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Anisotropy and Spatial Heterogeneity in Quantitative Ultrasound Parameters: Relevance to Study of the Human Cervix

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Abstract

Imaging biomarkers based on quantitative ultrasound (QUS) can offer valuable information about properties that inform tissue function and behavior such as microstructural organization (e.g. collagen alignment) and viscoelasticity (i.e. compliance). For example, the cervix feels softer as its microstructure remodels during pregnancy, an increase in compliance that can be objectively quantified with shear wave speed (SWS), and therefore SWS estimation is a potential biomarker of cervical remodeling. Other proposed biomarkers include parameters derived from the backscattered echo signal, e.g. attenuation and backscattered power loss, because such parameters can provide insight into tissue microstructural alignment/organization. Of these, attenuation values for the pregnant cervix have been reported, but large estimate variance reduces their clinical value. That said, parameter estimates based on the backscattered echo signal may be incorrect if assumptions they rely upon, such as tissue isotropy and homogeneity, are violated. For that reason, we explored backscatter and attenuation parameters as potential biomarkers of cervical remodeling via careful investigation of the assumptions of isotropy and homogeneity in cervical tissue. Specifically, we estimated the angle- and spatial-dependence of parameters of backscattered power and acoustic attenuation in the *ex vivo* human cervix using the Reference Phantom Method and electronic steering of the ultrasound beam. We found that estimates are anisotropic and spatially heterogeneous, presumably because the tissue itself is anisotropic and heterogeneous. We conclude that appropriate interpretation of imaging biomarkers of cervical remodeling must account for tissue anisotropy and heterogeneity.

Keywords

QUS; Cervical Assessment; Anisotropy; Attenuation; Reference Phantom Method

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Introduction

During pregnancy, the cervix undergoes a remarkable transformation from a rigid structure that supports a growing fetus *in utero* to one compliant enough to allow the fetus to pass through for delivery. This process involves extensive remodeling of its microstructure, including “ripening” (the apparent rapid acceleration of remodeling resulting in a marked increase in tissue compliance), that has been evidenced directly through animal models (Word et al., 2007; Timmons et al., 2010; Akins et al., 2011; Uldbjerg et al., 1983; Theobald et al., 1982) and is clinically palpable in women. However, precisely *what* changes, *how* changes happen, and especially *why* they happen to result in delivery at a particular time is unclear. Because answers to these questions could facilitate solutions to abnormal parturition such as pre- or post-term delivery, quantitative ultrasound (QUS) approaches to describe and quantitate properties of the pregnant cervix are emerging.

Prevailing thought is that the extracellular matrix (ECM), the microstructural scaffolding which directs mechanical behavior, is relatively homogeneous throughout the cervix, primarily (85–90%) comprised of relatively discrete layers of collagen with only a small amount of cellular content (10–15% smooth muscle cells, vascular cells, glandular cells, immune cells, etc.). (Danforth, 1983; Aspden, 1988) If that were true, QUS assessment would be straightforward. Unfortunately, it is not true; recent findings suggest that the cervix is extremely anisotropic and heterogeneous. A few of the many examples are that the proximal cervix, as compared to the distal, is (1) highly cellular, with 50–60% smooth muscle cells, (Vink and Feltovich, 2016) (2) contains significantly greater collagen crosslink heterogeneity, (Zork et al., 2015) and (3) demonstrates marked directionality of collagen fibers around the cervical canal. (Reusch et al., 2013; Fernandez et al., 2016; Yao et al., 2016)

Shear wave speed (SWS) estimation, a QUS technique based on shear wave elasticity imaging (SWEI), is a promising biomarker of cervical remodeling because it is an objective measure of cervical viscoelasticity that seems to distinguish the early (feels stiffer) from the late (feels softer) pregnant cervix. (Carlson et al., 2014c,b, 2017; Hernandez-Andrade et al., 2014) Animal studies combining microscopy and mechanical strength testing confirm that progressive disorganization and restructuring of cervical ECM leads to increased tissue homogeneity that is concomitant with increased compliance. (Akins et al., 2010) In pregnant women, SWS studies suggest that cervical tissue in late pregnancy is more homogeneous than in early pregnancy, consistent with tissue that feels softer in late, as compared to early, pregnancy. (Carlson et al., 2017) Other QUS parameters that could help quantify cervical remodeling include those based upon the backscattered echo signal, such as the backscatter coefficient, attenuation coefficient, and backscattered power difference. These parameters have not been well studied in the cervix, but it is established in many other tissues that acoustic properties such as backscattered power and attenuation have clear angle- and spatial-dependence that is consistent with homo/heterogeneity and (an)isotropy of the underlying tissue. (Nassiri et al., 1979; Topp and O’Brien, 2000; Insana et al., 1991; Wear, 2000; Milne et al., 2012; Guerrero et al., 2017) Simple attenuation estimation in pregnant rodents appears to detect remodeling, (Bigelow et al., 2008) and, in the human cervix, estimates decrease with increasing gestational age and with prostaglandin ripening (a

