Rayleigh wave propagation method for the characterization of a thin layer of biomaterials

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An experimental method based on Rayleigh wave propagation was developed for quantifying the frequency-dependent viscoelastic properties of a small volume of expensive biomaterials over a broad frequency range. Synthetic silicone rubber and gelatin materials were fabricated and tested to evaluate the proposed method. Planar harmonic Rayleigh waves at different frequencies, from 80 to 4000 Hz, were launched on the surface of a sample composed of a substrate with known material properties coated with a thin layer of the soft material to be characterized. A transfer function method was used to obtain the complex Rayleigh wavenumber. An inverse wave propagation problem was solved and a complex nonlinear dispersion equation was obtained. The complex shear and elastic moduli of the sample materials were then calculated through the numerical solution of the obtained dispersion equation using the measured wavenumbers. The results were in good agreement with those of a previous independent study. The proposed method was found to be reliable and cost effective for the measurement of viscoelastic properties of a thin layer of expensive biomaterials, such as phonosurgical biomaterials, over a wide frequency range. © 2013 Acoustical Society of America.
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I. INTRODUCTION

The characterization of the viscoelastic properties of soft materials at high frequencies is needed in many applications, in particular in the field of voice production. Vocal fold oscillation is significantly affected by the mechanical (viscoelastic) properties of the vocal fold mucosa. Human vocal folds oscillate at a fundamental frequency around 125, 200, 300, and 500 Hz in normal phonation for male, female, children, and infants, respectively. The sound produced by the modulated airflow through the glottis excites acoustic standing waves within the vocal tract. The associated sound radiated from the mouth is what we perceive as the human voice. Vowels are uniquely identified by the first two formants, the frequency of which usually falls between 200 Hz and 4 kHz (Titze, 1994).

Laser Doppler velocimetry of the human vocal folds during phonation has shown that there is significant vibration energy at frequencies up to 3 kHz (Chan *et al.*, 2013). Furthermore, the fundamental frequency of the vocal fold vibrations can reach up to a few kilohertz in singing (Titze, 1994). It is thus important to characterize viscoelastic properties of biomaterials at high frequencies. Implants, biomaterials, and tissue engineering scaffold materials designed for voice restoration should be tested over a wide frequency range to ensure that the viscoelastic properties of these materials match those of the surrounding tissue. This is a requirement to achieve a proper mucosal wave and phonatory function. Fine-tuning of the mechanical properties of biomaterials used for vocal fold augmentation and repair requires

the development of more sophisticated methods to characterize viscoelastic properties at frequencies up to 3 kHz.

Parallel-plate rheometry has been mostly used to characterize the viscoelastic properties of phonosurgical biomaterials (Chan and Titze, 1998, 1999; Klemuk and Titze, 2004; Titze *et al.*, 2004; Caton *et al.*, 2007; Kimura *et al.*, 2010). Chan and Titze (1998, 1999) employed a parallel-plate shear rheometer to measure the viscoelastic properties of commonly used phonosurgical biomaterials and hyaluronic acid. Measurements were performed at frequencies up to 10 Hz. Klemuk and Titze (2004) used a parallel-plate rheometer to measure the viscoelastic properties of three vocal fold injectable biomaterials at low frequencies, i.e., 0.1 to 100 Hz. Titze *et al.* (2004) introduced a method for the rheological characterization of biomaterials with approximate vocal fold tissue properties at low frequencies within the audible range, i.e., from 20 to 250 Hz. Sample geometry, phase corrections, and compensation for the system inertia were taken into account to extend the frequency range of a commercially available rheometer. Caton *et al.* (2007) measured the shear and viscous moduli of five commonly used vocal fold injectable biomaterials using a parallel-plate shear rheometer at frequencies up to 150 Hz. Results were compared with viscoelastic properties of cadaveric vocal fold tissue. Kimura *et al.* (2010) used a controlled-strain simple-shear rheometer to measure the viscoelastic properties of currently or potentially used vocal fold injectable biomaterials at frequencies up to 250 Hz. Based on the literature, methods developed so far for the quantification of the viscoelastic properties of vocal fold injectable biomaterials are limited to frequencies lower than 250 Hz.

Wave propagation methods have been recently introduced to measure the viscoelastic properties of soft materials and tissues (Greenleaf *et al.*, 2003; Catheline *et al.*, 2004;

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Chen *et al.*, 2004; Kanai, 2005; Sinkus *et al.*, 2005; Chen *et al.*, 2009; Vappou *et al.*, 2009; Nenadic *et al.*, 2011a; Nenadic *et al.*, 2011b). The excitation of the waves has been performed using either external or internal methods. The former has usually been done by a mechanical actuator, and the latter by means of the radiation force of ultrasound. Three general methods have been used for tracking and detecting the waves; namely acoustic, ultrasound, and magnetic resonance (MR) methods. Acoustic methods measure the sound produced by the vibrating sample. Ultrasound methods measure the displacement using either Doppler or pulse-echo methods. MR methods use a phase-sensitive MR machine to measure the displacement distribution (Greenleaf *et al.*, 2003). For example, Catheline *et al.* (2004) investigated an inverse problem algorithm to estimate the viscoelastic properties of soft materials. Transient elastography experiments were performed using plane shear wave propagations in the medium. Experimental results were compared to predictions from two simple rheological models.

