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High Frequency Measurements of Viscoelastic Properties of Hydrogels for Vocal Fold Regeneration

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Abstract

This report describes a torsional wave experiment used to measure the viscoelastic properties of vocal fold tissues and soft materials over the range of phonation frequencies. A thin cylindrical sample is mounted between two hexagonal plates. The assembly is enclosed in an environmental chamber to maintain the temperature and relative humidity at *in vivo* conditions. The bottom plate is subjected to small oscillations by means of a galvanometer driven by a frequency generator that steps through a sequence of frequencies. At each frequency, measured rotations of the top and bottom plates are used to determine the ratio of the amplitudes of the rotations of the two plates. Comparisons of the frequency dependence of this ratio with that predicted for torsional waves in a linear viscoelastic material allows the storage modulus and the loss angle, in shear, to be calculated by a best-fit procedure. Experimental results are presented for hydrogels that are being examined as potential materials for vocal fold regeneration.

Keywords

Viscoelastic properties; Torsional waves; Vocal folds; Hyaluronic acids; Torsional wave device

Introduction

Vocal folds are composed of twin folds of mucous membrane stretched horizontally across the larynx. Each vocal fold consists of a pliable vibratory layer of connective tissue, known as the lamina propria (LP), sandwiched between an epithelium membrane and a *vocalis* muscle [1, 2] or vocal cord. Great varieties of sounds are articulated by humans as the vocal folds move together and apart in a wavelike motion at frequencies of 75 to 1,000 Hz [3]. However, voice overuse and abuse can impose greater strain on this delicate system than it can sustain. As a result, damage occurs. Healing leads to the formation of scar tissue that disrupts the natural pliability of the LP, resulting in phonotrauma in the superficial lamina propria (SLP). [4,5]

Tissue engineering methods hold promise for the restoration of functional vocal folds. However, the unique biochemical composition, structural organization, and viscoelastic properties of vocal folds pose significant challenges for vocal fold regeneration. Because both natural vocal fold tissues and replacement biomaterials are soft and viscoelastic, the ability to measure—at phonation frequencies—the biomechanical properties of natural vocal fold tissues as well as candidate replacement materials is prerequisite to the development of workable tissue engineering approaches.

Over the past few years, several research efforts have addressed the measurement of the viscoelastic properties of vocal fold tissues. For vocal fold lamina propria (LP), the most important viscoelastic property to measure is its complex shear modulus because voice production involves primarily distortional vibration of the LP, not changes in its volume [6]. In an effort [7] using a commercial rheometer, the vocal fold LP was placed between two parallel plates and subjected to a precisely controlled sinusoidal torque from the top plate. However, because of instrument inertia, the upper frequency limit for making reliable measurements in such a stress-controlled rheometer was about 10 Hz; 15 Hz was deemed marginally acceptable. To overcome this limitation, a strain-controlled rheometer [8] was introduced to extend the upper frequency limit to 50 Hz. By further reducing the inertia of the instrument, Chen and Lake [9,10] developed a torsional apparatus that they used to measure the viscoelastic shearing response of materials at frequencies from 10^{-6} Hz to approximately 10^4 Hz. They attached one end of a cylindrical sample to a fixed framework and drove the other end by an electromagnetic torque acting on a permanent magnet. Rotation of the driven end is measured optically. By minimizing the inertia of the torque generator, and of the system used for the measurement of rotation, the fundamental resonant frequency of the system can be made quite large, comparable to the 10^4 Hz limitation on the apparatus.

However, the validity of all of these techniques is based on the assumption that the stress is nominally uniform through the thickness of the sample. [11,12] From consideration of elastic waves, this assumption holds when the time required for round trip transit of stress waves through the thickness of the sample is much less than the period of a single oscillation, i.e.

$$f \ll \frac{c_s}{2h} = \frac{\sqrt{G/\rho}}{2h} \quad (1)$$

where f is the frequency, c_s , G , ρ are, respectively, the shear wave speed, the shear modulus, and the density of the sample; h is the thickness of the sample. For vocal fold tissues, the sample thickness is approximately 0.3 mm, ρ is approximately $1,000 \text{ kg/m}^3$, and G may be as small as 100 Pa. Then, from equation (1), valid testing frequencies must be much less than 500 Hz, say 30 Hz.

In addition, there is a frequency limitation corresponding to a requirement that the stresses due to sample inertia be small relative to those due to sample viscosity [11]. This limitation can be expressed as [11–13]:

$$f \ll \frac{\eta}{\pi \rho h^2} \quad (2)$$

where η is the viscosity of the sample. For vocal folds, the frequency dependence of the viscosity has been described approximately by $\eta = \eta_0 f^{-0.85}$ with $\eta_0 = 1.0 \text{ Pa s}^{0.15}$ [7]. Substitution of this expression into equation (2) gives $f \ll 83 \text{ Hz}$ or, say, $f < 10 \text{ Hz}$. The accuracy of these limitations (1) and (2) is re-examined in “Discussion” of this paper.

As mentioned above, the human phonation regime is from 75 to 1,000 Hz. The highest frequencies for which rheometers can be used successfully for measuring the viscoelastic response of vocal fold tissues are near or below the lower limit of this range. Additional methods [14–16] have been introduced to measure the viscoelastic properties of materials at frequencies above the range covered by rheometers. However, these methods either do not determine the shear modulus directly, or the sample sizes required are not compatible with the small, thin geometries of vocal folds. Other methods have been used to measure the viscoelastic properties of vocal fold tissues [17] or hydrogels [18]. Despite several attractive features, all of these methods are subject to frequency limitations of the type mentioned above. Therefore, there is need for a technique to measure the viscoelastic properties of soft materials at phonation frequencies.

