Ex vivo ultrasound attenuation coefficient for human cervical and uterine tissue from 5 to 10 MHz

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1. Introduction

Several investigators have utilized variations in ultrasonic properties such as the propagation speed [1–4] and attenuation [2–14] to differentiate between normal and diseased tissue. Bamber and Hill [15], measured the ultrasonic attenuation and propagation speed in bovine and human soft tissues in the temperature range from 5–65 °C, while Worthington and Sherar [16] measured these properties in the porcine kidney. Both groups measured the attenuation using a broadband (transducer bandwidth of 50% or greater) substitution method. Literature results on the variation of the ultrasonic propagation speed and attenuation coefficients in human and animal tissues have been summarized in Wells [17], Goss et al. [18], and Duck [19].

One of the most common reasons for ultrasound imaging of the pelvis remains dysfunctional uterine bleeding [20,21]. Common causes include the presence of leiomyomas or fibroids [22–24], adenomyosis [20], endometrial polyps, endometrial cancer, endometrial hyperplasia among other conditions. However, conventional ultrasound imaging cannot reliably differentiate myometrial abnormalities such as adenomyosis and leiomyomas, or endometrial abnormalities such as hyperplasia, polyps, and cancer [21,25]. Measurement of the stiffness [26–29] and acoustic [24,30–34] properties of the cervix and uterus and associated pathologies may provide important insights on ability to differentiate between tissue types.

Pre-term labor and birth is associated with about 12% of all pregnancies in the United States. Ultrasound measurements of the cervical length have been utilized to determine the risk for pre-term labor [35,36]. Previous work has also indicated that ultrasound scattering may be related to the organization and density of collagen in the cervix. McFarlin et al. [32] report that with a progression in pregnancy in rats the collagen content of the cervix increases, however, the fibrils become more disorganized thereby increasing the spacing between the fibrils. They reported on the variation of quantitative ultrasound (QUS) parameters namely the scatterer diameter and acoustic concentration during pregnancy, with the scatterer diameter remaining within the standard deviation however the acoustic concentration decreased significantly (~130 to ~141 dB/mm³ over days 15–21) as the cervix ripened. This result was also corroborated by Feltovich et al. [37] who reported an increased spacing (from a mean spacing of 20 nm to 30 nm ± 2 nm) between the fibrils with progress in pregnancy while their diameter (from about 53 to 58 nm ± 3 nm standard error of the mean) remained unchanged. Bigelow et al. [33] hypothesized that as the spacing between the collagen fibrils increases the ultrasonic attenuation of the cervix would decrease, since the surrounding tissue is filled with water and other enzymes. Quantitative ultrasound imaging techniques that utilize attenuation
estimates have the potential to detect changes in the cervical microstructure that occur during pregnancy. During cervical ripening, the collagen fibers are not as organized altering the stiffness of the cervix and a reduction in the attenuation slope is expected [30]. Studies have also shown that the amount of collagen in the cervix is lower for women with cervical insufficiency [38].

Keshavarzi et al. [31] present the only peer-reviewed report on measurement of the attenuation coefficient in fresh human uterine fibroid and myometrial tissue before and after high-intensity focused ultrasound treatment in the 1–3 MHz frequency range. Their results indicate a linear increase in the attenuation coefficient of both the fibroids and myometrial tissue with frequency, with the attenuation increasing after high-intensity focused ultrasound treatments, from 0.9 to 2.2 and 1.8 to 3.9 dB/cm², respectively, for fibroids and 0.5 to 1.6 and 1.7 to 3.3 dB/cm², respectively, for myometrium. The increase in attenuation coefficient as reported is due possibly to the loss of fluid from the tissue and the presence of gas or vapor bubbles in the high-intensity focused ultrasound region.

In this paper, we present the ultrasonic attenuation coefficient in ex vivo human uterine and cervical tissue, in the 5–10 MHz frequency range, where the attenuation coefficient increases monotonically following a power law. The attenuation coefficient was measured using a narrowband substitution technique.

2. Materials and methods

Uterine and cervical tissue samples from 24 women ranging in age from 31 to 79 years (mean age 50.4 ± 10.8 years) were used to determine their ultrasound attenuation coefficient in the 5–10 MHz range. Tissue samples were obtained from the Department of Pathology in accordance with a protocol approved by the University of Wisconsin's Institutional Review Board (IRB). Each patient had been diagnosed with a pathology necessitating a complete hysterectomy, requiring removal of the entire uterus and cervix. All surgical procedures were performed at the University of Wisconsin hospitals and clinics in Madison, Wisconsin. Following surgical resection, the entire organ was transferred to the surgical pathology facility. Rectangular specimens approximately 25 mm on a side and approximately 5 mm thick were excised from the uterine and cervical walls by a pathologist and placed in saline to be transported to our laboratory for acoustic and tissue stiffness measurements [28,29].

Samples were tested typically within 2 h of the organ being transferred to the surgical pathology facility. The time delay was due to the availability of the pathologist to prepare samples, and for transporting the samples from the surgical pathology facility to our laboratory where the experiments were conducted. The specimens were kept immersed in saline in a refrigerator and

![Fig. 1. Schematic diagram of the uterus (a) the labeled boxes indicate the approximate location of the tissue samples, along with the fiber orientation (b) of the uterine tissue samples.](image-url)
allowed to come to room temperature prior to testing. All physical
dimensions were measured four times using a pair of dial calipers,
and the results averaged. From each patient, a part of the cervix
and two orthogonally oriented sections of the uterine wall were
obtained. In the uterine samples, the muscle fibers generally ran
either perpendicular or parallel to the short axis of the sample.
These orientations are denoted here by the terms uterus ⊥ and
uterus ||, respectively. This was not done with the cervical tissue
due to the limited amounts of material that could be obtained from
an intact cervix. However, the fibers in the cervical tissue sample
generally ran perpendicular to the short axis of the sample. Leiomyoma or uterine fibroid tissues were also obtained whenever
available. The leiomyomas had no distinct fiber orientation.