Chen *et al.* (2004, 2009) developed a method to calculate the viscoelastic properties of homogeneous materials via shear wave speed dispersion measurements. An ultrasound transducer was used to generate cylindrical shear waves at different frequencies in a medium. The propagation speed was determined from the phase shift of the propagating shear wave along the propagation direction. The shear elasticity and viscosity of the medium were calculated by fitting the Voigt viscoelastic model to the measured data. This method was used to measure the viscoelastic properties of gelatin phantoms, bovine muscle *in vitro*, and swine liver *in vivo*. Following a similar approach, Nenadic *et al.* (2011b) developed a measurement method based on Lamb wave propagation for quantifying the viscoelastic properties of soft materials and tissues with plate-like geometries. The viscoelastic properties of urethane rubber, gelatin, and excised porcine myocardium samples were measured. Dispersion equations for cylindrical Lamb and Rayleigh waves propagated in a viscoelastic plate submerged in a nonviscous fluid were presented by Nenadic *et al.* (2011a). Wave propagation velocities were measured in gelatin plates, excised porcine spleen, and left ventricular free-wall myocardium samples; the corresponding viscoelastic properties were determined. However, these methods use rheological models that assume *one constant value* for the shear modulus and viscosity and do not take the dependence of viscoelastic properties of the material on frequency into consideration. In these studies, only the phase of the propagating wave was measured at different locations along the propagation direction. The decrease in the amplitude of the propagating wave with distance, which takes place in viscoelastic media due to dissipation, was not measured and considered in the modeling. Thus real values were obtained for the wave propagation speeds. The wave speeds and consequently the wavenumbers in viscoelastic media should be complex (Borcherdt, 2009). In the end, an approximate rheological model was used to perform the regression of the measured data to obtain the complex shear modulus of the medium. This yields only a *constant value* for the shear elasticity and viscosity over the frequency range of interest.

A method for the characterization of *frequency-dependent* viscoelastic properties of soft biomaterials based on Rayleigh wave propagations was recently introduced in a previous, related study (Kazemirad *et al.*, 2013). In this study, both the phase and amplitude of the propagating surface (Rayleigh) waves were measured at different locations along the propagation direction. These two independent parameters were simultaneously considered in the modeling via the transfer function method, and thus complex values for the Rayleigh wavenumber (or propagation speed) were obtained at each excitation frequency. Therefore, it was not required to fit any approximate rheological model to the measured data. Eventually, the frequency-dependent complex shear and elastic moduli were calculated using a dispersion relation. This method was developed for the characterization of soft materials, specifically phonosurgical biomaterials and hydrogels, at high frequencies. It requires a relatively large volume of sample material, which is very expensive for many phonosurgical biomaterials. To reduce the volume of material needed, a novel model-independent characterization method based on Rayleigh wave propagation for quantifying the *frequency-dependent* viscoelastic properties of a *thin layer of biomaterials* was developed in the present study. A thin layer of the soft material under study was applied on a substrate made from another soft material with known viscoelastic properties. The viscoelastic properties of silicone rubber materials used as the substrate were measured and cross-validated using three different characterization methods, including Rayleigh and longitudinal wave propagation as well as an AFM-based nano-indentation method, in Kazemirad *et al.* (2013). Rayleigh waves were produced on the sample surface using an actuator connected to a shaker. A transfer function method was used to obtain the complex Rayleigh wavenumber and the velocity dispersion curves from measured vibration amplitudes and phases at different locations on the sample surface. An inverse wave propagation problem was solved, and a complex nonlinear dispersion equation for Rayleigh wave propagation in a layered media was obtained. The dispersion equation was solved numerically to calculate the complex shear and elastic moduli of the thin samples from the measured wavenumbers. The method was evaluated using synthetic silicone rubber materials and gelatin. The results were found to be in very good agreement with those presented by Kazemirad *et al.* (2013) and Nenadic *et al.* (2011a). Prospective applications for various hydrogels, biomaterials, and tissue engineering scaffolds are envisioned.

II. METHODS

A. Rayleigh wave propagation in layered media

The interactions between body waves (i.e., compressional and shear waves) propagating inside a layered medium may cause Rayleigh waves to propagate parallel to the stress-free boundary of the medium, as shown in Fig. 1. Rayleigh waves include motion in both the longitudinal and transverse directions, in particular a two-dimensional rolling motion along the x_1 - x_2 plane. The amplitude of the propagating surface wave decreases as x_2 increases in the substrate, while there is a standing wave in the top layer. The wave

FIG. 1. (Color online) Schematic of the Rayleigh wave propagation concept in (a) a uniform and (b) a layered half-space. In the substrate, the amplitude of the propagating surface wave decreases as x_2 increases. The amplitude decreases as x_1 increases because of the dissipative behavior of the viscoelastic medium. The particles undergo elliptical motion. The motion direction is counter clockwise when the wave propagates from left to right.

amplitude decreases as x_1 increases because of dissipation in the viscoelastic materials.

The material was assumed to be linear, isotropic, and macroscopically homogeneous viscoelastic. Navier's equation governs the wave motion in the medium. In the absence of body forces, the equation is written in the form (Achenbach, 1973)

$$\rho \frac{\partial^2 \mathbf{u}}{\partial t^2} = \hat{\mu} \nabla^2 \mathbf{u} + (\hat{\lambda} + \hat{\mu}) \nabla (\nabla \cdot \mathbf{u}), \quad (1)$$

where ρ is the material density, $\hat{\lambda}(\omega)$ and $\hat{\mu}(\omega)$ are complex, frequency-dependent Lamé functions, and $\mathbf{u}$ is the displacement vector. The Helmholtz decomposition theorem allows the displacement vector to be resolved in terms of the sum of the gradient of a scalar potential and the curl of a vector potential as

$$\mathbf{u} = \nabla \phi + \nabla \times \boldsymbol{\psi}, \quad (2)$$

with the conditions $\nabla \cdot \boldsymbol{\psi} = 0$, and $\boldsymbol{\psi} = (0, 0, \psi)$ due to the two-dimensional nature of the problem. Substitution of the above equation into the field equation of motion, Eq. (1),

after some manipulations, yields the classical Helmholtz equations

$$(\nabla^2 + \hat{k}_c^2) \phi = 0, \quad (3a)$$

$$(\nabla^2 + \hat{k}_s^2) \boldsymbol{\psi} = 0, \quad (3b)$$

in which ∇^2 is the Laplacian, ϕ and $\boldsymbol{\psi}$ are the compressional and the shear wave potentials, and $\hat{k}_c$ and $\hat{k}_s$ are the corresponding complex wavenumbers, known as (Graff, 1975)

