maximum strain were recorded at failure (point at which the specimen breaks apart). The strain energy density was calculated by taking the area underneath the stress-strain curve until the point of failure.

Data analysis and statistics

All statistical analyses were performed using statistical software (SPSS v. 17.0; SPSS Inc, Chicago, IL). Our sample size was calculated based on preliminary data from virgin rats. Based on previously determined collagen ratios, we calculated that 10 rats per group would have 80% power at the 2-sided .05 significance level to detect at least a 35% difference in the collagen I:V ratio and 50% difference in the ratio of collagen I:III between injured and uninjured animals. For mechanical data 6 rats per group would have 80% power at the 2-sided .05 significance level to detect at least a 45% difference could be observed in the tensile strength and a 30% difference in the tangent modulus. Since the values of the skewness and kurtosis statistics did not indicate a departure from symmetry in the data distribution, Student *t* tests and 1-way analysis of variance with a Sidak post hoc were used to evaluate differences in the mean collagen ratios (I–III and I–V), mechanical properties (tensile strength, maximum strain, tangent modulus, and strain-energy density), as well as the continuous variables of the anatomical and demographic data between the injured and uninjured and treated and untreated rats. Since the variances for vaginal diameter were not equal based on Levene test, post hoc *P* values were determined using Student *t* test for unequal variances and adjusted using the Bonferroni correction. Statistical tests were evaluated at the 2-sided significance level of .05.

RESULTS

Baseline characteristics obtained for the animals used in the study are listed in Table 1. Injury-induced changes were reflected by a 1.2- to 1.5-fold increase in vaginal diameter despite 4 weeks of healing in the injured untreated group when compared to the controls (*P* = .001). Treatment with SIS mitigated this consequence of simulated birth injury (*P* = .35). TVL was 28% shorter in the injured untreated group >4 weeks of healing compared to control animals (*P* < .001). This scarring effect was also obviated by application of SIS (*P* = .37) (Table 1). When compared to controls, vaginal wet weights demonstrated a 36% increase in the injured untreated group (*P* < .001) suggesting a global tissue response to the injury (Table 1). This increase in wet weight was mitigated with the addition of SIS, with vaginal weights returning to the values of the control animals (*P* = .24) (Table 1).

Histology

Sectioned and Masson trichrome–stained full-thickness vaginal tissues were examined for overall histomorphology with light microscopy. The 4 layers of the vagina were well delineated in the control rats. For injured animals, disruption of the fibromuscular layer was noted on histological examination. In the injured group treated with SIS, the fibromuscular layer demonstrated better organization than in untreated injured rats; however, the vaginal layers were still not as well delineated as in the control group. A cellular infiltrate was observed in the injured group and did not appear to resolve with the addition of SIS (Figure 2). The transverse diameter of the cross section of the midportion of the vagina, measured across from antiluminal side of the epithelium to the other, was significantly smaller (0.46 cm) in the control animals when compared to the untreated injured rats (0.71 cm) ($P = .001$).

Immunofluorescence

Scanning confocal microscopy was used to simultaneously examine and quantify the relative amounts of 2 different antigens present in a fluorescent micrograph (Figure 3). Using this technique, we demonstrated that the ratio of collagen I/V decreased 44% in rats after simulated birth injury relative to the controls ($P = .001$), indicating persistent injury >4 weeks of recovery. The ratio of collagen I/III, however, was not different 4 weeks postsimulated birth injury ($P = .98$) (Table 2). The application of SIS (both liquid and sheet forms) dramatically altered the healing response. In rats treated with SIS following simulated birth injury, the ratio of collagen I/V was 28% higher than the ratio of the nontreated injured group, approaching that of uninjured controls ($P = .11$). The ratio of collagen I/III was significantly higher in injured animals after treatment with SIS, even when compared to the controls ($P = .001$), which is likely a reflection of increased collagen I deposition under the influence of SIS. There was no difference in healing response to the sheet vs gel form of SIS.

