Complex Shear Modulus of Thermally-Damaged Liver

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***Abstract*—The complex shear modulus of fresh and thermally damaged porcine liver has been measured, in vitro, using an ultrasonic shear wave imaging technique. Measurements were compared to two constitutive models, Kelvin-Voigt and Zener, to estimate the complex modulus of liver for shear wave frequencies between 50 and 300 Hz. An axially vibrated needle placed in the liver excites harmonic shear waves that are imaged using a pulsed Doppler technique. Liver heated to 47^{o}C for 90 min was found to have little measurable cellular damage, and yet the elastic shear modulus increased by a factor of two and the viscous shear modulus increased by a factor of three in this frequency range. These observations imply that elastic properties, especially the viscous shear modulus, may be a sensitive indicator of thermal damage. Also, within the testing bandwidth for shear waves, the Zener model represented the data better than the Kelvin-Voigt model.**

I. Introduction

Our overall objective of the study reported below is to develop methods for estimating the complex shear modulus of 3-D cell culture gels over a large bandwidth of shear-wave frequencies. Our purpose is to study cell mechanobiology at the meso-scale (cell groups surrounded by an extracellular matrix) during cell transformation and malignant progression. We developed this vibrating needle approach by studying denatured collagen (gelatin) hydrogels, as reported in [1]. Gelatin gels are predominantly a linear-elastic continuum (weak viscous response) at this frequency range. To further develop our measurement technique for biphasic collagen gels used in cell culture, we selected ex vivo liver because of its nonlinear, viscoelastic response. While the results may have implications for hyperthermia and perhaps ablation, our specific goal is to use liver tissue as a phantom medium to developed the needle-based shear wave technique for estimating the complex shear modulus. Addition goals include identifying appropriate rheological models for modulus estimation and comparing our measurements with those in the literature for consistency.

II. Methods

A. Measuring the complex shear modulus

A mechanical actuator harmonically vibrates a needle along its axis at frequencies between 50 and 300 Hz and in steps of 50 Hz after it is placed in liver tissue. The oscillations generate sinusoidal shear waves that are measured via pulsed Doppler techniques as a function of slow time and within a spatial plane. Waves radiating from the needle have decreasing amplitude because of divergence and absorption by the liver. From particle velocity phase, shear speed is estimated via a phase-gradient approach. Wave speed measurements as a function of shear-wave frequency $\hat{c}_s(\omega)$ were compared with predictions obtained from rheological models $c_s(\omega)$. These comparisons require nonlinear regression techniques, where the complex modulus μ is a free parameter. The modulus value yielding the best fit between wave speed measurements and predictions is selected as the value for that shear-wave frequency. The Kelvin-Voigt model and a standard solid body (Zener) model were both fit to the data to study fresh and thermally-damaged liver samples.

B. Tissue preparation

A total of six pig livers were studies in pairs, one fresh and the other heated, during three separate experiments. Pairs of livers obtained at the time of slaughter were immediately stored in saline at 5^{o}C for transportation. Within 30 minutes, one of the livers was transferred to a container with saline at room temperature, 23^{o}C, for one hour before testing. Mechanical properties were measured after inserting the needle and applying the Doppler probe.

The second liver was removed from the cold saline and placed in heated saline at 47^{o}C for 90 minutes. The goal was to thermally damage the tissue and thus alter the viscoelastic properties [2]. Subsequently, the heated liver was placed in saline at 23^{o}C for one hour before measurements as described for fresh liver above. The entire process was repeated three time and the results averaged. All tests were completed within 8 hours of tissue harvesting to minimize the effects of outgassing from decomposition.

At the end of each experiment, liver samples were prepared for histological analysis. Samples were formalin fixed, paraffin embedded, microtome sectioned, stained with H&E, and mounted on glass slides. The slides were read by a pathologist for changes in structure that might explain any observed changes in the shear modulus. Figs 1 and 2 are examples of histology from fresh and heated liver samples. Both images are acquired with 20x magnification, which provides a pixel size of 0.3 mm.

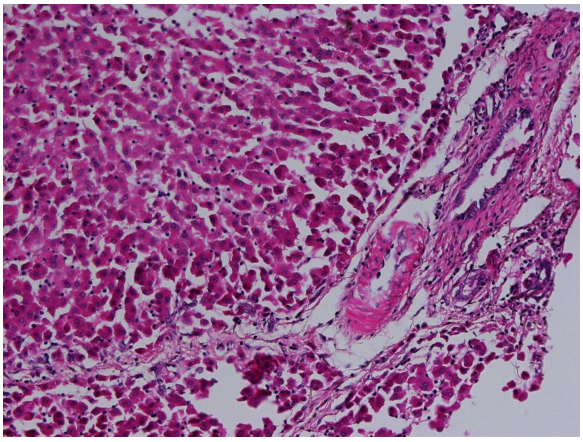

Fig. 1. Histological image of fresh porcine liver at 20x magnification.

