The Shear Modulus of the Human Vocal Fold in a Transverse Direction

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Summary: The aim of this study was to measure the shear modulus of the vocal fold in a human hemilarynx, such that the data can be related to direction of applied stress and anatomical context. Dynamic spring rate data were collected using a modified linear skin rheometer using human hemilarynges, and converted to estimated shear modulus via application of a simple shear model. The measurement probe was attached to the epithelial layer of the vocal fold cover using suction. A sinusoidal force of 3 g was applied to the epithelium, and the resultant displacement logged at a rate of 1 kHz. Force measurement accuracy was 20 μg and position measurement accuracy was 4 μm. The force was applied in a transverse direction at the midmembranous point between the vocal process and the anterior commissure. The shear modulus of the three female vocal folds ranged from 814 to 1232 Pa. The shear modulus of the three male vocal folds ranged from 1021 to 1796 Pa. These data demonstrate that it is possible to obtain estimates for the shear modulus of the vocal fold while preserving anatomical context. The modulus values reported here are higher than those reported using parallel plate rheometry. This is to be expected as the tissue is attached to surrounding structures, and is under natural tension.

Key Words: Elasticity–Vocal fold–Rheometry–Shear modulus.

INTRODUCTION

Knowledge of the biomechanical properties of the vocal fold is an essential requirement for researchers seeking to develop mathematical models of phonation, and to provide benchmarks against which novel medical and surgical procedures can be objectively assessed. Biomechanical properties can be expressed in many different ways, and one widely accepted property is the material’s shear modulus (Figure 1). A material under stress has two flat surfaces, typically a rectangle or a column; one surface is fixed. A force $F$ is applied to the upper surface such that the material deforms as shown. The stress ($\sigma$) is defined as the amount of force applied divided by the surface area to which it is applied.

$$\sigma = \frac{F}{A},$$  \hspace{1cm} (1)

where $A$ is the surface area.

The strain ($\varepsilon$) is defined as the maximum tissue displacement $X$ divided by the thickness of the tissue $H$.

$$\varepsilon = \frac{X}{H},$$  \hspace{1cm} (2)

The shear modulus $G$ is the ratio of stress to strain.

$$G = \frac{\sigma}{\varepsilon},$$  \hspace{1cm} (3)

The value of $G$ in effect defines how much a substance will deform when a force is applied to it. It is sometimes called “stiffness,” as a “stiff” material will distort less than a “soft” material. Another common measurement is the Young’s modulus ($E$), which defines how much a material is extended when a force is applied to it. There is a simple formula that relates $E$ to $G$.

$$G = \frac{E}{2(1 + \nu)}$$  \hspace{1cm} (4)

where $\nu$ is Poisson’s ratio, which is a measure of the compressibility of a material. Human tissue is almost incompressible; and $\nu$ is usually simplified to be 0.5 such that

$$G = \frac{E}{3}$$  \hspace{1cm} (5)

Shear and Young’s moduli are used to predict how the tissue moves when subjected to external forces. For example, the change in vocal fold length when muscular force is applied as a result of stimulation of the recurrent laryngeal nerve can be derived from knowledge of the Young’s modulus. Another important physical property is the efficiency of the transfer of energy across boundaries, such as from aerodynamic into mechanical vibratory energy; which is directly related to the materials’ shear modulus. Knowledge of a tissue’s modulus therefore enables us to predict how it will work, and to develop rigorous mathematical models of phonation.

Research teams in Europe, the USA, and Japan are actively developing techniques to repair vocal fold tissue that has been damaged by scarring and other pathologies. Current approaches involve the use of hyaluronic acid implants, growth factors, and mesenchymal stem cells to stimulate tissue regeneration and improve wound healing outcomes. The ability to measure the biomechanical properties of the vocal fold without dissecting it out of anatomical context is an essential pre-requisite to determining the viability of any new tissue repair technique. A valid and reliable solution to this measurement challenge could form the basis of an in vivo tool for the biomechanical assessment of vocal fold tissue injury and therapeutic outcomes.
Although many research teams have presented vocal fold viscoelasticity data obtained from excised vocal fold tissue, few have reported data obtained from human larynges. Further, most published approaches have inferred modulus from secondary phenomena. Kaneko et al.\textsuperscript{13} and Tamura et al.\textsuperscript{14} reported data collected with ultrasound technology, but did not derive elastic moduli. Hsiao et al.\textsuperscript{15} reported the use of color Doppler imaging. McGlashan et al.\textsuperscript{16} used stroboscopy to perform measurements of mucosal wave velocity. Only Tran et al.\textsuperscript{17–19} have deployed a method that directly measures vocal fold modulus \textit{in vivo}. Their groundbreaking series of studies were completed in 1993, but were limited by a cumbersome apparatus.