Biomechanics

Comparing the biomechanical properties of the vagina between uninjured controls and injured untreated rats revealed significantly inferior results in the later group, with a 44% decrease in the tensile strength (force per cross-sectional area required to disrupt the vagina) and a 38% decrease in the tangent modulus (a measurement of the resistance of the vagina to deformation) after simulated birth injury (Table 3). The control animals had an average tensile strength and tangent modulus of 2.09 ± 0.65 MPa and 25.05 ± 5.07 MPa, respectively. Each of these mechanical properties were significantly higher when compared to the injured untreated animals, which had a tensile strength of 1.16 ± 0.67 MPa ($P = .02$) and tangent modulus of 15.32 ± 8.59 MPa ($P = .03$). However, after treatment with SIS the tissue displayed an increase in both the tensile strength (1.67 ± 0.36 MPa) and tangent modulus (16.55 ± 4.18 MPa). This improvement in biomechanical properties returned the tissue properties to control values with no significant differences between animals treated with SIS compared to uninjured control for either the tensile strength ($P = .57$) or the tangent modulus ($P = .09$). There were no statistical differences in either the strain ($P = .52$) or the strain-energy density ($P = .11$).

COMMENT

The rodent model for simulating childbirth injury has been utilized extensively for the study of stress urinary incontinence.³⁷⁻⁴⁶⁻⁴⁷ Simulated birth injury has been also shown to result in hypoxic injury to the vagina.⁴⁸ However, no previous studies performed a comprehensive evaluation of the effect of simulated birth injury on the vaginal tissue by correlating the gross anatomical findings, biochemical transformations, and alterations in biomechanical behavior. In this study we also determined the impact of collagen scaffold application at the

time of birth injury on the healing response of the vagina. The primary findings of our experiments were that simulated birth injury resulted in long-term injury as measured by a combination of the above endpoints: an increased GH and persistently low ratios of collagen I/V despite 4 weeks of healing. The results of the biomechanical tests were consistent with the biochemical findings demonstrating persistent long-term injury (namely inferior biomechanical properties) in untreated injured animals. Significantly less force was required to disrupt the vagina in this group (decreased tensile strength) when compared to the controls. The vaginal tissue in the injured untreated rats was more compliant when compared to the controls (a decreased tangent modulus) indicating inferior tissue quality and increased vulnerability to deform in response to increases in force, eg, increases in intraabdominal pressure.

Multiple studies have shown an improvement in healing response of various tissues (Achilles tendon, urethra, MCL) after treatment with SIS.^{23,26,27} Consistent with previously published literature, the second major finding of our study was that application of SIS at the time of injury mitigated the negative impact of injury on the vagina. SIS application at the time of simulated birth injury led to a restoration of the gross anatomic changes as well as a significant increase in collagen I/III and collagen I/V ratios in treated animals, restoring the ratios to the level of uninjured controls. Recently, it has been shown that the mechanical properties of healing ligaments nearly doubled following SIS treatment compared to a nontreated control, corresponding with the increase in collagen I/V ratio.¹⁶ In our study, the improved biomechanical properties of the tissue in SIS treated rats, characterized by increase in maximum stress at failure (stronger) and higher tangent modulus (less likely to deform with the application of a force), mirrored the increase in collagen I/III and I/V ratios. Thus, our study demonstrated that SIS improved healing with near restoration of the tissue to the noninjured state.

SIS grafts have never been shown to improve outcomes of pelvic floor reconstruction surgeries.⁴⁹ This is perhaps due to the tendency of patients not to seek treatment for pelvic floor disorders until years, often decades, following the initial injury. At this point the tissue has been changed to new, likely inferior condition. In this way, SIS treatment at the time of birth injury is a novel approach. We show that SIS effectively negates the deleterious impact of the birth injury on vaginal mechanical properties long term. The current study establishes the foundation for future experiments of the impact of SIS application on vaginal tissue in the immediate postpartum period.