Fig. 1a is a schematic of the uterus showing the locations from
which each sample was obtained, and Fig. 1b indicates the fiber
orientation with respect to the sample’s short axis. Table 1 summar-
izes the number of samples tested by component. The difference
in the number of samples for each component is due to viability
of the tissue, as determined by the pathologist. For example, in
the case of one patient, no normal uterine tissue was obtained
due to the profusion of leiomyomas. However, that case did pro-
vide a viable cervical tissue sample.

Ultrasonic attenuation of the tissue samples were measured in
the frequency range 5–10 MHz using commercially available unfo-
cused single element transducers with a 7.5 MHz center frequency
with a −6 dB bandwidth of 6.348 MHz or 85% bandwidth and ele-
ment diameter of 0.125 mm (Valpey Fisher Corporation, MA, USA).
Two identical transducers were utilized for the through transmis-
sion measurements to ensure that they posses similar point spread
functions. Ultrasound frequencies in this range are frequently used
for real-time clinical imaging of uterine and cervical abnormalities.

Fig. 2 shows a schematic diagram of the apparatus used for the nar-
rowband substitution measurements. The testing chamber was an
acrylic cylinder, filled with room temperature de-ionized water in
two reservoirs. The upper reservoir held the sample and the receiv-
ing transducer was immersed in the water bath in the upper reser-
voir. A Saran film (thickness 25 μm) separated the two reservoirs.
The lower reservoir with a depth of 2 cm held only water, and it
ensured that the sample would always remain beyond the near-
field region of the transmitting transducer. The bottom was sealed
off with Saran film, underneath which the transmitting transducer
was situated. Ultrasound gel was used to couple the transmitting
transducer to the Saran film.

A narrowband through transmission substitution technique
[39] was utilized to measure the attenuation coefficient of the tis-
sue sample. We measure the reduction in signal amplitude with
and without the sample present in the acoustic path between the
transmitting and receiving transducers. A sinusooidal signal burst
in the desired frequency range with a 20-cycle duration was first
obtained using a function generator Agilent 33250A (Santa Clara,
CA, USA). This signal was amplified using a commercially available
signal amplifier (Model 75A250A, Amplifier Research, PA, USA) be-
fore being transmitted through the transducer. After propagating
through the water only, or the water and sample path, the refer-
ence and attenuated signal was picked up by the receiving trans-
ducer and sent it to the oscilloscope (LeCroy 9310L) for display
and subsequent measurement. Measurement of the time-shift for
sound speed measurements was performed at the center of the sig-
nal burst to avoid ambiguities due to signal loss due to attenuation,
along with the reduction in signal amplitude for attenuation
measurements.

Measurements were made on the water path without the sam-
ple before and after measuring the signal with the sample present.
The sample itself was measured five times in different locations in
order to obtain independent measurements. Given these inputs,
the attenuation coefficient is determined by measuring the ampli-
tude of the ultrasonic wave according to

\[
\text{Table 1}
\begin{array}{|c|c|}
\hline
\text{Component} & \text{Number of samples tested} \\
\hline
\text{Cervix} & 20 \\
\text{Uterus ⊥} & 19 \\
\text{Uterus ||} & 14 \\
\text{Leiomyoma} & 6 \\
\hline
\end{array}
\]

Fig. 2. Schematic diagram of the measurement apparatus.
\[ a \, [\text{dB/cm}] = \frac{20}{d} \log_{10} \left( \frac{V_0}{V_1} \right), \]

where \( V_0 \) and \( V_1 \) are the peak-to-peak voltages of the signal received at the oscilloscope of the attenuated ultrasonic wave through the water path and the sample, respectively, and \( d \) is the path thickness.

In order to obtain accurate measurements of the attenuation coefficient, the acoustic transmission properties of the Saran layers have to be considered [40]. Both the entrance and exit transmission coefficients for propagation through the Saran layers for the bottom reservoir are used to obtain the correction factor. Eqs. (1)–(3) in Wear et al. [40] describe the correction procedure for transmission through the Saran layer. Data for the frequency-dependent attenuation was acquired by varying the transmit center frequency using the function generator and reading the resulting change in voltage response directly off the oscilloscope display.

3. Results and discussion

The frequency dependence of the ultrasonic attenuation coefficient for the three tissue types is shown in Fig. 3. The data points are the mean observations and the error bars represent one standard deviation from the mean. Not all error bars are displayed indicating that fiber orientation influences ultrasound attenuation. However, the true separation may not be clear, given the size of the error bars.

The increase in cervical attenuation with increasing frequency when compared to that of the uterus is likely due to the tissue composition. According to Winkler and Rath (1999), the cervix consists mainly of collagen (70% Type I, 30% Type III, and a small amount of Type IV). It is only 5–10% smooth muscle. The uterus, on the other hand consists mainly of smooth muscle. The increased attenuation in the fibroid compared to normal uterine tissue (15 dB/cm vs. 8 dB/cm at 7.5 MHz) is possibly due to the increased disorder in fiber orientation, leading to higher extinction/scattering of the propagating ultrasound waves.

4. Summary

The results in this paper present ultrasonic attenuation coefficient variations in ex vivo human uterine and cervical tissue using a transmit center frequency of 7.5 MHz. The results show that in the 5–10 MHz range, the attenuation coefficient increases monotonically, and follow a power law. The results extend the knowledge acquired previously by other researchers in a lower frequency range. Quantitative measurement of the attenuation may also help in the development of noninvasive ultrasound based approaches for the characterization of uterine and cervical microstructure.

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References


