Acta Biomaterialia 57 (2017) 324–333

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Towards rebuilding vaginal support utilizing an extracellular matrix bioscaffold

Rui Liang [a,c], Katrina Knight [b], Deanna Easley [b], Stacy Palcsey [a], Steven Abramowitch [a,b], Pamela A. Moalli [a,b,c,*]

[a] Magee Women Research Institute, University of Pittsburgh, Pittsburgh, PA, USA
[b] Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA
[c] Department of Obstetrics, Gynecology, Reproductive Sciences, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

ARTICLE INFO

Article history:
Received 28 September 2016
Received in revised form 25 April 2017
Accepted 5 May 2017
Available online 6 May 2017

Keywords:
Prolapse
Extracellular matrix
Vaginal incision
Pelvic support
Regeneration

ABSTRACT

As an alternative to polypropylene mesh, we explored an extracellular matrix (ECM) bioscaffold derived from urinary bladder matrix (MatriStem™) in the repair of vaginal prolapse. We aimed to restore disrupted vaginal support simulating application via transvaginal and transabdominal approaches in a macaque model focusing on the impact on vaginal structure, function, and the host immune response. In 16 macaques, after laparotomy, the uterosacral ligaments and paravaginal attachments to pelvic side wall were completely transected (IACUC# 13081928). 6-ply MatriStem was cut into posterior and anterior templates with a portion covering the vagina and arms simulating uterosacral ligaments and paravaginal attachments, respectively. After surgically exposing the correct anatomical sites, in 8 animals, a vaginal incision was made on the anterior and posterior vagina and the respective scaffolds were passed into the vagina via these incisions (transvaginal insertion) prior to placement. The remaining 8 animals underwent the same surgery without vaginal incisions (transabdominal insertion). Three months post implantation, firm tissue bands extending from vagina to pelvic side wall appeared in both MatriStem groups. Experimental endpoints examining impact of MatriStem on the vagina demonstrated that vaginal biochemical and biomechanical parameters, smooth muscle thickness and contractility, and immune responses were similar in the MatriStem no incision group and sham-operated controls. In the MatriStem incision group, a 41% decrease in vaginal stiffness ($P = 0.042$), a 22% decrease in collagen content ($P = 0.008$) and a 25% increase in collagen subtypes III/I was observed vs. Sham. Active MMP2 was increased in both Matristem groups vs. Sham (both $P = 0.002$). This study presents a novel application of ECM bioscaffolds as a first step towards the rebuilding of vaginal support.

Statement of Significance

Pelvic organ prolapse is a common condition related to failure of the supportive soft tissues of the vagina; particularly at the apex and mid-vagina. Few studies have investigated methods to regenerate these failed structures. The overall goal of the study was to determine the feasibility of utilizing a regenerative bioscaffold in prolapse applications to restore apical (level I) and lateral (level II) support to the vagina without negatively impacting vaginal structure and function. The significance of our findings is two fold: 1. Implantation of properly constructed extracellular matrix grafts promoted rebuilding of level I and level II support to the vagina and did not negatively impact the overall functional, morphological and biochemical properties of the vagina. 2. The presence of vaginal incisions in the transvaginal insertion of bioscaffolds may compromise vaginal structural integrity in the short term.

* Corresponding author at: Magee Women Research Institute, Department of Obstetrics and Gynecology, 204 Craft Ave., Pittsburgh, PA 15219, USA.
E-mail addresses: rul3@pitt.edu (R. Liang), knightkm@mwri.magee.edu (K. Knight), deanna.christine.easley@gmail.com (D. Easley), tokarsl@mwri.magee.edu (S. Palcsey), sdast9@pitt.edu (S. Abramowitch), moalpa@mail.magee.edu (P.A. Moalli).

http://dx.doi.org/10.1016/j.actbio.2017.05.015
1742-7061/

1. Introduction

Vaginal prolapse also known as pelvic organ prolapse (POP), is a common condition in which loss of soft tissue support to the vagina leads the organs supported by it (bladder, uterus, small bowel and rectum) to herniate or fall into the vaginal lumen causing significant physical discomfort and psychological distress to affected women. Support to the upper and mid-vagina is provided by the uterosacral ligaments and paravaginal attachments to the pelvic side-wall, respectively. The vagina and supportive tissues of women with prolapse have been shown to be structurally and functionally compromised with altered collagen and elastin content [1–3], increased tissue degrading proteases [2], disorganization and atrophy of smooth muscle [4,5] and inferior mechanical properties [6]. Up to 12.6% of women will undergo a surgery to repair prolapse by age 80 [7]. Of those who undergo a native tissue repair, 40% will fail by 2 years [8,9][10].

In response to the high failure rates associated with native tissue repairs, surgeons have turned to lightweight polypropylene mesh. In spite of fairly good anatomical outcomes, polypropylene prolapse meshes have been associated with numerous complications, most commonly mesh exposure through the vaginal epithelium, pain, and erosion into adjacent structures [11], prompting 2 FDA warnings and an upclassification of meshes from Class II to Class III devices [12]. In nonhuman primates, Gynemesh PS - the prototype and most widely implanted polypropylene prolapse mesh, has been shown to have a negative impact on the vagina inducing a robust long-term foreign body response associated with degeneration and atrophy, and a loss of functional integrity [13,14]. Thus, regenerative techniques that direct the host to rebuild and restore damaged vaginal supportive structures represent a viable alternative approach.

