Characterization of material properties of soft solid thin layers with acoustic radiation force and wave propagation

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Evaluation of tissue engineering constructs is performed by a series of different tests. In many cases it is important to match the mechanical properties of these constructs to those of native tissues. However, many mechanical testing methods are destructive in nature which increases cost for evaluation because of the need for additional samples reserved for these assessments. A wave propagation method is proposed for characterizing the shear elasticity of thin layers bounded by a rigid substrate and fluid-loading, similar to the configuration for many tissue engineering applications. An analytic wave propagation model was derived for this configuration and compared against finite element model simulations and numerical solutions from the software package Disperse. The results from the different models found very good agreement. Experiments were performed in tissue-mimicking gelatin phantoms with thicknesses of 1 and 4 mm and found that the wave propagation method could resolve the shear modulus with very good accuracy, no more than 4.10% error. This method could be used in tissue engineering applications to monitor tissue engineering construct maturation with a nondestructive wave propagation method to evaluate the shear modulus of a material. © 2015 Acoustical Society of America.

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I. INTRODUCTION

In the field of tissue engineering, monitoring the elasticity of developing tissue cultures is important for evaluating their growth and maturation. Many types of methods and indicators have been employed to accomplish this task including measurement of collagen content, protein and DNA levels, expression of certain genes, and tissue stiffness.^{1,2} Most of these methods involve the destruction of the tissue cultures, preventing further growth of the culture. This type of destructive evaluation also leads to higher costs because more tissue cultures are needed to evaluate growth to a certain temporal endpoint. It is desirable to have a method that could test the development of the tissue culture *in situ* and be applied at multiple time points to facilitate a longitudinal study of the same sample as it grows.

One of the metrics that is used to evaluate the development of tissue cultures is their elasticity. The elasticity of the tissue of engineered cartilage is directly related to its maturation in culture.^{1,2} The elasticity of the cultured tissue should be close to that of *in vivo* tissue so that a transplant of the grown tissue might be efficacious for substitution.

Elasticity of tissue cultures can be evaluated with a number of different methods, most of which are destructive to the tissue samples. These methods include compressive mechanical testing, atomic force microscopy, magnetic

resonance elastography (MRE), and ultrasound-based methods.^{3–14} Ultrasound-based methods are attractive because they can typically be applied to the tissue without destroying the culture. Strain-based ultrasound elasticity imaging has been used to investigate the stiffness of tissue engineering constructs by applying compression with the ultrasound transducer and measuring the resulting strain in the sample under investigation.^{11,12} Another ultrasound-based method used acoustic radiation force (ARF) to induce vibration in thin tissue-mimicking constructs.¹⁴ A forced harmonic oscillator model was used to extract the viscoelastic properties of the constructs. A second ultrasound-based model used ARF excitation and a model-based inversion approach to solve for the model-free complex moduli.¹³ Magnetic resonance elastography uses external vibration to create propagating waves in soft tissues and magnetic resonance imaging techniques to measure the motion of the waves.¹⁵ MRE has been used on cartilage mimicking samples⁷ and cartilage samples that were modified with enzymatic exposure.⁸ Additional MRE work has been done on measurement of engineered samples implanted in mice.^{9,10}

While the elasticity measurement methods are effective, the ultrasound-based methods discussed above need a model and knowledge of the stress distribution provided by the ARF or by a transducer compressing the sample. The MRE methods use wave propagation so the stress magnitude does not need to be known. However, the technology is not widely available and very high propagation frequencies were used which attenuate very quickly.

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An ultrasound-based wave propagation method would combine the nondestructive nature of the ultrasound measurements with the advantages of a wave propagation method like MRE where the stress distribution is not needed for the material property estimation. Similar investigations have been reported in dealing with thin layers bounded on each side by fluids which generates Lamb waves.^{16,17} In this paper, we propose an ultrasound-based method that uses ARF to induce propagating waves in thin gelatin layers bounded by a solid substrate and overlying fluid. These types of soft gelatin polymers have been characterized by many different methods and are useful for mimicking soft tissues.^{18–22} We have derived an analytic solution for the configuration with a soft solid layer bounded by fluid on one side and a rigid substrate on the other. We compared the results from the analytic model with results from a finite element simulation and a numerical solution using a software package called Disperse. Experimentally, we measure the resulting motion and use the wave velocity dispersion to solve for the elasticity of the layer, which may be a growing tissue. The thickness of the layer must be measured accurately, but that can be accomplished with ultrasound imaging techniques.

