Acta Biomaterialia xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

The mechanical response of the mouse cervix to tensile cyclic loading in term and preterm pregnancy

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ARTICLE INFO

Article history:
Received 15 March 2018
Received in revised form 21 June 2018
Accepted 10 July 2018
Available online xxxx

Keywords:
Preterm birth
Cervical remodeling
Cyclic tensile test
LPS
RU486

ABSTRACT

A well-timed modification of both the collagen and elastic fiber network in the cervix during pregnancy accompanies the evolution of tissue mechanical parameters that are key to a successful pregnancy. Understanding of the cervical mechanical behaviour along normal and abnormal pregnancy is crucial to define the molecular events that regulate remodeling in term and preterm birth (PTB). In this study, we measured the mechanical response of mouse cervical tissue to a history of cyclic loading and quantified the tissue's ability to recover from small and large deformations. Assessments were made in non-pregnant, pregnant (gestation days 6, 12, 15 and 18) and mouse models of infection mediated PTB treated with lipopolysaccharide on gestation d15 (LPS treated) and hormone withdrawal mediated PTB on gestation d15 (RU486 treated). The current study uncovers the contributions of collagen and elastic fiber networks to the progressive change in mechanical function of the cervix through pregnancy. Premature cervical remodeling induced on gestation day 15 in the LPS infection model is characterized by distinct mechanical properties that are similar but not identical to mechanical properties at term ripening on day 18. Remodeling in the LPS infection model results in a weaker cervix, unable to withstand high loads. In contrast, the RU486 preterm model resembles the cyclic mechanical behaviour seen for term d18 cervix, where the extremely compliant tissue is able to withstand multiple cycles under large deformations without breaking. The distinct material responses to load-unload cycles in the two PTB models matches the differing microstructural changes in collagen and elastic fibers in these two models of preterm birth. Improved understanding of the impact of microstructural changes to mechanical performance of the cervix will provide insights to aid in the development of therapies for prevention of preterm birth.

Statement of significance

Preterm Birth (PTB) still represents a serious challenge to be overcome, considering its implications on infant mortality and lifelong health consequences. While the causes and etiologies of PTB are diverse and yet to be fully elucidated, a common pathway leading to a preterm delivery is premature cervical remodeling. Throughout pregnancy, the cervix remodels through changes of its microstructure, thus altering its mechanical properties. An appropriate timing for these transformations is critical for a healthy pregnancy and avoidance of PTB. Hence, this study aims at understanding how the mechanical function of the cervix evolves during a normal and preterm pregnancy. By performing cyclic mechanical testing on cervix samples from animal models, we assess the cervix's ability to recover from moderate and severe loading. The developed methodology links mechanical parameters to specific microstructural components. This work identifies a distinct biomechanical signature associated with inflammation mediated PTB that differs from PTB induced by hormone withdrawal and from normal term remodeling.

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1. Introduction

Preterm Birth (PTB), defined as a delivery before 37 weeks of gestation, remains an important clinical challenge. It impacts approximately 12% of all births [1] (9.6% in the US in 2015 [2]),

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https://doi.org/10.1016/j.actbio.2018.07.017
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Please cite this article in press as: C. Jayyosi et al., The mechanical response of the mouse cervix to tensile cyclic loading in term and preterm pregnancy, Acta Biomater. (2018), https://doi.org/10.1016/j.actbio.2018.07.017

with over 15 million babies affected every year worldwide[3]. The PTB rate significantly contributes to infant mortality and lifelong health consequences in some survivors [4]. An understanding of causes and etiologies remains incomplete and few studies link biologic, chemical, and mechanical factors to overall tissue function. Thus, there is still much to be learned in order to implement successful therapies or improve the ones currently used [5].

Over the course of pregnancy, the uterine cervix undergoes important structural and mechanical changes [6]. This process, known as cervical remodeling, ensures the transformation of the cervix from a strong barrier, which protects and prevents the fetus from passing into the birth canal, to a compliant structure that can significantly dilate to allow for a successful delivery [7]. Understanding the functional mechanisms of cervical remodeling is then required for the development of early, accurate diagnostic methods for PTB and improved targeted therapies addressing patient-specific etiologies.