One of the limitations of our study is a relatively short (4 weeks) recovery period that is roughly equivalent to 3 years in human beings. We chose this endpoint based on previous qualitative studies examining ultrastructure of the vaginal fibromuscular layer in a rodent model, in which it was demonstrated that collagen structure recovered 4 weeks after normal vaginal delivery.⁵⁰ The latter study, however, focused on the fibril area fraction (the amount of matrix relative to collagen fibers) and did not distinguish between collagen subtypes. Studies examining the healing response of the MCL after injury have demonstrated a decrease in collagen I/V ratio during the early healing phase that persists even >52 weeks, indicating that this change may be a permanent indication of prior injury.⁵¹ Some studies examining the effects of SIS applied to injured knee ligaments allowed 12 weeks of healing.⁵² At these longer time points significant improvements in the tissues biomechanical properties have been observed. Although the rats are known to heal quickly, at our early time points, we may be quantifying a small window that captures only the beginning of this augmented healing response, which is demonstrated by the significant biochemical changes in the vaginal tissue of the treated animals; however, the remodeling process might have not been completed. Thus, the biomechanical and biochemical outcomes may continue to improve beyond this study's findings.

A second limitation was the method of uniaxial testing. Even though it allowed us to characterize and compare the longitudinal properties of the vagina and observe changes due to a simulated birth injury, it may not have been sensitive enough to characterize the full response of the vagina to treatment with SIS. The final limitation of this study was the wide variation in the tangent modulus, which is likely due to the low sensitivity of the uniaxial testing. Due to this wide variation in values, the means do not always represent the trends in the data. Despite no statistically significant difference between SIS-treated and control animals, only a small difference was found between the tangent modulus of the SIS-treated and injured untreated groups. However, the tangent modulus of the SIS-treated group tended to logically fall between the injured untreated and control groups values. This is consistent with previously published research and the overall findings of the current study.

To overcome the above limitations, we have currently begun to examine an in vivo testing as well as multiple axial testing methods that would allow us to more fully characterize vaginal behavior in the longitudinal and circumferential axes of control, injured untreated, and treated animals.

In conclusion, birth injury persists long term; however, collagen scaffold treatment mitigates its impact by altering healing response and enhancing tissue quality.

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References

1. Allen RE, Hosker GL, Smith ARB, Warrell DW. Pelvic floor damage and childbirth: a neurophysiological study. *Br J Obstet Gynaecol* 1990;97:770–9. [PubMed: 2242361]
2. Carley ME, Turner RJ, Scott DE, Alexander JM. Obstetric history in women with surgically corrected adult urinary incontinence or pelvic organ prolapse. *J Am Assoc Gynecol Laparosc* 1999;6:85–9. [PubMed: 9971858]
3. DeLancey JO. Pelvic organ prolapse: clinical management and scientific foundations. *Clin Obstet Gynecol* 1993;36:895–6.
4. DeLancey JO, Kearney R, Chou Q, Speights S, Binno S. The appearance of levator ani muscle abnormalities in magnetic resonance images after vaginal delivery. *Obstet Gynecol* 2003;101:46–53. [PubMed: 12517644]
5. Dietz HP, Bennett MJ. The effect of childbirth on pelvic organ mobility. *Obstet Gynecol* 2003;102:223–8. [PubMed: 12907092]
6. Gurel H, Gurel SA. Pelvic relaxation and associated risk factors: the results of logistic regression analysis. *Acta Obstet Gynecol Scand* 1999;78:290–3. [PubMed: 10203294]
7. Moalli PA, Jones IS, Meyn LA, Zyczynski HM. Risk factors associated with pelvic floor disorders in women undergoing surgical repair. *Obstet Gynecol* 2003;101:869–74. [PubMed: 12738142]
8. Sheiner E, Sarid L, Levy A, Seidman DS, Hallak M. Obstetric risk factors and outcome of pregnancies complicated with early postpartum hemorrhage: a population-based study. *J Matern Fetal Neonatal Med* 2005;18:149–54. [PubMed: 16272036]
9. Olsen AL, Smith VJ, Bergstrom JO, Colling JC, Clark AL. Epidemiology of surgically managed pelvic organ prolapse and urinary incontinence. *Obstet Gynecol* 1997;89:501–6. [PubMed: 9083302]
10. Popovic JR, Kozak LJ. National hospital discharge survey: annual summary, 1998. *Vital Health Stat* 13 2000;148:1–194. [PubMed: 11077892]

