Ultrasound in Med. & Biol., Vol. ■, No. ■, pp. 1–14, 2017

0301-5629/$ - see front matter

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http://dx.doi.org/10.1016/j.ultrasmedbio.2016.12.006

● *Original Contribution*

ASSESSMENT OF STRUCTURAL HETEROGENEITY AND VISCOSITY IN THE CERVIX USING SHEAR WAVE ELASTICITY IMAGING: INITIAL RESULTS FROM A RHESUS MACAQUE MODEL

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(*Received* 22 *July* 2016; *revised* 2 *November* 2016; *in final form* 14 *December* 2016)

Abstract—Shear wave elasticity imaging has shown promise in evaluation of the pregnant cervix. Changes in shear wave group velocity have been attributed exclusively to changes in stiffness. This assumes homogeneity within the region of interest and purely elastic tissue behavior. However, the cervix is structurally/microstructurally heterogeneous and viscoelastic. We therefore developed strategies to investigate these complex tissue properties. Shear wave elasticity imaging was performed *ex vivo* on 14 unripened and 13 misoprostol-ripened cervix specimens from rhesus macaques. After tests of significant and uniform shear wave displacement, as well as reliability of estimates, group velocity decreased significantly from the distal (vaginal) to proximal (uterine) end of unripened, but not ripened, specimens. Viscosity was quantified by the slope of the phase velocity versus frequency. Dispersion was observed in both groups (median: 5.5 m/s/kHz, interquartile range: 1.5–12.0 m/s/kHz), also decreasing toward the proximal cervix. This work suggests that comprehensive assessment of complex tissues such as cervix requires consideration of structural heterogeneity and viscosity. (E-mail: rosadomendez@wisc.edu) © 2016 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Cervix, Shear Wave Elasticity, Viscoelasticity.**

INTRODUCTION

During pregnancy, the cervix must prepare to allow eventual delivery of the fetus. Accordingly, dramatic remodeling of cervical microstructure causes progressive softening, shortening, and dilation. This normal process appears to be accelerated in spontaneous preterm birth (sPTB, birth before 37 wk of gestation), which is the greatest global cause of neonatal mortality worldwide and an important risk factor for long-term health complications (Behrman et al. 2007; Liu et al. 2015; Shapiro-Mendoza and Lackritz 2012) The epidemiology of sPTB is complex and multifactorial (Goldenberg et al. 2008), but premature cervical change always occurs (Vink et al. 2016), making the cervix an excellent target for investigation.

Shear wave elasticity imaging (SWEI) has been investigated as a non-invasive approach to quantification of cervical softening (Carlson et al. 2014a; Gennisson et al. 2011; Muller et al. 2015). The parameter used most commonly is shear wave speed group velocity (c_g, the propagation speed of the shear disturbance). Using c_g, Peralta et al. (2015a) reported an increase in cervical softness after labor induction in ewes. Although compelling, mechanical and anatomical differences between sheep and humans may limit clinical applicability of the results. We use a rhesus macaque model. Unlike sheep and other animal models used for preterm birth studies (*e.g.*, rodents), macaques are bipeds/quadripeds instead of obligate quadripeds, which makes the mechanical forces on the macaque cervix more similar to those in humans (obligate bipeds). Also, macaques and humans share similar maternal–fetal immunology and physiology (Hafez and Jaszczak 1972; Haluska et al. 1990). Although mechanical forces on the macaque's cervix may not completely match those on the human cervix (because of climbing and jumping behavior, for example), the macaque is closer to humans in terms of ambulation than are sheep or rodents.

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We have used SWEI to assess the spatial distribution of stiffness within the cervix and the effects of ripening (via administration of misoprostol, a prostaglandin used clinically to soften the cervix in preparation for labor induction) in *ex vivo* macaque specimens (Huang et al. 2016). We noted a trend of decreasing c_g from the distal (vaginal) end to the proximal (uterine) end of the cervix, suggesting spatial variability, but measurement variation was large. We found no difference in stiffness between unripened and ripened cervices. Although this could be due to a number of factors, including that there is no difference with ripening, it could be that simple c_g is not sensitive to these differences.

