Direct ultrasonic velocity measurements of mammalian collagen threads

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Direct ultrasonic velocity measurements have been made in fresh collagen threads from mammalian tendon at a frequency of 100 MHz using the scanning laser acoustic microscope. Tendon is of interest since it contains an unusually large amount of collagen, a structural protein thought to be important to the echographic visualizability of normal and pathologic tissues in medical ultrasound. Results indicate that ultrasonic velocity is appreciably higher than in soft tissues, lending support to the view that tendon, as well as collagen, is markedly distinguished ultrasonically from other biological tissues.

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INTRODUCTION

Collagen is the most abundant single protein in the human body and the most common protein in the entire animal kingdom. Closely associated with the connective tissue of vertebrates, collagen comprises between 25%–33% of the total protein in the human body, and therefore about six percent of the total body weight. There is some evidence to suggest that it is the elastic properties of soft tissues, determined primarily by the content of collagen and other structural proteins, which define acoustic contrast during echographic visualization. This hypothesis is based on the fact that the static or low-frequency elastic modulus (Young’s) of collagenous fibers is at least 100 times greater than those of the parenchymal tissues. Since the ultrasonic velocity is proportional to the square root of the elastic modulus, collagenous tissues are thought to introduce a greater impedance mismatch than would be the case for a tissue interface of similar elastic modulus, thereby increasing the acoustic reflectivity of soft (parenchymal) tissue–collagenous tissue boundaries.

While a correlation suggests that tissues with higher collagen content appear to exhibit ultrasonic velocities greater than those of lesser amounts of collagen, the quantitative determination of ultrasonic velocity in tissues which predominate comprise collagen fibers has received little attention. Acoustic microscopy has been used in this study to examine ultrasonic propagation properties in collagen fibers at 100 MHz. Specifically, quantitative determination of the ultrasonic velocity and qualitative evaluation of ultrasonic attenuation have been made.

I. METHOD

A scanning laser acoustic microscope (Sonomicroscope 100®, Sonoscan Inc., Bensenville, Illinois), operating at a frequency of 100 MHz, is employed in both the examination and quantitative velocity determination of the collagen specimens. The operational details of this instrument may be found elsewhere, and are only summarized here. The specimen is placed on a sonically activated fused silica stage and is covered with a mirrored coverslip which also provides for a small amount of light transmission. Mechanical perturbations of the coverslip surface due to the acoustic energy transmitted through the specimen are detected by a focused, scanning laser beam probe. These disturbances occur at the acoustic frequency of 100 MHz and are proportional to the acoustic amplitude in each region. The laser light transmitted through the coverslip and specimen allows for the formation of an optical image electronically in perfect register with the acoustic image.

Velocity determinations in small tissue components are made by detecting variations in the acoustic index of refraction using the acoustic interferometry mode. Here, the displacement of acoustic interference fringe lines, measured graphically, is related to regional variations in acoustic index of refraction, as described in detail in the Appendix. The velocity of sound in the specimen can be found from

\[ c_s = \frac{c_0}{\sin \theta_0} \left[ \tan^{-1} \left( \frac{1}{\tan \theta_0} - \frac{1}{N \lambda_p T \sin \theta_0} \right) \right] \]

where \( c_0 \) is the velocity of sound in the medium surrounding the specimen, \( \theta_0 \) is the angle from the normal of acoustic beam in the medium surrounding the specimen, \( N \) is the normalized lateral fringe shift, \( T \) is the thickness of the specimen, and \( \lambda_p \) is the wavelength of sound in the medium surrounding the specimen. The medium surrounding the collagen specimens is 0.9% saline, for which \( c_0 \) is 1507 m/s at 22 °C. Velocity variations on the order of 1%–2% may be discerned using this technique, assuming a specimen thickness of approximately 100 µm and an achievable accuracy of the fringe displacement measurement of 0.1 fringe. Specimen thickness introduced perhaps the greatest single source of error, as this could only be measured to within ±10% using either a calibrated light microscope, the unperturbed fringe spacing, or a grid cut onto the coverslip to "calibrate" the acoustic micrograph.

II. SPECIMEN PREPARATION

Tail tendons were surgically removed within minutes post mortem from 10-month-old female, and 12-month-
old male LAFi/J mice (Jackson Labs), and placed im-
mediately in 0.9% saline solution at room temperature
(22°C). Single tendon fibers, sometimes referred to
as primary fiber bundles,10 ranging in size from 42–
115 μm in diameter, were isolated, placed on the acous-
tic microscope stage under no tension, and again bathed
with 0.9% saline solution. Care was taken to assure that
the specimen did not dry out. A doughnut shaped spacer
was placed between the stage and coverslip to prevent the
coverslip from compressing or crushing the small fiber.
Acoustic measurements were performed at room tem-
perature, usually within 1–2 h post mortem.