Bioscaffolds derived from extracellular matrix (ECM) have been widely adopted in tissue engineering applications and are considered a novel tool in tissue regeneration. They have been shown to promote regeneration in different tissues, e.g. skeletal muscle, tendon, fibrocartilage, and digits, in numerous preclinical animal studies and human clinical applications by facilitating a site-specific constructive remodeling response [15]. When placed in the appropriate *in vivo* mechanical loading environment, the remodeling of ECM bioscaffolds is directed to promote the formation of a site specific tissue with robust mechanical properties [16–18]: a scenario seemingly perfectly aligned with the goals of urogynecological patients and surgeons – that is, to repair the damaged/impaired vagina and its supportive tissues in patients with prolapse.

Restoration of apical (level I) and lateral (level II) support to the vagina has been shown to be key in achieving successful anatomical outcomes in the long term [19–21]. Therefore, the overall goal of this study was to determine the feasibility of utilizing a regenerative ECM bioscaffold in prolapse applications to restore uterosacral ligaments (level I) and paravaginal attachments into the pelvic side wall (level II) without negatively impacting vaginal structure and function (Fig. 1). Secondly, since the approach by which surgical material is delivered to the host has been shown to impact outcomes with transvaginal applications associated with higher rates of complications than transabdominal applications [22–24], we modeled and compared these two approaches for bioscaffold placement. We chose MatriStem (porcine urinary bladder matrix, ACELL), a noncrosslinked degradable acellular ECM bioscaffold because of its ability to promote the growth of tissue containing both smooth muscle and matrix [25–28], two critical components of the vagina and the soft tissues that support it. We used a 6-ply scaffold since we arguably needed a graft that had similar initial mechanical integrity to commonly used prolapse meshes [13,29] and its strength would persist as it was slowly replaced with host newly formed tissues. Since complications following prolapse procedures typically involve the vagina (exposure and pain), we sought to focus on the impact of MatriStem on vaginal morphology, biochemical composition, and function (passive and active mechanics) in the current study. Moreover, we were concerned about stress shielding effects given that the 6-ply MatriStem bioscaffold had an initial structural stiffness similar to synthetic prolapse meshes and therefore, could potentially exert a negative impact on the vagina due to stress shielding [13]. The rhesus macaque model was used due to its marked similarities to humans. Previous studies have demonstrated a prolonged foreign body response with synthetic meshes and crosslinked biologic materials; thus, the host immune response was also assessed [22–24].

2. Materials and methods

2.1. Animals

Rhesus macaques (macacca mulatta) used in this study were maintained and treated according to experimental protocols approved by the Institutional Animal Care Use Committee of the University of Pittsburgh (IACUC #13081928) and in adherence to the National Institutes of Health Guidelines for the use of primates as "an acutely scarce resource" such that a minimum number were used for meaningful results. Routine laboratory tests and regular examinations by veterinarians during a quarantine period were used to certify that these experimental animals were pathogen-free and in good physical condition. Animals were maintained in standard cages with ad libitum water and a scheduled monkey nutritious diet. A 12-h light/dark cycle (7 am to 7 pm) was used, and menstrual cycle patterns were recorded daily. Age, weight, and parity were collected prior to and after surgery.

2.2. Surgical procedures

For comparison of our experimental endpoints, we used previously published data for Sham (n = 12). These animals had been operated in the same way as our MatriStem implanted animals with surgical exposures affording the implantation of a vaginal graft but did not undergo disruption of level I and II support or the application of the bioscaffold [13,14]. Our decision to use control animals without disrupted support is based on our previous finding that the prolapsed unsupported vagina has inferior mechanical properties, altered collagen ratios and increased active MMP-9 [30,31] relative to the vagina with intact support. In this way, by comparing to normally supported animals, we would be comparing our MatriStem implanted vagina to the "gold standard". After laparotomy, 16 rhesus macaques underwent a hysterectomy followed by complete transection of the uterosacral ligaments (level I) and paravaginal attachments to pelvic sidewall (level II) (Fig. 2). The bladder and rectum were sharply dissected off of the vagina to expose the full thickness vaginal wall. A 6-ply MatriStem scaffold was cut into posterior and anterior templates with a vaginal portion and arms to simulate uterosacral ligaments and paravaginal attachments, respectively (Fig. 3). To model transvaginal insertion (N = 8), a 3 cm vaginal incision was made on both the anterior and posterior vaginal walls and the respective scaffolds were placed into the vaginal lumen via these incisions where they were held for ~ 5 min before pulling back into the pelvic cavity. Vaginal incisions were then repaired with 2–0 Vicryl. The remaining animals (N = 8) underwent the same surgery without vaginal incisions to model a transabdominal insertion. The scaffolds were