clinical procedure in which prostaglandin is administered to prepare the cervix for labor). (McFarlin et al., 2010, 2015) However, the usefulness of attenuation as a biomarker of cervical remodeling is limited because inter-subject variability is greater than the average reduction in attenuation from either ripening or advancing pregnancy. (McFarlin et al., 2010, 2015) That said, in studies to date, attenuation has been estimated under the assumptions of tissue isotropy and homogeneity, but violating these assumptions can lead to erroneously high variances. (Nassiri et al., 1979; Topp and O'Brien, 2000; Nam et al., 2012)

This work evaluates the validity of the assumptions of isotropy and spatial homogeneity in the acoustic properties of the cervix, and explores how departures from these assumptions might affect the accuracy of parameter estimates. For this, we obtained specimens from women undergoing hysterectomy for benign conditions. Some cervixes were ripened exogenously prior to surgery (with a prostaglandin), others ripened endogenously (uterine bleeding is associated with increased serum levels of prostaglandins and clinically apparent softening), and others left unripened. To test the assumption of isotropy, we measured the angle-dependence of backscattered power (Guerrero et al., 2017) because this parameter has been shown to detect anisotropy in tissue with aligned microstructure (e.g. skeletal muscle (Nassiri et al., 1979; Topp and O'Brien, 2000)). To test the assumption of spatial homogeneity, we compared measurements along the length and perimeter of the cervix. Taken together, our results suggest that cervical tissue anisotropy and heterogeneity affects backscatter and attenuation, and therefore accurate biomarkers of cervical remodeling must account for these properties in both acoustic parameters and in the tissue itself.

Materials and Methods

Data Acquisition

Tissue Acquisition and Preparation—Hysterectomy specimens were obtained from premenopausal women undergoing surgery for benign conditions not involving cervical pathology ($n=14$). All subjects provided informed consent, and all work was HIPAA compliant and approved by Institutional Review Boards at Intermountain Healthcare and the University of Wisconsin. Pregnancy history, age, date of last menstrual period, and indication for hysterectomy were documented. Exclusion criteria were history of preterm birth or previous cervical surgery.

Prior to surgery, women were classified into two groups: unripened ($n_u=4$) and ripened ($n_r=10$). The ripened group consisted of both endogenously ripened ($n_{en}=6$) and exogenously ripened ($n_{ex}=4$) cervixes. The rationale behind combining endogenously and exogenously ripened cervixes is that ripening (softening) is associated with endogenous prostaglandin production (e.g. from pregnancy or uterine bleeding) (Salamonsen and Findlay, 1990) or exogenous prostaglandin administration (e.g. from prostaglandins such as misoprostol which are commonly used in practice to soften the cervix for induction of labor or gynecologic procedures). (Calder, 1991; Allen and O'Brien, 2009)

Women in the exogenously ripened group were given misoprostol in the standard fashion (400 μg misoprostol per vagina) 10–12 hours before surgery. (Allen and O'Brien, 2009; Crane and Healey, 2006) Of the 14 subjects, two were nulliparous (never had a baby), one

of them in the ripened group and the other in the unripened group. It is logical to analyze the nulliparous cervix, which has never undergone pregnancy-associated remodeling, separately from the multiparous cervix. Because we had so few nulliparous cervixes ($n_{\text{nulli}}=2$), however, we could not confidently explore differences. Therefore, the two nulliparous samples were discarded from analysis, leaving $n=12$ subjects: 3 unripened ($n_u=3$) and 9 ripened ($n_r=9$).

Directly after excision, hysterectomy specimens were placed in saline and allowed to stabilize to room temperature (approximately 1 hour). The sample was bisected and attached to sound absorbing rubber to prevent reverberation and minimize spurious motion. To avoid distortion, the tissue was attached to the rubber by pinning along the contour of the cervix within the outer serosa (see Fig. 1 of Carlson *et al.* 2014 for experimental set-up). A diagram of the experimental setup is shown in Fig. 1(a).

