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Innovative methods of cervical assessment and potential for novel treatment

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Abstract

A multitude of pathophysiologic pathways culminate in the final common denominator of cervical softening, shortening and dilation that lead to preterm birth. Currently, a variety of emerging technology aims to objectively quantify critical cervical parameters such as microstructural organization and softening of the cervix. If the nature and timing of cervical changes can be precisely identified, it should be possible to identify the causative upstream molecular processes and resultant biomechanical events associated with each unique pathway. This would promote molecular studies, ultimately leading to novel approaches to preterm birth prediction, novel treatments, and prevention.

Keywords

Preterm birth; short cervix; cervical microstructural assessment; quantitative ultrasound; novel treatments for cervical insufficiency

THE CERVIX AND PRETERM BIRTH

Spontaneous preterm birth (sPTB) is the leading global cause of neonatal death. sPTB is poorly understood and multifactorial, involving factors such as infection/inflammation, bleeding, genetics, poor nutrition, social status, stress, ethnicity/race, and others.¹ Despite intense research, studies of genomics, proteomics, transcriptomics and metabolomics have not yet provided definitive associations with sPTB, and thus no biomarkers have as yet emerged as reliable for prediction of preterm birth.²

The pathways to sPTB dovetail into final unifying processes such as cervical remodeling.² Gravett *et al.* recently stated “The finding that heterogeneous origins result in common downstream biological pathways and outcomes ... provides the opportunity to develop rational treatment strategies that target the upstream initiators ... ”¹ In other words, if we could identify cervical microstructural changes prior to preterm birth, it would promote study and understanding of specific molecular events, which would in turn allow conceiving

of novel approach to prediction, treatment and ultimately prevention of sPTB through identification of both imaging and molecular biomarkers.

The inverse relationship between a short cervix and preterm birth risk is well established, and vaginal progesterone supplementation has emerged as a viable treatment. However, this treatment is imperfect; the risk reduction is modest (less than 50%) and the mechanism is unclear, making it difficult to choose the best candidates for the treatment. This is because most women with a short cervix in the midtrimester but no prior history of sPTB deliver at term without treatment, and most preterm births in low risk women occur in those with a normal midtrimester cervical length.³

The immensely complex cervix is an investigational challenge. It has layers of collagen that remodel differently, likely because of independent molecular processes, and cause softening and shortening.⁴ Extensive microstructural change has already occurred by the time shortening is evident; this means that cervical softening, which begins soon after conception and continues progressively throughout pregnancy, is likely more critical than shortening.⁵ Yet clinical assessment of softening is entirely subjective; the cervix is labeled “soft, medium or firm” based solely on digital examination.

Many technologies are emerging to assess objectively the softness and microstructure of the pregnant cervix. In order to understand them, it is important to emphasize the central relationship between cervical softness and the organization and composition of the cervical extracellular matrix (ECM) because the pathogenesis of cervical softening and shortening likely relates to dysfunctional remodeling of the ECM.⁶ Approaches to evaluating ECM microstructure and softening of the pregnant cervix include elastography, acoustic attenuation, light-induced fluorescence, Raman spectroscopy, cervical consistency index, aspiration, quantitative ultrasound and shear wave speed estimation, among others. These are too numerous to cover in this brief review, so here we focus on a few ultrasound-based techniques that have seen recent attention in the literature. We conclude by discussing an approach to modify the cervical ECM using an injectable biomaterial with the ultimate goal of a possible alternative cervical treatment.