11. Subak LL, Waetjen LE, Van Den Eeden S, Thom DH, Vittinghoff E, Brown JS. Cost of pelvic organ prolapse surgery in the United States. *Obstet Gynecol* 2001;98:646–51. [PubMed: 11576582]
12. Anderson AE, Peters CL, Tuttle BD, Weiss JA. Subject-specific finite element model of the pelvis: development, validation and sensitivity studies. *J Biomech Eng* 2005;127:364–73. [PubMed: 16060343]
13. Fernandez JW, Pandy MG. Integrating modeling and experiments to assess dynamic musculoskeletal function. *Exp Physiol* 2006;91:371–82. [PubMed: 16407475]
14. Yarnitzky G, Yizhar Z, Gefen A. Real-time subject-specific monitoring of internal deformations and stresses in the soft tissues of the foot: a new approach in gait analysis. *J Biomech* 2006;39:2673–89. [PubMed: 16212969]
15. Kearney R, Miller JM, Ashton Miller JA, DeLancey JOL. Obstetric factors associated with levator ani muscle injury after vaginal birth. *Obstet Gynecol* 2006;107:144–9. [PubMed: 16394052]
16. Musahl V, Abramowitch SD, Gilbert TW, et al. The use of porcine small intestinal submucosa to enhance the healing of the medial collateral ligament—a functional tissue engineering study in rabbits. *J Orthop Res* 2004;22:214–20. [PubMed: 14656683]
17. Badylak SF, Wu CC, Bible M, McPherson E. Host protection against deliberate bacterial contamination of an extracellular matrix bioscaffold versus Dacron mesh in a dog model of orthopedic soft tissue repair. *J Biomed Mater Res B Appl Biomater* 2003;67:648–54. [PubMed: 14528463]
18. Badylak S, Liang A, Record R, Tullius R, Hodde J. Endothelial cell adherence to small intestinal submucosa: an acellular bioscaffold. *Biomaterials* 1999;20:2257–63. [PubMed: 10614932]
19. Hodde JP, Record RD, Liang HA, Badylak SF. Vascular endothelial growth factor in porcine-derived extracellular matrix. *Endothelium* 2001;8:11–24. [PubMed: 11409848]
20. McPherson TB, Liang H, Record RD, Badylak SF. Galalpha(1,3)Gal eptope in porcine small intestinal submucosa. *Tissue Eng* 2000;6:233–9. [PubMed: 10941218]
21. Voytik-Harbin SL, Brightman AO, Kraine MR, Waisner B, Badylak SF. Identification of extractable growth factors from small intestinal submucosa. *J Cell Biochem* 1997;67:478–91. [PubMed: 9383707]
22. Thomas WG, Stewart-Akers AM, Simmons-Byrd A, Badylak SF. Degradation and remodeling of small intestinal submucosa in canine Achilles tendon repair. *J Bone Joint Surg* 2007;89:621–30. [PubMed: 17332112]
23. Badylak S, Arnoczky S, Plouhar P, et al. Naturally occurring extracellular matrix as a scaffold for musculoskeletal repair. *Clin Orthop Relat Res* 1999;367:S333–43. [PubMed: 10546657]
24. Edelman DS. Laparoscopic herniorrhaphy with porcine small intestinal submucosa: a preliminary study. *JSLs* 2002;6:203–5. [PubMed: 12166756]
25. Franklin ME, Gonzalez JJ, Glass JL. Use of porcine small intestinal submucosa as a prosthetic device for laparoscopic repair of hernias in contaminated fields: 2-year follow-up. *Hernia* 2004;8:186–9. [PubMed: 14991410]
26. Kropp BP, Ludlow JK, Spicer D, et al. Rabbit urethral regeneration using small intestinal submucosa onlay grafts. *Urology* 1998;52:138–42. [PubMed: 9671888]
27. Badylak SF, Tullius R, Kokini K, et al. The use of xenogeneic small intestinal submucosa as a biomaterial for Achilles tendon repair in a dog model. *J Biomed Mater Res* 1995;29:977–85. [PubMed: 7593041]
28. Moalli PA, Shand SH, Zyczynski HM, Gordy SC, Meyn LA. Remodeling of vaginal connective tissue in patients with prolapse. *Obstet Gynecol* 2005;106:953–63. [PubMed: 16260512]
29. Frank C, McDonald D, Bray D, et al. Collagen fibril diameters in the healing adult rabbit medial collateral ligament. *Connect Tissue Res* 1992;27:251–63. [PubMed: 1576825]
30. Oakes, B.; Parker, A.; Norman, J. Changes in collagen fiber populations in young rat cruciate ligaments in response to an intensive one month's exercise program. In: Russo, P.; Gass, G., editors. *Human adaptation*. Williamsberg: Cumberland College of Health Sciences; 1981. p. 223–30.
31. Frank C, Woo SL-Y, Amiel D. Medial collateral ligament healing: a multidisciplinary assessment in rabbits. *Am J Sports Med* 1983;11:379–89. [PubMed: 6650715]