Principle of Torsional Wave Experiment

To overcome the frequency limitations imposed by the assumption of uniform deformation, the underlying concept of the torsional wave experiment described here is to treat the deformation of the sample as a wave propagation problem.

To this end, consider a cylindrical, viscoelastic sample attached to two rigid plates [11,12], as shown in Fig. 1. When the bottom plate rotates at angular velocity $\Omega_0(t)$, a torsional wave propagates through the thickness of the sample and causes the top plate to rotate at an angular velocity of $\Omega_h(t)$. The torsional moment at position z at time t can be represented as

$$T(z, t) = 2\pi \int_0^a r^2 \tau(z, r, t) dr \quad (3)$$

where a is the radius of the sample and $\tau(z, r, t)$ is the shear stress corresponding to the strain rate history $\dot{\gamma}(z; r; t)$. Like many other soft biological tissues, vocal fold tissues exhibit linear viscoelastic properties when subjected to small strains [19]. Therefore, $\tau(z, r, t)$ can be expressed as

$$\tau(z, r, t) = \int_0^t G(t - t') \dot{\gamma}(z, r, t') dt' \quad (4)$$

where $G(t)$ is the viscoelastic relaxation modulus in shear. The deformation within the sample is assumed to be described adequately as pure torsion in which planes of constant z rotate about the axis of symmetry by an amount $\theta(z, t)$ so that the condition of kinematical compatibility becomes

$$\begin{aligned} \dot{\gamma}(z, r, t) &= \frac{\partial v(z, r, t)}{\partial z} \\ &= \frac{\partial \dot{\theta}(z, t)}{\partial z} r \\ &= r \frac{\partial \dot{\Omega}(z, t)}{\partial z} \end{aligned} \quad (5)$$

where $v(z, r, t)$ is the (circumferential) particle velocity and $\Omega(z, t)$ is the angular velocity of the cross-section. Substitution of equations (5) and (4) into equation (3), gives

$$T(z, t) = J \int_0^t G(t - t') \frac{\partial \Omega(z, t')}{\partial z} dt' \quad (6)$$

where $J = \frac{\pi a^4}{2}$ is the polar moment inertia of the cross-sectional area. Consideration of the rate of change of angular momentum for a circular slice of the sample gives the dynamical equation

$$\rho J \frac{\partial \Omega(z, t)}{\partial t} = \frac{\partial T(z, t)}{\partial z}. \quad (7)$$

Substitution of equation (6) into equation (7) gives the following integro-partial-differential equation for $\Omega(z, t)$

$$\begin{aligned} \rho \frac{\partial \Omega(z, t)}{\partial t} \\ = \int_0^t G(t - t') \frac{\partial^2 \Omega(z, t')}{\partial z^2} dt'. \end{aligned} \quad (8)$$

We seek a solution of equation (8) that corresponds to imposed rotation $\theta_0(t)$ at $z=0$ and attachment to a free-to-rotate, rigid plate at $z=h$. For harmonic excitation, the boundary conditions for this problem are

$$\theta(0, t) = \theta_0 \exp(i\omega_0 t) \quad \text{at } z=0 \quad (9)$$

$$-J \int_0^t G(t - t') \frac{\partial \Omega(z, t')}{\partial z} \Big|_{z=h} dt' = \rho_0 I_0 \frac{\partial \Omega(h, t)}{\partial t} \quad (10)$$

where θ_0 and ω_0 are, respectively, the amplitude and frequency of the excitation; $\rho_0 I_0$ is the polar moment of inertia of the top plate. For a hexagonal plate, I_0 is given by $I_0 = \frac{5\sqrt{3}}{8} l^4 h$, where l and h are the side length and thickness, respectively. Solving equations (8)–(10), one obtains the rotation in the form (See Appendix)

$$\theta(z, t) = M(\omega_0, z) \theta_0 \cos(\omega_0 t - \phi(\omega_0, z)) \quad (11)$$

in which $M(\omega_0, z)$ is the amplification factor and $\phi(\omega_0, z)$ is the phase shift. At $z=h$, $M(\omega_0, h)$ can be expressed as

$$M = \frac{c}{\sqrt{c^2 [\cos^2(\xi\alpha) + \sinh^2(\xi\beta)] + d^2 [\cosh^2(\xi\beta) - \cos^2(\xi\alpha)] + cd(\beta \sinh(2\xi\beta) - \alpha \sin(2\xi\alpha))}} \quad (12)$$

and $\phi(\omega_0, h)$ is given by

$$\tan(\phi) = \frac{(c \sin(\xi\alpha) + d\alpha \cos(\xi\alpha)) \sinh(\xi\beta) + d\beta \sin(\xi\alpha) \cosh(\xi\beta)}{(c \cos(\xi\alpha) - d\alpha \sin(\xi\alpha)) \cosh(\xi\beta) + d\beta \cos(\xi\alpha) \sinh(\xi\beta)} \quad (13)$$

where

$$c = c(\omega_0) \equiv J \sqrt{|G^*(\omega_0)|} / \rho \quad (14a)$$

$$d = d(\omega_0) \equiv \rho_0 I_0 \omega_0 \quad (14b)$$

$$\xi = \omega_0 h \sqrt{\frac{\rho}{|G^*(\omega_0)|}} \quad (14c)$$

$$\alpha = \cos\left(\frac{\delta(\omega_0)}{2}\right), \quad \text{and} \quad \beta = \sin\left(\frac{\delta(\omega_0)}{2}\right) \quad (14d)$$

in which the complex modulus $G^*(\omega)$ is expressed in terms of its magnitude $|G^*(\omega)|$ and its phase shift $\delta(\omega)$ (errors in expressions (12) and (13) in [11] and [12] have been corrected here).