Ultrasound Data Acquisition—We used a Siemens Acuson S2000 ultrasound system (Siemens Healthcare, Ultrasound Business Unit, Mountain View, CA, USA) equipped with an 18L6 linear array transducer operating at a nominal frequency of 10 MHz to acquire radiofrequency (RF) echo signal data from each cervix specimen. We used the Axius Direct Ultrasound Research Interface (Brunke et al., 2007) for beamsteering and collection of raw RF echo signals (sampled at 40 MHz). RF echo data were collected with the linear acoustic beams steered from -28° to $+28^\circ$ in steps of 4° (the beamsteering geometry is shown in the diagram of Fig. 1(a)). By convention of the Axius Direct Ultrasound Research Interface, negative beamsteering angles refer to beams that travel towards the left-hand side of the B-mode image on the screen and positive angles refer to beams that travel towards the right.

The transducer was secured to a positioning stage with its face aligned parallel to the endocervical canal, but not in contact with the tissue. The focus was set at 1 cm, and the specimen centered at the focal depth. Using the thyroid preset and a transmit power of 63% we had a mechanical index of 1.1 for all scans. The acoustic beam was electronically steered, and a frame of RF echo data (456 parallel beam lines) was collected at each angular step. Negative beamsteered angles were directed towards the distal end of the cervix and positive towards the proximal end. (Of note, the 18L6 transducer is long enough to scan the entire length of the human cervix in a single acquisition, as shown in Figure 1.)

An analogous process was used to acquire RF echo signal data from a reference phantom, with system settings identical to those used for cervix specimen data acquisition. The reference phantom was composed of a water-based gel (4.5 g/L; BD Bacto™ Dehydrated Agar; Becton, Dickinson and Company, Franklin Lakes, NJ) containing graphite powder (50 g/L) and glass beads (4 g/L, 3000E beads; $\sim 5\text{--}20\ \mu\text{m}$; Potter's Industries, Malvern, PA). Twenty sets of beamsteered frames of RF echo data were acquired from independent planes of the reference phantom by translating the transducer perpendicular to the image plane between frames of data. The phantom had a speed of sound of 1560 m/s and a linear attenuation near $0.67\ \text{dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$ in the 4–9 MHz bandwidth.

Data from both the specimen and phantom were downloaded and analyzed off-line using MATLAB (Mathworks, Natick, MA).

Data Analysis

We used Thomson's multitaper method (Thomson, 1982) to estimate power spectra for both tissue and reference phantom echo signal analysis. The multitaper method provides the lowest variance estimate of attenuation when the size of the power spectral estimation region is limited, and the trade-off in spatial resolution versus bias and variance of spectral estimates can be optimized using the acoustic pulse length and lateral correlation length. (Rosado-Mendez et al., 2013) Using methods described by Thijssen, (Thijssen, 2003) the axial and lateral RF echo signal correlation lengths were estimated to be 234 and 287 μm , respectively, in the reference phantom (similar results were obtained in the cervix tissue). Based on previous work, (Rosado-Mendez et al., 2013) for these correlation cell sizes the multitaper method (4 tapers with 209 RF time samples for 5.2 μs signal segments (Thomson, 1982)) called for the following: a 4 mm $\times$ 4 mm (axial $\times$ lateral) power spectral estimation region (PSER), 4 mm $\times$ 4 mm BSPD parameter estimation region (PER) (since only a single PSER is needed for BSPD parameter estimation) and 10 mm $\times$ 4 mm PER for attenuation estimation. All power spectral estimation regions were estimated using a 90% overlap, therefore, each power spectral estimation region was estimated after a 400 μm axial or lateral shift.

The bandwidth selected for data analysis was the range that had power at least 10 dB above the spectral noise floor. The noise floor was estimated by averaging the echo signal power between 13–20 MHz, a range far outside the observed echo signal bandwidth. Based on these criteria, the 4–9 MHz frequency range was chosen for all data analysis. This frequency range was tested for each power spectrum and any frequency that fell below the noise floor estimate was excluded from further analysis. The average bandwidth for all attenuation estimates was 4.9 MHz, with the average bandwidth falling to 4.3 MHz in some cervixes with very high attenuation. Any attenuation estimate with a usable bandwidth lower than 4.5 MHz was removed from analysis.

In all cases, PERs were defined at least one aperture away from the lateral edges of the RF data frame to avoid incomplete aperture artifacts.