$$\hat{k}_c = \frac{\omega}{\hat{c}_c} = \frac{\omega}{\sqrt{(\hat{\lambda} + 2\hat{\mu})/\rho}}, \quad (4a)$$

$$\hat{k}_s = \frac{\omega}{\hat{c}_s} = \frac{\omega}{\sqrt{\hat{\mu}/\rho}}, \quad (4b)$$

where ω is the angular frequency, and $\hat{c}_c$ and $\hat{c}_s$ are complex compressional and shear wave speeds, respectively. Considering the fundamental field equations in a Cartesian coordinate system, the displacement components are defined as

$$u_1 = \frac{\partial \phi}{\partial x_1} + \frac{\partial \psi}{\partial x_2}, \quad (5a)$$

$$u_2 = \frac{\partial \phi}{\partial x_2} - \frac{\partial \psi}{\partial x_1}, \quad (5b)$$

and the corresponding stress components are

$$\sigma_{11} = 2\hat{\mu} \frac{\partial u_1}{\partial x_1} + \hat{\lambda} \varepsilon, \quad (6a)$$

$$\sigma_{22} = 2\hat{\mu} \frac{\partial u_2}{\partial x_2} + \hat{\lambda} \varepsilon, \quad (6b)$$

$$\sigma_{12} = \hat{\mu} \left(\frac{\partial u_1}{\partial x_2} + \frac{\partial u_2}{\partial x_1} \right), \quad (6c)$$

in which $\varepsilon = \nabla \cdot \mathbf{u} = \nabla^2 \phi = -\hat{k}_c^2 \phi$.

The specific boundary conditions that had to be satisfied on the stress-free boundary, $x_2 = 0$, and at the interface of the two media, $x_2 = h$, are

$$\sigma_{12}^1|_{x_2=0} = 0, \quad \sigma_{22}^1|_{x_2=0} = 0, \quad (7a)$$

$$\begin{aligned} \sigma_{12}^1|_{x_2=h} &= \sigma_{12}^2|_{x_2=h}, & \sigma_{22}^1|_{x_2=h} &= \sigma_{22}^2|_{x_2=h}, \\ u_1^1|_{x_2=h} &= u_1^2|_{x_2=h}, & u_2^1|_{x_2=h} &= u_2^2|_{x_2=h}, \end{aligned} \quad (7b)$$

where superscripts 1 and 2 refer to the parameters associated with the top layer and the substrate, respectively. One proper set of compressional and shear wave potentials propagating inside the substrate and the top layer, which satisfies the boundary conditions, is (Kundu, 2004)

$$\phi_1 = (A_1 e^{-ik_1 x_2} + A_2 e^{ik_1 x_2}) e^{i\hat{k}_R x_1}, \quad (8a)$$

$$\psi_1 = (B_1 e^{-ik_2 x_2} + B_2 e^{ik_2 x_2}) e^{i\hat{k}_R x_1}, \quad (8b)$$

$$\phi_2 = C_1 e^{i\hat{k}_R x_1 - k_3 x_2}, \quad (8c)$$

$$\psi_2 = D_1 e^{i\hat{k}_R x_1 - k_4 x_2}, \quad (8d)$$

where $\hat{k}_R$ is the complex Rayleigh wavenumber, and A_1, A_2, B_1, B_2, C_1 , and D_1 are the unknown amplitudes of the corresponding potentials. The harmonic time variation factor, $e^{-i\omega t}$, is omitted throughout the formulations for simplicity. Substitution of the field potentials, Eqs. (8), into the Helmholtz equations, Eqs. (3), yields

$$k_1^2 = \hat{k}_{c1}^2 - \hat{k}_R^2, \quad k_2^2 = \hat{k}_{s1}^2 - \hat{k}_R^2, \quad (9a)$$

$$k_3^2 = \hat{k}_R^2 - \hat{k}_{c2}^2, \quad k_4^2 = \hat{k}_R^2 - \hat{k}_{s2}^2. \quad (9b)$$

Substitution of Eqs. (8) and (9) into the boundary conditions, Eqs. (7), yields

$$2\hat{k}_R k_1 (A_1 - A_2) + (2\hat{k}_R^2 - \hat{k}_{s1}^2) (B_1 - B_2) = 0, \quad (10a)$$

$$(2\hat{k}_R^2 - \hat{k}_{s1}^2) (A_1 + A_2) + 2\hat{k}_R k_2 (B_2 - B_1) = 0, \quad (10b)$$

$$\begin{aligned} & 2\hat{k}_R k_1 \hat{\mu}_1 (A_1 e^{-ik_1 h} - A_2 e^{ik_1 h}) \\ & + (2\hat{k}_R^2 - \hat{k}_{s1}^2) \hat{\mu}_1 (B_1 e^{-ik_2 h} + B_2 e^{ik_2 h}) \\ & + 2i\hat{k}_R k_3 \hat{\mu}_2 C_1 e^{-k_3 h} - (2\hat{k}_R^2 - \hat{k}_{s2}^2) \hat{\mu}_2 D_1 e^{-k_4 h} = 0, \end{aligned} \quad (10c)$$

$$\begin{aligned} & (2\hat{k}_R^2 - \hat{k}_{s1}^2) \hat{\mu}_1 (A_1 e^{-ik_1 h} + A_2 e^{ik_1 h}) \\ & + 2\hat{k}_R k_2 (B_2 e^{ik_2 h} - B_1 e^{-ik_2 h}) - (2\hat{k}_R^2 - \hat{k}_{s2}^2) \hat{\mu}_2 C_1 e^{-k_3 h} \\ & - 2i\hat{k}_R k_4 \hat{\mu}_2 D_1 e^{-k_4 h} = 0, \end{aligned} \quad (10d)$$

$$\begin{aligned} & i\hat{k}_R (A_1 e^{-ik_1 h} + A_2 e^{ik_1 h}) + ik_2 (B_2 e^{ik_2 h} - B_1 e^{-ik_2 h}) \\ & - i\hat{k}_R C_1 e^{-k_3 h} + k_4 D_1 e^{-k_4 h} = 0, \end{aligned} \quad (10e)$$

$$\begin{aligned} & ik_1 (A_2 e^{ik_1 h} - A_1 e^{-ik_1 h}) - i\hat{k}_R (B_1 e^{-ik_2 h} + B_2 e^{ik_2 h}) \\ & + k_3 C_1 e^{-k_3 h} + i\hat{k}_R D_1 e^{-k_4 h} = 0. \end{aligned} \quad (10f)$$

In a situation where the Rayleigh wavenumber is measured and the viscoelastic properties of the media are known, Equations (10) form a set of six coupled equations with six unknown; i.e., the amplitudes of the field potentials. The unknown amplitudes, and subsequently the displacement field and the corresponding stress and strain components, can be defined within the two media. In the current study, however, the viscoelastic properties of the substrate are known, but those of the top layer material are not.