Elastography

Elastography is based on determining relative motion in areas of the cervix compared to other areas, described by a color map. Typically, the cervix is scanned in the usual manner, slight pressure is applied with the transducer to deform the tissue and specialized software is utilized to produce a color map that describes deformation of the tissue relative to neighboring areas. In an early study, elastography showed a statistically significant difference before and after induction of labor at term in individual women, but the procedure could not be standardized among women and the authors noted that standardization would be required if elastography was to be clinically useful.⁷ Molina et al attempted a reproducibility study in 112 women at all points in gestation, and noted no statistically significant differences between cervixes except in the precise area that received the force from the transducer, which led them to the conclusion that elastography measurements may be “a mere reflection of the force being applied by the transducer”.⁸ Many groups are working diligently on the problem of standardizing the transducer force so that

measurements can be meaningfully interpreted between patients. For instance, Hernandez-Andrade et al., using a semiquantitative technique involving deriving strain values from elastographic images, evaluated 189 women between 16–24 weeks, finding that strain values from a cross-sectional view near the internal os were significantly associated with sPTB < 37 weeks.⁹ Fruscalzo et al. evaluated another parameter derived from elastography, cervical tissue strain (TS), which uses Tissue Doppler imaging, in 77 women undergoing induction of labor at term.¹⁰ Their preliminary data suggest that TS may be useful for predicting success of induction of labor. Hee et al. are developing a reference cap which fits over a transvaginal transducer and could possibly standardize measurements between women for evaluation of preterm birth risk and prediction of success of labor induction.¹¹

Aspiration and Cervical consistency index (CCI)

Tissue compliance is related to tissue softness because soft tissues compress more than stiff tissues. Two groups exploit this principle, one by using aspiration, and the other by the cervical consistency index (CCI). To obtain an aspiration measurement, a 10mm diameter tube is pushed against the distal end of the cervix while a vacuum sucks the tissue into it and an optical fiber (connected to an external light source) quantifies the amount of aspirated tissue, which correlates with tissue softness. To obtain the CCI, an image of the cervix is obtained in the standard manner, then a 2nd image is obtained after pressure is applied with the transducer until no more shortening of the anterior-posterior diameter is observed (eg the cervix will not compress further). The ratio of the AP diameter on the pre- and post-deformation image is the CCI, and a lower CCI indicates softer tissue. A recent study demonstrated that both aspiration measurements and the CCI show an increase in compliance with gestational age, suggesting that this combined technique is promising for biomechanical description of cervical change and softening.¹²

Quantitative ultrasound with beamsteering

The ECM and its relationship to cervical softening may be assessed through quantitative ultrasound with beamsteering (QUS). QUS is based on the relationship of the amount of ultrasound wave that gets lost versus that which comes back to the transducer when an ultrasound wave encounters a scatterer (such as collagen in the cervix). The echo signal changes based on the shape of the scatterer and the angle at which the ultrasound beam hits the scatterer. Therefore, measuring backscattered power loss (BSPL) as a function of the beam angle provides information about the shape and organization of scatterers in a medium, such as organized, aligned collagen versus disorganized, unaligned collagen. We have used this technique to assess cervical collagen microstructure. We correlated the backscatter measurements to cervical softening in human hysterectomy specimens, and have compared the findings to second harmonic generation (SHG) microscopy (which provides micron-scale maps of collagen distribution and alignment in the cervix) to make sure our assumptions about what the ultrasound waves are encountering (eg collagen) are correct. Feasibility studies suggest that BSPL is sensitive to the collagen disorganization that occurs with cervical softening in the human cervix.¹³

Shear wave speed (SWS)

Shear waves are caused by sending a long duration, high-frequency ultrasound pulse into tissue, and watching how the tissue responds. The shear (transverse) waves that are created in response to the high-frequency pulse travel slowly enough to monitor with typical ultrasound imaging. These shear wave speeds (SWS) objectively describe tissue softness because shear waves travel more slowly in softer tissue. Also, they provide information about tissue microstructure because shear modulus (stiffness) is dependent on fiber (eg collagen) orientation and organization. In ripened versus unripened hysterectomy specimens, SWS are sensitive to the softness and microstructural disorganization that occurs with cervical remodeling.¹⁴ In a preliminary study of women undergoing induction of labor at term, statistically significant differences in pre- and post-ripening measurements were found in individual subjects as well as between groups. SWS estimates were also significantly correlated with digitally assessed cervical softness. (data submitted for publication)

