Published in final edited form as:

Semin Perinatol. 2009 October ; 33(5): 300–307. doi:10.1053/j.semperi.2009.06.002.

Relationships between Mechanical Properties and Extracellular Matrix Constituents of the Cervical Stroma during Pregnancy

Michael House, MD^a, David L. Kaplan, PhD^b, and Simona Socrate, PhD^c

^a Department of Obstetrics and Gynecology, Tufts Medical Center, Boston, MA

^b Department of Biomedical Engineering, Tufts University, Medford, MA

^c Harvard – MIT Division of Health Sciences & Technology, Cambridge, MA From Tufts Medical Center, Boston, MA

Abstract

In normal pregnancy, the cervix maintains its shape during a period of substantial fetal and uterine growth. Hence, maintenance of biomechanical integrity is an important aspect of cervical function. It is known that cervical mechanical properties arise from the extracellular matrix. The most important constituent of the cervical extracellular matrix is fibrillar collagen – it is from collagen protein that the cervix derives its “strength.” Other matrix molecules known to affect the collagen network include water, proteoglycans, hyaluronan and elastin. The objective of this review is to discuss relationships between biochemical constituents and macroscopic mechanical properties. The individual constituents of the extracellular matrix will be discussed, especially in regard to collagen remodeling during pregnancy. In addition, the macroscopic mechanical properties of cervical tissue will be reviewed. An improved understanding of the biochemistry of cervical “strength” will shed light into how the cervix maintains its shape in normal pregnancy and shortens in preterm birth.

INTRODUCTION

The structural integrity of the cervix is a key feature of normal pregnancy and abnormalities of cervical structure are associated with spontaneous preterm birth.^{1, 2} In normal pregnancy, the cervix maintains its anatomic shape despite substantial uterine growth. Spontaneous preterm birth is associated with undesired cervical changes, which manifest clinically as cervical shortening, effacement and dilation. Although the causes of spontaneous preterm birth are heterogeneous, in some patients, undesired cervical changes appear to be caused by impaired mechanical properties of the cervical tissue. The strongest evidence for a primary role for impaired cervical mechanical properties is that cervical cerclage appears to benefit a select group of patients,^{3–5} especially when infection or hemorrhage is absent.^{6, 7}

The mechanical properties of cervical tissue are derived from its extracellular matrix (ECM).⁸ The most important constituent of the cervical ECM is fibrillar collagen – it is from collagen protein that the ECM derives its mechanical strength. Other important constituents include proteoglycans, hyaluronan, elastin, and water. These constituents undergo a complex

Address reprint requests to Michael House, MD, Assistant Professor, Tufts Medical Center #360, 800 Washington Street, Boston, MA 02111. mhouse@tuftsmedicalcenter.org.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

remodeling process in preparation for labor. The mechanisms of remodeling in the cervical ECM have been the subject of much prior work (recently reviewed in Word, 2007⁹). Less is known about the mechanical properties of cervical tissue.

This review will emphasize the relationships between biochemical constituents and mechanical properties of human cervical tissue during pregnancy. The following will be discussed: 1) biochemical constituents of the cervical ECM and 2) mechanical properties of the cervical stroma. Due to the wide breadth of previous work in this area, emphasis will be placed on studies involving tissue from human sources supplemented by notable animal models.

Cervical Remodeling Prior to Labor: Softening vs. Ripening

When describing cervical remodeling prior to labor, investigators classify the process into two phases: cervical “softening” and cervical “ripening.”^{9–11} This terminology is somewhat misleading as these two phases could be more carefully described as “softening of the cervical tissue” and “ripening of the cervix.” For the purposes of this review, “cervical softening” refers to *cervical tissue* ECM remodeling that occurs slowly over the course of gestation and occurs prior effacement and dilation. It is a process that relates to changes in the biochemical composition and mechanical properties of the *material* that constitutes the cervix. Conversely, “cervical ripening” refers to changes in the *cervix* as a load bearing *structure*: it is clinically identified by an elevated Bishop score,¹² and associated with a loss of ability to remain closed.. Cervical softening is likely related to local matrix remodeling by cells native to cervical stroma (fibroblasts, myofibroblasts, epithelial cells).¹⁰ In contrast, ripening is known to be associated with migration of leukocytes into the cervical stroma* and release of inflammatory mediators.¹⁵ While cervical softening is simply defined in terms of changes in tissue properties and composition, cervical ripening is more complex because it is defined in terms of loss of structural function of the cervix and therefore involves not only tissue softening but also shape changes (i.e. effacement, dilation, shortening). As any “structural failure”, cervical ripening is due to the combination of external loading conditions (i.e. uterine contractions, uterine growth, intrauterine pressure, membrane adhesion), structure geometry (cervical anatomy, i.e. cervical length)¹⁶ and material (tissue) properties. It is important to emphasize that, due to the complexity of these interactions, relative contributions of tissue softening to cervical “weakness” and the explicit connection between “weakness” and adverse pregnancy outcome is not well understood.

BIOCHEMICAL CONSTITUENTS

Histologic studies indicate that 80 – 85% of the cervical stroma is fibrous connective tissue.¹⁷ Smooth muscle constitutes approximately 10% of the cervix, though the amount of smooth muscle appears to increase in cervical insufficiency.¹⁸ The strength of the stroma is due to fibrous ECM - its passive biomechanical strength is substantially higher than the contribution due to the contractile activity of cervical muscle.¹⁹ The primary constituents of the cervical ECM are water, collagen, proteoglycans, hyaluronan and elastin. These constituents will be discussed individually, especially in regard to tissue mechanical properties.