B. The transfer function method

The transfer function is the complex ratio of the vibration displacements of two different locations of the sample along the wave propagation direction. The transfer function method was developed and used along with a standing longitudinal wave propagation method to obtain the complex modulus of rod-like specimens (Pritz, 1982). It has never

been used in characterization methods based on surface wave propagations. In this study, the transfer function method was employed to calculate the complex Rayleigh wavenumber from the measured amplitude and phase of the propagated wave at different locations. The transfer function between the vertical displacement components, u_2 , at two different locations along the propagation direction, x_1 , on the surface of the sample is defined as

$$\hat{H}(i\omega) = |\hat{H}(i\omega)| e^{i\varphi} = \frac{\hat{u}_2(i\omega)|_{x_1=x_{12}}}{\hat{u}_2(i\omega)|_{x_1=x_{11}}}, \quad (11)$$

where $|\hat{H}(i\omega)|$ and φ are the amplitude and phase of the transfer function. Substitution of the field potentials, Eq. (8), into Eq. (5) yields the displacement component in the top layer as

$$\hat{u}_1(i\omega, x_1)|_{x_2=0} = [i\hat{k}_R (A_1 + A_2) + ik_2 (B_2 - B_1)] e^{i\hat{k}_R x_1}, \quad (12a)$$

$$\hat{u}_2(i\omega, x_1)|_{x_2=0} = [ik_1 (A_2 - A_1) - i\hat{k}_R (B_1 + B_2)] e^{i\hat{k}_R x_1}. \quad (12b)$$

Consequently, the real and imaginary parts of the Rayleigh wavenumber were calculated from substitution of Eq. (12b) into Eq. (11) as

$$\begin{aligned} \hat{H}(i\omega) &= \frac{\hat{u}_2(i\omega)|_{x_1=x_{12}}}{\hat{u}_2(i\omega)|_{x_1=x_{11}}} = e^{i\hat{k}_R (x_{12} - x_{11})} \\ &= e^{\gamma L} e^{i\lambda L} \Rightarrow |\hat{H}(i\omega)| = e^{\gamma L}, \quad \varphi = \lambda L, \end{aligned} \quad (13)$$

where $\hat{k}_R = \lambda - \gamma i$, and L is the distance between two locations on the surface of the sample; i.e., $x_{12} - x_{11} = L$.

Considering the following relation between the shear and compressional wavenumbers for each of the media

$$\begin{aligned} \kappa^2 &= \left(\frac{\hat{c}_{ci}}{\hat{c}_{si}} \right)^2 = \left(\frac{\hat{k}_{si}}{\hat{k}_{ci}} \right)^2 = \frac{\hat{\lambda}_i + 2\hat{\mu}_i}{\hat{\mu}_i} = \frac{2 - 2\nu_i}{1 - 2\nu_i}, \\ i &= 1, 2, \end{aligned} \quad (14)$$

in which ν is Poisson's ratio, the components of the coefficient matrix of the system of equations developed in Eq. (10) become $C_{ij} = f_{ij}(\hat{k}_R, \hat{k}_{s1}, \hat{k}_{s2}, \mu_2, \nu_1, \nu_2, \rho, \omega, h)$; $i, j = 1, 2, \dots, 6$. The coefficient matrix, $\mathbf{C}$, is presented in the Appendix. To ensure nontrivial solutions for the unknown coefficients in Eq. (10), the determinant of the coefficient matrix must vanish. Accordingly, a complex dispersion equation for the propagating Rayleigh waves was obtained by equating the determinant of the coefficient matrix with zero. The dispersion equation relates the measured Rayleigh wavenumbers to shear wavenumbers. In contrast to a uniform (non-layered) medium, the Rayleigh wave is dispersive in a layered media. It means that the relation between the Rayleigh and shear wavenumbers depends on the frequency, and thus the dispersion equation must be solved separately for each set of data at each frequency. The real and imaginary parts of the dispersion equation form two coupled

nonlinear equations. The real and imaginary parts of the shear wavenumber propagated in the top layer were calculated from the numerical solution of these equations:

$$\text{Re}\{\det(\mathbf{C})\} = 0, \quad (15a)$$

$$\text{Im}\{\det(\mathbf{C})\} = 0, \quad (15b)$$

where “det” represents the determinant of matrix $\mathbf{C}$, and “Re” and “Im” are the real and imaginary parts of the determinant. The subroutines of commercial computational softwares, such as MATLAB, for solving nonlinear algebraic equations did not yield accurate solutions, due to the presence of many local minima. Thus, the results depended on the initial guess. A code based on the iterative Rayleigh-Ritz method was developed in MATHEMATICA and numerical analysis tools were used, yielding reliable robust results for different initial guesses. Finally, the complex shear and elastic moduli and the loss factor of the medium (top layer) were calculated from (Pao and Mow, 1973; Graff, 1975; Lakes, 2009)

$$\hat{G}_1 = \hat{\mu}_1 = \frac{\omega^2 \rho_1}{\hat{k}_{s1}^2}, \quad (16a)$$

$$\hat{E}_1 = 2(1 + \nu_1)\hat{G}_1, \quad (16b)$$

$$\eta_1 = \frac{\text{Im}\{\hat{E}_1\}}{\text{Re}\{\hat{E}_1\}} = \frac{\text{Im}\{\hat{G}_1\}}{\text{Re}\{\hat{G}_1\}}. \quad (16c)$$