II. METHODS

A. Analytic model

We consider a model with a thin layer of thickness, h , in contact with a rigid half space on one side and fluid coupled to the upper surface as shown in Fig. 1. The ensuing theoretical development follows nomenclature and derivations of some of our previously reported work.^{23,24}

We can write the potential functions for the wave motion

$$\begin{aligned}\phi_1 &= (a_1 e^{-i\eta_1 x_2} + b_1 e^{i\eta_1 x_2}) e^{ikx_1}, \\ \psi_1 &= (c_1 e^{-i\beta_1 x_2} + d_1 e^{i\beta_1 x_2}) e^{ikx_1}, \\ \phi_2 &= b_2 e^{-\eta_2 x_2} e^{ikx_1}, \\ \psi_2 &= d_2 e^{-\beta_2 x_2} e^{ikx_1}, \\ \phi_f &= m e^{\eta_f x_2} e^{ikx_1},\end{aligned}\quad (1)$$

where $\eta_1 = \sqrt{k_{p1}^2 - k^2}$, $\beta_1 = \sqrt{k_{s1}^2 - k^2}$, $\eta_2 = \sqrt{k^2 - k_{p2}^2}$, $\beta_2 = \sqrt{k^2 - k_{s2}^2}$, $\eta_f = \sqrt{k^2 - k_f^2}$, and k is the wavenumber, subscript p denotes the primary or compression wave, s subscript denotes the shear wave, f denotes the fluid. Here, ψ and ϕ are potential functions of medium deformation, and a ,

FIG. 1. (Color online) Model of layer of thickness h on semi-infinite elastic medium and fluid-loading.

b , c , d , and m are constants. The superscripts correspond to the media which are the thin layer, semi-infinite elastic medium, and fluid (1, 2, and f, respectively) and subscript to the axes (x_1 or x_2) along which the displacement or stress is observed in Fig. 1. We assume that the phase velocities of the guided waves are much smaller than the phase velocities of the bulk waves in the fluid and substrate. This model also assumes plane strain and that in the x_3 dimension the plate is of infinite extent.

We apply the following boundary conditions at the interface between the layer and rigid half-space at $x_2 = 0$,

$$\begin{aligned}\sigma_{22}^1 &= \sigma_{22}^2, \\ \sigma_{12}^1 &= \sigma_{12}^2, \\ u_1^1 &= u_1^2, \\ u_2^1 &= u_2^2,\end{aligned}\quad (2)$$

and the following boundary conditions at the interface between the layer and fluid at $x_2 = h$,

$$\begin{aligned}\sigma_{22}^1 &= \sigma_{22}^f, \\ \sigma_{12}^1 &= 0, \\ u_2^1 &= u_2^f.\end{aligned}\quad (3)$$

Applying the boundary conditions above with the potential functions in Eq. (1), we can write the plane stress in the rigid substrate at $x_2 = h$ where $E_1 = e^{i\eta_1 h}$, $B_1 = e^{i\beta_1 h}$, $E_2 = e^{-\eta_2 h}$, $B_2 = e^{-\beta_2 h}$:

$$\begin{aligned}\sigma_{22}^1 &= \sigma_{22}^2, \\ \sigma_{22}^1 &= -\mu_1 \{k_{s1}^2 \phi_1 + 2(\psi_{1,12} + \phi_{1,11})\}, \\ \sigma_{22}^2 &= -\mu_2 \{k_{s2}^2 \phi_2 + 2(\psi_{2,12} + \phi_{2,11})\}, \\ \sigma_{22}^1 &= -\mu_1 \{[k_{s1}^2 - k^2](a_1 E_1^{-1} + b_1 E_1) \\ &\quad + 2k\beta_1 (c_1 B_1^{-1} - d_1 B_1)\} e^{ikx_1}, \\ \sigma_{22}^2 &= -\mu_2 \{[k_{s2}^2 - k^2]b_2 E_2 - 2k\beta_2 d_2 B_2\} e^{ikx_1}.\end{aligned}\quad (4)$$