Water

The cervix is a hydrated soft tissue. Nonpregnant and first trimester cervical tissue is 75 – 80% water, a value that increases by approximately 5% in the third trimester.^{20–22} Tissue hydration affects cervical mechanical properties because interstitial fluid flow is thought to control the

* Although causal relationships are still a matter of some controversy, evidence of studies on a mouse model suggest that inflammatory cells do not contribute to the processes necessary for initiation of cervical ripening, and are primarily recruited, in response to dramatic tissue deformation, to orchestrate postpartum remodeling.^{13, 14}

short-term, nonequilibrium properties of soft tissue.⁸ Flow of interstitial fluid occurs according to pressure gradients within the tissue and contributes to the transient response to deformation. For example, when non-pregnant cervical tissue was tested in confined compression, the peak stress during the loading ramp was an order of magnitude higher than the equilibrium stress after a period of relaxation. In contrast, pregnant cervical tissue demonstrated peak stresses similar to equilibrium stresses, a response consistent with less organized morphology of pregnant tissue, which can be associated higher hydraulic permeability.⁸ More subtle effects of hydration levels are related to the influence of volumetric swelling on the mechanical response of the collagen network: in previous studies we found that altering the hydration level of cervical tissue samples substantially modified the stiffness of the material response.⁸ These findings are similar to effects observed in cartilage and other hydrated tissues.^{23, 24}

Collagen

The “strength” of cervical ECM is determined by the content and organization of its collagenous ECM. Collagen protein constitutes at least 54 – 77 % of the non-pregnant cervical dry weight (Table 1). Fibrillar collagens type 1 and type 3 are the primary cervical collagens²⁵ and a polymorphism associated collagen type 1 has been linked to familial cervical insufficiency.²⁶ The collagen molecule is composed of three polypeptide chains (α – chains) wound in a tight, triple helix. The α – chains exhibit a characteristic triplet sequence (Gly – X – Y)_n where glycine is required at every third residue to accommodate close packing of the three α – chains. Frequently, proline is found at the X position and 4-hydroxyproline is found at the Y position. Hydroxyproline is important for helix stability and is found in few proteins aside from collagen. Hence, measurement of hydroxyproline is an accepted method of assessing cervical collagen (Table 1). A collagen molecule is secreted from the cell in a precursor form (procollagen) where its terminal procollagen peptides undergo proteolytic processing by specific proteinases to form a mature collagen molecule. In the extracellular space, collagen molecules of the fibrillar type self assemble along the helical axis in a staggered array to form a cross-striated collagen fibril. Further stabilization of the fibrils occurs by intermolecular and intramolecular cross-links catalyzed by lysyl oxidase and collagen cross-links are essential for normal mechanical properties of the ECM.^{27, 28}

Cervical softening is associated with disorganization of the collagen network - a process characterized by increased collagen solubility and decreased collagen concentration (Table 1). Collagen solubility measures the organization and stability of collagen fibrils. Typically, a solvent system containing 0.5 M acetic acid and pepsin is employed, which degrades the cross-linked portion of the fibril allowing extraction of collagen molecules.²⁹ Quantification is performed by hydrolyzing the collagen molecule to constituent amino acids and measuring hydroxyproline concentration.³⁰ Newly synthesized collagen is characterized by fewer cross-links and increased solubility. Mature collagen is characterized by more stable cross-links and decreased solubility. In pregnancy, collagen solubility increases as early as 10 weeks gestational age.²⁰ In the third trimester, 80 – 90% of cervical collagen is soluble, and collagen concentration falls by nearly 50% compared to non-pregnant values. In parallel studies of biomechanical and biochemical properties, increased collagen solubility correlated most strongly with cervical softening in both human^{8, 22} and mouse¹⁰ cervical tissue. In addition, increased collagen solubility has been correlated to shorter labors,³¹ and cervical insufficiency.^{21, 32}

Morphological studies using multiple imaging modalities corroborate biochemical evidence of decreased collagen organization during cervical softening. Collagen fibrils are not randomly oriented within the cervix. Using non-pregnant cervical tissue, X-ray diffraction studies demonstrated evidence of preferred fibril orientation according to anatomic location (anisotropy).³³ Fibril anisotropy of non-pregnant tissue was also seen with diffusion tensor

imaging, which showed an outer circumferential layer and an inner layer aligned in a longitudinal orientation.³⁴ Qualitative comparisons of human cervical tissue from pregnant and non-pregnant sources using both second harmonic imaging and polarized light microscopy also demonstrated preferential alignment of the collagen network along the circumferential direction. In addition, pregnant tissue showed decreased collagen organization, especially when viewed at a length scale of 10 μm .²² Immunofluorescent staining against type 1 collagen demonstrated a dense, fibrillar signal in the first trimester compared to diffuse signal in the third trimester.³⁵ This study also reported relative decreased type 1 collagen gene expression in the third trimester, though others have reported stable collagen gene expression during pregnancy.³⁶ In a rat model of cervical ripening, decreased tensile strength was correlated with decreased proportion of organized collagen, as seen with transmission electron microscopy.³⁷

Taken together, it appears that decreased collagen organization is primarily responsible for the biochemical, morphological and physical changes observed during cervical softening. Recent studies highlight the importance of collagen remodeling in the postpartum cervix as well.³⁸ Collagen organization is affected not only by collagen self assembly in the ECM but also by other matrix molecules. In the following sections, molecules thought to be important for regulating collagen assembly during pregnancy are discussed.

Proteoglycans

Proteoglycans are macromolecules containing a core protein with one or more covalently linked glycosaminoglycan (GAG) chains. Proteoglycans can be classified into families according location (i.e. cell surface vs. ECM) and core protein properties. The proteoglycans most extensively studied in the cervix (i.e. decorin) are located in the ECM and belong to the family of small-leucine-rich-proteoglycans (SLRP). This family is characterized by a relatively small core protein (32 – 39 kDa) and a centrally located leucine-rich domain. Prior to knowledge of core protein amino acid sequence, proteoglycans were classified according to the GAG chain. GAGs are linear polymers of repeating disaccharide units, which may be sulfated in different positions. There are essentially three types of sulfated GAG chains: 1) chondroitin/dermatan sulfate, 2) heparin and heparan sulfate and 3) keratan sulfate. A single GAG chain of the chondroitin/dermatan sulfate type is attached to the decorin core protein. Hyaluronan is a free GAG chain with no sulfate groups and no core protein (see below).³⁹