III. RESULTS AND DISCUSSION

The acoustic micrographic image of the tendon fibers,
shown in Fig. 1, is indicative of seemingly an acoustical-
lly homogeneous thread, with few attenuating structures
detectable from within the fiber. This is in contrast to
the optical image of the same fiber (Fig. 2), which is
seen to be opaque. The relatively low acoustic attenua-
tion noted here could be the result of either the specimen
size (42–115 μm) or the attenuating properties of the
thread or a combination thereof owing to the approximate
limit of detectability of 5-dB attenuation as determined
by attenuation measurements of water with the micro-
scope. For example, the ultrasonic attenuation at a
frequency of 1 MHz is about 1 cm−1 in high collagen con-
tent tissue,4 and assuming a linear frequency dependency
and extrapolating to 100 MHz yields 100 cm−1. For a 60-
μm-thick specimen, this results in a total attenuation
around 5 dB. However, for the thicker specimens the
total attenuation remained within the limits of detectabil-
ity, suggesting that the actual attenuation for the collagen
thread may be less than 5 dB per 115 μm at 100 MHz. Fur-
ther studies will be required to define the nature of this
observation.

Velocity measurements in fresh mouse tendon threads
(Table I) at 100 MHz yield a value of 1733 ± 56 m/s, for
eight specimens taken from two different animals.
Figure 3 shows a typical interferogram from which the ve-
locity data are obtained. To assure that fluid loss from
the specimen during mounting on the stage did not result
in abnormally high measured values of velocity, these
same specimens were dried in air for 30–127 min, then
placed on the microscope stage in 0.9% saline for sev-
eral minutes and remeasured, yielding a value of 1747

TABLE I. Summary of ultrasonic velocity data in mouse tail
tendon fibers at 100 MHz. Average value for wet fibers is
1733 ± 56 m/s, while for rehydrated 1747 ± 66 m/s.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Twet (μm)</th>
<th>cwet (m/s)</th>
<th>Time Dried (min)</th>
<th>Trehydrated (μm)</th>
<th>crorehydrated (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–2</td>
<td>63–65</td>
<td>1716–1723</td>
<td>30</td>
<td>60–75</td>
<td>1740–1810</td>
</tr>
<tr>
<td>10–18</td>
<td>70–75</td>
<td>1751–1771</td>
<td>95</td>
<td>75–89</td>
<td>1736–1754</td>
</tr>
<tr>
<td>10–25</td>
<td>65–75</td>
<td>1740–1782</td>
<td>122</td>
<td>75–90</td>
<td>1697–1740</td>
</tr>
<tr>
<td>10–33</td>
<td>42–50</td>
<td>1798–1867</td>
<td>127</td>
<td>50–70</td>
<td>1757–1890</td>
</tr>
<tr>
<td>11–14</td>
<td>90–100</td>
<td>1657–1675</td>
<td>55</td>
<td>75–83</td>
<td>1738–1946</td>
</tr>
<tr>
<td>11–25</td>
<td>55–75</td>
<td>1697–1778</td>
<td>60</td>
<td>75–90</td>
<td>1688–1704</td>
</tr>
<tr>
<td>11–35</td>
<td>100–105</td>
<td>1689–1710</td>
<td>63</td>
<td>100–115</td>
<td>1647–1670</td>
</tr>
<tr>
<td>12–14</td>
<td>99–107</td>
<td>1664–1699</td>
<td>67</td>
<td>79–95</td>
<td>1712–1799</td>
</tr>
</tbody>
</table>
While the mean velocity of the rehydrated specimens is somewhat greater than that of the fresh samples, the variation (less than 1%) is within the 3%–4% standard deviation among all of the specimens, and is not considered significant.

Velocity measurements with the scanning laser acoustic microscope on dental\textsuperscript{11} and myocardial\textsuperscript{12} tissues have been in agreement with independent, lower frequency measurements. In addition velocity measurements with the microscope used in this study on soft polyethylene sheets are in agreement with handbook values.\textsuperscript{3}