C. Sample preparation

Silicone rubber and gelatin samples were fabricated and tested. Silicone rubber samples were made from Ecoflex 10 Platinum Cure Silicone Rubber (Smooth-On, Inc., Easton, PA). The mixing ratio of the two parts (part A and part B) was unity. Silicone thinner was added to achieve viscoelastic properties similar to values reported for commercial phonosurgical biomaterials at low frequencies (Kimura *et al.*, 2010). A vacuum pump was used to evacuate the mixture and remove air bubbles after mixing of the components and after casting. It was cured at room temperature for around 10 to 12 h. Two rubber samples with component ratios 1:1:0.5 and 1:1:1 (i.e., part A: part B: silicone thinner), all by weight, were used as the test specimens. Gelatin samples were fabricated from 70% water, 15% glycerol, and 15% 300 Bloom gelatin (Sigma-Aldrich, St. Louis, MO), all by volume. The mixture used in Nenadic *et al.* (2011a) was fabricated to allow for the comparison of the viscoelastic

TABLE I. Dimensions and properties of the layered sample for the case with the silicone rubber with a component ratio 1:1:0.5 as the material to be characterized.

Material	Length [10 ⁻³ m]	Width [10 ⁻³ m]	Height [10 ⁻³ m]	Mass (m) [10 ⁻³ kg]	Density ρ [kg/m ³]
Silicone rubber 1:1:0.5	102	51	5	28.17	1083
Silicone rubber 1:1:1	102	51	46	241.9	1011

TABLE II. Dimensions and properties of the layered sample for the case with the silicone rubber with a component ratio 1:1:1 as the material to be characterized.

Material	Length [10 ⁻³ m]	Width [10 ⁻³ m]	Height [10 ⁻³ m]	Mass (m) [10 ⁻³ kg]	Density ρ [kg/m ³]
Silicone rubber 1:1:0.5	102	51	46	259.9	1086
Silicone rubber 1:1:1	102	51	5	26.27	1010

properties of gelatin samples found in this study with those previously reported. The mixture was molded and evacuated in the same manner as for the silicone rubber samples. It was cured at room temperature shortly after casting, but was kept under water to ensure constant mechanical properties over a 24 h period prior to the experiments. The specimens' dimensions and properties are given in Tables I–III.

The substrate was made of silicone rubber with a component ratio 1:1:1. A thin layer of either gelatin or silicone rubber with a component ratio 1:1:0.5 was added on the top. A silicone rubber sample with a component ratio 1:1:0.5 was used as the substrate for the case with a top layer of silicone rubber with a component ratio 1:1:1.

D. Experiments

A schematic of the experimental apparatus is shown in Fig. 2. A linear actuator, with a width of 2.54 cm, connected to a shaker was used to generate a planar harmonic Rayleigh wave with a known frequency on the surface of a sample, as shown in Fig. 2. The sample was mounted on an optical table to reduce the influence of extraneous seismic vibration. The input vibrations of the actuator and the motion of the sample surface were measured using an attached accelerometer and the laser Doppler vibrometer (LDV), respectively. The reflection of the laser beam from some of the samples was poor. To enhance the reflection, a very small amount of a titanium dioxide powder was deposited onto the samples at measurement locations where the strength of the reflected signal was not sufficient. The titanium dioxide is a very fine powder that did not change the mechanical properties of the medium. The frequency of the excitation was increased step by step from 20 Hz to 4 kHz. The amplitude and phase of the propagating wave were measured at each excitation frequency at several locations along the propagation direction. These measurement locations were 1 and 0.5 cm apart for the frequency ranges of 20 to 500 Hz and 500 to 4000 Hz, respectively. The measured data from at least 1000 cycles of excitation were saved. The data from the first 100 cycles were removed to exclude possible transient effects. Each experiment was repeated at least five times. The real and

TABLE III. Dimensions and properties of the layered sample for the case with the gelatin as the material to be characterized.

Material	Length [10 ⁻³ m]	Width [10 ⁻³ m]	Height [10 ⁻³ m]	Mass (m) [10 ⁻³ kg]	Density ρ [kg/m ³]
Gelatin	102	51	5	27.96	1075
Silicone rubber 1:1:1	102	51	46	242.2	1012

FIG. 2. (Color online) Schematic of the test setup. Single frequency harmonic excitation signals were generated using a signal generator (Data Translation) and amplified before being fed to a shaker. A linear actuator connected to the shaker was used to produce planar Rayleigh waves on the surface of a sample fixed, mounted on an optical table. A PCB accelerometer and a Polytec laser Doppler vibrometer were used to measure the vibrations of the actuator and the sample surface, respectively.

imaginary parts of the Rayleigh wavenumber were calculated from the transfer function between vertical displacements at two different locations on the surface of the sample, using Eq. (13).

The real part of the wavenumber, calculated from the phase difference between any two locations from Eq. (13), was consistent; i.e., the standard deviation of obtained values was within 8%. The imaginary part of the wavenumber has a significant effect on the value of the imaginary part of the complex shear and elastic moduli and consequently the loss factor. The results did vary with the measurement location in this case. To deal with this variation, measurements were performed at several locations (at least five) along the propagation direction and an exponential decay function was used to obtain a regression of the oscillation amplitudes. The imaginary part of the wavenumber was calculated from the regression and Eq. (13). In the following, the shear and elastic moduli mean the real part of the complex moduli (storage moduli).

In isotropic (non-layered) half-space *elastic solid* media, the amplitudes of compressional and shear components of Rayleigh waves decrease with depth exponentially by a factor of $e^{k_1 x_2}$ and $e^{k_2 x_2}$, respectively. In layered half-space *elastic solid* media, these amplitudes decrease exponentially as x_2 increases in the substrate (the same as in the previous case), while there is a standing wave in the top layer. In *viscoelastic* media, either isotropic or layered, these amplitudes decrease even further by a factor of $e^{\lambda x_1}$ and $e^{\lambda x_2}$ in the x_1 and x_2 directions, respectively, where λ is the imaginary part of the Rayleigh wavenumber, $\hat{k}_R$.