Among the myriad biological functions ascribed to proteoglycans, several are relevant to cervical structural function. First, glycosaminoglycans possess a fixed negative charge, which renders them hydrophilic. Hydrophilic GAG chains attract water into the tissue causing an osmotic swelling pressure, which contributes to tissue physical properties. For example, the ability of cartilage to withstand compressive loading is due to the hydrophilic GAG side chains of aggrecan and hyaluronan. As cartilage is compressed uniaxially, the osmotic swelling pressure due to GAGs limits the decrease in tissue volume, recruiting the collagen resistance to transverse stretching. Mechanical properties of cervical tissue are asymmetric in tension and compression (see below), and are controlled by complex interactions between the volumetric compressive resistance of the hydrophilic proteoglycans and the tensile properties of the collagen constituents. Second, proteoglycans present in the cervix are known to influence ECM assembly, which may affect mechanical properties. Decorin is the dominant proteoglycan⁴⁰ and the core protein of decorin has been shown to bind to collagen fibrils and regulate fibril formation.⁴¹ Decorin knockout mice demonstrate skin fragility due to abnormal collagen morphology.⁴² A shift in proteoglycan composition during pregnancy is an attractive hypothesis for explaining altered cervical mechanical properties associated with softening and/or ripening.⁴³

Pregnancy is associated with changes in cervical proteoglycan composition and metabolism. Decorin constitutes 90% of cervical proteoglycan content. Biglycan, versican and heparan

sulfate proteoglycans together constitute less than 10% of cervical proteoglycan.⁴⁴ Term pregnancy is associated with a 40 – 50 % decrease in decorin concentration, which is postulated to decrease stability of the collagen network. It is interesting to note that the rate of proteoglycan synthesis increases 2 fold in term cervical tissue compared to non-pregnant tissue, in spite of falling concentrations.⁴⁵ This data has been interpreted to indicate that term pregnancy is associated with increased matrix degradation and turnover. Biglycan also interacts with collagen fibrils⁴⁶ and may regulate matrix assembly. Versican is in the same family as aggrecan and may play a role in regulating tissue hydration.

Hyaluronan

Hyaluronan (HA) is a large, negatively charged polysaccharide that exists as a free GAG in the ECM. Local tissue HA concentration is controlled by the balance of HA synthesis (HA synthase enzymes), HA degradation (hyaluronidases) and HA binding (CD44, proteoglycans).⁴⁷ Early labor is associated with a significant increase in HA concentration in the cervical stroma⁴⁸ and mucus⁴⁹, which has sparked interest in the link between HA and cervical ripening. HA has been implicated in two important features of cervical ripening: 1) increased tissue hydration and 2) increased cervical inflammation. A key role for HA in regulating cervical hydration has been postulated because large aggregates of HA and aggrecan are known to play a central role in maintaining cartilage hydration and mechanical properties. The cervix is 80 – 85% water in the third trimester, an increase of 5% from the non-pregnant cervix. Increased cervical hydration is thought to contribute to collagen disorganization, which would decrease barriers to molecular reorganization and decrease stiffness. In human studies, term pregnancy was associated with increased distribution of HA in cervical connective tissue⁵⁰ and term labor was associated with increased expression of hyaluronan synthase 2 (HAS2).⁵¹ In a rabbit model, HA administration using vaginal suppositories was associated with increased cervical water content and morphological evidence of decreased collagen organization,⁵⁰ an effect augmented by dehydroepiandrosterone 3-sulphate.⁵² In the mouse, HAS2 was shown to control HA concentration at parturition and HAS2 was regulated by progesterone.⁵¹

Cervical HA increases production of inflammatory cytokines, which may signal migration of inflammatory cells into the cervical stroma, an important feature of cervical ripening.¹⁵ In primary uterine cell cultures, production of IL-8,⁵⁰ IL-1 β and TNF- α ⁵³ were increased by stimulation with HA, an effect mediated by the cell surface HA binding protein CD44. These studies were extended by showing that cyclic mechanical stretch of human cervical fibroblasts increased HA production, thereby demonstrating that inflammatory changes associated with cervical stretch during labor may be mediated in part by HA.⁵⁴ In addition, inflammatory cytokines increased production of HA⁵⁵ and HA binding proteins^{56, 57} in primary culture of cervical fibroblasts, demonstrating significant cross talk between local inflammatory cytokines and HA concentration.

Finally, the importance of HA has been highlighted by several clinical studies. Among women presenting for first trimester termination of pregnancy, administration of prostaglandin E2 was associated with a 50% rise in HA concentration in cervical biopsy specimens.⁵⁸ A recent randomized, double blinded, placebo controlled trial demonstrated that intracervical injection of hyaluronidase, a HA degrading enzyme, was associated with improved Bishop score, decreased duration of labor and increased likelihood of vaginal delivery.⁵⁹ Taken together, evidence for an important role for HA in cervical ripening is strong; its role in cervical softening is unknown.

Thrombospondin 2

Thrombospondin 2 is a glycoprotein located in the extracellular matrix that may be important in regulating collagen fibril morphology and structural function.⁶⁰ In a knockout mouse model,

the cervix of thrombospondin 2 – deficient mice was abnormally soft in the second half of pregnancy. In the wild-type mouse, thrombospondin 2 expression was initiated in the second half of pregnancy, which correlated with improved cervical mechanical properties compared to the knockout mouse.⁶¹ Hence, thrombospondin 2 expression may be necessary to maintain collagen integrity, though its role in human pregnancy has not been investigated.

Elastin

The elastic fiber is composed of an amorphous core of elastin protein surrounded by a sheet of microfibrils.⁶² Elastic fibers confer elasticity to the ECM, allowing both stretch and elastic recoil. Cervical elastic fibers are present in the walls of blood vessels and parallel to collagen fibers in the stroma.⁶³ The elastin concentration is 0.9 – 2.9 % of cervical dry weight,⁶⁴ a percentage that is suggested to decrease in cervical insufficiency.⁶⁵ In addition, cervical insufficiency was associated with decreased visualization of elastic fibers by histologic staining and light microscopy.⁶⁵ Regarding the structural role of elastic fibers, there are several reasons to think it is unlikely that elastic fibers contribute significantly to the initial strength of the collagenous matrix. First, single elastic fibers display Young's moduli at least several orders of magnitude lower than collagen fibrils (0.3 – 1.5 MPa for elastic fibers⁶⁶ vs. 400 – 500 MPa for hydrated collagen fibrils⁶⁷). Second, the volume fraction of collagen protein in the cervical ECM is over 20 times higher than elastin protein. Rather, it is more likely that elastic fibers play a complementary role to collagen. Whereas the collagen network determines the initial stiffness and resistance to deformation of the cervix, elastic fibers are likely important for restoring cervical shape following marked deformation associated with vaginal delivery.