32. Frank, CB. Soft tissue healing. In: Fu, FH.; Harner, CD.; Vince, KG., editors. Knee surgery. Baltimore: Williams and Wilkins; 1994. p. 189-230.
33. Aspden RM. Collagen organization in the cervix and its relation to mechanical function. *Coll Relat Res* 1988;8:103–12. [PubMed: 3378391]
34. Harkness ML, Harkness HD. Changes in the physical properties of the uterine cervix of the rat during pregnancy. *J Physiol* 1959;148:524–47. [PubMed: 14399825]
35. Hulmes D. The collagen superfamily—diverse structures and assemblies. *Essays Biochem* 1992;27:46–67.
36. Kokenyesi R, Woessner JF. Effects of hormonal perturbations on the small dermatan sulfate proteoglycan and mechanical properties of the uterine cervix of late pregnant rats. *Connect Tissue Res* 1991;26:199–205. [PubMed: 1769238]
37. Lin AS, Carrier S, Morgan DM, Lue TF. Effect of simulated birth trauma on the urinary continence mechanism in the rat. *Urology* 1998;52:143–51. [PubMed: 9671889]
38. Resplande J, Gholami SS, Graziottin TM, et al. Long-term effect of ovariectomy and simulated birth trauma on the lower urinary tract of female rats. *J Urol* 2002;168:323–30. [PubMed: 12050564]
39. Sievert KD, Bakircioglu ME, Tsai T, Dahms SE, Nunes L, Lue TF. The effect of simulated birth trauma and/or ovariectomy on rodent continence mechanism, part I: functional and structural change. *J Urol* 2001;166:311–7. [PubMed: 11435892]
40. Freytes DO, Martin J, Velankar SS, Lee AS, Badylak SF. Preparation and rheological characterization of a gel form of the porcine urinary bladder matrix. *Biomaterials* 2008;29:1630–7. [PubMed: 18201760]
41. Rubod C, Boukerrou M, Brieu M, Dubois P, Cosson M. Biomechanical properties of vaginal tissue, part 1: new experimental protocol. *J Urol* 2007;178:320–5. [PubMed: 17499792]
42. Woo SL-Y, Orlando CA, Camp JF, Akeson WH. Effects of postmortem storage by freezing on ligament tensile behavior. *J Biomech* 1986;19:399–404. [PubMed: 3733765]
43. Abramowitch SD, Woo SL-Y, Clineff TD, Debski RE. An evaluation of the quasi-linear viscoelastic properties of the healing medial collateral ligament in a goat model. *Ann Biomed Eng* 2004;32:329–35. [PubMed: 15098537]
44. Woo SL-Y, Gomez MA, Sites TJ, Newton PO, Orlando CA, Akeson WH. The biomechanical and morphological changes in the medial collateral ligament of the rabbit after immobilization and remobilization. *J Bone Joint Surg Am* 1987;69:1200–11. [PubMed: 3667649]
45. Lee TQ, Woo SL. A new method for determining cross-sectional shape and area of soft tissues. *J Biomech Eng* 1988;110:110–4. [PubMed: 3379932]
46. Cannon TW, Wojcik EM, Ferguson C, Saraga S, Thomas C, Damaser MS. Effects of vaginal distension on urethral anatomy and function. *BJU Int* 2002;90:403–7. [PubMed: 12175397]
47. Woo LL, Hijaz A, Pan HQ, Kuang M, Rackley RR, Damaser MS. Simulated childbirth injuries in an inbred rat strain. *Neurourol Urodyn* 2009;28:356–61. [PubMed: 18973147]
48. Damaser MS, Whitbeck C, Chichester P, Levin RM. Effect of vaginal distension on blood flow and hypoxia of urogenital organs of the female rat. *J Applied Physiol* 2005;98:1884–90. [PubMed: 15591288]
49. Paraiso MF, Barber MD, Muir TW, Walters MD. Rectocele repair: a randomized trial of three surgical techniques including graft augmentation. *Am J Obstet Gynecol* 2006;195:1762–71. [PubMed: 17132479]
50. Daucher JA, Clark KA, Stolz DB, Meyn LA, Moalli PA. Adaptations of the rat vagina in pregnancy to accommodate delivery. *Obstet Gynecol* 2007;109:128–35. [PubMed: 17197598]
51. Niyibizi C, Kavalkovich K, Yamaji T, Woo SL-Y. Type V collagen is increased during rabbit medial collateral ligament healing. *Knee Surg Sports Traumatol Arthrosc* 2000;8:281–5. [PubMed: 11061296]
52. Liang R, Woo SL-Y, Takakura Y, Moon DK, Jia F, Abramowitch SD. Long-term effects of porcine small intestine submucosa on the healing of medial collateral ligament: a functional tissue engineering study. *J Orthop Res* 2006;24:811–9. [PubMed: 16514641]