Backscattered Power Difference (BSPD) Parameters—Summary measures of backscattered echo signal power for investigating anisotropy were calculated as previously described. (Guerrero et al., 2017) Parameters include normalization angle (θ_{norm}), normalized BSPD (nBSPD), asymmetric BSPD (*a*BSPD), and mean BSPD (*m*BSPD) and are summarized in Table 1. Briefly, after calculating the echo signal power spectrum of the sample and reference phantom, we compute the backscattered power difference (BSPD) by integrating the log ratio of sample power spectra to reference phantom power spectra in the usable bandwidth for a set of beam-steering angles (-28° to $+28^\circ$). The angle of highest BSPD is the normalization angle (θ_{norm}). Assuming there is an underlying aligned structure in the tissue that contributes substantially to the echo signal, the θ_{norm} will indicate the angle at which that structure is perpendicular to the beam. The normalized BSPD (nBSPD) is calculated by subtracting the BSPD estimate at the normalization beamsteering angle from the BSPD estimates at all other angles and is used to compute the asymmetric BSPD (*a*BSPD) and the mean BSPD (*m*BSPD). The *a*BSPD is the difference between the average

nBSPD among positive beamsteering angles and negative beamsteering angles. It will be non-zero in the presence of anisotropic scattering structures, and zero when the scattering is symmetric about the axis normal to the transducer aperture (which includes isotropic scattering sources or anisotropic structure that is parallel to the transducer face). The *m*BSPD is estimated by averaging nBSPD among all beamsteering angles. It describes the magnitude of anisotropy, and will increase in concordance with insonification angle-dependence in either the backscatter coefficient or attenuation coefficient.

Attenuation Parameters—The attenuation coefficient was estimated by the Spectral Difference approach, which entails computing the ratio of the sample power spectral density (PSD) and reference phantom PSD, fitting a line to the logarithm of the PSD ratio versus depth at each frequency within the usable bandwidth, and determining the attenuation coefficient from the slope of the linear fit. (Yao et al., 1990) The specific attenuation coefficient (IEC 61391-2: 2010, 2010) (SAC) was estimated at all beamsteering angles by computing the linear least-squares fit to the attenuation coefficients in the usable bandwidth (4–9 MHz).

Attenuation anisotropy (AA), the slope of SAC vs. beamsteering angle, was estimated by linear regression (Figure 2). AA values significantly different from zero indicate anisotropy.

Statistical Analysis

Standardizing Measurement Location—To standardize measurements among cervixes of different lengths and maintain consistency with previous work involving SWS estimation in the same specimens, (Carlson et al., 2014a,b) cervixes were partitioned based on fractional distance from the internal os (uterine end of the cervix). First, the internal and external ostia were located in B-mode images of the cervix. Then, the distance from the internal to the external os was expressed as a fraction of the cervical length and divided into five regions: 0–25% (proximal), 26–44% (mid-proximal), 45–55% (middle), 56–75% (mid-distal), and 76–100% (distal). (Carlson et al., 2014a,b) QUS parameter estimates were then grouped into those regions (see Fig. 1(b) for an example of spatial partitioning).

Mixed Effects Regression Model—A multivariate mixed effects regression model (see, for example, Bates *et al.* 2015) was used to test for evidence of anisotropy in QUS parameters (θ_{norm} , *a*BSPD, *m*BSPD, AA, SAC(0°) and SAC(θ_{norm})). The models used explanatory variables of categorical type (ripening state and cervix side) and continuous type (location along the length of the cervix, allowing for linear and quadratic dependence). Inter-sample variability was included as a random effect. A sequential model-building strategy was performed for model selection: two nested mixed-effect models were compared by likelihood ratio test to assess the improvement in the fit between sequential models (models with additional explanatory variables). The final nested model included the least number of statistically significant variables; variables which did not improve the fit were removed. Statistical analyses were performed in R (R Core Team, 2013) with the lme4 package. (Bates et al., 2015) Residual and Q-Q plots were used to assess any possible violations in model assumptions.

Results

Backscattered Power Difference

All BSPD parameters (θ_{norm} , $a\text{BSPD}$, $m\text{BSPD}$), irrespective of state of ripening or cervix side, demonstrated anisotropy (i.e. angle-dependence) and were spatially dependent (i.e. dependent upon the location in the cervix where the data were acquired). This is shown in Figure 3. The box plots show the θ_{norm} , $a\text{BSPD}$, and $m\text{BSPD}$ vs. position along the length of the cervix for both sides and both ripening states. BSPD parameters showed less variation in the middle regions of the cervix (mid-distal, mid, and mid-proximal) than at the distal and proximal ends. This trend persisted in ripened samples, although differences were smaller. Table 2 demonstrates these findings, summarizing $m\text{BSPD}$ values at all regions, ripened states, and cervix sides.