MECHANICAL PROPERTIES OF HUMAN CERVICAL STROMA

The macroscopic mechanical properties of cervical stroma arise from its complex hierarchical structure.⁶⁸ Structural hierarchy refers to the way in which the organization of collagen molecules (nanometer scale) influences properties of collagen fibrils (micrometer scale), which determines macroscopic mechanical properties (centimeter scale). Opportunities for tissue remodeling occur at each length scale (or hierarchy) to meet mechanical needs at a particular moment in time. For example, the biochemical variable most consistently associated with cervical softening is increased collagen solubility - marked differences in collagen concentration, proteoglycan constituents or hyaluronan have not been detected during cervical softening. Hence, it is likely that softening is due to subtle changes in collagen fibril structure, which leads to increase susceptibility to pepsin digestion and markedly modified mechanical properties.

Compared to studies of cervical biochemical properties, studies of the mechanical properties are relatively scarce. Although investigators acknowledge that mechanical properties of the cervix are derived from its ECM, there are few studies that measure both mechanical and biochemical properties on human tissue. When reviewing prior literature, it is difficult to make direct comparisons between studies because investigators use different testing configurations and loading protocols. In addition, samples of human cervix of sufficient size to permit mechanical characterization are difficult to obtain. Last, investigators have noted marked mechanical heterogeneity, even among samples from the same patient. With these limitations in mind, the essential features of the mechanical behavior of cervical tissue are summarized below (Table 2).

Nonlinear, time-dependent stress response that is asymmetric in tension and compression

The stress – strain behavior of cervical stroma is nonlinear both in tension^{8, 19, 69} and compression,⁸ with a stiffer response and reduced extensibility in tension, and a more compliant response in compression. It is not surprising that cervical tissue displays nonlinear behavior

given that the collagen fibril is known to display a nonlinear stress response with differences in tension and compression.⁷⁰ Collagen fibrils display “rope-like” mechanical behavior. When pulled along the axis of its triple helix, the fibril is remarkably stiff. Yet, when compressed, the fibril buckles under small load. Extrapolating from mechanics of fibrils to 3D tissue behavior is complex.⁷¹ However, by drawing an analogy to the mechanics of cartilage, it can be inferred that the mechanical response of cervical tissue is controlled by the complex interplay between the tensile response of the collagen network and the compressive response of the hydrated GAGs.⁷²

Nonpregnant vs. pregnant tissue

In vitro mechanical testing of cervical stroma uniformly demonstrates more compliant tissue response during pregnancy. Whether the tissue is tested after vaginal delivery^{21, 73} or in the third trimester prior to delivery,⁸ tissue associated with pregnancy is at least an order of magnitude more compliant than non-pregnant tissue. Less clear is the relative contributions of baseline tissue strength, cervical softening and cervical ripening to mechanical properties at a particular moment in time.

Collagen cross-linking is correlated to obstetric outcome and obstetric history

Although it is well known that collagen fibrils require chemical cross-links for mechanical stability, there have been no direct measurements of cervical collagen cross-links to date. Rather, investigators using two different methodologies have focused on indirect assessment of collagen cross-links. First, the Collascope measures intrinsic cervical fluorescence – the most important intrinsic fluorophor is suspected to be pyridinoline, a cross-link present in mature collagenous tissue.⁷⁴ The Collascope detected decreased cervical fluorescence, which presumably correlates with decreased collagen cross-links, over the course of gestation⁷⁵ and after prostaglandin application.⁷⁶ Second, in a mechanical modeling study, material parameters associated with increased collagen cross-linking were increased in non-pregnant compared to pregnant tissue.⁷² More detailed studies of collagen cross-links in human cervical tissue would appear to be a promising avenue of investigation in the future.

Clinical studies of cervical mechanics

Studies of cervical mechanical properties during pregnancy confirm that tissue associated with pregnancy is 5 – 10 times more compliant than non-pregnant tissue. Two instruments have been developed to assess cervical mechanical properties in vivo: the cervicotonometer⁷⁷ and an aspiration device.⁷⁸ The cervicotonometer used a calibrated tip placed in the cervical canal to open the cervix. Both the force required to open (“distend”) the cervix and the distance of travel were used to define a “cervical distensibility index” (CDI). Studies using the cervicotonometer demonstrated the CDI at term was 5.9 times larger than the non-pregnant value. In addition, the CDI increased gradually as gestational age increased and preterm delivery was associated with an elevated CDI. The aspiration device applied a time-varying vacuum to the portio vaginalis part of the cervix such that a small amount of tissue was aspirated into the device. A mirror was used to capture the side-view profile of the tissue as a function of applied vacuum. By measuring the amount of tissue aspirated into the device, a stiffness parameter was defined, which is a quantitative measurement of cervical softness. The aspiration device demonstrated that measurements performed in vitro and in vivo are similar.⁷⁹ In addition, the mechanical response of pregnant cervixes was considerably softer than non-pregnant cervixes and pregnancy was associated with gradual decreasing stiffness over a time interval of four weeks.⁷⁸

Complementing studies involving pregnant women, investigators have shown that non-pregnant women with a history of spontaneous preterm birth demonstrate cervical abnormalities. To quantify the mechanical properties of the cervical stroma, investigators used

either an intracervical balloon^{69, 80, 81} or calibrated dilator.⁸² The diameter of the cervical canal was assessed either radiographically⁸⁰ or with dilators.⁸¹ Although there is overlap between cases and controls, it appears that a degree of cervical insufficiency is detectable even when the patient is not pregnant.

Connection Between Impaired Cervical Mechanical Properties and Adverse Outcome—Establishing a link between impaired cervical mechanical properties and adverse pregnancy outcome is difficult to prove. Progress has been hampered by the lack of a widely accepted method to assess ECM integrity during pregnancy. Patients presenting with what clinically appears to be a “weak” cervix (i.e. short) may have subclinical chorioamnionitis or decidual hemorrhage.^{7, 83} Conversely, impaired cervical mechanical properties may lead to cervical shortening and lower the barrier to infection. A noninvasive technique to assess cervical mechanical properties during pregnancy would enable studies to test the hypothesis that a “weak” cervix leads to subsequent cervical shortening and spontaneous preterm birth. In recent years, the clinical importance of a short cervix has motivated investigators to develop new, noninvasive techniques to assess cervical mechanical properties. Techniques using fluorescence,⁷⁴ tonometry⁷⁸ and ultrasound⁸⁴ have been developed to investigate different aspects of the cervical ECM and are in preliminary stages of clinical testing.