If the responses $M(\omega_0, h)$ and $\phi(\omega_0, h)$ are measured over a range of frequencies, then the moduli $|G^*(\omega_0)|$ and $\delta(\omega_0)$ can be estimated by means of regression analysis. To simplify the calculations, we take the viscoelastic moduli at the frequency of the resonance peak to be $|G^*(\omega_0)|$ and $\delta(\omega_0)$. Values for these two parameters are readily obtained from the regression analysis using an Excel spreadsheet and minimizing the differences between calculated and measured values of the amplification factor.

Experimental Setup

Figure 2 shows the experimental setup [12]. A thin, cylindrical sample of soft material is placed between two hexagonal plates: the ‘top plate’ and ‘bottom plate’ shown in the inset of Fig. 2. These two plates are vertically aligned and share a common axis. The bottom plate is mounted on the drive shaft of a galvanometer (GSI Lumonics, optical scanner Model 000-G112) that can rotate back and forth at angles up to $\pm 6^\circ$ and frequencies up to 2,500 Hz. To ensure that the shear strains in the sample are sufficiently small for linear viscoelasticity to be a satisfactory approximation, the imposed rotations are kept less than $\pm 0.2^\circ$. This assembly is enclosed in an environmental chamber in order to provide *in vivo* conditions. Compared to the soft viscoelastic sample, the acrylic bottom and top plates can be considered to be rigid. Therefore, when the bottom plate is oscillated, a torsional wave propagates up and down the sample. From the wave analysis described above, the complex modulus of a particular sample can be readily calculated from the measured rotations of the top and bottom plates.

Rotations of the top and bottom plates are measured by an optical lever technique as shown in Fig. 2 and Fig. 3 [11,12]. One facet of each plate is mirror-polished and aluminized. Two laser

beams (laser is from Coherent, Model INNOVA 300) are brought into the chamber and reflected off the aluminized facets of the two plates (Fig. 2 shows only the optical setup for the rotation of the top plate). The reflected beam first goes through a spherical lens, becomes focused on a point and continues to expand horizontally and vertically. Subsequently, the beam goes through a cylindrical lens and begins to focus horizontally. Finally, a thin vertical sheet of light forms at the butterfly-shaped mask, as shown in the 'End View' of Fig. 3. When the top plate oscillates, the vertical line of laser light moves back and forth on the mask. As the line moves away from the center of the mask, more light passes through the mask and is collected by the photodiode located behind the mask. As a result, the output of the photodiode is proportional to the angular rotation of the plate. A similar optical layout is used to measure the rotation of the bottom plate.

The oscillation of the galvanometer is driven by a frequency generator (Agilent, Model 33120A) and an amplifier controlled by a computer. The frequency generator steps through a sequence of frequencies from a minimum frequency, $f_{\min}$, to a maximum frequency, $f_{\max}$, with steps of Δf . At each frequency, the amplitude of the rotation of each plate is obtained as the average amplitude over a fixed number of cycles, usually 10, after transients from the change in frequency die out. The output of the photodiodes is monitored by an oscilloscope (Agilent, Model 54622A) that provides direct measurement of the average amplitude of the signal and its minimum value. The difference between these two quantities is proportional to the rotation of the respective plate. The experimentally determined amplification factor is obtained as the ratio of the amplitude of the rotation of the top plate divided by that of the bottom plate. Calibration differences between the recorded outputs for the two plates are accounted for by adjusting the experimentally determined amplification factor to approach the required value of unity as the frequency goes to zero.

Figure 4 shows a typical output of the amplification factors at successive frequencies, with records of the wave traces at several frequencies. The top trace is the measured rotation of top plate and the bottom one is the measured rotation of the bottom plate. Every two periods of the sinusoidal wave forms corresponds to one period of the rotation of the corresponding plate. Figure 4 clearly shows that as the frequency is stepped up the amplitude of the rotation of the top plate increases and that of the bottom plate stays almost the same or even decreases a little. Eventually the amplification factor reaches a peak value at the resonance frequency, f_{peak} , and then decreases.

Amplification factors for each frequency, determined from the experimental records as described above, are compared with those predicted by the viscoelastic model described in the previous section. For that model, the viscoelastic description of the materials is expressed in terms of the amplitude of the complex shear modulus, $|G^*(\omega)|$, and the loss angle, $\delta(\omega)$. The height and diameter of the sample, required for the wave analysis, are obtained from digital images of the sample in place. For each test, values of $|G^*|$ and δ are obtained that provide the best fit between the model and the experimental results over a range of frequencies spanning the frequency f_{peak} . Once $|G^*|$ and δ are determined, the storage modulus G' and the loss modulus G'' can be easily calculated from $G' = |G^*| \cos(\delta)$ and $G'' = |G^*| \sin(\delta)$.

