Correlation of Corneal Acoustic and Elastic Properties in a Canine Eye Model

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PURPOSE. To examine the correlation between corneal acoustic impedance and Young’s modulus in a canine eye model.

METHODS. Twenty canine globes were recovered from healthy animals. Corneal acoustic impedance was measured in the intact globes using two methods: a quantitative ultrasound spectroscopy method and the reflection amplitude method. The intraocular pressure was maintained at 10 mm Hg during the ultrasound measurements. Corneal strips were then prepared for standard uniaxial tensile tests. Young’s moduli at various strain levels and those at a loading level equivalent to that for ultrasound measurements were compared with the acoustic impedance of the same cornea.

RESULTS. The mean acoustic impedance of the canine corneas was $1.72 \pm 0.05$ MPa $\cdot$ m/s using the quantitative ultrasound spectroscopy method and $1.71 \pm 0.04$ MPa $\cdot$ m/s using the reflection amplitude method. Young’s secant modulus was $1.07 \pm 0.08$ MPa at 1% strain and $2.01 \pm 0.98$ MPa at 5% strain, and the tangent modulus was $1.28 \pm 0.69$ and $3.16 \pm 0.71$ MPa, respectively. Significant linear correlations between acoustic impedance and Young’s modulus (at 1%-5% strains) were found in the measured canine corneas. The correlation remained strong when comparing the two parameters measured under equivalent loading.

CONCLUSIONS. This study suggests a potentially strong correlation between corneal acoustic impedance and Young’s modulus at low strain levels. If such correlation also exists in the human eye, it may allow the noninvasively determined acoustic impedance to be used as a surrogate for Young’s modulus, which is difficult to obtain in vivo. (Invest Ophthalmol Vis Sci. 2011;52:731–736) DOI:10.1167/iovs.10-5723

There has been a growing interest in measuring and understanding corneal biomechanical properties, because these properties may play an important role in the normal function and the pathophysiology of the eye. For example, keratoconus is associated with corneal thinning and softening. Ablative refractive surgery may induce significant changes in corneal biomechanical properties, leading to complications. Corneal stiffness also may be confounded in the tonometric measurement of intraocular pressure (IOP), in that stiffer corneas may lead to overestimation, while more compliant corneas lead to underestimation of IOP. Recent studies have also indicated that corneal stiffness may affect the eye’s ability to dampen IOP spikes. Determination and monitoring of corneal biomechanical properties will thus enable us to better study the mechanisms of these ocular conditions, and facilitate the development of new technologies for their detection and diagnosis.

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Tensile and inflation tests have been performed in the past on postmortem corneas, and these studies have yielded important results for understanding the biomechanical behavior of the cornea. It is still desirable to characterize corneal biomechanical properties noninvasively. In vivo biomechanical characterization has also been explored by examining corneal deformation under the force of a rapid air puff (Ocular Response Analyzer [ORA]; Reichert, Inc., Depew, NY). Corneal hysteresis and the resistance factor measured by ORA may provide new diagnostic information, but it is generally believed that these parameters do not always represent the elastic properties of the cornea.

We have investigated an ultrasound method that measures corneal properties noninvasively and has the potential to translate into in vivo settings. Ultrasound is mechanical energy, and its propagation causes microscopic deformations within the supporting medium. Acoustic parameters, including acoustic impedance, speed of sound, and attenuation are affected by the microstructure, density, and elasticity of the material in which the sound waves propagate. For example, the characteristic acoustic impedance of a lossless medium is a function of aggregate modulus and density:

$$Z = \sqrt{\frac{M}{\rho}}$$

where $Z$ is the acoustic impedance, $M$ is the aggregate modulus, and $\rho$ is the density.

Acoustic methods have been established since the 1970s to determine elastic properties of bones. To our best knowledge, the relationship between corneal acoustic properties and Young’s modulus has not been studied before. The goal of this study was to investigate whether there is a correlation between corneal acoustic impedance (determined by ultrasonic measurements) and corneal Young’s modulus (determined by tensile tests) in a canine eye model. The acoustic impedance of the cornea can be determined from the amplitude of the reflected ultrasound signals calibrated to that from a material with known properties. It can also be calculated from aggregate modulus and density by applying equation 1. A secondary goal of this study is to compare the two methods of measuring corneal acoustic impedance: (1) the reflection amplitude method, and (2) the quantitative ultrasound spectroscopy method that provides an estimation of corneal aggregate modulus and density.