CONCLUSION

Although significant progress has been made in identifying and treating women at high risk for preterm birth, there is still much left to do. We advocate for a more fundamental understanding of the relationships between cervical biochemical properties and mechanical properties (structure – function relationships). Investigation of structure – function relationships, together with improved understanding of cervical loading during pregnancy, will reveal insight into how the cervix maintains its shape in normal pregnancy and shortens in preterm birth. Coming to terms with the biochemistry of cervical “strength” is a necessary first step for the long term goal of developing rational therapies that aim to prevent undesired cervical changes during pregnancy.

Acknowledgments

Supported in part by The Reproductive Scientist Development Program (2K12HD000849-21) and The March of Dimes Birth Defects Foundation

References

1. Iams JD, Goldenberg RL, Meis PJ, et al. The length of the cervix and the risk of spontaneous premature delivery. *NEJM* 1996;334:567–572. [PubMed: 8569824]
2. Owen J, Yost N, Berghella V, et al. Mid-trimester endovaginal sonography in women at high risk for spontaneous preterm birth. *JAMA* 2001;286:1340–8. [PubMed: 11560539]
3. Daskalakis G, Papantoniou N, Mesogitis S, Antsaklis A. Management of cervical insufficiency and bulging fetal membranes. *Obstet Gynecol* 2006;107:221–6. [PubMed: 16449104]
4. Owen J. Multicenter randomized trial of cerclage for preterm birth prevention in high-risk women with shortened mid-trimester cervical length. *Am J Obstet Gynecol* 2008;199:S3.
5. Berghella V, Odibo AO, To MS, Rust OA, Althuisius SM. Cerclage for short cervix on ultrasonography: meta-analysis of trials using individual patient-level data. *Obstet Gynecol* 2005;106:181–9. [PubMed: 15994635]
6. Lee KY, Jun HA, Kim HB, Kang SW. Interleukin-6, but not relaxin, predicts outcome of rescue cerclage in women with cervical incompetence. *Am J Obstet Gynecol* 2004;191:784–9. [PubMed: 15467541]
7. Weiner CP, Lee KY, Buhimschi CS, Christner R, Buhimschi IA. Proteomic biomarkers that predict the clinical success of rescue cerclage. *Am J Obstet Gynecol* 2005;192:710–8. [PubMed: 15746662]

8. Myers KM, Paskaleva AP, House M, Socrate S. Mechanical and biochemical properties of human cervical tissue. *Acta Biomater* 2008;4:104–16. [PubMed: 17904431]
9. Word RA, Li XH, Hnat M, Carrick K. Dynamics of cervical remodeling during pregnancy and parturition: mechanisms and current concepts. *Semin Reprod Med* 2007;25:69–79. [PubMed: 17205425]
10. Read CP, Word RA, Ruscheinsky MA, Timmons BC, Mahendroo MS. Cervical remodeling during pregnancy and parturition: molecular characterization of the softening phase in mice. *Reproduction* 2007;134:327–40. [PubMed: 17660242]
11. Sennstrom MB, Ekman G, Westergren-Thorsson G, et al. Human cervical ripening, an inflammatory process mediated by cytokines. *Mol Hum Reprod* 2000;6:375–81. [PubMed: 10729321]
12. Bishop EH. Pelvic Scoring for Elective Induction. *Obstet Gynecol* 1964;244:266–8. [PubMed: 14199536]
13. Timmons BC, Mahendroo MS. Timing of neutrophil activation and expression of proinflammatory markers do not support a role for neutrophils in cervical ripening in the mouse. *Biol Reprod* 2006;74:236–45. [PubMed: 16237151]
14. Timmons BC, Fairhurst AM, Mahendroo MS. Temporal changes in myeloid cells in the cervix during pregnancy and parturition. *J Immunol* 2009;182:2700–7. [PubMed: 19234164]
15. Junqueira LC, Zugaib M, Montes GS, Toledo OM, Krisztan RM, Shigihara KM. Morphologic and histochemical evidence for the occurrence of collagenolysis and for the role of neutrophilic polymorphonuclear leukocytes during cervical dilation. *Am J Obstet Gynecol* 1980;138:273–81. [PubMed: 7416217]
16. Paskaleva, A.; Socrate, S.; House, M. Biomechanics of Cervical Funneling: Case of Cervical Incompetence. Proceedings of the Summer Bioengineering Conference. 2005 ASME Summer Bioengineering Conference; Vail CO.
17. Danforth D. The distribution and functional activity of the cervical musculature. *Am J Obstet Gynecol* 1954;68:1261–71. [PubMed: 13207218]
18. Buckingham JC, Buethe RA Jr, Danforth DN. Collagen-Muscle Ratio in Clinically Normal and Clinically Incompetent Cervices. *Am J Obstet Gynecol* 1965;91:232–7. [PubMed: 14258025]
19. Petersen LK, Oxlund H, Uldbjerg N, Forman A. In vitro analysis of muscular contractile ability and passive biomechanical properties of uterine cervical samples from nonpregnant women. *Obstet Gynecol* 1991;77:772–6. [PubMed: 2014094]
20. Uldbjerg N, Ekman G, Malmstrom A, Olsson K, Ulmsten U. Ripening of the human uterine cervix related to changes in collagen, glycosaminoglycans, and collagenolytic activity. *Am J Obstet Gynecol* 1983;147:662–666. [PubMed: 6638110]
21. Rechberger T, Uldbjerg N, Oxlund H. Connective tissue changes in the cervix during normal pregnancy and pregnancy complicated by cervical incompetence. *Obstetrics & Gynecology* 1988;71:563–7. [PubMed: 3353047]
22. Myers K, Socrate S, Tzeranis D, House M. Changes in the biochemical constituents and morphologic appearance of the human cervical stroma during pregnancy. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 2009;144:S82–S89. [PubMed: 19303693]
23. Chahine NO, Wang CC, Hung CT, Ateshian GA. Anisotropic strain-dependent material properties of bovine articular cartilage in the transitional range from tension to compression. *J Biomech* 2004;37:1251–61. [PubMed: 15212931]
24. Bischoff JE, Arruda EM, Grosh K. Finite element modeling of human skin using an isotropic, nonlinear elastic constitutive model. *J Biomech* 2000;33:645–52. [PubMed: 10807984]
25. Minamoto T, Arai K, Hirakawa S, Nagai Y. Immunohistochemical studies on collagen types in the uterine cervix in pregnant and nonpregnant states. *Am J Obstet Gynecol* 1987;156:138–44. [PubMed: 3541616]
26. Warren JE, Silver RM, Dalton J, Nelson LT, Branch DW, Porter TF. Collagen 1Alpha1 and transforming growth factor-beta polymorphisms in women with cervical insufficiency. *Obstet Gynecol* 2007;110:619–24. [PubMed: 17766609]
27. Linsenmayer, TF. Collagen. In: Hay, E., editor. *Cell Biology of the Extracellular Matrix*. Vol. 2. New York, NY: Plenum Press; 1991. p. 7-44.