Recently, a team based at Universitat Klinic Eppendorf (UKE) Hamburg successfully used novel instrumentation developed at DeMontfort University to obtain rheometric data from hemilarynges and human patients \textit{in vivo}.\textsuperscript{20–22} These devices are known as the linear skin rheometer (LSR) and the laryngeal tensiometer. The LSR has also been deployed at Harvard Medical School\textsuperscript{23} and the University of Wisconsin School of Medicine and Public Health\textsuperscript{24} to develop isocontour maps showing the variation of modulus over the surface of the vocal fold of a hemilarynx. These mapping studies used a needle to attach a probe to the vocal fold, and were therefore only capable of producing reliable relative data, not absolute measurements of tissue elastic modulus. This limitation was due to the lack of a constitutive equation to transform stress/strain data into a value for modulus. This paper reports additional data from measurement of the transverse shear modulus of a group of six larynges using a modified suction attachment placed against the vocal fold epithelium. We demonstrate that this modified attachment technique enhances measurement reliability and provides a recognizable geometric framework (a simple shear model) that can form the basis of a credible deduction for shear modulus.

**MATERIALS AND METHODS**

Measurements were taken from the right vocal folds of six excised human larynges (three males and three females; age range 33–89 years). Data were obtained using a modified LSR instrument.

All larynges were harvested from autopsy cases within 12 hours after death, quick-frozen in liquid N\(_2\) and stored at \(-80\) °C until use. The larynges were thawed at \(4\) °C one day before experimentation. Once thawed, a midline incision was made to create two hemilarynges, and the right hemilarynx was mounted horizontally to expose the right vocal fold. All samples were secured using pins, with care taken not to place tension on the \textit{in situ} vocal fold. Measurements were taken typically within 2 minutes of mounting, and the larynx was moistened using saline solution.

The LSR is a programmable tensiometer device capable of measuring displacement to a resolution of 4 \(\mu\)m, and force to a resolution of 20 \(\mu\)g.\textsuperscript{25} The force sensor can be attached to the tissue under test using a variety of specialist attachments. For this study, a 2-mm bore cannula was used to attach the force sensor to the vocal fold epithelium, using a right-angled opening and 50 mbar of negative pressure was applied using a vacuum pump. The vacuum pump was disengaged during data collection.

The experimental setup is shown in Figure 2. The hemilarynx was mounted to expose the vocal fold. The LSR probe was attached to the epithelium such that resultant shear force was applied in the transverse direction, similar to the direction of airflow from the lungs. Using the simple shear model described in the introduction of this article, the geometry of this setup can be defined as follows. The shear force \(F\) applied by the LSR is transmitted into the vocal fold over the area determined by the probe diameter \(A\). The displacement \(X\) is the resultant shear strain, which is tangential to the epithelial surface, and is measured by the LSR. The thickness of the tissue \(H\) is obtained from published data. This setup allowed derivation of an estimate for the shear modulus \(G\).

The suction probe was attached to the LSR, which is mounted rigidly, and positioned such that the there was no gap between the suction probe and the epithelium, at which time 50 mbar of suction was applied. Although we cannot guarantee that this applies no stress to the vocal fold, this arrangement minimizes the effect. In addition, as the LSR operates by applying a cyclical force, any DC offset due to initial loading is removed. The force sensor and suction attachment were gently cycled in a sinusoidal...
manner such that a cyclical shear force of 1 g was applied to the vocal fold. Five measurements were taken from the same position without removing the suction probe. The LSR is mounted such that the probe rests in the tissue thus minimizing the applied vertical force. The built-to-order load cell used in the LSR was supplied by Maywood (Reading, UK), and has a full-scale reading of 50 g, with an overall accuracy of 0.02 g. The linear variable displacement transducer was used to derive dynamic spring rate (DSR), defined below. DSR is a mechanical engineering term. It is defined as the change in length of a material when a unit of force is applied to it so that it is either stretched or compressed. It is not a time-dependent term. By applying knowledge of the geometry of the experimental setup, it is possible to estimate the shear modulus of the vocal fold.

Conversion of DSR data to an estimate of shear modulus is achieved by applying a simple shear model, with a correction to take account of effects due to adjacent attached tissue. The DSR is a measure of the amount of shear force required to achieve a unit of shear displacement.

A sinusoidal force $F$ is applied to the material under test and the resultant displacement $X$ is logged.

$$F = F_{\text{max}} \sin(t)$$

$$X = X_{\text{max}} \sin(t + \tau)$$

where $F_{\text{max}}$ is the maximum force, $t$ is the time, $X_{\text{max}}$ is the maximum displacement, and $\tau$ is the phase shift in radians.

The DSR of the tissue is defined as $F_{\text{max}}/X_{\text{max}}$, and is expressed in grams per millimeter. As we are not using the time-dependent information associated with the sinusoidal nature of the applied force, we can substitute $F$ for $F_{\text{max}}$ and $X$ for $X_{\text{max}}$. DSR can then be used to estimate the shear modulus of the displaced vocal fold tissue using knowledge of the geometry of the test site, as follows.