Canine eyes were used in this study because of the following considerations. First, they are readily available from a local animal shelter and can be tested within a short postmortem time because of the scheduled euthanization and immediate recovery of the globes. Second, both corneal thickness and modulus in dogs have been shown to be similar to those in humans in our initial testing results on canine corneas and the reported data on human corneas. Third, the variance in the tissue properties of the natural-born animals are likely to be more representative of the general population than laboratory animals or those raised for slaughter whose age and genetic background could be narrow within a short sampling period.

Disclosure: He X, None; Liu J, None

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MATERIALS AND METHODS

Sample Preparation
Twenty fresh canine globes were collected from a local animal shelter. The globes were recovered from healthy dogs that were humanely euthanatized for population control purposes. All globes were collected within 1 hour of death and stored in a moist chamber at 4°C before experimental use. Ultrasound reflections of the cornea were measured by A-mode ultrasound, while the globe was immersed in a saline bath. Three readings were taken for each sample during a brief immersion (less than 2 minutes) of the eye. Data from the three measurements were processed, and the average was used for further analysis. During the ultrasound measurements, the IOP of the canine eyes was maintained at approximately 10 mm Hg, with a saline column of 10 mm in height. The height of the saline column was adjusted such that a saline column was used to couple the acoustic waves between the cornea and the saline bath. After the height of the saline column was adjusted, the IOP was confirmed by hand-held tonometer (Tonopen XL, Reichert, Inc.). After the ultrasound measurement, the corneal strips were dissected, and corneal strips of 6.5 mm in width that spanned from limbus to limbus were prepared. The strips were preserved in mineral oil after dissection, to prevent dehydration.

Quantitative Ultrasound Spectroscopy
The details of the quantitative ultrasound method and its validation have been described previously. Briefly, the ultrasonic measurement system is composed of a transducer, a pulser-receiver, a digitizer, and a computer (Fig. 1a). During the ultrasonic measurements, the globes were immersed in a saline bath, which served as the coupling medium for the ultrasound pulses to transfer from the transducer to the globe. The globes were immersed in a saline bath. Three readings were taken for each sample during a brief immersion (less than 2 minutes) of the eye. Data from the three measurements were processed, and the average was used for further analysis. During the ultrasound measurements, the IOP of the canine eyes was maintained at approximately 10 mm Hg, with a saline column of 10 mm in height. The height of the saline column was adjusted such that a saline column was used to couple the acoustic waves between the cornea and the saline bath. After the height of the saline column was adjusted, the IOP was confirmed by hand-held tonometer (Tonopen XL, Reichert, Inc.). After the ultrasound measurement, the corneal strips were dissected, and corneal strips of 6.5 mm in width that spanned from limbus to limbus were prepared. The strips were preserved in mineral oil after dissection, to prevent dehydration.

Determination of Acoustic Impedance from Reflection Amplitudes
Assuming that a sample is immersed in water and the angle of the ultrasonic beam is perpendicular to the sample surface (normal incidence), the reflection coefficient (R) can be calculated:

\[ R = \frac{A_s}{A_0} \frac{Z_s - Z_w}{Z_s + Z_w} \]  

Figure 2, the peak-to-peak value of the reflected signals can be used to determine the amplitude of the reflection. In this study, the mean amplitude (A_r, which is the average of the reflection amplitudes from anterior and posterior surfaces of the cornea) was used to calculate corneal acoustic impedance. The reference signal was measured from a material (Soflens-59; Bausch & Lomb, Rochester, NY) with a density of 1.14 g/mL and a speed of sound of 1675 m/s (measured on the bulk material). The acoustic impedance of the reference material was calculated as the product of density and speed of sound. The amplitude of the incident acoustic waves (A_0) can be calculated:

\[ \frac{A_r}{A_0} = \frac{Z_s - Z_w}{Z_s + Z_w} \]
Ultrasound Transducer

FIGURE 2. Illustration of acoustic impedance calculation from the reflection amplitudes. The mean amplitude (A$_{1}$) of the reflection from the anterior (A$_{a}$) and the posterior surfaces (A$_{p}$) of the cornea was used.

where A$_{1}$ is the amplitude of the reflected signal at the reference/water interface, Z$_{c}$ is the acoustic impedance of the reference material, and Z$_{w}$ is the acoustic impedance of water. With the calculated A$_{a}$ and the measured A$_{c}$, the acoustic impedance of the cornea, Z$_{c}$, can be calculated:

\[
\frac{A_{1}}{A_{a}} = \frac{Z_{c} - Z_{w}}{Z_{c} + Z_{w}} \tag{4}
\]