Attenuation

Like the BSPD parameters, the attenuation parameters demonstrated anisotropy and spatial heterogeneity. For example, $\text{SAC}(0^\circ)$ was spatially dependent, again with estimates greatest in the middle regions of the cervix. Figure 4 shows $\text{SAC}(0^\circ)$ as a function of position along the length of the cervix for each ripening state and cervix side. With SAC estimated at the normalization angle $\text{SAC}(\theta_{\text{norm}})$, to remove the angle dependence of attenuation by taking into account the local dominant fiber orientation, estimates still demonstrated spatial dependence.

Attenuation also demonstrated anisotropy and spatial heterogeneity. Figure 5 shows AA (slope of SAC vs. beamsteering angle) as a function of location along the length of the cervix for each ripening state and cervix side. Similar to the BSPD parameters, attenuation showed less anisotropy at the distal and proximal ends than in the middle regions of the cervix. As with BSPD parameters, these differences persisted, but were smaller, in ripened samples. Table 3 summarizes AA values at all regions, ripened states, and cervix sides.

Statistical Analysis

As noted above, all parameters displayed anisotropy and spatial heterogeneity (i.e. dependence upon location along the length of the cervix) in the mixed effects regression model. Comprehensive results are summarized in Table 4. θ_{norm} , $a\text{BSPD}$ and $\text{SAC}(\theta_{\text{norm}})$ demonstrated significant linear dependence on location, and all parameters (θ_{norm} , $a\text{BSPD}$, $m\text{BSPD}$, slope of AA, $\text{SAC}(0^\circ)$ and $\text{SAC}(\theta_{\text{norm}})$) demonstrated significant quadratic dependence on location. $a\text{BSPD}$ and $m\text{BSPD}$ were significantly different between unripened and ripened tissue.

Discussion

The cervix continuously remodels throughout pregnancy in preparation for delivery of a fetus. This process, which involves progressive disorganization of the extracellular matrix, results in increasing tissue isotropy and homogeneity in animal models (Akens et al., 2011) and thus presumably also in women. The purpose of our study was to investigate whether and how anisotropy and spatial heterogeneity in human cervical tissue affects QUS

parameters. We found that (1) QUS parameter estimates of both backscattered power loss and attenuation indicate anisotropy and spatial heterogeneity in cervical tissue, and (2) anisotropy and spatial heterogeneity persisted, although to a lesser degree, in ripened tissue.

Unless otherwise specified, values reported below are from the anterior mid locations of the cervix; this region is most relevant to us because it is where we find parameter values to be most reliable in this study, consistent with our previous SWS studies, (Carlson et al., 2014a,b) and therefore is where we sample in our ongoing clinical studies in pregnant women.

Anisotropy was clearly demonstrated in all tissue irrespective of ripening state. As discussed above, if a tissue contains an aligned, anisotropic scattering structure, the θ_{norm} will indicate the angle of closest perpendicular incidence with this dominant structure, $a\text{BSPD}$ values will be non-zero (except in the special case that the dominant scattering structure is parallel to the transducer), and both $m\text{BSPD}$ and AA values will be significantly different from zero. In our study, the θ_{norm} and $a\text{BSPD}$ were non-zero for every location along the length of the cervix for both unripened and ripened tissue. Median $m\text{BSPD}$ values were also consistent with anisotropy. For example, median $m\text{BSPD}$ in unripened samples was 9.09 dB, comparable to bicep muscle imaged along its dominant axis (≈ 9 dB) (Guerrero et al., 2017), a tissue that is well described as anisotropic. (Nassiri et al., 1979; Topp and O'Brien, 2000) In ripened tissue, median $m\text{BSPD}$ was smaller (7.45 dB), but still more than twice that of bicep muscle imaged in transverse cross-section (≈ 3 dB; i.e. more isotropic) and well above the <1 dB found in homogeneous, isotropic phantoms. (Guerrero et al., 2017) Further, the AA was significantly different from zero in all tissue. This makes it appropriate to assume that attenuation estimates will differ with varying angles of insonation, and in fact that was the case. For example, at a beamsteering angle of $+28^\circ$ compared to -28° , $\text{SAC}(0^\circ)$ estimates were $2.13 \text{ dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$ higher in unripened, and $1.46 \text{ dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$ higher in ripened, tissue; both are large compared to values in other soft tissues, e.g. liver ($\approx 0.5 \text{ dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$). (Maklad et al., 1984) As with BSPD parameters, smaller SAC values were observed in ripened, as compared to unripened, tissue; median (25th – 75th percentiles) $\text{SAC}(0^\circ)$ was 3.27 ($2.63 - 3.42$) $\text{dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$ in ripened and 4.17 ($4.05 - 4.61$) $\text{dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$ in unripened tissue. In summary, although median parameter values were smaller in ripened tissue, perhaps suggesting reduced anisotropy, the critical point is that ripening did not eliminate anisotropy.