To ensure that this experimental technique can capture the real viscoelastic properties of soft materials, we have measured the viscoelastic properties of a silicone putty (polydimethylsiloxane) [20]. This material is often used as a standard material to calibrate rheometers. Its viscoelastic properties under standard conditions are well known. Figure 5 shows the measured results of storage modulus, loss modulus and $\tan(\delta)$ for the silicone putty. The results measured by the torsional wave device are compared with those measured by a strain controlled rheometer [8] for a sample with the same gel point (as provided by TA instruments). Figure 5 shows that the storage modulus, loss modulus and $\tan(\delta)$ determined by

torsional wave experiments follow the natural trend of the measured results at low frequencies. The frequency range covered by the torsional wave measurements is from 200 to over 1,000 Hz, which is an order of magnitude higher than the upper limit achieved by rheometers.

Materials

Our strategies for voice restoration include two parallel approaches. The first involves the creation of injectable materials to improve pliability of damaged vocal folds. The second is to use tissue engineering methods to “grow” new vocal fold tissue [21]. The extracellular matrix (ECM) of vocal fold LP is composed of two families of macro-molecules, the fibrous proteins (mainly elastin and collagen) and ground substance (glycosaminoglycans). Of the fibrous portion of the vocal fold ECM, collagen constitutes approximately $43\pm 3\%$ of LP total protein [22]. It plays a pivotal role in withstanding shear stress during phonation. Of the amorphous ground substances, hyaluronic acid (HA) is of primary importance in modulating vocal fold behavior and properties. HA is a non-sulfated glycosaminoglycan found throughout the ECM of the lamina propria. HA likely contributes to the maintenance of an optimal tissue viscosity to facilitate phonation, and an optimal tissue stiffness that may be important for controlling vocal fundamental frequency [23]. Therefore, collagen/HA-based composite hydrogels are attractive scaffolding materials for vocal fold tissue engineering.

Material Compositions and Synthesis Procedure

Prior to hydrogel preparation, HA was chemically modified with varying amounts of sodium periodate (NaIO_4) following reported procedures [24]. The resulting polymers are designated as HACHO1:4, HACHO1:2 and HACHO1:1 respectively, with the numbers indicating the molar ratio of NaIO_4 to the HA repeating unit during chemical modification. To prepare the composite hydrogels, HACHO was dissolved in a HEPES solution at a concentration of 20 mg/mL. Type I collagen from rat tail tendon (9.37 mg/mL, BD Biosciences) was neutralized with NaOH (1 M), $10\times$ DPBS (Gibco), and DI H_2O to reach a concentration of 8.33 mg/mL. Phenol red was added to both solutions as a pH indicator. The ice-cold collagen and HA solutions were subsequently mixed and quickly vortexed. The final mixture contains 5 mg/mL collagen and 8 mg/mL HA, respectively. Hydrogel disks were obtained after incubating the solution (0.2 mL each in 1 cc syringe) at 37°C for 20 h. The as-synthesized hydrogel samples were snap-frozen with liquid nitrogen and stored at -80°C . Samples were thawed with running tap water prior to testing.

Swelling Ratio

The as-synthesized gels were dehydrated by passing through graded ethanol solutions followed by drying under vacuum. After measuring their dry weight (W_d), the hydrogels were placed in DPBS for 24 h. The gels were then placed on tilted glass slides to remove excess water, and their wet weight (W_w) was measured. The swelling ratio (SW) was obtained by taking the ratio of the wet weight to the dry weight.

Experimental Results

The environmental chamber was maintained at 34°C to 37°C , with the relative humidity at greater than 94%. Samples of thin cylindrical discs were cut with a dermal punch, with diameters ranging from 3.5 to 5 mm and thicknesses from 0.2 to 0.9 mm. The location of the resonance peak varies as the sample geometry is changed.

Figure 6 shows the frequency dependence of amplification factors for the HA/collagen gels investigated. Best-fit models are shown as curves, while the experimental results are shown as symbols. Also included in Fig. 6 are optical images of the samples (the thin discs) sandwiched

between the plates. The sample dimensions (height h and radius a), and the viscoelastic moduli G' and δ that provide the best fit between model predictions and experimental results are summarized in the figure caption. It is obvious that the linear viscoelastic wave analysis provides a remarkably good fit to the observed frequency dependence of the amplification factor over a range of frequencies spanning the peak.

Figure 7 shows the measured storage modulus G' and $\tan(\delta)$ as a function of resonance frequency. Storage moduli are shown as solid symbols and $\tan(\delta)$ values are shown as hollow symbols. The frequency range of these tests varies from 80 to 170 Hz—about the typical physiological frequency range of voice production for men [3]. Figure 7 clearly shows that for these three materials, both storage modulus and $\tan(\delta)$ stay almost constant over the range of frequencies tested.

Since the viscoelasticity of these three materials does not change much with frequency in the frequency range of 80 to 170 Hz, we could ignore the frequency dependence over this range and compare the dependence of their viscoelasticity on their composition. Figure 8 shows the storage modulus, $\tan(\delta)$, and the swelling ratio of the collagen/HA gels as a function of the relative amount of NaIO_4 used during HA modification. It is clear that the storage modulus increases with the increase of NaIO_4 . Increasing NaIO_4 concentration probably leads to an increase of aldehyde content in HA, resulting in a higher degree of crosslinking in the hydrogels. However, loss angle and swelling ratio decreases with increasing NaIO_4 . The stiffness of hydrogels is understood to be proportional to the crosslink density, which is inversely proportional to swelling ratio. From Fig. 8, the measured correspondence of storage modulus with swelling ratio appears to be consistent with such understanding.

Discussion

From the Appendix, we have the full, explicit solution for the problem of torsional waves propagating along the axis of a cylindrical, linear-viscoelastic sample subjected to harmonic oscillation at one end. This solution can be used to examine the upper limits on frequency for which valid property measurements can be made using a rheometer for which the interpretation of the measurements is based on the assumption that the deformation is uniform through the thickness of the sample. Limitations on the validity of this assumption can be examined by considering the variation of the amplification factor M through the thickness of the sample.