Uniaxial Tensile Tests

Standard uniaxial tensile tests were applied on corneal strips with a rheological analyzer (Rheometrics System Analyzer III; Rheometrics Scientific, New Castle, DE). The sample was coupled between a motor and a transducer that measures the resultant force generated by sample deformation. The initial sample length between the two gripping jaws was approximately 10 mm. Sample geometric information, including width and thickness, was input into the analyzer’s control panel. The sample thickness was measured by using the A-scan ultrasound. Stress and strain were computed by the device by using the initial sample geometry. A preload of 20 mN was applied to each sample to precondition and flatten the tissue. After preloading, the sample length was recorded automatically by the RSA device. The sample was then subjected to a constant strain rate of 0.1% per second until strain reached approximately 6%. The data were stored on the hard disc of the computer for further processing. The strain rate was selected from the typical values used in the literature.$^{22-24}$

Statistical Analysis

Data are presented as the mean ± SD. The Pearson correlation coefficients were calculated (SAS software; ver. 9.1, SAS Institute Inc., NC) to analyze the correlations (1) between corneal acoustic impedance and Young’s modulus at different strain levels and (2) between the two corneal acoustic impedance values measured by reflection amplitudes and the quantitative spectroscopy method.

RESULTS

The mean canine corneal acoustic impedance obtained from the quantitative ultrasound spectroscopy method was $1.72 ± 0.05$ MPa·s/m with a range from 1.61 to 1.81 MPa·s/m. The mean acoustic impedance calculated from reflection amplitudes was $1.71 ± 0.04$ MPa·s/m with a range from 1.63 to 1.76 MPa·s/m. Strong linear correlation was observed between the two acoustic impedances measured by the reflection amplitude method and the quantitative spectroscopy method ($R = 0.97$, $P < 0.001$). Figure 3 shows the comparison of the acoustic impedance obtained from these two methods.

Young’s moduli were calculated from the stress-strain curves obtained from uniaxial tensile tests at different strain levels. The secant modulus was calculated as the ratio of stress and strain, and the tangent modulus was estimated based on curve (i.e., exponential) fitting. The mean secant modulus increased with increased strain levels from 1.07 ± 0.48 MPa at 1% strain to 2.01 ± 0.98 MPa at 5% strain. The mean tangent modulus increased from 1.28 ± 0.69 to 3.16 ± 0.71 MPa. As shown in Figure 4, the nonlinearity of the stress-strain relationship was evident in all tested corneal samples. The mean and the SD of Young’s moduli at different strain levels and their correlation with the acoustic impedance are summarized in Table 1. When compared at a specific strain level, the correlation decreased at higher strains. For example, the Pearson correlation between secant modulus and acoustic impedance measured by the quantitative ultrasound spectroscopy method was 0.93 at 1% strain and decreased to 0.80 at 5% strain. The same trend was observed for the correlation between the modulus and acoustic impedance measured by reflection amplitudes.

It is noted that both secant modulus and tangent modulus showed a large variance in the canine corneas. For example, at 1% strain, the range was from 0.21 to 2.05 MPa for secant modulus and 0.20 to 2.69 MPa for tangent modulus. The ratio between the lowest and the highest modulus was about 10 for all strain levels tested.

Figure 5 presents the correlation between acoustic impedance measured by the ultrasound spectroscopy method and secant modulus at different strain levels (for clarity, up to 3% strain was plotted). Linear regression lines are also shown, with

\[
\text{Acoustic Impedance} = \frac{Z_{c} - Z_{w}}{Z_{c} + Z_{w}}
\]

\[
\text{Quantitative ultrasound spectroscopy method} = \frac{Z_{c} - Z_{w}}{Z_{c} + Z_{w}}
\]

$\text{Reflection amplitude method} = \frac{Z_{c} - Z_{w}}{Z_{c} + Z_{w}}$

$\text{Linear Regression Line}$

FIGURE 3. Comparison of corneal acoustic impedance determined from the quantitative ultrasound spectroscopy method and the reflection amplitude method. Strong correlation was observed ($R = 0.97$, $P < 0.001$).
slopes increasing at higher strains because of the higher modulus values at higher strains.