Here, μ is the general shear modulus so μ_1 and μ_2 are the shear moduli of elasticity of the thin layer and semi-infinite elastic media, respectively (Fig. 1). The shear stress at $x_2 = 0$ is

$$\begin{aligned}\sigma_{12}^1 &= 0, \\ \sigma_{12}^1 &= \mu_1 \{2\phi_{1,12} + \psi_{1,22} - \psi_{1,11}\}, \\ \sigma_{12}^1 &= \mu_1 \{2k\eta_1 (a_1 - b_1) + (k^2 - \beta_1^2)(c_1 + d_1)\} e^{ikx_1} = 0.\end{aligned}\quad (5)$$

The shear stress at $x_2 = h$ is

$$\begin{aligned}\sigma_{12}^1 &= \sigma_{12}^2, \\ \sigma_{12}^1 &= \mu_1 \{2\phi_{1,12} + \psi_{1,22} - \psi_{1,11}\}, \\ \sigma_{12}^2 &= \mu_2 \{2\phi_{2,12} + \psi_{2,22} - \psi_{2,11}\}, \\ \sigma_{12}^1 &= \mu_1 \{2k\eta_1 (a_1 E_1^{-1} - b_1 E_1) \\ &\quad + (k^2 - \beta_1^2)(c_1 B_1^{-1} + d_1 B_1)\} e^{ikx_1}, \\ \sigma_{12}^1 &= \mu_2 \{-2ik\eta_2 b_2 E_2 + (k^2 + \beta_2^2)d_2 B_2\} e^{ikx_1}.\end{aligned}\quad (6)$$

The subscript following the commas in the potential functions correspond to partial derivatives with respect to the x_1 and x_2 axis. For example, $\phi_{1,1}$ corresponds to the partial derivative of ϕ_1 with respect to x_1 and $\phi_{1,2}$ corresponds to the partial derivative of ϕ_1 with respect to x_2 . Similarly, $\phi_{1,12}$ is the partial derivative of $\phi_{1,1}$ with respect to x_2 and $\phi_{2,11}$ would be the second partial derivative of ϕ_2 with respect to x_1 . The displacements along the x_1 direction due to the boundary condition at $x_2 = h$,

$$\begin{aligned} u_1^1 &= u_1^2, \\ u_1^1 &= \{\phi_{1,1} + \psi_{1,2}\}, \\ u_1^2 &= \{\phi_{2,1} + \psi_{2,2}\}, \\ u_1^1 &= \{ik(a_1E_1^{-1} + b_1E_1) + i\beta_1(-c_1B_1^{-1} + d_1B_1)\}e^{ikx_1}, \\ u_1^2 &= \{ikb_2E_2 - \beta_2d_2B_2\}e^{ikx_1}. \end{aligned} \quad (7)$$

The plane stress in the substrate due to fluid loading at $x_2 = 0$ is

$$\begin{aligned} \sigma_{22}^1 &= \sigma_{22}^f, \\ \sigma_{22}^1 &= -\mu_1 \{k_{s1}^2\phi_1 + 2(\psi_{1,12} + \phi_{1,11})\}, \\ \sigma_{22}^f &= -\mu_f \{\phi_{2,11}\}, \\ \sigma_{22}^1 &= -\mu_1 \{[k_{s1}^2 - k^2](a_1 + b_1) + 2k\beta_1(c_1 - d_1)\}e^{ikx_1}, \\ \sigma_{22}^f &= \rho_f \omega^2 m e^{n_f x_2} e^{ikx_1} = \frac{\mu_1 \rho_f k_{s1}^2}{\rho_1} m e^{ikx_1}. \end{aligned} \quad (8)$$

The medium densities ρ_1 and ρ_f correspond to the thin layer and fluid, respectively. The displacement in the x_2 direction due to fluid loading at $x_2 = 0$ is

$$\begin{aligned} u_2^1 &= u_2^f, \\ u_2^1 &= \{\phi_{1,2} - \psi_{1,1}\}, \\ u_2^f &= \phi_{2,2}, \\ u_2^1 &= \{i\eta_1(-a_1 + b_1) + ik(c_1 + d_1)\}e^{ikx_1}, \\ u_2^f &= n_f m e^{n_f x_2} e^{ikx_1} = n_f m e^{ikx_1}. \end{aligned} \quad (9)$$