28. Kadler KE, Holmes DF, Trotter JA, Chapman JA. Collagen fibril formation. *Biochem J* 1996;316 (Pt 1):1–11. [PubMed: 8645190]
29. Miller EJ, Rhodes RK. Preparation and characterization of the different types of collagen. *Methods Enzymol* 1982;82(Pt A):33–64. [PubMed: 7078441]
30. Woessner, JF. Determination of hydroxyproline in connective tissues. In: Hall, DA., editor. *Methodology of Connective Tissue Research*. Oxford: Joynson-Bruvvers Ltd; 1976. p. 227–33.
31. Ekman G, Malmstrom A, Uldbjerg N, Ulmsten U. Cervical collagen: an important regulator of cervical function in term labor. *Obstetrics & Gynecology* 1986;67:633–6. [PubMed: 3457329]
32. Petersen LK, Uldbjerg N. Cervical collagen in non-pregnant women with previous cervical incompetence. *Eur J Obstet Gynecol Reprod Biol* 1996;67:41–45. [PubMed: 8789748]
33. Aspden RM. Collagen organisation in the cervix and its relation to mechanical function. *Coll Relat Res* 1988;8:103–12. [PubMed: 3378391]
34. Weiss S, Jaermann T, Schmid P, et al. Three-dimensional fiber architecture of the nonpregnant human uterus determined ex vivo using magnetic resonance diffusion tensor imaging. *Anat Rec A Discov Mol Cell Evol Biol* 2006;288:84–90. [PubMed: 16345078]
35. Iwahashi M, Muragaki Y, Ooshima A, Umesaki N. Decreased type I collagen expression in human uterine cervix during pregnancy. *J Clin Endocrinol Metab* 2003;88:2231–5. [PubMed: 12727979]
36. Malmstrom E, Sennstrom M, Holmberg A, et al. The importance of fibroblasts in remodelling of the human uterine cervix during pregnancy and parturition. *Mol Hum Reprod*. 2007
37. Feltovich H, Ji H, Janowski JW, Delance NC, Moran CC, Chien EK. Effects of selective and nonselective PGE2 receptor agonists on cervical tensile strength and collagen organization and microstructure in the pregnant rat at term. *Am J Obstet Gynecol* 2005;192:753–60. [PubMed: 15746668]
38. Buhimschi CS, Sora N, Zhao G, Buhimschi IA. Genetic background affects the biomechanical behavior of the postpartum mouse cervix. *Am J Obstet Gynecol* 2009;200:434:e1–7.
39. Haralson, MA.; Hassell, JR. The Extracellular Matrix - An Overview. In: Haralson, MA.; Hassell, JR., editors. *Extracellular Matrix*. New York, NY: Oxford University Press Inc; 1995. p. 1–24.
40. Uldbjerg N, Malmstrom A, Ekman G, Sheehan J, Ulmsten U, Wingerup L. Isolation and characterization of dermatan sulphate proteoglycan from human uterine cervix. *Biochemical Journal* 1983;209:497–503. [PubMed: 6847631]
41. Scott JE. Proteoglycan-fibrillar collagen interactions. *Biochem J* 1988;252:313–23. [PubMed: 3046606]
42. Danielson KG, Baribault H, Holmes DF, Graham H, Kadler KE, Iozzo RV. Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. *J Cell Biol* 1997;136:729–43. [PubMed: 9024701]
43. Uldbjerg N, Malmstrom A. The role of proteoglycans in cervical dilatation. *Seminars in Perinatology* 1991;15:127–32. [PubMed: 1876867]
44. Westergren-Thorsson G, Norman M, Bjornsson S, et al. Differential expressions of mRNA for proteoglycans, collagens and transforming growth factor-beta in the human cervix during pregnancy and involution. *Biochim Biophys Acta* 1998;1406:203–13. [PubMed: 9573366]
45. Norman M, Ekman G, Ulmsten U, Barchan K, Malmstrom A. Proteoglycan metabolism in the connective tissue of pregnant and non-pregnant human cervix. An in vitro study. *Biochem J* 1991;275 (Pt 2):515–20. [PubMed: 2025230]
46. Schonherr E, Witsch-Prehm P, Harrach B, Robenek H, Rauterberg J, Kresse H. Interaction of biglycan with type I collagen. *J Biol Chem* 1995;270:2776–83. [PubMed: 7852349]
47. Ruschinsky M, De la Motte C, Mahendroo M. Hyaluronan and its binding proteins during cervical ripening and parturition: dynamic changes in size, distribution and temporal sequence. *Matrix Biol* 2008;27:487–97. [PubMed: 18353623]
48. Osmers R, Rath W, Pflanz MA, Kuhn W, Stuhlsatz HW, Szevenyi M. Glycosaminoglycans in cervical connective tissue during pregnancy and parturition. *Obstetrics & Gynecology* 1993;81:88–92. [PubMed: 8416467]
49. Obara M, Hirano H, Ogawa M, et al. Changes in molecular weight of hyaluronan and hyaluronidase activity in uterine cervical mucus in cervical ripening. *Acta Obstet Gynecol Scand* 2001;80:492–6. [PubMed: 11380283]