POTENTIAL FOR NOVEL TREATMENT

Closely coupled with innovative methods of cervical assessment are efforts to develop novel therapies for cervical dysfunction. The pathophysiology most commonly linked to cervical dysfunction is ascending intrauterine infection. It is known that ascending infection can lead to an inflammatory response in the lower uterine segment, decidual activation and the cascade of events resulting in parturition. However, what is not clear from previous studies is whether 1) infection/inflammation initiates cervical shortening or 2) infection/inflammation occurs as a consequence of a short cervix. It is possible that excessive tissue softening is the key event resulting in cervical shortening.¹⁵ An excessively soft cervix could shorten as a physical response to uterine growth. As the cervix shortens, there is reduced barrier function and increased risk of ascending infection and inflammation. Thus, preventing cervical shortening could improve cervical barrier function, reduce the risk of ascending infection and delay the natural history of preterm birth. This concept has guided our efforts to develop an injectable biomaterial for cervical augmentation. It is important to note a critical distinction between a therapy for cervical augmentation and existing therapies that support the cervix (i.e. cerclage or pessary). Cerclage and pessary may fail because they have no effect on the properties of cervical tissue. A therapy that improves the properties of the cervical tissue, without having an adverse effect on labor, could be more efficacious than cerclage or pessary.

The design goals of a biomaterial for cervical injection are particularly stringent. The injected biomaterial must be biocompatible, biodegradable and augment cervical tissue without having an adverse effect on pregnancy. The mechanical properties of the gel should be tunable so that the properties of the gel match the properties of the cervical stroma. The ability to control degradation times is also critical so that the gel is mostly degraded by the late third trimester. Here we discuss several pilot studies with an injectable silk-based gel for cervical augmentation during pregnancy. Silk protein was chosen as the basis for the biomaterial because silk is FDA approved for human use and demonstrates a range of functionalities in terms of degradation and gelation.¹⁶

The initial biomaterial for cervical augmentation was a two component silk-based biomaterial that gelled upon mixing.¹⁷ Purified silk protein was mixed with a commercially available gelation system based on polyethylene glycol (PEG) functionalized with reactive chemical groups. The rationale for adding silk was to increase mechanical strength to the gelled biomaterial. We showed that the silk-PEG biomaterial increased stiffness of non-pregnant cervical tissue in vitro and was not cytotoxic to cervical fibroblasts. However, the clinical applicability was limited because it was necessary to add exogenous alcohol to achieve adequate gelation.

In the next iteration, the PEG system was eliminated and silk gelation was achieved using high intensity ultrasound.¹⁸ Using rats as a model for pregnancy, the cervical inflammatory response to an injected silk gel was similar to the response seen with suture controls. Cervical fibroblasts were seen to be viable, metabolically active, and proliferating on a silk surface. Also, inflammatory cytokine release was similar when cervical fibroblasts were cultured on silk surfaces and tissue culture plastic.

There are numerous challenges inherent in developing a novel treatment for the cervical component of preterm birth. Leaving aside the regulatory hurdles, the studies discussed above highlighted the lack of an accepted animal model for studying cervical treatment. Not all cervical injections in the rat were visualized on histology, likely because of the small target size. In addition, the short gestation length makes long term studies challenging. Nevertheless, there is a strong need to develop alternatives to existing therapies. Indeed there are populations of patients (i.e. twins with a short cervix, failed cerclage) for whom there are no good treatment options. Viable, efficacious alternatives that address the cervical component of preterm birth would be an exciting clinical development.

CONCLUSIONS

Techniques to evaluate softening and the ECM component, including ones not discussed in this brief review, are under active development currently and many are very promising. It is likely that a combination of methods will provide the best approach for understanding the molecular and microstructural processes within the extracellular matrix that cause premature softening and shortening of the cervix. Novel methods of treatment, such as injectable biomaterials for augmentation of cervical strength, can be based on this information. The next decade will be an exciting time for investigating new approaches to the problem of preterm birth.

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