The displacement in the x_2 direction due to fluid loading at $x_2 = h$ is

$$\begin{aligned} u_2^1 &= u_2^2, \\ u_2^1 &= \{\phi_{1,2} - \psi_{1,1}\}, \\ u_2^2 &= \{\phi_{2,2} + \psi_{2,1}\}, \\ u_2^1 &= \{i\eta_1(-a_1E_1^{-1} + b_1E_1) + ik(c_1B_1^{-1} + d_1B_1)\}e^{ikx_1}, \\ u_2^2 &= \{-\eta_2b_2E_2 - ikd_2B_2\}e^{ikx_1}. \end{aligned} \quad (10)$$

Assembling these equations into a system of equations we come to the matrix A . To obtain non-trivial results, the determinant of A is set to zero. $[\det(A) = 0]$. Using these results we can plot the dispersion curves for specified frequency ranges:

$$A = \begin{bmatrix} (k_{s1}^2 - k^2) & (k_{s1}^2 - k^2) & 2k\beta_1 & -2k\beta_1 & 0 & 0 & k_{s1}^2 \\ -i\eta_1 & i\eta_1 & -ik & -ik & 0 & 0 & n_f \\ 2k\eta_1 & -2k\eta_1 & (2k^2 - k_{s1}^2) & (2k^2 - k_{s1}^2) & 0 & 0 & 0 \\ (2k^2 - k_{s1}^2)\mu_1 E_1^{-1} & (2k^2 - k_{s1}^2)\mu_1 E_1 & -2k\beta_1 B_1^{-1} & -2k\beta_1 B_1 & -(2k^2 - k_{s2}^2)\mu_2 B_2 & -2ik\beta_2 \mu_2 B_2 & 0 \\ 2k\eta_1 \mu_1 E_1^{-1} & -2k\eta_1 \mu_1 E_1 & (2k^2 - k_{s1}^2)\mu_1 B_1^{-1} & (2k^2 - k_{s1}^2)\mu_1 B_1 & 2k\eta_2 \mu_2 E_2 & -(2k^2 - k_{s2}^2)\mu_2 B_2 & 0 \\ ikE_1^{-1} & ikE_1 & -i\beta_1 B_1^{-1} & i\beta_1 B_1 & -ikE_2 & \beta_2 B_2 & 0 \\ -i\eta_1 E_1^{-1} & i\eta_1 E_1 & -ikB_1^{-1} & -ikB_1 & \eta_2 E_2 & ikB_2 & 0 \end{bmatrix}. \quad (11)$$

For calculation purposes in this study, the semi-infinite medium was approximated with parameters for plastic with $c_{s2} = 1330$ m/s and $c_{p2} = 2740$ m/s. The fluid was assumed to be water with a density of $\rho_f = 1000$ kg/m³ and the density of the thin layer was also $\rho_1 = 1000$ kg/m³, similar to most soft tissues. The compressional speed of the thin layer was assumed to be $c_{p1} = 1540$ m/s.

Regarding the method we used to compute the dispersion curves from the determinant, we calculated the values of the determinant for a range of frequencies and speeds to generate a matrix of the determinant. We then performed a vertical and horizontal search through that matrix to identify

zero-crossings. Once these were identified we extracted those dispersion curves from the matrix and plotted them.

B. Finite element modeling

To validate the analytic solution derived above, we performed a finite element model (FEM) simulation to understand the wave motion and the wave speed dispersion. The finite element model was constructed using ABAQUS/CAE 6.8-3 (SIMULIA, Providence, RI). The FEM was set up as an axisymmetric model in the shape of cylinder, with a radius of 100 mm and thickness of 5 mm. A fixed boundary condition was prescribed to the bottom surface of the

phantom and its top surface is covered by a 10 mm thickness of acoustic medium to simulate the water. The excitation was applied to the center region of the phantom and was introduced as nodal displacements. The excitation was a cylindrical source that is 2.5 mm in radius and also extends 2 mm deep from the phantom's top surface to mimic the experimental ARF push. The duration of the displacement was 600 μ s. Applying the excitation through the whole thickness presented numerical issues so this was a compromise made to be deeper than just a surface excitation while preserving the integrity of the model. The entire model was meshed with square elements that are 0.25 mm $\times$ 0.25 mm. For the FEM, the elements were linear quadrilateral type with reduced integration (CAX4R). For the water on top of the phantom, the elements were linear quadrilateral acoustic type (ACAX4). The model was solved by the implicit dynamic solver of Abaqus and nodal displacements were extracted and analyzed. The thin layer was elastic and two shear moduli were simulated $\mu_1 = 10$ and 20 kPa.