50. El Maradny E, Kanayama N, Kobayashi H, et al. The role of hyaluronic acid as a mediator and regulator of cervical ripening. *Hum Reprod* 1997;12:1080–8. [PubMed: 9194670]
51. Straach KJ, Shelton JM, Richardson JA, Hascall VC, Mahendroo MS. Regulation of hyaluronan expression during cervical ripening. *Glycobiology* 2005;15:55–65. [PubMed: 15317739]
52. Belayet HM, Kanayama N, Khatun S, et al. Dehydroepiandrosterone sulphate promotes hyaluronic acid-induced cervical ripening in rabbits. *Hum Reprod* 1999;14:1361–7. [PubMed: 10325294]
53. Kobayashi H, Terao T. Hyaluronic acid-specific regulation of cytokines by human uterine fibroblasts. *Am J Physiol* 1997;273:C1151–9. [PubMed: 9357758]
54. Takemura M, Itoh H, Sagawa N, et al. Cyclic mechanical stretch augments hyaluronan production in cultured human uterine cervical fibroblast cells. *Mol Hum Reprod* 2005;11:659–65. [PubMed: 16199413]
55. Ogawa M, Hirano H, Tsubaki H, Kodama H, Tanaka T. The role of cytokines in cervical ripening: correlations between the concentrations of cytokines and hyaluronic acid in cervical mucus and the induction of hyaluronic acid production by inflammatory cytokines by human cervical fibroblasts. *Am J Obstet Gynecol* 1998;179:105–10. [PubMed: 9704773]
56. Tsubaki H, Ogawa M, Hosoya N, et al. Expression of CD44 mRNA induced by interleukin-1beta in human cultured uterine cervical fibroblasts. *Eur J Obstet Gynecol Reprod Biol* 2005;122:156–61. [PubMed: 16219515]
57. Shimizu D, Hosoya N, Ogawa M, et al. Expression of tumor necrosis factor-alpha stimulated gene-6 mRNA in cultured human uterine cervical fibroblasts. *Acta Obstet Gynecol Scand* 2005;84:780–7. [PubMed: 16026405]
58. Rath W, Osmers R, Adelman-Grill BC, Stuhlsatz HW, Szevereny M, Kuhn W. Biochemical changes in human cervical connective tissue after intracervical application of prostaglandin E2. *Prostaglandins* 1993;45:375–84. [PubMed: 8493359]
59. Spallicci MD, Chiea MA, Singer JM, Albuquerque PB, Bittar RE, Zugaib M. Use of hyaluronidase for cervical ripening: a randomized trial. *Eur J Obstet Gynecol Reprod Biol* 2007;130:46–50. [PubMed: 16324780]
60. Kyriakides TR, Zhu YH, Smith LT, et al. Mice that lack thrombospondin 2 display connective tissue abnormalities that are associated with disordered collagen fibrillogenesis, an increased vascular density, and a bleeding diathesis. *J Cell Biol* 1998;140:419–30. [PubMed: 9442117]
61. Kokenyesi R, Armstrong LC, Agah A, Artal R, Bornstein P. Thrombospondin 2 deficiency in pregnant mice results in premature softening of the uterine cervix. *Biol Reprod* 2004;70:385–90. [PubMed: 14561659]
62. Rosenbloom J, Abrams WR, Mecham R. Extracellular matrix 4: the elastic fiber. *Faseb J* 1993;7:1208–18. [PubMed: 8405806]
63. Leppert PC, Cerreta JM, Mandl I. Orientation of elastic fibers in the human cervix. *American Journal of Obstetrics & Gynecology* 1986;155:219–24. [PubMed: 3728591]
64. Leppert PC, Keller S, Cerreta J, Hosannah Y, Mandl I. The content of elastin in the uterine cervix. *Archives of Biochemistry & Biophysics* 1983;222:53–8. [PubMed: 6838229]
65. Leppert PC, Yu SY, Keller S, Cerreta J, Mandl I. Decreased elastic fibers and desmosine content in incompetent cervix. *American Journal of Obstetrics & Gynecology* 1987;157:1134–9. [PubMed: 3688067]
66. Koenders MM, Yang L, Wismans RG, et al. Microscale mechanical properties of single elastic fibers: the role of fibrillin-microfibrils. *Biomaterials* 2009;30:2425–32. [PubMed: 19217657]
67. Eppell SJ, Smith BN, Kahn H, Ballarini R. Nano measurements with micro-devices: mechanical properties of hydrated collagen fibrils. *J R Soc Interface* 2006;3:117–21. [PubMed: 16849223]
68. Fratzl, P. Collagen: Structure and Mechanics, an Introduction. In: Fratzl, P., editor. *Collagen Structure and Mechanics*. New York, NY: Springer; 2008. p. 1–13.
69. van Duyl WA, van der Zon AT, Drogendijk AC. Stress relaxation of the human cervix: a new tool for diagnosis of cervical incompetence. *Clinical Physics & Physiological Measurement* 1984;5:207–18. [PubMed: 6488727]
70. Buehler, MJ. Hierarchical Nanomechanics of Collagen Fibrils: Atomistic and Molecular Modeling. In: Fratzl, P., editor. *Collagen Structure and Mechanics*. New York, NY: Springer; 2008. p. 175–247.