The method developed for this study is based on the assumption of no reflection; hence, the propagated waves should dissipate before reaching the boundaries. The dissipation is a function of frequency (wavelength), the distance across which the wave is propagated (i.e., sample size), and the loss factor (intrinsic dissipation coefficient) of the medium. The propagated surface waves are generally dissipated over a shorter propagation distance at higher frequencies, where the wavelength is smaller. Therefore, the sample size can be smaller if the characterization at higher frequencies is the aim or the sample material has a greater loss factor. Experiments were performed for evaluation purposes. The motion of the sample surface was measured at different frequencies and locations to ensure that the propagating waves dissipated before reaching the sample boundaries. The

motion of the mold was also measured at different frequencies to determine if the propagating waves have reached the bottom or lateral boundaries of the sample. The motion of the sample surface at locations near the boundaries and that of the mold were negligible at frequencies greater than 80 Hz, proving no reflection from the boundaries at those frequencies. Therefore, the assumed model is accurate for frequencies greater than 80 Hz. Therefore, the frequency range of validity of the experimental results was between 80 and 4000 Hz. This frequency limit might be slightly different for different materials. The motion of the sample surface was negligible in directions other than the assumed propagation direction (i.e., perpendicular to the actuator, x_1 direction). This confirms the accuracy of the proposed model for a planar Rayleigh wave propagation.

The top layer of the sample to be characterized can be very thin, as long as the precise measurement of the thickness, h , is possible. However, the length and the width of the sample cannot be smaller than a certain limit for accurate measurements (no reflection) based on the frequency range of interest and the loss factor of the media.

III. RESULTS AND DISCUSSION

The mean values and standard deviations of the measured data were calculated. The standard deviation values of the measured shear and elastic moduli and loss factor were less than 10% of the mean values except for the loss factor of the silicone rubber sample with a component ratio 1:1:0.5 at 500 Hz, where it was 12%. Thus, only mean values are shown.

Rayleigh and shear wave propagation speeds versus frequency for silicone rubber samples with component ratios 1:1:0.5 and 1:1:1 and a gelatin sample in the frequency range from 80 Hz to 4 kHz are shown in Figs. 3–5, respectively. The results obtained from layered samples were compared to those from uniform (non-layered) ones (Kazemirad *et al.*, 2013). Shear wave speeds obtained from layered samples were those of the top layer, $\hat{c}_{s1}$. As seen in these figures, the wave propagation speeds increased with the frequency. This is consistent with the expected trend of greater elastic and shear moduli at higher frequencies. The measured wave speeds for the silicone rubber sample with a component ratio 1:1:1 were smaller than those for a component ratio 1:1:0.5 at low frequencies (below 300 Hz). This is consistent with the fact that the addition of silicone thinner yields lowers

FIG. 3. Wave propagation speed versus frequency for a silicone rubber sample with a component ratio 1:1:0.5 in the frequency range from 80 Hz to 4 kHz. $\circ$: Rayleigh wave speed from a uniform sample; $\square$: Shear wave speed from a uniform sample; $\diamond$: Rayleigh wave speed from a layered sample; $\triangle$: Shear wave speed within the top layer of a layered sample;: Regression for Rayleigh wave speed from a uniform sample; —: Regression for shear wave speed from a uniform sample; ---: Regression for Rayleigh wave speed from a layered sample; —·—: Regression for shear wave speed from a layered sample. (Regressions are to guide the eyes.)

shear and elastic moduli at low frequencies. In contrast, wave speeds for the silicone rubber sample with a component ratio 1:1:1 were greater than those with a component ratio 1:1:0.5 at high frequencies. One possible reason for this observation is that the shear and elastic moduli of the two samples with different ratios are nearly equal at high frequencies. Therefore, the smaller density of the sample with a component ratio 1:1:1 resulted in greater wave speeds.

The results obtained from the layered samples were slightly smaller than those from uniform samples, the

FIG. 4. Wave propagation speed versus frequency for a silicone rubber sample with a component ratio 1:1:1 in the frequency range from 80 Hz to 4 kHz. $\circ$: Rayleigh wave speed from a uniform sample; $\square$: Shear wave speed from a uniform sample; $\diamond$: Rayleigh wave speed from a layered sample; $\triangle$: Shear wave speed within the top layer of a layered sample;: Regression for Rayleigh wave speed from a uniform sample; —: Regression for shear wave speed from a uniform sample; ---: Regression for Rayleigh wave speed from a layered sample; —·—: Regression for shear wave speed from a layered sample. (Regressions are to guide the eyes.)

FIG. 5. Wave propagation speed versus frequency for a gelatin sample in the frequency range from 80 Hz to 4 kHz. $\circ$: Rayleigh wave speed from a uniform sample; $\square$: Shear wave speed from a uniform sample; $\diamond$: Rayleigh wave speed from a layered sample; $\triangle$: Shear wave speed within the top layer of a layered sample; ∇ : Rayleigh wave speed (Nenadic *et al.*, 2011a);: Regression for Rayleigh wave speed from a uniform sample; —: Regression for shear wave speed from a uniform sample; ---: Regression for Rayleigh wave speed from a layered sample; —·—: Regression for shear wave speed from a layered sample; —: Regression for Rayleigh wave speed (Nenadic *et al.*, 2011a). (Regressions are to guide the eyes.)

difference being less than 15%. It is noteworthy that the Rayleigh wave speeds in a layered medium are not necessarily the same as those in a uniform medium. But the shear wave speed and shear and elastic moduli are only *frequency-dependent*, at a fixed experiment temperature, and should be the same for uniform and layered samples. However, there were differences between shear wave speeds obtained from a layered sample and those from a non-layered sample on the order of 10% or less. This discrepancy was the source of differences between the calculated values for the shear and elastic moduli and loss factor from layered and non-layered samples. The discrepancy is believed to be caused by experimental errors in the data and variability in the fabrication procedures and the resulting elastomer mechanical properties.