For the FEM data we recorded both the particle displacement and velocity at many locations over a 60 mm span away from the original excitation for 20 ms. To extract the wave velocities at multiple frequencies we performed a two-dimensional fast Fourier transform (2D-FFT) on the motion data to obtain a frequency-domain representation with the temporal frequency, f , on the horizontal axis and the wavenumber, k , on the vertical axis.^{25,26} To extract the wave velocities we examine the 2D-FFT representation and find where the energy is concentrated, or the peaks in the 2D-FFT. To find these peaks we search horizontally (at a constant wavenumber) and vertically (at a constant frequency). Once these peaks are identified we use the corresponding frequency and wavenumber and use the relationship $c = f/k = f\lambda$ to calculate the wave velocities.

C. Disperse modeling

A commercial software package called Disperse can calculate the wave velocity dispersion for certain multilayered geometries.²⁷ Information regarding the Disperse software can be found at <http://www3.imperial.ac.uk/nde/products%20and%20services/disperse>. We used Version 2.0.16B released in 2003. We modeled the same configurations as described above for the FEM tests. The water had a density of 1000 kg/m³ and a longitudinal wave velocity of 1500 m/s. The layer had a thickness of 5 mm, density of 1000 kg/m³, Young's modulus of 10 or 20 kPa and Poisson's ratio of 0.499979. The substrate was modeled with Perspex with a density of 1180 kg/m³, Young's modulus of 6.33 GPa and Poisson's ratio of 0.31.

D. Experiments

We made three phantoms from the same gelatin mixture with 10% 300 Bloom gelatin and 10% glycerol (Sigma Aldrich, Inc., St. Louis, MO) where the concentrations were measured by volume. One phantom was a reference phantom with large thickness to make a shear wave measurement. The other phantoms were 1 and 4 mm thick poured into a plastic circular plate. The thickness was measured using

ultrasound pulse-echo techniques assuming a sound speed of 1540 m/s. The propagating waves were produced by ARF from an unfocused push, which is accomplished by turning on a certain number of consecutive elements,²⁸ for a time duration of 600 μ s using a linear array transducer (L7-4, Philips Healthcare, Andover, MA) and Verasonics V-1 system (Verasonics, Kirkland, WA). The unfocused push used 16 elements which was a total width of 4.8 mm. The unfocused nature of the push comes from not applying any electronic focusing delays to any of the signals transmitted. Therefore, the elements that are turned on are transmitting a "plane wave" similar to that used for detection of the guided waves. Propagation of waves were imaged using 10 kHz pulse repetition frequency with plane wave transmissions.²⁹ Motion was calculated using an autocorrelation technique.³⁰

The group velocity of the reference phantom was measured using a time-of-flight measurement technique and ρ was assumed to be 1000 kg/m³. A 2D-FFT was applied to the motion data from the 1 and 4 mm phantoms, and the wave velocity dispersion was extracted. The analytic model was used to fit the data and the shear modulus providing the best fit was reported. The fitting process was driven by evaluating the error between the data and the analytic curves. Only the lowest-order mode was fit because that consistently generated in the experiments.

III. RESULTS

A. Simulations

We compared the results of the FEM, Disperse, and the analytic solution for the 5 mm thick layer on the rigid substrate with shear moduli of 10 and 20 kPa. Figure 2 shows the velocity and displacement motion in the left and right columns, respectively. The 2D-FFT representations for the particle velocity and displacement are shown in the middle row of Fig. 2. The wave velocities for the particle velocity and displacement motion are shown in bottom row of Fig. 2.

Figure 3 shows a comparison of the dispersion curves derived from the FEM displacement data, the Disperse software package, and the analytic solution for the layer with $\mu_1 = 10$ kPa. Figure 4 shows the same comparison for the layer with $\mu_1 = 20$ kPa.