Spatial heterogeneity was also clearly demonstrated in all samples irrespective of ripening state. As noted above, the θ_{norm} , $a\text{BSPD}$ and $m\text{BSPD}$ changed along the length of the cervix, particularly at the distal and proximal ends, and, similarly, SAC was dependent upon location within the cervix, in both unripened and ripened tissue. For instance, in unripened tissue, $\text{SAC}(0^\circ)$ estimates between the anterior mid and the proximal cervix (regions separated by 5.15 ± 0.98 mm) differed by $1.16 \text{ dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$, which is comparable to the difference between ripened and unripened cervixes at the anterior mid location ($0.9 \text{ dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$). Our SAC estimates in ripened tissue (which can be expected to be more similar to the remodeling pregnant cervix than unripened tissue) were higher than those reported in pregnancy; for example, $\text{SAC}(0^\circ)$ median (25th – 75th percentiles) was 3.27 ($2.63 - 3.42$) $\text{dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$ as compared to $\approx 0.5 - 2 \pm 0.4 \text{ dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$ in pregnant women. (McFarlin

et al., 2010, 2015) The difference can be attributed at least partly to differences in perfusion, tissue temperature, and other factors related to evaluating *ex vivo* vs. *in vivo* tissue, but the important point is that the variances in both studies are large.

As discussed in the Introduction, published attenuation estimates in the human cervix are “noisy”, with variances as large as the reported difference in attenuation between the early and late pregnant cervix.(McFarlin et al., 2015) We expected that estimating SAC at the normalization angle would reduce this large estimate variance by accounting for some of the anisotropy and spatial heterogeneity in the cervix. Our findings certainly suggested microstructural anisotropy; θ_{norm} values demonstrated that, at the spatial scale of the acoustic pulse ($\approx 200\text{--}300\ \mu\text{m}$), the dominant scattering structure has a changing preferential directionality along the length of the cervix. This finding is consistent with microscopy evaluation of cervical tissue, which confirms a thin layer of collagen fibers running longitudinally to the cervical canal, and a thicker band encircling the canal (this is the dominant scattering structure we suspect we are detecting). (Reusch et al., 2013; Weiss et al., 2006; Fernandez et al., 2016) Our findings also confirm that violation of the assumptions of isotropy and/or spatial heterogeneity can cause erroneous values; for example, a 10° deviation in beam angle at the anterior mid region could cause a $0.27\ \text{dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$ difference in attenuation, which is on par with the reported $0.4\ \text{dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$ difference in attenuation between the early (first trimester) and late (3rd trimester) cervix. (McFarlin et al., 2010) We did not, however, expect to find significant variations of SAC along the length of the cervix after SAC estimation at the normalization angle. The unexpected finding that $\text{SAC}(\theta_{\text{norm}})$ exhibits as much variance as $\text{SAC}(0^\circ)$ merits further investigation, because it suggests there are sources of anisotropy and spatial heterogeneity besides collagen alignment that have not yet been elucidated. The critical finding is that SAC estimates are relatively “noisy” and thus of questionable clinical usefulness for characterizing the remodeling cervix.