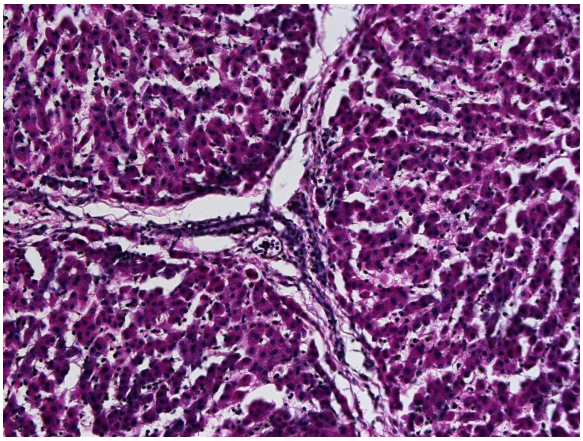

Fig. 2. Histological image of heated porcine liver at 20x magnification.

C. Shear wave imaging

Shear wave properties of viscoelastic media may be characterized by a complex shear wave number, $k_s = (\rho\omega^2/\mu)^{1/2}$, where ρ is mass density, ω is the angular shear-wave frequency, and μ is the complex shear modulus of liver. Different rheological models predict the response of the liver to the applied vibrational stress through μ in different ways, and therefore μ can be estimated by fitting measured values to predicted dispersion curves. In this study we examined two rheological models.

First we examined the Kelvin-Voigt model illustrated in Fig 3a. It is most often used in formulating a constitutive equation describing creep experiments [4]. The complex modulus obtained from the Kelvin-Voigt model is $\mu^K(\omega) = \mu_1 - i\omega\eta_K$, where μ_1 is the relaxed elastic modulus ($\mu^K|_{\omega\to 0} = \mu_1$) and η_K is the viscous modulus. Shear wave propagation is influenced by viscous dissipation of the wave energy. Viscous dissipation can be quantified by the quality factor $Q(\omega) = -\Re\{k_s^2\}/\Im\{k_s^2\}$, whose inverse Q^{-1}, is called the dissipation factor. For the Kelvin-Voigt model, it can be shown that $Q^K(\omega) = 1/(\omega\tau)$ where $\tau = \eta_K/\mu_1$. The frequency

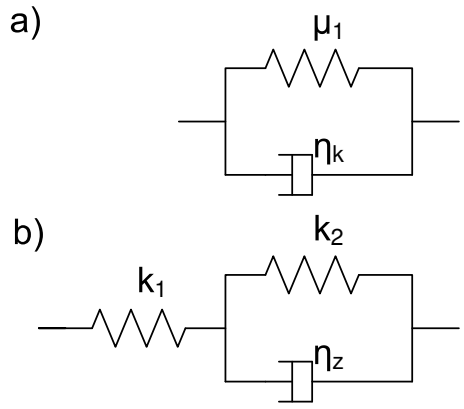

Fig. 3. In the upper half of the figure, a), Kelvin-Voigt model is diagramed. Model consists of a elastic component μ_1 in parallel with the viscous component η_k. In the lower half of the figure, b), we illustrated a standard linear solid or Zener model, which is represented by a series of an elastic component and a Kelvin-Voigt model.

dependance of Q^{-1} shows that K-V dissipation is unbounded and increasing with frequency. Thus liver behaves as a low-pass filter of shear wave energy.

The Zener model is illustrated in Fig 3b. It can describe the effects of creep and stress relaxation, which are both factors in our measurements, and therefore it could provide a better representation of the viscoelastic response [4]. The Zener model has been shown to accurately represent the viscoelastic behavior of human liver, in vivo, in the shear frequency range of 25 to 62.5 Hz [5]. The complex modulus obtained from the Zener model is

$$\mu^Z(\omega) = \mu_1 \frac{1+\omega^2\tau_\sigma\tau_\epsilon}{1+\omega^2\tau_\sigma^2} - i\omega\mu_1\frac{\tau_\epsilon-\tau_\sigma}{1+\omega^2\tau_\sigma^2}, \qquad (1)$$