The agreement among the three methods is very good. The analytic and Disperse results provide data for more of the possible modes than the FEM results, more than likely because the FEM excitation did not elicit the higher order modes with this particular type of excitation used.

With this validated analytical method, we performed a parametric study to explore the ranges of wave velocities for the lowest order mode that the FEM agreed. We varied the thickness of the layer from 1 to 5 mm in 1 mm increments and use different values of the shear modulus, $\mu_1 = 5, 10, 20, 50$, and 100 kPa. The results are shown in Fig. 5. These results demonstrate that as the shear modulus increases, the modes separate more for the different thicknesses and shift to higher frequencies. Higher bandwidths are needed characterization of thinner layers for a given value of μ_1 .

FIG. 2. (Color online) FEM motion analysis for a layer with $h = 5$ mm and $\mu_1 = 10$ kPa. (a) Particle displacement, (b) Particle velocity, (c) 2D-FFT of particle displacement, (d) 2D-FFT of particle velocity, (e) wave velocity dispersion from particle displacement, (f) wave velocity dispersion from particle velocity. The black and cyan symbols are from the horizontal and vertical searches, respectively.

B. Experiments

The measurements from the reference sample using a shear wave measurement gave a shear modulus of 7.56 kPa. Figure 6 shows the particle displacement of the surface of the 4 mm thick phantom, the 2D-FFT, and the wave velocity dispersion extracted from the 2D-FFT using a horizontal and vertical search scheme. Figure 7 shows the fitting of the dispersion data with $\mu_1 = 7.50$ kPa. Figure 8 shows the particle velocity of the 1 mm thick phantom, the 2D-FFT, and extracted wave speeds. Figure 9 shows the wave speed dispersion data with the analytic model fit with $\mu_1 = 7.25$ kPa.

The experimental results for the different phantoms compared very well as demonstrated in Table I where the percent errors were at most 4.10%.

IV. DISCUSSION

The analytic wave propagation model was found to be in good agreement with the FEM results and the results from the Disperse software package. This agreement validated the model for fluid-loaded elastic layers on a rigid substrate. The analytic model was fast and was used to fit the experimental results. The parametric study results in Fig. 5 show that high bandwidths are needed for proper measurement as the thickness of the layer decreases. The Disperse model used a plastic layer and not a rigid boundary. For this case, with the stiff plastic, the results from the FEM with the rigid boundary condition and the Disperse model are very similar. Using a rigid boundary could simplify the model, but we decided

FIG. 3. (Color online) Wave velocity dispersion curves from particle displacement FEM data, Disperse, and the analytic model for $h = 5$ mm and $\mu_1 = 10$ kPa.

FIG. 4. (Color online) Wave velocity dispersion curves from particle displacement FEM data, Disperse, and the analytic model for $h = 5$ mm and $\mu_1 = 20$ kPa.

FIG. 5. (Color online) Parametric study of lowest order mode for layers of different thicknesses ($h = 1\text{--}5\text{ mm}$) and values of the shear modulus. The black solid line represents c_{s1} for each case. (a) $\mu_1 = 5\text{ kPa}$, (b) $\mu_1 = 10\text{ kPa}$, (c) $\mu_1 = 20\text{ kPa}$, (d) $\mu_1 = 50\text{ kPa}$, (e) $\mu_1 = 100\text{ kPa}$.

to make it more general so that any type of elastic substrate could be used.

The experimental and FEM data generally had good agreement with the lowest order mode, but higher order modes did not have sufficient energy to be compared to the analytic model. This may also be due to the type of excitation that is performed with the ARF stimulation. Figure 7 showed that we could excite two modes but Fig. 8 only depicted the lowest order mode. We need to explore further on the conditions in which higher order modes may be produced. The waves that travel often have high bandwidth. The ARF excitation could be further optimized to produce higher bandwidths by using shorter excitation pulses and tightly focused beams. However, for a tighter focus more fluid offset may be necessary for some transducers. In addition, if the motion has sufficient signal-to-noise ratio, the particle acceleration of the motion could be analyzed instead of the particle velocity. This yields a higher bandwidth, because the acceleration is a time derivative of velocity which in the frequency domain will multiply the magnitude distribution by a factor of $i\omega$.