Taking advantage of the similarities between the human and rodent cervix [8,7,9], the ability to obtain cervical samples at all time points in pregnancy, and the relatively short gestational period in rodents (19–21 days), numerous mechanical testing methodologies have been implemented in mice and rats to characterize the highly hyperviscoelastic mechanical behavior of the cervix through pregnancy [10–13]. In mice, cervical softening, defined by a drop in tissue stiffness relative to non-pregnant (NP), is detectable by gestation day 12 and thereafter declines such that cervical stiffness is 20 times reduced at term before labor on gestation day 18 as compared to the NP cervix [14,12,15]. This softening is not homogeneous during pregnancy, since the rate of stiffness decline is most pronounced between day 6 and day 12, followed by a less drastic continuous softening until day 15, while a minor stiffness drop is observed in late pregnancy between day 15 and day 18 [16,17,12]. While the tangent stiffness of the mechanical response between day 15 and day 18 is not altered much, the swelling behavior of the tissue is postulated to change significantly given the important increase in hyaluronan (HA a non-sulfated glycosaminoglycan of the extracellular matrix) during this time [18]. Hence, the material property changes between day 15 and day 18 remain to be determined. Similar to cervical stiffness, the mechanical strength of pregnant mouse cervix undergoes a dramatic decrease by day 12 with a less pronounced decrease in late gestation [12,15]. As a load-bearing soft tissue, the mechanical properties of the cervix are linked to the composition and organization of its extracellular matrix (ECM) [19,17]. Significant remodeling of the cervical ECM over the course of pregnancy is achieved, in part by modification of the collagen fiber network [20]. Microscopic imaging of the collagen network during pregnancy reveal a dynamic remodeling from straight fibers for non-pregnant tissue to thicker, crimped fibers for pregnant tissues [21,22], in both human and mouse cervices. Cervical collagen also undergoes a turnover during pregnancy where mature cross-linked collagen is progressively replaced by immature less cross-linked collagen [17] while total collagen content remains the same [23,14].

While the evolution of cervical mechanical parameters through pregnancy is primarily equated with the progressive change in processing, assembly and turnover of collagen fibers [8], recent studies from our group suggest that elastic fiber remodeling may also contribute to mechanical behavior in cervical remodeling [15]. Elastic fiber ultrastructure is observed to change from long and straight fibers in the NP to progressively shorter and randomly oriented fibers on gestation day 18. Uni-axial tensile tests on cervices of mice with structural defects in elastic fibers exhibit overall degraded mechanical properties [15]. The importance of understanding the collective contributions of both collagen and elastic fibers to the mechanical behavior of the cervix is also supported by our recent studies demonstrating a change in collagen fiber morphology by second harmonic generation (SHG) and disruption of elastic fiber ultrastructure by transmission electron microscopy, in the subepithelial stroma of mice undergoing inflammation-induced premature cervical ripening [24,25](see figure in discussion).

Two established mouse models of premature cervical remodeling and PTB are infection-mediated and progesterone withdrawal-mediated, achieved by the administration of lipopolysaccharide (LPS) or mifepristone (RU486), respectively. Cervical ripening induced by LPS treatment is achieved by a transcriptional pathway that is distinct from term ripening [26,24], and noted structural changes in collagen and elastic fibers are hypothesized to result from the increased expression of ECM-degrading proteases and proinflammatory genes. In contrast, RU486-mediated ripening is most similar to term ripening [26]. Since mifepristone is a progesterone antagonist, mice treated with RU486 experience a withdrawal of progesterone similar to the pathway for term ripening on day 18.

Previously published uni-axial load-to-break mechanical tests suggest a decline in tissue stiffness in LPS and RU486-treated mice similar to term before labor [27]. Given the difficulty in conducting mechanical tests on such a small, curved tissue sample, these load-to-break tests provide the necessary starting point for mechanical characterization. However, these tests provide an incomplete picture of the individual contributions of collagen and elastic fiber remodeling to the hyperviscoelastic mechanical behavior of the non-pregnant and pregnant cervix. In addition, they do not accurately reflect the repetitive load-unload that the cervix experiences with acute loading from the fetus or with labor. Therefore, we developed a load-unload cyclic mechanical test for the mouse cervix to tease out specific contributions of the collagen or elastic fibers to the mechanical behavior [28]. Further, we investigate the evolving damage state of the ECM and its ability to recoil back to an undeformed state after loading [29,30].

Therefore, the aims of this study are: (1) to provide a detailed characterization of cervical mechanical properties at different time points during a normal pregnancy with a focus on parameters that quantify the ability of the cervix to recover from loading; (2) to characterize the mechanical differences between normal day 15, day 18 tissues and premature remodeling induced on day 15 by LPS or RU486, and link those mechanical parameters to microstructural considerations to better identify key factors in the appearance of PTB disorders. Here, we report the biomechanical load-unload signature of normal tissue remodeling in a murine pregnancy and show that infection-mediated premature cervical remodeling is functionally distinct from term and hormone withdrawal-mediated preterm remodeling.

2. Material and methods

2.1. Mouse cervix collection

All animal studies were approved by the University of Texas Southwestern Medical Center Institutional Animal Care and Research Advisory Committee. The breeding and tissue collection were performed at the University of Texas Southwestern Medical Center (Dallas, TX). C57B6/129sv wild-type (WT) mice were housed under a 12 h light/12 h dark cycle at 22 °C. Pregnancy was detected with the presence of a vaginal plug (considered day 0), with birth usually occurring on early morning of day 19. Non-pregnant (NP), gestation day 6 (d6), day 12 (d12), day 15 (d15) and day 18 (d18) reproductive tracts were collected (n = 5 per gestation group) and immediately frozen to be shipped overnight to Columbia University on dry ice. Tissues were then stored at −80 °C until test day.

PTB models were generated following the procedure described in Holt et al. [26]. Briefly, for the infection mediated treatment,

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