Our initial studies, like all studies of SWEI in the cervix to date (Carlson et al. 2014a; Gennisson et al. 2011; Muller et al. 2015; Peralta et al. 2015a), ignored two important issues: (i) potential structural/microstructural heterogeneity and (ii) viscosity. Regarding the former, c_g estimation assumes that tissue is homogeneous within the analyzed region of interest (ROI). Although this may be true within small ROIs, no robust tests of homogeneity of shear wave propagation have been previously applied. This is an issue because cervical microstructure contains three overlapping and interwoven quasi-layers of collagen (Akins et al. 2010), and it is thus possible that inappropriate estimates have been included in reports of c_g. Another type of heterogeneity that could affect interpretation of results between individuals, and especially species, is differences in gross cervical structure. Specifically, in our previous macaque study, we assumed that c_g estimates could be compared between cervices of different sizes by simple linear scaling of fractional length, as we have successfully done in human studies (Carlson et al. 2014a). Unlike humans, however, the distal end of the rhesus cervix is bulbous, with a tortuous central canal. Between this bulb and the internal os (where the proximal cervix ends and the uterus begins), the macaque cervix abruptly changes to resemble the human in a region we have named the *transition zone*; specifically, it becomes cylindrical with a relatively straight central canal. Analyzing the entire macaque cervix, including the distal bulb, likely compromised our analysis because accurate estimates most likely could not be obtained from such grossly heterogeneous tissue. Furthermore, because humans do not share this structural feature, applicability of these results to women is compromised. Finally, and perhaps most importantly in terms of translation to the bedside, our evaluation in humans ignores the distal cervix because we have found assessment there to be less clinically relevant (regarding detection of ripening) than that of the mid- and proximal cervix (Carlson et al. 2014b), and therefore, it seems prudent to ignore the distal cervix in our macaque model as well.

The second issue is that previous estimates of c_g in the cervix have assumed that differences are defined primarily by purely elastic stiffness, with strain independent of the rate of the induced stress. However, *ex vivo* studies in rodents (Barone et al. 2010; Buhimschi et al. 2004) and humans (DeWall et al. 2010; Myers et al. 2008, 2009) have clearly indicated that the cervix behaves like a viscoelastic solid in which the magnitude of the response depends also on the rate of the applied strain (Lakes 2009).

To overcome these potential sources of measurement imprecision, we attempted to (i) account for structural/microstructural tissue heterogeneity in a robust manner and (ii) assess the viscous component of the cervix. Three steps were required for (i). First, we analyzed only the region of the macaque cervix between the transition zone and internal os, both to reduce potential measurement inaccuracy resulting from extreme structural heterogeneity and to make the results more generalizable to humans. Second, we developed a test for significant and uniform shear wave-induced displacement to confirm that the region of measurement is sufficiently homogeneous for accurate evaluation. Third, we developed a test of reliability of individual measurements (*e.g.*, rejection of spurious data).

To achieve (ii), we quantified shear wave dispersion, a direct effect of viscoelasticity on shear wave propagation that results in variation of the phase velocity c_p (the velocity of each spectral component of the wave) with shear wave frequency content (Lakes 2009). Shear wave dispersion has been used to characterize viscoelasticity in the breast, liver, and muscle (Barry et al. 2012, 2014; Gennisson et al. 2010; Muller et al. 2009; Tanter et al. 2008). As reported by Barry et al. (2012), Deffieux et al. (2013), and Nightingale et al. (2015), the frequency dependence of c_p can be represented as a linear function of frequency defined by an intercept c_0 and a slope dc_p/df:

$$c_p(f) = c_0 + \frac{dc_p}{df} f \quad (1)$$

Thus, an increase in the viscous response is associated with an increase in the slope dc_p/df.

We explored these two issues because comprehensive identification and quantification of key changes in the pregnant cervix should help uncover molecular mechanisms of sPTB, in turn leading to novel solutions for its prediction and prevention.

METHODS

Data acquisition

Cervical specimens were obtained from 27 rhesus macaques from the Wisconsin National Primate Research