Figure 1. Representative photograph taken after immediately simulated birth injury
Urethra is located at 12 o'clock. Full-thickness vaginal tear is visible, extending from 7–10 o'clock.

Figure 2.

Light micrographs of vaginal wall of **A**, control, demonstrating well-delineated layers of vagina; **B**, 4 weeks after simulated birth injury, demonstrating disruption of fibromuscular layer; and **C**, 4 weeks after simulated birth injury and small intestinal submucosa treatment, showing better organization of fibromuscular layer than untreated injured rats. Masson trichrome stain, magnification $\times 5$.

M, muscularis; *SE*, subepithelium.

Figure 3. Representative fluorographs of collagen subtypes

Collagen I (*green*) and V (*red*) in vaginas of **A**, uninjured; **B**, injured; and **C**, injured treated with small intestinal submucosa (SIS) rats. *Blue* = smooth muscle F-actin. **A**, The 5–7 μ m sections of full-thickness vagina were labeled with primary antibodies against collagen I, V, and smooth muscle actin. Secondary antibodies linked to 3 different fluorophores were added at appropriate dilutions. Quantitative analysis was performed using scanning confocal microscopy showing that ratio of collagen I/V is decreased after simulated birth injury and recovers to uninjured level with application of SIS. **B** and **C**, Prepared similarly.