where $\mu_1 = \mu^Z|_{\omega\to 0} = k_1k_2/(k_1+k_2)$ is the relaxed modulus and $\tau_\sigma = \eta_Z/(k_1+k_2)$ and $\tau_\epsilon = \eta_Z/k_2 \geq \tau_\sigma$ are associated relaxation times. Q^{-1} for the Zener model is found from $Q^Z(\omega) = (1+\omega^2\tau_\epsilon\tau_\sigma)/\omega(\tau_\epsilon-\tau_\sigma))$. Systems represented by the Zener model exhibit purely elastic behavior, $Q^{-1} = 0$ on both ends of frequency spectrum. Moreover, there exists a relaxation peak at $\omega_0 = 1/\tau_0$, where $\tau_0 = \sqrt{\tau_\epsilon\tau_\sigma}$. The relaxation peak indicated the frequency where viscous losses are greatest.

The complex wave number can be written as a function of the shear wave speed and shear wave attenuation constant $k_s = \omega/c_s - i\alpha_s$. An analytical equation describing shear wave dispersion for both models can be characterized by the general expression

$$c_s^{K,Z}(\omega) = \omega/\Re\{k_s^{K,Z}\} = \sqrt{\frac{2(\Re\{\mu^{K,Z}\}^2+\Im\{\mu^{K,Z}\}^2)}{\rho(\Re\{\mu^{K,Z}\}+\sqrt{\Re\{\mu^{K,Z}\}^2+\Im\{\mu^{K,Z}\}^2})}} \qquad (2)$$

where indexes K or Z denote the Kelvin-Voigt or Zener models, respectively. For both models, shear wave speed approaches the elastic limit as $\omega \to 0$, given by $c_s^{K,Z}(0) =$

TABLE I
ESTIMATED DENSITY OF FRESH AND HEATED PORCINE LIVER

Fresh tissue [g/cm^3]	TD tissue [g/cm^3]
1.0541	1.0602
1.0668	1.0537
1.0515	1.0544
1.0575 ± 0.0082	1.0561 ± 0.0036

$\sqrt{\mu_1/\rho}$. Both models predict an increase in speed with frequency, but the K-V model is unbounded while the Zener model is bounded by the unrelaxed modulus. The unrelaxed modulus is defined as $\mu_U = \mu_1(\tau_\epsilon/\tau_\sigma)$ and the upper bound for shear wave speed for a Zener material is $c_s^Z(\omega) \mid_{\omega\to\infty} = \sqrt{\mu_U/\rho}$.

D. Rheometer Testing

Samples of the porcine liver tissue were used in shear stress relaxation tests on an AR-G2 rhoemeter (TA Instruments, New Castle, USA). To provide a no slip surface for the tissue testing, water-proof sandpaper was glued to the upper and lower surfaces of a 25 mm diameter parallel plate fixture. To prepare tissue samples, the liver was cut into sections approximately 5 mm wide. The sections were laid flat and 25 mm discs were cut using a circular punch. Tissue samples were stored in cool water until testing. After being placed in the fixture a small normal load was applied to each sample (< 0.1 N) to ensure contact with the sandpaper and minimize slippage. Each disc was tested in a shear relaxation test with 5% strain applied for 30 min after a one second ramp in strain. Stress data were recorded for the duration of the experiment and were used to determine the relaxation modulus. The relaxation behavior was fit with a 3rd order generalized Maxwell model to determine the viscoelastic relaxation constants and time constants for each test. The parameter values for similar tests were averaged.

The density of each liver sample was measured. Results reported in Table I are in close agreement with those from the literature [6].

III. RESULTS

In Fig. 4, we summarize our measured and best-fit modeled dispersion curves for fresh and heated liver. Results for the Kelvin-Voigt and Zener models are given. Best-fit modulus parameters are listed in Table II for both models along with rheometer measurements.

Fig 5 shows how the dissipation factor predicted for the estimated modulus values varies with frequency between 0 to 10^4 Hz. There is little dissipation for either model at low frequency, but the models diverge in their predictions at high frequencies. The Zener model predicts maximum dissipation for fresh and heated liver frequencies between 100 to 150 Hz. Heated liver peaks at a slightly lower frequency and has a higher peak dissipation value.

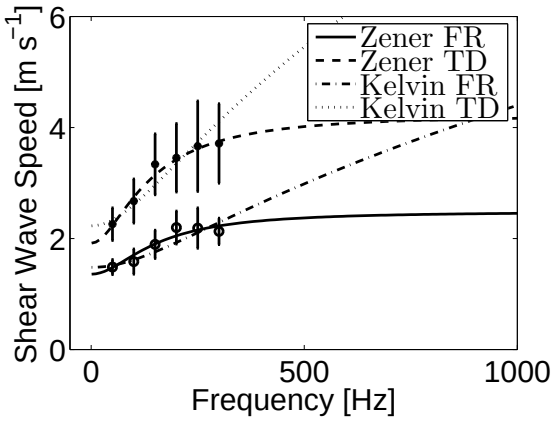

Fig. 4. Comparison of the shear wave speeds as a function of frequency for the Kelvin-Voigt model and the Zener model for both fresh and thermally-damaged porcine liver.