We used a horizontal and vertical search for extracting the wave speed dispersion curves from the k-space distributions. Ideally the horizontal and vertical search would provide the same results. However, when there is a feature in the k-space that is vertically oriented at a given frequency,

then the speed is changing rapidly and that would be difficult to identify with a vertical search, but a horizontal search would be sensitive to that type of feature. Therefore, the vertical and horizontal searches can provide complementary information due to their preferred sensitivities when looking for zero-crossings.

Because the waves can be very high speed we wanted to optimize the frame rate of the motion detection which was 10 kHz in this case. One way to maximize this is to reduce the distance between the sample and the transducer. However, in doing this a focused push with a low f-number becomes difficult to employ. Alternatively, we used an unfocused push which can push on the material from the surface of the transducer to a reasonable depth. A focused push does have the advantage of producing more motion in typical circumstances which would provide a higher signal-to-noise ratio, but the flexibility for shallow measurements determined our choice to use the unfocused push. The signal-to-noise ratio plays a significant role in the shear modulus estimation, but we did have k-space with good fidelity to estimate the dispersion curves.

We would have to perform more tests to understand the extreme bounds which the method can be used in terms of the layer thickness and layer stiffness. One way to analyze this is to normalize the family of dispersion curves generated by the analytic method by the frequency-thickness product, and the shear wave speed of the layer. This produces a set of

FIG. 6. (Color online) Experimental results from gelatin layer with $h=4$ mm. (a) Particle displacement data, (b) 2D-FFT, (c) wave velocities extracted from horizontal and vertical searches.

curves shown in Fig. 10, which can then be used to understand the parameter space that could be explored with this method. In general the results shown in this study range from a frequency-thickness product of 0–4 Hz m and normalized speed (c/c_s) ranging from 1 to 6. These types of curves

FIG. 7. (Color online) Wave velocity dispersion and analytic model fit with $\mu_1 = 7.50$ kPa for phantom with $h = 4$ mm layer.

FIG. 8. (Color online) Experimental results from gelatin layer with $h=1$ mm. (a) Particle velocity data, (b) 2D-FFT, (c) wave velocities extracted from horizontal and vertical searches.

FIG. 9. (Color online) Wave velocity dispersion and analytic model fit with $\mu_1 = 7.25$ kPa for phantom with $h = 1$ mm layer.

TABLE I. Comparison of shear modulus values for phantoms.

Phantom	μ , kPa	Percent error, %
Reference	7.56	–
$h = 1$ mm	7.25	–4.10
$h = 4$ mm	7.50	–0.79

could be used to analyze the effects of errors in the thickness estimate for modulus estimation. As an example if a layer of $h = 1$ mm and $\mu = 5$ kPa are the true values, but a thickness of $h = 1.1$ mm is used, then the estimated modulus rises to 6 kPa so a 10% error in thickness measurement could lead to an overestimation of the shear modulus.

In this work we assumed that the thin layers were elastic materials, but it is well known that most soft tissues are viscoelastic in nature.³¹ In previous work, we have incorporated the viscoelastic nature by making the shear modulus complex.²³ An extension of this work would be to insert that type of material definition to characterize the viscoelastic properties of a soft, thin layer.

The wave propagation method only requires measurement of the layer thickness and motion of the excited waves, both of which can be measured nondestructively by ultrasound pulse-echo methods. Additionally, because of the non-destructive nature of the measurement, the progression of the layer stiffness could be measured in a longitudinal study with fewer samples and lower cost than current study designs. All that is physically needed is a sufficient fluid standoff for proper focusing of the ARF push beam to generate the propagating waves. Also, the model inversion is independent of knowing the stress magnitude, which is often difficult to model or measure.

V. CONCLUSIONS

We have presented a wave propagation method for characterizing the material properties of thin layers bounded by a rigid substrate and fluid-loading. We derived an analytic

FIG. 10. Normalized analytic wave velocity dispersion curves. The horizontal line at $c/c_s = 1$ is the shear wave speed of the material.

method for this model and compared against FEM simulations and simulations from the software package Disperse and found very good agreement. We performed experiments in tissue-mimicking gelatin and found that the wave propagation method could resolve the elastic material properties with very good accuracy. This method could be used in tissue engineering applications to monitor culture growth as evaluated using the shear modulus.

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