While these collagen thread velocity measurements are about 10–20% greater than those obtained for parenchymal tissues at much lower frequencies, they are commensurate with the value of velocity in beef tendon (whole, measured parallel to fiber direction) reported by Dussik and Fritch, of 1750 m/s at 1 MHz.\textsuperscript{14} O’Brien\textsuperscript{4} has shown that the following relation exists between ultrasonic velocity $v$, and the wet weight percentage of tissue collagen $C$, for nine tissues in the 1–10-MHz frequency range

$$v = 1588 + 32 \ln C$$

and, for tendon collagen (30% wet weight\textsuperscript{15,16}) this expression yields about 1700 m/s which is within 2% of the measurements reported herein. Similar measurements of ultrasonic velocity in liver tissue at 100 MHz with the acoustic microscope\textsuperscript{17} have identified little difference from that reported at much lower frequencies,\textsuperscript{18} indicating that if a velocity dispersion does exist over the 1–100 MHz frequency range in this tissue, it is much smaller than the differences identified in the present study between parenchymal tissues and collagenous fibers. Since networks of similar collagenous fibers are interspersed in varying amounts throughout all body tissues and organs, such velocity differences may be responsible, at least in part, for variations in the ultrasonic impedance, and thus the subsequent visualizability, of tissues and organs. Tissue density, however, also contributes to the acoustic impedance. Results from the literature\textsuperscript{19,20} indicate that the density of molecular collagen in the native state is 1.16–1.33 g/cm$^3$, which is appreciably greater than that found in soft tissues, which is usually assumed to be similar to that of water. It appears then that the density, as well as the ultrasonic velocity is greater than that found in soft tissues, and suggests that the echographic visualizability of tissue is dependent, to some degree, on the amount and arrangement of collagen within a particular tissue.\textsuperscript{2}

\textbf{ACKNOWLEDGMENTS}

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\textbf{APPENDIX}

The following derivation is based upon unpublished results of L. W. Kessler and P. R. Palermo (Sonoscan, Inc., Bensenville, Illinois) and is provided to clarify the phenomenon and process responsible for obtaining acoustic velocity measurements.

Figure A1 schematically depicts the acoustic, constant phase wavefronts and their relationship to the interference pattern displayed on the Sonomicroscope 100© monitor.

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figureA1.png}
\caption{Schematic representation of acoustic, constant phase wavefronts and their relationship to the interference pattern displayed on the Sonomicroscope 100© monitor.}
\end{figure}

The following derivation is based upon unpublished results of L. W. Kessler and P. R. Palermo (Sonoscan, Inc., Bensenville, Illinois) and is provided to clarify the
This phenomenon permits the quantitative determination of ultrasonic velocity and is further described in Fig. A2 wherein the acoustic energy is incident upon the stage surface-specimen interface at the angle $\theta_s$. Two separate but superimposed transmitted wavefronts are shown, one into a known medium at an angle $\theta_0$ and the other into an unknown medium at an angle $\theta_\alpha$, the relationship being described by Snell's Law, viz.,

$$\sin \theta_\alpha = \frac{c_\alpha}{c_0} \sin \theta_0$$

(A1)

or, as a single frequency prevails,

$$\sin \theta_\alpha = \frac{\sin \theta_0}{\lambda_\alpha} = \frac{\sin \theta_\alpha}{\lambda_\alpha}$$

(A2)

In Fig. A2, the unknown medium is shown to have an ultrasonic velocity greater than the known medium, and both have the same thickness $T$. The normalized lateral fringe shift is defined as the distance the fringe shifts (in this case to the right) from the known fringe to the unknown fringe, divided by the distance between two adjacent known fringes, that is,

$$N = \frac{\text{line segment } ad}{\text{line segment } ab}$$

(A3)

From triangle $abc$, where line segment $bc$ is perpendicular to and at the distance $\lambda_\alpha$ from the wavefront $ae$, it can be seen that

$$\text{line segment } ab = \frac{\lambda_\alpha}{\sin \theta_\alpha}$$

(A4)

and, similarly, from triangle $ade$,

$$\text{line segment } ad = \frac{T}{\tan \theta_s} - \frac{T}{\tan \theta_\alpha}$$

(A5)

yielding

$$N = \frac{T}{\lambda_\alpha} \left[ \frac{1}{\tan \theta_\alpha} - \frac{1}{\tan \theta_s} \right]$$

(A6)

Therefore, the ultrasonic velocity $c_\alpha$ in the unknown specimen is determined by first calculating $\theta_\alpha$ from Eq. (A6), and substituting this value into Eq. (A1).

For example, if the known medium is water at 25 °C ($c_0 = 1497$ m/s) and the acoustic microscope stage is fused silica ($c_\alpha = 5968$ m/s), $\theta_0 = 10.22^\circ$ for $\theta_s = 45^\circ$. Given a normalized lateral fringe shift of 0.5 and a specimen thickness of 100 μm, $\theta_\alpha$ is calculated to be (at an ultrasonic frequency of 100 MHz) $11.04^\circ$, which yields $c_\alpha = 1616$ m/s.

17W. O. O'Brien, Jr., "Ultrasonic velocity and qualitative...