To maximize the usefulness of our analysis of the uniformity of the deformation we use dimensionless variables. We use ξ , defined in equation (14c), as a measure of the driving frequency relative to the natural frequency of the sample and δ as a measure of the damping of the sample. From equations (14a) and (14b), we have

$$\frac{d}{c} = \omega_0 h \sqrt{\frac{\rho}{|G^*|}} \frac{\rho_0 I_0}{J \rho h} = \xi \frac{\rho_0 I_0}{J \rho h} \equiv \xi \mu \quad (15)$$

where μ is the ratio of the polar moment of inertia of the top plate to that of the sample.

Substitution of equation (15) into equation (12), shows that the amplification factor is a function only of ξ , δ , μ , and z/h . Figure 9 shows, for the same loss angle ($\delta=0.1$), and the same ratio of the polar moments of inertia ($\mu=1.67$) how the amplification factor M changes with z/h at different ξ . For sufficiently small ξ , M increases proportionally with z/h , corresponding to uniform deformation of the sample. As ξ increases to approximately $\xi=0.24$, the deformation becomes appreciably nonlinear. Further increases in ξ result in further deviations from uniform deformation so that, for example, when $\xi=0.3$, the deformation is highly nonuniform.

Therefore, we do not expect valid rheometric interpretations for dimensionless frequencies in the range, say, of $\xi > 0.24$.

To understand the limitation of $\xi \leq 0.24$, consider the dimensionless frequency at which the resonance peak occurs. When we express $M(\omega_0, h)$ in terms of the dimensionless variables ξ , δ , μ —and note that μ is unchanged as the frequency is increased in the experiments for which the resonance peaks are observed—the resonance peaks occur for

$$\frac{\partial M}{\partial \omega_0} = \frac{\partial M}{\partial \xi} \frac{\partial \xi}{\partial \omega_0} + \frac{\partial M}{\partial \delta} \frac{\partial \delta}{\partial \omega_0} = 0 \quad (16)$$

If we assume that the viscoelastic properties of the sample vary slowly with frequency in a narrow range of frequencies near the resonance peak, then equation (16) can be rewritten as

$$\frac{\partial M}{\partial \omega_0} = \frac{\partial M}{\partial \xi} \frac{\partial \xi}{\partial \omega_0} = 0 \quad (17)$$

Because $\frac{\partial \xi}{\partial \omega_0} \neq 0$, the resonance peak occurs for $\frac{\partial M}{\partial \xi} = 0$. Figure 10 is a contour plot of $\frac{\partial M}{\partial \xi}$ as a function of ξ and δ for $\mu = 1.67$. The arrow points to the curve along which the resonance peak occurs. From this curve, we see that, for $\delta = 0.1$, $\mu = 1.67$, the resonance peak occurs at $\xi = 0.242$. Therefore, the curve with $\xi = 0.24$ in Fig. 9 describes the deformation of the sample at a frequency very close to resonance.

To quantify how much error would be introduced if uniform deformation were assumed and wave propagation in the sample were neglected, as in the interpretation of rheometric tests, consider an error measure defined by

$$\text{err}(\delta, \xi) = \frac{\frac{M(\delta, \xi, h) - M(\delta, \xi, 0)}{h} - \left. \frac{\partial M(\delta, \xi, z)}{\partial z} \right|_{z=h}}{\left. \frac{\partial M(\delta, \xi, z)}{\partial z} \right|_{z=h}} \quad (18)$$

The first term in the numerator of this quotient is proportional to the torque that would be inferred as acting on the top plate if the deformation were assumed to be uniform through the thickness of the sample. The second term is proportional to the torque that is actually applied to the top plate. The difference between the two terms represents an error that would be introduced by an assumption of uniform deformation. Figure 11 is a contour plot of $\text{err}(\delta, \xi)$ as a function of δ and ξ . Comparison of Fig. 10 and Fig. 11, shows that, for $\delta = 0.1$, $\mu = 1.67$, the error measure (18) is less than 20%. Moreover, Fig. 11 shows how the error increases with increasing ξ and δ .

Now that we have the more quantitative measure of error shown in Fig. 11 it is of interest to refine the two frequency limitations for rheometric tests that were presented in the introduction as a means for motivating the need for wave-based interpretations. From the definition of ξ in equation (14c), with ω_0 replaced by $2\pi f$, one finds that the frequency limit, equation (1), based on round-trip times for elastic waves has the form, in the elastic limit as $\delta \rightarrow 0$:

$$f_{\max} = \frac{\sqrt{G'/\rho}}{2\pi h} \xi_{\max} \quad (19)$$

where $\xi_{\max}$ is the maximum value for the admissible error in the determination of the storage modulus from measurements of the motion of the top plate, assuming uniform deformation of the sample. If, for example, one assumes an admissible error of 20%, then from Fig. 11, $\xi_{\max} = 0.255$ as $\delta \rightarrow 0$ and the corresponding value for $f_{\max}$ would be (again for $G' = 100$ Pa, $\rho = 1000$ kg/m³, $h = 0.3$ mm as in “Introduction”) $f_{\max} \approx 43$ Hz which is similar to the initial estimate of a limiting frequency of approximately 30 Hz. Similarly, consider the frequency limit of equation (2)—making use of the definition of the complex viscosity $\eta^* \equiv G^* = (i\omega_0)$ and taking the limit of a liquid (i.e. $\delta \rightarrow \pi/2$). Then, the limiting frequency based on the assumption that the stresses due to sample inertia are small relative to those due to sample viscosity has the form:

$$\begin{aligned} f_{\max} &= \frac{\sqrt{G_2/\rho}}{2\pi h} \xi_{\max} \\ &= \frac{\sqrt{\eta_0 \omega_0/\rho}}{2\pi h} \xi_{\max} \\ &= \frac{\sqrt{\eta_0 f_{\max}^{-0.85} 2\pi f_{\max}/\rho}}{2\pi h} \xi_{\max}, \end{aligned} \quad (20)$$

or,

$$f_{\max} = \left[\frac{\sqrt{\eta_0 2\pi/\rho}}{2\pi h} \xi_{\max} \right]^{(1/0.925)}$$

where $\eta_0 = 1.0 \text{ Pa} \cdot \text{s}^{0.15}$ is a representative value from viscosity measurements at lower frequencies. If, for example, one again assumes an admissible error of 20%, then from Fig. 11, $\xi_{\max} = 0.04$ as $\delta \rightarrow \pi/2$ and the corresponding value for $f_{\max}$ would be (again for $\rho = 1000$ kg/m³, $h = 0.3$ mm as in the “Introduction”) $f_{\max} \approx 1.75$ Hz, which is somewhat smaller than the initial estimate of a limiting frequency of approximately 10 Hz. Overall, this detailed analysis supports the earlier estimates of limiting frequencies for rheometric measurements.

The numerical examples above are based on a ‘machine inertia’ value $\mu = 1.67$ corresponding to the ratio of the polar moment of inertia of the top plate to the polar moment of inertia of the sample. This ‘machine inertia’ is relatively small for the torsional wave experiment described here. To get an indication of the effect of a larger machine inertia, as would be expected for a commercial rheometer, the calculations leading to Fig. 11 have been repeated for a larger top plate for which the corresponding ‘machine inertia’ parameter has the value $\mu = 41.2$. The analogous contour plots are shown in Fig. 12. Comparisons of Fig. 11 and Fig. 12 show that the values of the dimensionless frequency ξ at which a given level of error is introduced are reduced substantially. For example, the intercept of the 20% contour with the axis for the limiting case of elastic response (i.e. $\delta \rightarrow 0$) is reduced from 0.255 to 0.054. For the limiting case of fluid response (i.e. $\delta \rightarrow \pi/2$) the intercept is reduced from 0.04 to 0.01. Thus, the limiting frequencies for valid rheometric tests are expected to be quite low for rheometers with greater machine inertia than that of the torsional wave device described here.

The frequency range for the tests reported here for hydrogel samples is from 70 to 180 Hz, which falls in the typical physiological frequency range (80 to 180 Hz) of voice production for men and approaches the conversational frequency range (200 to 250 Hz) for women. However, these frequencies are well below the frequency range (say 300 to 1,000 Hz) of the high pitch singing voice. As described in the analysis herein, the frequency of the resonance peak depends not only on the mechanical properties of the sample, but also on the geometry of the sample and the top plate. For the same sample material, the resonance frequency can be increased by decreasing the machine inertia parameter μ by, for example, reducing the polar moment of

inertia of the top plate. Such a modification appears to be possible and should enable measurements over much of the upper range of phonation frequencies.

Concluding Remarks

A torsional wave experiment has been developed to measure the viscoelastic properties of soft materials at high frequencies. Because the complex modulus is obtained by a best fit of experimental results with those based on an analysis of torsional wave propagation in the sample, this experiment can be used to measure viscoelastic properties at higher frequencies than in existing rheometric approaches where the stress state is assumed to be uniform through the thickness of the sample. In particular, for studies of vocal fold lamina propria this method makes it possible to measure viscoelastic properties in the range of phonation frequencies. Experiments on hydrogels being assessed for their potential for vocal fold regeneration demonstrate the effectiveness of this torsional wave approach. For the collagen/HA-based composite hydrogels studied, both the storage modulus and the loss modulus are nearly constant over the range of frequencies tested, i.e. 80 to 170 Hz. Values for the storage modulus appear to be larger than those obtained in our initial tests on human tissues. Systematic comparison between replacement materials and the natural vocal fold lamina propria will follow. Comparisons of the measured storage moduli with swelling ratios for different compositions suggest that materials with lower crosslink density are required.

From an experimental mechanics perspective, the analysis presented here may have significant implications for designing experiments to measure the high-frequency response of viscoelastic materials. In particular, the analysis shows the combined importance of sample geometry, machine inertia, and the material properties of the sample. This analysis shows not only what the frequency limitation is for obtaining reliable results for a given configuration and material combination, but also what can be done to the configuration to extend the upper limit of the usable frequency range. For the work reported here, this analysis indicates that the frequency range can be extended significantly by reducing the polar moment of inertia of the top plate.