Because of the nonlinear nature of corneal stress/strain relationship, the modulus is dependent on the strain level. During ultrasonic measurements, the corneas were subject to an IOP of 10 mm Hg, which would yield different stresses/strains in different corneas due to the difference in their geometric properties (i.e., thickness and radius of curvature) and material properties. To examine whether nonlinearity affects the correlation, the data were further analyzed to compare acoustic impedance and modulus obtained under equivalent stress/strain levels. The stress that each cornea experienced during ultrasound measurements was estimated. As shown in Figure 6, the circumferential stress \( \sigma \) caused by IOP \( P \) can be estimated by Laplace’s Law:

\[
\sigma = \frac{P \cdot r}{2t}
\]  

assuming an average radius of curvature \( r \) of 8.5 mm for canine corneas (based on reports in the literature for medium to large dogs)\(^{25}\) and using the measured corneal thickness \( t \) from analysis of the ultrasound data. The modulus was then obtained from the stress–strain relationship at the corresponding stress.

Figure 7 shows the correlation between corneal acoustic impedance measured by the quantitative ultrasound spectroscopy method and the secant modulus at different strain \( \epsilon \) levels. The correlation coefficient and the secant modulus at equivalent loading \((R = 0.93, P < 0.001)\). The linear regression equation is

\[
y = 6.95x - 10.90
\]  

where \( x \) is corneal acoustic impedance, and \( y \) is corneal secant modulus.

It is noted that the strains corresponding to the same IOP varied significantly across the tested corneas, due to the difference in corneal geometrical and mechanical properties. In this study, the range of corneal strains corresponding to a 10 mm Hg IOP loading was from 0.5% to 3.7%.

**DISCUSSION**

Corneal acoustic impedance was measured by both the quantitative ultrasound spectroscopy method and the reflection amplitude method in canine eyes. The results showed a strong correlation between corneal acoustic impedance and the well-studied corneal Young’s modulus obtained from uniaxial tensile tests.

Corneas are known to exhibit nonlinear mechanical behavior.\(^{10}\) We have analyzed the potential effect of nonlinearity and found it did not significantly alter the observed correlation at low

**TABLE 1. Summary of the Canine Corneal Moduli and the Correlations between Corneal Modulus and Acoustic Impedance**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Modulus (MPa) (Mean ± SD)</th>
<th>Correlation Coefficient ( R ) Modulus vs. Acoustic Impedance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Secant</td>
<td>Tangent</td>
</tr>
<tr>
<td>1%</td>
<td>1.07 ± 0.48</td>
<td>1.28 ± 0.69</td>
</tr>
<tr>
<td>2%</td>
<td>1.28 ± 0.61</td>
<td>1.80 ± 0.90</td>
</tr>
<tr>
<td>3%</td>
<td>1.54 ± 0.74</td>
<td>2.28 ± 1.14</td>
</tr>
<tr>
<td>4%</td>
<td>1.80 ± 0.88</td>
<td>2.74 ± 1.41</td>
</tr>
<tr>
<td>5%</td>
<td>2.01 ± 0.98</td>
<td>3.16 ± 1.71</td>
</tr>
<tr>
<td>Equivalent loading</td>
<td>1.05 ± 0.40</td>
<td>1.28 ± 0.47</td>
</tr>
</tbody>
</table>

The last row presents data calculated at stresses corresponding to a 10-mm Hg IOP loading. Spec, spectroscopy method; Amp, amplitude method.
strains levels, which are typically what the cornea experiences under physiological pressure loadings. At higher strains (>4%), the correlation was still significant but weaker, most likely because of the nonlinear effect and the larger variance of modulus at higher strains. The strong correlation may reflect the rather simple composition of cornea. As a collagenous tissue, corneal collagen content may have similar influence on its density and Young’s modulus. For instance, higher collagen content is likely to be associated with a higher density and a higher stiffness. Acoustic impedance represents the resistance to sound passing through the material. A stiffer and denser material usually exerts greater resistance and thus has higher acoustic impedance. Other tissue properties such as collagen cross-linking may also affect the elastic properties and the ultrasound reflectance in similar ways.