Theoretically the Rayleigh waves propagation speed is slightly smaller than the shear waves propagation speed at each frequency, which is consistent with the results presented in this work. For a uniform sample, the ratio of the Rayleigh wave speed to shear wave speed is a constant value that is a function of the Poisson's ratio of the medium. In this case, the approximate relation between the Rayleigh and shear wave speeds (or the corresponding wavenumbers) obtained from the Rayleigh wave model is expressed as (Achenbach, 1973)

$$\hat{c}_R = \frac{0.862 + 1.14\nu}{1 + \nu} \hat{c}_s, \quad (17)$$

where c_R is the Rayleigh wave speed. The Poisson's ratio of the used silicone rubber was assumed to be 0.4 (Zörner *et al.*, 2010), while that of the gelatin sample was assumed to be 0.495 (Nenadic *et al.*, 2011a). Equation (17) yields wave speed ratios of 0.94 and 0.95 for silicone rubber and gelatin

samples, respectively. As shown in Figs. 3–5, the wave speeds measured in a uniform sample are in good agreement with the calculated values. In the case of a layered sample, the measured wave speed ratio was found to vary for different materials and different frequencies. For example, for the silicone rubber sample with a component ratio 1:1:0.5, this ratio was 0.93 and 0.90 at 3 and 4 kHz, respectively.

The measured Rayleigh and shear wave propagation speeds in the uniform and layered gelatin samples are shown in Fig. 5. The measured wave speeds were slightly greater than those measured by Nenadic *et al.* (2011a) in the range of 6% to 17%. This discrepancy can be due to several reasons. First, the measurements were performed on samples with slightly different properties. Attempts were made to use gelatin samples similar to those in Nenadic *et al.* (2011a); however, the necessary fabrication procedures are unpublished. For example, it was unknown whether Nenadic *et al.* (2011a) evacuated the mixture to remove air bubbles after component mixing and casting. If not, the samples would likely have been less stiff, yielding slightly lower wave propagation speeds than found in the present study, where evacuation was performed. The sample dimensions can also be another source of error. Nenadic *et al.* (2011a) used gelatin samples with a plate-shaped geometry, which is not the most appropriate geometry for Rayleigh wave propagation modeling.

The shear and elastic moduli and loss factor of silicone rubber samples with component ratios 1:1:0.5 and 1:1:1 and the gelatin sample are shown in the frequency range from 80 Hz to 4 kHz in Figs. 6–8, respectively. The values obtained from the layered samples were compared with those of the corresponding uniform sample (Kazemirad *et al.*, 2013). The difference between the results was generally less than 10% for the shear and elastic moduli, and 20% for the loss factor except at few frequencies. The shear and elastic moduli appeared to be more consistent, maybe because the real part of the wavenumbers was more accurately measured than the imaginary part.

The shear and elastic moduli of all materials tested increased with frequency, as illustrated in Figs. 6–8. The rate of change increased beyond a specific frequency, where the materials undergo a transition from a rubber- to glass-like consistency (glass transition phenomenon). The loss factor peak, which is an important characteristic of the dissipative behavior of viscoelastic materials, occurred within the transition zone. A comparison between Figs. 6 and 7 suggests that the addition of silicone thinner yielded a lower elastic modulus at low frequencies. Thinning also resulted in a slight decrease in the frequency of the peak loss factor, which corresponds to the onset of a rapid elastic modulus increase. As a result, the elastic moduli for the two samples of different ratios were nearly equivalent at high frequencies. This is consistent with the hypothesis that the wave speed and, consequently, the shear and elastic moduli are governed by the matrix at low frequencies (rubber-like consistency) and by the polymer fibers at high frequencies (glass-like consistency). A comprehensive discussion of the viscoelastic behavior of rubbers and its dependency on their molecular entanglement; i.e., the coupled configurational motion of neighboring molecules, is available in the literature (Ferry, 1980; Lakes, 2009; Kazemirad *et al.*, 2013).

FIG. 6. (a) Elastic modulus and (b) loss factor versus frequency for a silicone rubber sample with a component ratio 1:1:0.5 in the frequency range from 80 Hz to 4 kHz. $\circ$: A uniform sample; $\diamond$: A layered sample; $\cdots$: Regression for a uniform sample; $---$: Regression for a layered sample. (Regressions are to guide the eyes.)

The shear modulus and loss factor of the gelatin sample were obtained from a layered and a uniform sample and compared with those obtained by Nenadic *et al.* (2011a) in Figs. 8(a) and 8(b). The mechanical properties of soft biomaterials are highly dependent on frequency. For instance, the shear modulus of the gelatin sample at high frequencies (a few kilohertz) was 2 orders of magnitude greater than that at low frequencies. This accentuates the importance of the characterization of frequency-dependent viscoelastic properties of soft biomaterials. In the study by Nenadic *et al.* (2011a), a Voigt material frequency response with a complex shear modulus $\hat{\mu} = \mu' + i\omega\mu''$ was fitted to the measured wave speeds, where μ' and μ'' are the shear elasticity (modulus) and viscosity of the medium, respectively. This method yields one constant value for the shear modulus and viscosity of the material over the entire frequency range of interest. As Fig. 8(a) shows, the constant value in Nenadic *et al.* (2011a) is approximately equivalent to the mean shear modulus of the gelatin sample in the present study, obtained for the same frequency range (i.e., 40 to 500 Hz) using the Rayleigh wave propagation method. As seen in Fig. 8(b), the loss factor

(a)

(b)

FIG. 7. (a) Elastic modulus and (b) loss factor versus frequency for a silicone rubber sample with a component ratio 1:1:1 in the frequency range from 80 Hz to 4 kHz. $\circ$: A uniform sample; $\diamond$: A layered sample; $\cdots$: Regression for a uniform sample; $---$: Regression for a layered sample. (Regressions are to guide the eyes.)