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Appendix

As discussed in the body of this paper, solving equations (8)–(10) gives the rotation of the cross-section at any position z . To solve this problem, we take the Laplace transform of the field equation (8) and the boundary condition (10) to obtain

$$\rho s \tilde{\theta}(z, s) = \tilde{G}(s) \frac{\partial^2 \tilde{\theta}(z, s)}{\partial z^2} \quad (21)$$

and

$$-J \tilde{G}(s) = \frac{\partial \tilde{\theta}(h, s)}{\partial z} = \rho_0 I_0 s \theta(h, s) \quad (22)$$

respectively, where a superposed $\sim$ denotes the Laplace transform of the underlying function and Ω is replaced by $s\theta$. Equation (21) has a solution of the form

$$\tilde{\theta}(z, s) = A \cosh(\lambda z) + B \sinh(\lambda z) \quad (23)$$

where

$$\lambda(s) = \sqrt{\frac{\rho s}{\tilde{G}(s)}}. \quad (24)$$

The coefficients A and B are to be determined from the boundary conditions (22) and equation (9). The coefficient A obtained by the substitution of (23) into the Laplace transform of equation (9) is

$$A(s) = \frac{\theta_0}{s - i\omega_0} \quad (25)$$

From the boundary condition (22), and the relation (25), the coefficient B is

$$\begin{aligned} B &= -\frac{J\tilde{G}(s)\lambda(s) \sinh[\lambda(s)h] + \rho_0 l_0 s \cosh[\lambda(s)h]}{J\tilde{G}(s)\lambda(s) \cosh[\lambda(s)h] + \rho_0 l_0 s \sinh[\lambda(s)h]} \\ &\quad \times \frac{\theta_0}{(s - i\omega_0)} \\ &= -B \frac{\theta_0}{s - i\omega_0}. \end{aligned} \quad (26)$$

To invert the Laplace transform of $\tilde{\theta}(z; s)$, we used the definition of the inverse transform:

$$\theta(z, t) = \frac{1}{2\pi i} \int_{\varepsilon - i\infty}^{\varepsilon + i\infty} \exp(st) \tilde{\theta}(h, s) ds. \quad (27)$$

The parameter ε is a positive real number so the integration path is to the right of the imaginary axis in the s -plane. The integration contour can be closed in the left half plane. No contribution is obtained from the contour at ∞ . Therefore, by the Residue Theorem, the integral (27) is simply equal to $2\pi i$ times the sum of the residues of the integrand at poles to the left of $s = \varepsilon$. Possible contributions from integrals along branch cuts can be shown to be zero. The only pole is at $s = i\omega_0$. Here

$$\lambda(i\omega_0) = \sqrt{\frac{\rho i\omega_0}{\tilde{G}(i\omega_0)}} = i \sqrt{\frac{\rho \omega_0^2}{G^*(\omega_0)}} \quad (28)$$

in which

$$G^*(\omega_0) \equiv i\omega_0 \tilde{G}(i\omega_0) \quad (29)$$

is the complex modulus for the viscoelastic response of the sample material in simple shear at circular frequency ω_0 . Characterization of the viscoelastic response can be put into more readily interpretable form by representing the complex modulus in terms of its magnitude $|G^*(\omega)|$ and its phase shift $\delta(\omega)$:

$$G^*(\omega) = |G^*(\omega)| e^{i\delta(\omega)}. \quad (30)$$

In writing the solution it is helpful to simplify the argument to

$$\lambda z = i\xi (\widehat{\alpha} - i\widehat{\beta}) \quad (31)$$

and

$$\lambda h = i\xi (\alpha - i\beta) \quad (32)$$

where, as presented partially before,

$$\xi = \omega_0 h \sqrt{\frac{\rho}{|G^*(\omega_0)|}}, \quad \widehat{\alpha} = \frac{z}{h} \alpha = \frac{z}{h} \cos\left(\frac{\delta(\omega_0)}{2}\right), \\ \widehat{\beta} = \frac{z}{h} \beta = \frac{z}{h} \sin\left(\frac{\delta(\omega_0)}{2}\right).$$

From equations (23) to (26), the rotation can be written in the form

$$\theta(z, t) = \theta_0 \left\{ \cosh(\lambda z) - \bar{B} \sinh(\lambda z) \right\} e^{i\omega_0 t} \quad (33)$$

where $\bar{B}$ is a complex number that can be written as

$$\bar{B} = \frac{N_r + iN_i}{D_r + iD_i} = \frac{N_r D_r + N_i D_i}{D_r^2 + D_i^2} + i \frac{N_i D_r - N_r D_i}{D_r^2 + D_i^2} \\ = \bar{B}_r + i\bar{B}_i \quad (34)$$

in which

$$N_r = \alpha c \cos(\xi \alpha) \sinh(\xi \beta) - \beta c \sin(\xi \alpha) \cosh(\xi \beta) \\ - d \sin(\xi \alpha) \sinh(\xi \beta) \quad (35)$$

$$N_i = \beta c \cos(\xi \alpha) \sinh(\xi \beta) + \alpha c \sin(\xi \alpha) \cosh(\xi \beta) \\ + d \cos(\xi \alpha) \cosh(\xi \beta) \quad (36)$$

$$D_r = \alpha c \cos(\xi \alpha) \cosh(\xi \beta) - \beta c \sin(\xi \alpha) \sinh(\xi \beta) \\ - d \sin(\xi \alpha) \cosh(\xi \beta) \quad (37)$$

$$D_i = \beta c \cos(\xi \alpha) \cosh(\xi \beta) + \alpha c \sin(\xi \alpha) \sinh(\xi \beta) \\ + d \cos(\xi \alpha) \sinh(\xi \beta). \quad (38)$$