The linear regression equation (equation 6) shows that a rather small difference in acoustic impedance could correspond to a large difference in Young’s modulus with an amplification factor of about 7. This result may be interpreted as follows: First, the measurement of acoustic impedance should be accurate if we use it to determine Young’s modulus (i.e., measurement error could be amplified). Accuracy should be readily achievable in an in vitro setting where precision devices can be used for positioning the ultrasound transducer with respect to the cornea. It would be more challenging to implement that in vivo; nonetheless, it is feasible since the cornea is a superficial layer of tissue and the measurement is not complicated by any anatomic barriers. Second, although corneal acoustic impedance may only vary by several percentage points, the modulus could vary by severalfold, which is consistent with previous findings that the corneal modulus has significant variance across subjects. Third, although in general the value of the modulus for a given cornea is sensitive to strain levels, it was fairly constant at low strain levels (Fig. 4). Thus, the correlation between acoustic impedance and modulus could hold at low strains regardless of the strain levels.

This study also showed that the quantitative spectroscopy method and the reflection amplitude method had good agreement in terms of measuring acoustic impedance. For the present study, with a goal of estimating Young’s modulus based on acoustic impedance, the reflection amplitude method would be sufficient and might be advantageous, because it involves only direct analysis of the radiofrequency ultrasound data without needing a wave propagation model. The quantitative spectroscopy method, however, offers more detailed information about corneal properties.

The correlation between corneal acoustic impedance and Young’s modulus may have implications for measuring and understanding corneal biomechanics, because the former can be determined noninvasively, either in the enucleated globe or in the living eye. For ex vivo characterization, the ultrasound measurement can be performed without tissue dissection, allowing simultaneous measurement of corneal elasticity and other ocular parameters that require the structural integrity of the whole globe. One can also envision using the acoustic impedance as a surrogate and thus implement an approach that allows quantitative determination and longitudinal monitoring of corneal elastic properties in vivo. This approach may be useful for screening refractive surgery candidates and diagnosing and monitoring treatment of keratoconus. In addition, the information of corneal elastic properties may be incorporated into biomechanical models to correct tonometric measurements of IOP.

Direct measurement of corneal stiffness (e.g., elastic modulus) in live eyes remains a challenge. Corneal hysteresis and
corneal resistance factor, measured by ORA, are two examples of the few biomechanical parameters that are currently available for clinical use. The ORA measurements are quick (within seconds) and convenient (noncontact), ideal for clinical use. In comparison, the ultrasound measurements require a water bath to couple the acoustic waves to the cornea. In addition, accurate measurement of acoustic impedance requires precise positioning and alignment of the ultrasound probe with respect to the cornea. Thus, the clinical use of the ultrasound method is likely more technically demanding than that of ORA. The ultrasound method, however, could provide an estimation of corneal modulus based on the strong correlation with acoustic impedance as observed in this study. On the contrary, corneal hysteresis and corneal resistance factor may not have a definitive correlation with modulus, because they are influenced by several corneal factors including both viscoelastic properties and geometric parameters.

Limitations of the present study include the following: Besides being nonlinear, the cornea has been shown to be viscoelastic. Thus, the strain rates used in the tensile tests may affect the measured properties. Higher strain rates tended to yield higher modulus for a viscoelastic material. Although the effect is less prominent at low strain levels, such as those used in the present study, the effect of strain rate should be investigated in the future. In addition, the acoustic impedance was only measured at one intraocular loading (which corresponded to various strain rates). Future studies are needed to examine whether corneal acoustic impedance is dependent on strain levels. The measurements were performed at room temperature, which differs from the in vivo situation. Temperature (25–40°C) is known to affect both mechanical and acoustical properties. Future studies are needed to compare the properties measured at body temperature, which are more representative of in vivo measurements. Importantly, the studies were performed in canine corneas, which may differ from human corneas. Future studies are needed to confirm the correlation in human eyes. In the present study, the cornea was treated as a single homogeneous layer and the through-thickness average of the acoustic properties was correlated with the mechanical properties obtained from strip testing. The sublayers such as corneal epithelium, Bowman’s, Descemet’s, and endothelium are not differentiable at the ultrasound frequencies used in the present study. However, it is possible to separately analyze the signals of the anterior and the posterior cornea, which may be of clinical interest, for example in the noninvasive measurement of biomechanical changes in the anterior stroma after cross-linking treatment. Corneas also have a strong anisotropy because the collagen fibers are mainly aligned parallel to the surface. Ultrasonic techniques have been developed in the past to characterize multilayered anisotropic composites. These approaches may be adopted for characterizing corneal anisotropy if the unique challenges related to cornea (such as its significant fluid content and curvature) can be successfully addressed.

In summary, the present study demonstrated a potentially strong correlation between acoustic impedance and Young’s modulus in the cornea. This correlation may be exploited to provide useful information for corneal biomechanical studies and noninvasive in vivo determination of corneal elastic properties.

References