71. Paskaleva, AP. Ph.D. Thesis. Massachusetts Institute of Technology; 2007. Biomechanics of Cervical Function in Pregnancy - Case of Cervical Insufficiency.
72. Myers KM, Socrate S, Paskaleva AP, House M. A Study of the Anisotropy and Tension/Compression Behavior of Human Cervical Tissue. *Journal of Biomechanics* (accepted for publication).
73. Conrad JT, Ueland K. Reduction of the stretch modulus of human cervical tissue by prostaglandin E2. *Am J Obstet Gynecol* 1976;126:218–23. [PubMed: 961760]
74. Schlembach D, Mackay L, Shi L, Maner WL, Garfield RE, Maul H. Cervical ripening and insufficiency: from biochemical and molecular studies to in vivo clinical examination. *Eur J Obstet Gynecol Reprod Biol* 2009;144 (Suppl 1):S70–6. [PubMed: 19303692]
75. Maul H, Saade G, Garfield RE. Prediction of term and preterm parturition and treatment monitoring by measurement of cervical cross-linked collagen using light-induced fluorescence. *Acta Obstet Gynecol Scand* 2005;84:534–6. [PubMed: 15901259]
76. Fittkow CT, Maul H, Olson G, et al. Light-induced fluorescence of the human cervix decreases after prostaglandin application for induction of labor at term. *Eur J Obstet Gynecol Reprod Biol* 2005;123:62–6. [PubMed: 15916844]
77. Cabrol D. Cervical distensibility changes in pregnancy, term and preterm labor. *Semin Perinatol* 1991;15:133–9. [PubMed: 1876868]
78. Bauer M, Mazza E, Jabareen M, et al. Assessment of the in vivo biomechanical properties of the human uterine cervix in pregnancy using the aspiration test: a feasibility study. *Eur J Obstet Gynecol Reprod Biol* 2009;144 (Suppl 1):S77–81. [PubMed: 19285777]
79. Mazza E, Nava A, Bauer M, Winter R, Bajka M, Holzapfel GA. Mechanical properties of the human uterine cervix: an in vivo study. *Med Image Anal* 2006;10:125–36. [PubMed: 16143559]
80. Zlatnik FJ, Burmeister LF. Interval evaluation of the cervix for predicting pregnancy outcome and diagnosing cervical incompetence. *Journal of Reproductive Medicine* 1993;38:365–9. [PubMed: 8320673]
81. Kiwi R, Neuman MR, Merkatz IR, Selim MA, Lysikiewicz A. Determination of the elastic properties of the cervix. *Obstetrics & Gynecology* 1988;71:568–74. [PubMed: 3353048]
82. Anthony GS, Walker RG, Robins JB, Cameron AD, Calder AA. Management of cervical weakness based on the measurement of cervical resistance index. *Eur J Obstet Gynecol Reprod Biol* 2007;134:174–8. [PubMed: 17123693]
83. Gomez R, Romero R, Nien JK, et al. A short cervix in women with preterm labor and intact membranes: a risk factor for microbial invasion of the amniotic cavity. *Am J Obstet Gynecol* 2005;192:678–89. [PubMed: 15746658]
84. McFarlin B, O'Brien WD, Oelze ML, Zachary JF, White-Traut R. Quantitative Ultrasound Predicts Cervical Ripening in the Rat. *Am J Obstet Gynecol* 2005;193(534 Suppl):S153–S153.
85. Ito A, Kitamura K, Mori Y, Hirakawa S. The change in solubility of type I collagen in human uterine cervix in pregnancy at term. *Biochem Med* 1979;21:262–70. [PubMed: 496919]
86. Norman M, Ekman G, Malmstrom A. Changed proteoglycan metabolism in human cervix immediately after spontaneous vaginal delivery. *Obstet Gynecol* 1993;81:217–23. [PubMed: 8423954]
87. Granstrom L, Ekman G, Ulmsten U, Malmstrom A. Changes in the connective tissue of corpus and cervix uteri during ripening and labour in term pregnancy. *Br J Obstet Gynaecol* 1989;96:1198–202. [PubMed: 2590655]
88. Granstrom L, Ekman G, Malmstrom A. Insufficient remodelling of the uterine connective tissue in women with protracted labour. *Br J Obstet Gynaecol* 1991;98:1212–6. [PubMed: 1777452]
89. Stjernholm Y, Sahlin L, Malmstrom A, Barchan K, Eriksson HA, Ekman G. Potential roles for gonadal steroids and insulin-like growth factor I during final cervical ripening. *Obstet Gynecol* 1997;90:375–80. [PubMed: 9277647]
90. Neuman RE, Logan MA. The determination of hydroxyproline. *J Biol Chem* 1950;184:299–306. [PubMed: 15421999]
91. Zlatnik FJ, Burmeister LF. Interval evaluation of the cervix for predicting pregnancy outcome and diagnosing cervical incompetence. *J Reprod Med* 1993;38:365–9. [PubMed: 8320673]

Table 1

Collagen concentration and extractability of human cervical tissue.

OBSTETRIC HISTORY	HYDROXYPROLINE CONCENTRATION IN CERVICAL BIOPSY		COLLAGEN EXTRACT-ABILITY** (%)	REFERENCES
	µg/mg wet weight	µg/µg dry weight (% collagen)*		
Non pregnant	18.9 – 20.8	54 – 77 %	18 – 67 %	20–22, 32, 44, 45, 85–87
Non pregnant, clinical history of cervical insufficiency		41.3%	80.2	32
10 weeks pregnant		48.4 %	77 %	20
13 – 15 weeks with cervical insufficiency		47.3 %	87.7 %	21
Third Trimester				
Labor absent	10	23 – 36%	79 – 94 %	20, 44, 85, 87
Labor present	5.6 – 13.1	26 – 40 %	72 – 93 %	20, 31, 45, 86–89
Post partum, 2 – 4 days	9		77	44

Data are presented as a range of values from multiple studies.

* Collagen concentration per tissue dry weight was estimated by the following ratio: 1 ug hydroxyproline : 7.46 ug collagen.⁹⁰

** Represents percent of collagen solubilized after acetic acid extraction followed by extraction with acetic acid + pepsin (1mg/ml).

Table 2**Mechanical Properties of Human Cervical Tissue**

PROPERTY	MEASUREMENT PROTOCOL	REFERENCE
Cervical collagen is anisotropic	X-ray diffraction, Diffusion Tensor Imaging, Second Harmonic Generation, Mechanical Testing	22· 33· 34· 72
Cervical stroma demonstrates a nonlinear, time-dependent stress response	Mechanical Testing	8· 19· 69
Compared to nonpregnant tissue, cervical tissue is less stiff:		
- after vaginal delivery	Tangent Modulus	21· 73
- in the third trimester	Mechanical Testing	8
The large strain response in tension is stiffer compared to compression	Mechanical Testing	8
Collagen crosslinking is correlated to obstetric outcome and obstetric history	Light Induced Fluorescence	74
	Mechanical Modeling	72
Progressive cervical softening occurs during the second and third trimester	Cervicotonometer	77
	Aspiration Device	78
Mechanical properties of the cervix of non- pregnant women with history of spontaneous preterm birth are impaired	Stress Relaxation	69
	Compliance Score	91
	Elastance	81
	Resistance Index	82