Substitution of equations (31) and (32) into equation (33) gives

$$\theta(z, t) = \theta_0 [E \cos(\omega_0 t) + F \sin(\omega_0 t)] + i\theta_0 [P \cos(\omega_0 t) + Q \sin(\omega_0 t)] \quad (39)$$

where

$$E = \cos(\xi\alpha) \cosh(\xi\beta) - \bar{B}_r (\cos(\xi\alpha) \sinh(\xi\beta)) + \bar{B}_i (\sin(\xi\alpha) \cosh(\xi\beta)) \quad (40)$$

$$F = -\sin(\xi\alpha) \sinh(\xi\beta) + \bar{B}_r (\sin(\xi\alpha) \cosh(\xi\beta)) - \bar{B}_i (\cos(\xi\alpha) \sinh(\xi\beta)) \quad (41)$$

$$P = \sin(\xi\alpha) \sinh(\xi\beta) - \bar{B}_r (\sin(\xi\alpha) \cosh(\xi\beta)) - \bar{B}_i (\cos(\xi\alpha) \sinh(\xi\beta)) \quad (42)$$

$$Q = \cos(\xi\alpha) \cosh(\xi\beta) - \bar{B}_r (\cos(\xi\alpha) \sinh(\xi\beta)) + \bar{B}_i (\sin(\xi\alpha) \cosh(\xi\beta)). \quad (43)$$

To extract information on the complex modulus from the recorded rotations of the ends of the sample it is helpful to consider in more detail the solution for the case in which the forced motion is the real part of the harmonic motion (9). Then, the physical solution of interest is the real part of equation (39), which can be written as

$$\theta(z, t) = \theta_0 M(z, \omega_0) \cos(\omega_0 t - \phi(z, \omega_0)) \quad (44)$$

where $M(z, \omega_0)$ is the amplification factor

$$M(z, \omega_0) = \sqrt{E^2 + F^2} \quad (45)$$

and $\phi(z, \omega_0)$ is the phase shift defined by

$$\tan(\phi(z, \omega_0)) = \frac{F}{E}. \quad (46)$$

At $z=h$, the amplification factor $M(h, \omega_0)$ simplifies to

$$M(h, \omega_0) = \frac{c}{\sqrt{D_r^2 + D_i^2}} \quad (47)$$

and the phase shift simplifies to

$$\begin{aligned} & \tan(\phi(z, \omega_0)) \\ &= \frac{-\beta D_r + \alpha D_i}{\alpha D_r + \beta D_i} \\ &= \frac{c \sin(\xi\alpha) \sinh(\xi\beta) + d\beta \sin(\xi\alpha) \cosh(\xi\beta) + d\alpha \cos(\xi\alpha) \sinh(\xi\beta)}{c \cos(\xi\alpha) \cosh(\xi\beta) - d\alpha \sin(\xi\alpha) \cosh(\xi\beta) + d\beta \cos(\xi\alpha) \sinh(\xi\beta)}. \end{aligned} \quad (48)$$

Equations (47) and (48) appear as the solutions, equations (13) and (14a–d).

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Fig. 1.
Sample geometry for torsional wave analysis

Fig. 2.
Experimental setup for torsional wave experiments

Fig. 3.

Top view, side view and end view of the optical layout for the torsional wave experiments

Fig. 4.
Typical output of the amplification factors at different frequencies, with records of wave traces at some frequencies

Fig. 5.
Test results for silicone putty (*asterisk*, [8])

Fig. 6.

Frequency dependence of amplification factors for one sample of each of three collagen/HA gels, with the molar ratio of NaIO₄ to HA repeat indicated in the parentheses. The sample dimensions and the viscoelastic moduli that provide the best fit between model predictions (*curves*) and experimental results (*symbols*) for the respective materials are: Collagen/HACHO with HACHO 1:1; $h=0.597$ mm, $a=2.067$ mm, $G'=3,545$ Pa, $\delta=0.392$, Collagen/HACHO with HACHO 1:2; $h=0.356$ mm, $a=2.151$ mm, $G'=3,249$ Pa, $\delta=0.539$, Collagen/HACHO with HACHO 1:4; $h=0.463$ mm, $a=2.081$ mm, $G'=1,672$ Pa, $\delta=0.622$

Fig. 7.
Frequency-dependence of storage modulus and $\tan(\delta)$ of collagen/HA gels

Fig. 8. Dependence of storage modulus, $\tan(\delta)$, and swelling ratio of collagen/HA gels on the amount of NaIO₄ (relative to the HA repeating unit) use during the chemical modification of HA

Fig. 9.

Variation of amplification factor M with z/h at different ξ , for $\delta=0.1$, $\mu=1.67$. For the sample, radius $a=0.002520413$ m, thickness $h=0.0003$ m, density $\rho=1040$ kg/m³. For the top plate, radius $a_0=0.003$ m, thickness $h_0=0.002$ m, density $\rho_0=1040$ kg/m³

Fig. 10.

Contour plot of $\frac{\partial M}{\partial \xi}$ as a function of ξ and δ . For the sample, radius $a=0.00252$ m, thickness $h=0.0003$ m, density $\rho=1040$ kg/m³. For the top plate, radius $a_0=0.003$ m, thickness $h_0=0.002$ m, density $\rho_0=1040$ kg/m³

Fig. 11.

Contour plot of the error defined in equation (18), as a function ξ and δ . For the sample, radius $a=0.00252$ m, thickness $h=0.0003$ m, density $\rho=1040$ kg/m³. For the top plate, radius $a_0=0.003$ m, thickness $h_0=0.002$ m, density $\rho_0=1040$ kg/m³

Fig. 12.

Contour plot of the error defined in equation (18), as a function ξ and δ for a different top plate geometry. For the sample, radius $a=0.00252$ m, thickness $h=0.0003$ m, density $\rho=1,040$ kg/m³. For the top plate, radius $a_0=0.006$ m, thickness $h_0=0.004$ m, density $\rho_0=1,040$ kg/m³.