Table 1

Variables at time of sacrifice, demonstrating significant increase in vaginal wet weights, genital hiatus, and vaginal diameter after injury, mitigated by treatment with small intestinal submucosa, when compared to controls

Group	n	Weight, g ^a	Vaginal weight, g ^a	TVL, cm ^a	GH, cm ^a	Vaginal diameter, cm ^a
Control	10	235.52 ± 21.98	0.25 ± 0.03	2.25 ± 0.24	0.28 ± 0.08	0.46 ± 0.07
Injured untreated	10	236.24 ± 14.05	0.34 ± 0.04	1.61 ± 0.22	0.61 ± 0.10	0.71 ± 0.22
Injured + SIS	10	257.90 ± 14.12	0.22 ± 0.03	2.11 ± 0.15	0.31 ± 0.07	0.56 ± 0.07
Overall <i>P</i> value ^b		.01	< .001	< .001	< .001	< .001
Control vs injured untreated ^c		> .99	< .001	< .001	< .001	.02 ^d
Control vs injured + SIS ^c		.02	.24	.37	.82	.02 ^d
Injured untreated vs injured + SIS ^c		.03	< .001	< .001	< .001	.16 ^d

GH, genital hiatus; SIS, small intestinal submucosa; TVL, total vaginal length.

^aData presented as mean ± SD;

^b*P* value from 1-way analysis of variance;

^c*P* value from Sidak multiple comparison procedure;

^d*P* value from Student *t* test for unequal variances and adjusted using Bonferroni correction.

Table 2

Comparison of ratio of collagen I/III and I/V in vaginal tissue of uninjured control, injured untreated, and injured treated with small intestinal submucosa rats

Group	n	Ratio I/III ^a	Ratio I/V ^a
Control	10	2.01 ± 0.63	4.95 ± 1.56
Injured untreated	10	1.88 ± 0.91	2.75 ± 0.96
Injured + SIS	10	3.51 ± 0.91	3.82 ± 0.78
Overall <i>P</i> value ^b		< .001	.001
Control vs injured untreated ^c		.98	.001
Control vs injured + SIS ^c		.001	.11
Injured untreated vs injured + SIS ^c		< .001	.14

Simulated birth injury resulted in significant decrease in collagen I/V at 4 weeks suggesting persistently injured tissue with inferior mechanical properties. These changes were mitigated by application of SIS.

SIS, small intestinal submucosa.

^aData presented as mean ± SD;

^b*P* value from 1-way analysis of variance;

^c*P* value from Sidak multiple comparison procedure.

Biomechanical properties of vaginal tissue, demonstrating significant decrease in tensile strength and tangent modulus after simulated birth injury

Table 3

Group	n	Tensile strength (MPa) ^a	Maximum strain ^a	Tangent modulus (MPa) ^d	Strain-energy density (MPa) ^d
Control	8	2.09 ± 0.65	0.14 ± 0.04	25.05 ± 5.07	0.12 ± 0.06
Injured untreated	8	1.16 ± 0.67	0.14 ± 0.05	15.32 ± 8.59	0.06 ± 0.03
Injured + SIS	6	1.67 ± 0.36	0.17 ± 0.05	16.55 ± 4.18	0.11 ± 0.05
Overall <i>P</i> value ^b		.03	.52	.02	.11
Control vs injured untreated ^c		.03	> .99	.03	.13
Control vs injured + SIS ^c		.57	.65	.09	.98
Injured untreated vs injured + SIS ^c		.42	.70	.98	.36

SIS, small intestinal submucosa.

^aData presented as mean ± SD;

^b*P* value from 1-way analysis of variance;

^c*P* value from Sidak multiple comparison procedure.