The parameters based on BSPD, however, were more reliable. They were also consistent with previous studies of shear wave speed (SWS) estimation in the cervix. As discussed in the Introduction, SWS estimation is a QUS technique that measures cervical viscoelasticity and can distinguish the early (feels stiffer) from the late (feels softer) pregnant cervix. (Carlson et al., 2014c,b, 2017; Hernandez-Andrade et al., 2014) Animal studies combining microscopy and mechanical strength testing confirm that progressive disorganization and restructuring of cervical ECM leads to increased tissue microstructural homogeneity that is concomitant with increased compliance,(Akins et al., 2010) and SWS/microscopy studies in the same tissue at the same locations reported in this study confirm that, while SWS are slower (suggesting softer tissue), microstructural anisotropy and heterogeneity are maintained after ripening.(Carlson et al., 2014c) Very recent SWS studies in pregnant women suggest that cervical tissue in late pregnancy (3rd trimester) is more homogeneous than in early pregnancy (1st trimester).(Carlson et al., 2017) We are currently analyzing BSPD parameters in these cohorts to explore whether they suggest greater microstructural organization/alignment in the early vs. late pregnant cervix.

Clinical translation of our findings is limited by two factors: we studied the (unperfused and room temperature) *ex vivo* cervix and we used a hand-held high frequency linear array

transducer that would be inappropriate for *in vivo* cervical imaging. In addition, our small sample size limits the generalizability of our results. Repeatability and reproducibility of these parameter estimates will need to be demonstrated prior to large-scale clinical trials. Reproducibility of parameter estimates will require standardization of estimate procedures among a variety of systems from different manufacturers. In particular, similar ranges for the usable bandwidth and angular range used in analysis to directly compare QUS parameters will need to be established. That said, the goal of this exploratory study was to determine whether anisotropy and spatial heterogeneity can be ignored in QUS evaluation of the cervix. Our results suggest that they cannot; anisotropy and spatial heterogeneity were clearly evidenced in both ripened and unripened specimens. Further, our anisotropy results are based on the limited angular range of $\pm 28^\circ$ with respect to the cervical canal, and therefore the anisotropic behavior of all other angular ranges within a full 360° analysis is unknown. These limitations, however, do not impact the primary finding of this study, which is that anisotropy and heterogeneity of tissue, and QUS estimates to describe that tissue, should be taken into account when evaluating the cervix.

Conclusions

The main goal of this study was to determine whether estimates of backscatter and attenuation parameters in cervix tissue were consistent with the typical assumptions of isotropy and homogeneity. We found that both backscattered power and attenuation are anisotropic (angle-dependent) and spatially heterogeneous (dependent upon spatial location) in the cervix, irrespective of ripening state. Of these, attenuation was highly variable, even after removing potential variability due to dominant fiber alignment. Parameters derived from the backscattered power difference, however, may be useful biomarkers to quantify cervical remodeling.

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Figure 1.

(a) Diagram showing the experimental setup and acoustic beam steering geometry. (b) B-mode image (at 0° beamsteering angle) of an anterior cervix sample pinned to sound absorbing rubber. Solid lines demarcate the beginning and ending of the fractionally defined spatial locations. D–distal, MD–mid-distal, M–middle, MP–mid-proximal, and P–proximal.

Figure 2.

An example of estimating attenuation anisotropy. X's represent the average SAC and error bars the standard deviation among all samples at that spatial location. The solid line represents the linear least-squares fit to the average SAC vs. beamsteering angle. Attenuation anisotropy is the slope of the linear least-squares fit to SAC versus beamsteering angle.

Figure 3.

Average normalization angle, $aBSPD$, and $mBSPD$ vs. position along the length of the cervix for (a)–(c) anterior and (d)–(f) posterior. The boxes represent the interquartile range (IQR) among parameter estimates, the horizontal line near the middle of each box is the median value for that group, the whiskers represent the maxima and minima within $1.5 \times IQR$, and dots are outliers outside of $1.5 \times IQR$. D-distal, MD-mid-distal, M-middle, MP-mid-proximal, P-proximal. The curved lines represent the result of the mixed effects model. Solid lines separate ripening states.

Figure 4.

The specific attenuation coefficient estimated with acoustic beams normal to the cervix vs. position along the length of the cervix as a function of ripening state for (a)&(c) anterior and (b)&(d) posterior side of the cervix. D-distal, MD-mid distal, M-mid, MP-mid proximal, P-proximal. The curved lines represent the result of the mixed effects model. Solid vertical lines separate ripening states.

Figure 5.

Plots of AA vs. location along the length of the cervix for anterior (a) and posterior (b).

Labels represent the following locations: D-distal, MD-mid distal, M-mid, MP-mid proximal, P-proximal. The curved line in each plot represents the result of the mixed effects model. Solid vertical lines separate ripening states.

Table 1

Summary of QUS parameters used in this manuscript.