The stress $\sigma$ is the applied force $F_{\text{max}}$ per unit area $A$ given by

$$\sigma = \frac{F_{\text{max}}}{A}$$

The resultant strain $\varepsilon$ is given by tangential displacement $X_{\text{max}}$ per material thickness $H$.

$$\varepsilon = \frac{X_{\text{max}}}{H}.$$ 

Shear modulus $G$ is defined as stress per unit strain.

$$G = \frac{\sigma}{\varepsilon}.$$ 

As $DSR = F_{\text{max}}/X_{\text{max}}$ then

$$G = DSR \frac{H}{A}. \quad (11)$$

The thickness of the adult human vocal fold lamina propria is typically 1 mm, which is the figure used for $H$. The area of attachment is obtained by measuring the bore of the suction attachment, which in this study was 2 mm.

It is important to note that this simple shear model does not make any allowance for the attached tissue, which is also subjected to shear stresses due to displacement of the tissue directly underneath and surrounding the suction attachment. This effect drops off rapidly as the force transmitted through a solid is inversely related to distance. However, a rigorous mathematical solution to describe the elastic processes involved has not been published. In the absence of a mathematical solution for the shear modulus of tissue attached to other tissue, we incorporated a simple correction derived experimentally based on a widely accepted mathematical model developed by W.C. Hayes. This model derives shear modulus from indentation data. We first evaluated this correction methodology using data collected from 40 human hemilarynges at UKE, which were tested using both the Hayes indentation method and the LSR. The data sets from the two methods correlated well when the surface area of attachment used to analyze the LSR data was increased by 0.75 mm in all dimensions. Based on these results, we used a comparable correction to the data in this study by increasing the diameter of the area of attachment from 2 mm by 1.5 mm to 3.5 m.

RESULTS

Table 1 contains demographic, DSR, and estimated shear modulus data. The shear modulus of the three female vocal folds ranged from 814 to 1232 Pa. The shear modulus of the three male vocal folds ranged from 1021 to 1796 Pa. The sample size in this study is too small to allow any generalized conclusions; however, these data are similar in magnitude to those obtained in the 40 larynges studied at UKE.

DISCUSSION

Finding similar published results to compare these findings with is challenging, as most of the published vocal fold viscoelasticity data are obtained from excised vocal fold covers. Chan and Titze, jointly and separately, have published extensive data using results obtained with a parallel plate rheometer taken

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**TABLE 1.**

Estimated Shear Modulus of the Human Vocal Fold Using a Modified Simple Shear Model
at the same frequency as the LSR. Their results ranged from 10 to 100 Pa. Our estimates of modulus are higher as we are measuring the same tissue, but in anatomical context; thus, the vocal fold is tensioned and anchored, resulting in higher values for modulus. The only other data set in the literature addressing vocal fold shear modulus in anatomical context was published by Tran et al., who reported in vivo data from four subjects ranging from 2450 to 29 400 Pa. These values exceed those reported using excised mucosa with parallel plate rheometry, and hemilynges with the LSR.

Of key interest are the results for the coefficient of variance (CoFV) in the data set, which averaged 3.8%. This contrasts with previous work that used adhesives, rather than suction, to attach the LSR probe to the vocal fold epithelium, and yielded a mean CoFV of 8.4%. This demonstrates that suction, as opposed to a needle or adhesive, appears to be a superior means of attachment.

CONCLUSIONS

The objective of this study was to measure the shear modulus of the vocal fold without dissecting the tissue out of a hemilynx. This was successfully achieved using the LSR device, which appears to be a potentially useful tool for making point-specific measurements of vocal fold shear modulus in whole larynges. The coefficients of variation obtained with the suction attachment in this study are superior to those obtained with alternative modes of attachment, such as pins or adhesives.

A major obstacle that needs to be overcome is the lack of a mathematical model for deriving the shear modulus that takes account of the tissue that is attached to the area that is under direct stress. However, the results and methods under development are of great value. The LSR technique can be reliably used to measure relative changes in tissue modulus, and as such, holds value for comparative studies. Thus, it can be used to measure changes due to the application of tissue augmentation materials, or to quantify tissue stiffness resulting from vocal fold scarring. The ability to measure relative change will be used in a new study that will attempt to quantify change in vocal fold tension with respect to stimulation of the recurrent laryngeal nerve.

We are now pursuing development of an in vivo device to apply this methodology in the intraoperative setting, which can be used to measure relative change in modulus, but also requires a rigorous mathematical solution to enable derivation of absolute values. A mathematical derivation of shear modulus that takes account of attached tissue therefore remains an important area for theoretical progress. Although the analytical solution presented here appears to hold value in yielding a credible estimate of shear modulus, these data require further mathematical qualification. Regardless of this, however, the application of the LSR methodology to derive point-specific measurements with respect to anatomical position and direction of stress means that this technique is presently most suited to obtaining relative, rather than absolute, measurements of modulus. To this end, we are presently collecting data using a ratiometric technique to quantify the anisotropic nature of the vocal fold, and initiating further work aiming to map variation in modulus with respect to anatomical position.

REFERENCES