predicted by the Voigt model increased linearly with frequency, such as $\eta = \omega\mu''/\mu'$, and did not capture the loss factor peak obtained from the present method. The loss factor peak is an important feature of the viscoelastic response. This highlights limitations of rheological models to describe the viscoelastic behavior of soft biomaterials accurately. Therefore, development of model-independent characterization methods, such as the Rayleigh wave propagation method presented in this study, is required to accurately quantify the frequency-dependent viscoelastic properties of such materials. For evaluation purposes, the Voigt model was fitted to the wave speeds measured in the present study at frequencies up to 500 Hz; i.e., the maximum measurement frequency in Nenadic *et al.* (2011a). The obtained shear modulus and viscosity were found to be in good agreement with those of Nenadic *et al.* (2011a). The differences between the values obtained in the present study, $\hat{\mu} = 11\,750 + 3.26i\omega$, and those obtained in Nenadic *et al.* (2011a), $\hat{\mu} = 10\,700 + 3.7i\omega$, are 10% for the shear modulus (elasticity) and 12% for the viscosity.

(a)

(b)

FIG. 8. (a) Shear modulus and (b) loss factor versus frequency for a gelatin sample in the frequency range from 80 Hz to 4 kHz. $\circ$: A uniform sample; $\diamond$: A layered sample; ∇ : Nenadic *et al.* (2011a); $\cdots$: Regression for a uniform sample; $---$: Regression for a layered sample; $—$: Regression for Nenadic *et al.* (2011a). (Regressions are to guide the eyes.)

IV. CONCLUSIONS

A cost-effective and model-independent characterization method based on Rayleigh wave propagation was developed for quantifying the frequency-dependent viscoelastic properties of a small volume of soft biomaterials at high frequencies. A thin layer of the sample material, only a few millimeters thick, was required, in contrast with several centimeters for a method presented in a previous, related study (Kazemirad *et al.*, 2013). This reduces the required sample volume, and consequently the cost of experiments, by a factor of 25 (96% saving). The method presented in this study has the potential to be used for the characterization of injectable biomaterials used for vocal fold augmentation and repair such as commercial phonosurgical biomaterials (Cymetra, Radiesse, Juvederm, and Restylane) and hydrogels designed in our group (Heris *et al.*, 2012). Layered silicone rubber and gelatin samples consisting of a substrate with known properties under a thin layer of a soft material with unknown properties were fabricated and tested. Measurements were performed at frequencies up to 4 kHz. The results were found

to be in good agreement with and improved upon those of Nenadic *et al.* (2011a).

The measured propagation wave speeds increased with frequency at a greater rate at high frequencies, which confirms the rapid increase of shear and elastic moduli beyond the transition zone. The results emphasize the need for the characterization of *frequency-dependent* viscoelastic properties of soft biomaterials over a broad frequency range. This is needed to accurately characterize the viscoelastic properties of injectable phonosurgical biomaterials and hydrogels used for vocal fold treatment.

This characterization method is based on the assumption of no reflection from the boundaries of the sample and cannot, therefore, be used to quantify the mechanical properties of materials with a negligible loss factor, such as elastic materials. In addition, the propagated surface waves are generally dissipated over a greater propagation distance at low

frequencies, where the wavelength is greater. Samples with small dimensions do not allow accurate measurements at low frequencies. In the present study, there was no reflection from the boundaries at frequencies higher than 80 Hz, and thus results were presented in the frequency range of 80 to 4000 Hz.

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APPENDIX

The coefficient matrix of the system of equations developed in Eq. (10) is as follows:

$$\mathbf{C} = \begin{bmatrix} (2\hat{k}_R^2 - \hat{k}_{s1}^2) & (2\hat{k}_R^2 - \hat{k}_{s1}^2) & -2\hat{k}_R k_2 & 2\hat{k}_R k_2 & 0 & 0 \\ 2\hat{k}_R k_1 & -2\hat{k}_R k_1 & (2\hat{k}_R^2 - \hat{k}_{s1}^2) & (2\hat{k}_R^2 - \hat{k}_{s1}^2) & 0 & 0 \\ (2\hat{k}_R^2 - \hat{k}_{s1}^2)\hat{\mu}_1 c_1^{-1} & (2\hat{k}_R^2 - \hat{k}_{s1}^2)\hat{\mu}_1 c_1 & -2\hat{k}_R k_2 c_2^{-1} & 2\hat{k}_R k_2 c_2 & -(2\hat{k}_R^2 - \hat{k}_{s2}^2)\hat{\mu}_2 c_3^{-1} & -2i\hat{k}_R k_4 \hat{\mu}_2 c_4 \\ 2\hat{k}_R k_1 \hat{\mu}_1 c_1^{-1} & -2\hat{k}_R k_1 \hat{\mu}_1 c_1 & (2\hat{k}_R^2 - \hat{k}_{s1}^2)\hat{\mu}_1 c_2^{-1} & (2\hat{k}_R^2 - \hat{k}_{s1}^2)\hat{\mu}_1 c_2 & 2i\hat{k}_R k_3 \hat{\mu}_2 c_3 & -(2\hat{k}_R^2 - \hat{k}_{s2}^2)\hat{\mu}_2 c_4^{-1} \\ i\hat{k}_R c_1^{-1} & i\hat{k}_R c_1 & -ik_2 c_2^{-1} & ik_2 c_2 & -i\hat{k}_R c_3 & k_4 c_4 \\ -ik_1 c_1^{-1} & ik_1 c_1 & -i\hat{k}_R c_2^{-1} & -i\hat{k}_R c_2 & k_3 c_3 & i\hat{k}_R c_4 \end{bmatrix} \quad (\text{A1})$$

where

$$c_1 = e^{ik_1 h}, \quad c_2 = e^{ik_2 h}, \quad c_3 = e^{ik_3 h}, \quad c_4 = e^{ik_4 h}. \quad (\text{A2})$$

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