Parameter Name	Acronym	Description
Backscattered Power Difference	BSPD	The average of the log ratio of sample to reference phantom power spectra in the usable bandwidth. Quantifies the difference in attenuation and backscatter coefficients between the sample and an isotropic reference material.
Normalized Backscattered Power Difference	nBSPD	The difference between the maximum value of the BSPD in the beamsteering range and the BSPD at any single beamsteering angle. Fundamental for calculation of <i>a</i> BSPD and <i>m</i> BSPD.
Normalization Angle	θ_{norm}	The angle at which the maximum value of the BSPD in the beamsteering range occurs. Indicates the angle of normal incidence between the acoustic pulse and the major axis of the aligned structure in the tissue (assuming presence of aligned scatterers).
Asymmetric Backscattered Power Difference	<i>a</i> BSPD	The difference between the average nBSPD among all negative beamsteering angles and the average nBSPD among all positive beamsteering angles. Indicates asymmetry in BSPD with respect to 0° beamsteering angle.
Mean Backscattered Power Difference	<i>m</i> BSPD	The average nBSPD among all beamsteering angles. Quantifies the average power lost among all beamsteering angles. Indicates presence of anisotropic scatterer (e.g. aligned structure in tissue) as value tends from 0.
Specific Attenuation Coefficient	SAC	The slope of a linear least-squares fit to the attenuation coefficient vs. frequency within the analysis bandwidth. (IEC 61391-2: 2010, 2010)
Attenuation Anisotropy	AA	The slope of the linear least-squares fit of SAC vs. beamsteering angle. Values significantly different from 0 suggest anisotropy in the tissue.

Table 2

Mean BSPD — Median (IQR lower – IQR upper)

		<i>m</i> BSPD (dB)	
		Anterior	Posterior
Unripened	Dist	4.96 (3.36 – 6.14)	6.09 (4.02 – 6.84)
	Mid-Dist	8.17 (5.74 – 9.34)	5.40 (4.05 – 5.42)
	Mid	9.09 (8.80 – 10.70)	6.21 (5.20 – 6.62)
	Mid-Prox	8.65 (7.11 – 9.32)	5.97 (3.86 – 6.03)
	Prox	3.54 (3.04 – 4.11)	3.41 (2.67 – 3.63)
Ripened	Dist	3.58 (3.13 – 4.05)	3.70 (3.10 – 4.27)
	Mid-Dist	6.26 (3.98 – 7.41)	3.71 (3.47 – 5.39)
	Mid	7.45 (5.02 – 8.51)	4.34 (3.46 – 7.33)
	Mid-Prox	6.21 (4.50 – 6.84)	4.30 (3.49 – 4.97)
	Prox	3.60 (2.50 – 4.33)	2.55 (2.22 – 4.84)

Table 3

Attenuation Anisotropy — Median (IQR lower – IQR upper)

		AA (dB·cm ⁻¹ MHz ⁻¹ degree ⁻¹)	
		Anterior	Posterior
Unripened	Dist	−0.006 (−0.030 – 0.001)	−0.015 (−0.030 – −0.009)
	Mid-Dist	0.022 (0.013 – 0.056)	0.020 (0.011 – 0.036)
	Mid	0.038 (0.035 – 0.041)	0.023 (0.021 – 0.032)
	Mid-Prox	0.019 (0.010 – 0.032)	−0.002 (−0.002 – 0.027)
	Prox	0.001 (−0.003 – 0.010)	−0.004 (−0.007 – 0.000)
Ripened	Dist	0.000 (−0.012 – 0.008)	0.007 (0.003 – 0.010)
	Mid-Dist	0.025 (0.007 – 0.031)	0.022 (0.011 – 0.027)
	Mid	0.026 (0.012 – 0.034)	0.019 (0.011 – 0.035)
	Mid-Prox	0.022 (0.008 – 0.031)	0.018 (0.005 – 0.034)
	Prox	0.010 (−0.004 – 0.022)	0.005 (−0.003 – 0.017)

Table 4

Parameters from the mixed effects regression model. Statistical significance is defined as p -values < 0.05 . NC means the parameter did not contribute to the best-fit model.

<i>Multivariate Mixed Effects Regression Model</i>						
	θ_{norm}	αBSPD	$m\text{BSPD}$	AA	SAC(θ°)	SAC(θ_{norm})
Location	<0.001	0.001	0.309	0.161	0.055	0.02
Location ²	<0.001	<0.001	<0.001	<0.001	<0.001	0.032
Side	NC	0.004	NC	NC	NC	NC
Ripened State	NC	0.048	0.025	NC	NC	NC