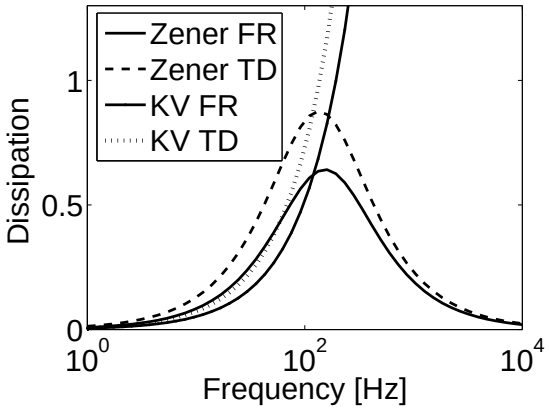

Fig. 5. Dissipation factor as a function of frequency for the Kelvin-Voigt and Zener models for fresh and thermally-damaged porcine liver.

TABLE II
ESTIMATED VISCOELASTIC PARAMETERS

Model	Tissue	μ_1 [Pa]	η_K [Pa s]		
KV	$Fresh$	2191	1.8		
KV	TD	4965	5.8		
			k_1 [Pa]	k_2 [Pa]	η_Z [Pa s]
$Zen.$	$Fresh$	1841	6170	2624	4.99
$Zen.$	TD	3668	17838	4618	12
$Rh.$ 2%	$Fresh$	59 ± 29			
$Rh.$ 5%	$Fresh$	90 ± 24			
$Rh.$ 2%	TD	140 ± 11			
$Rh.$ 5%	TD	154 ± 59			

IV. DISCUSSION

Although tissue boundaries can have a significant effect on material properties, we believe the sample sizes and shear absorption were sufficiently large to ignore boundary effects. However, our livers were not perfused, which was found to affect measured properties [3].

Tissues heated as we describe have been reported to result in coagulative necrosis and protein denaturation, thus resulting in changes in the elastic shear modulus of liver. The relaxed modulus of heated liver is approximately twice the value for fresh liver. Others have reported increases as high as four times the unheated modulus value and correlate with heating time and rate [7].

Several authors proposed that elastography techniques could successfully be used to track ablative changes in tissue [7], [8], [9], [10]. Their observations were based on apparent increases in stiffness or decreases in displacement amplitude and did not explicitly consider viscoelastic responses. Through constitutive modeling of the material response, we found that the viscous modulus triples in heated liver compared with fresh tissue for the heating technique described. This result implies that the viscous modulus could provide more contrast for tracking thermal lesion growth using shear wave imaging.

The Zener model provide a consistently better fit to the measurements than the single-unit Kelvin-Voigt model. The relaxed modulus of both models agrees well with literature values for fresh porcine liver, and there is good agreement between the estimated viscous modulus of Kelvin-Voigt model as well [11]. With the Zener model, one can predict from material properties the frequency of maximum viscous loss. This information suggests the shear-wave frequency bandwidth where the thermal lesion contrast is maximum for viscous shear modulus estimates. Results for heated liver are scaled versions of fresh tissue in the 50-300 Hz range for the given experimental conditions. The greatest contrast is predicted to be near 100-150 Hz.

Our elastic modulus measurements agree with values reported by others using similar techniques, but they exceed our rheometer measurements by up to two orders of magnitude. There are several possible reasons for such a result. Most likely is our assumption that liver is a linear-isotropic viscoelastic material, as required to apply shear wave imaging technique in estimating the complex shear modulus. The literature suggests that liver is non-linear and viscoelastic in its response; specifically that the modulus depends on the strain. We demonstrated this property by testing liver samples with a rheometer at two different strains. As shown in Table II, the relaxed modulus from the rheometer test increase with strain. However, shear wave imaging applied a comparable strain to the liver across the frequencies, and thus we believe the rheometer measurements should not agree with shear wave measurements as observed.

V. Conclusion

Material estimation via shear wave imaging was successfully implemented for estimation of the complex shear modulus of fresh and heated porcine liver.

We found that the elastic modulus increases two-fold after heating and the viscous modulus tripled in value. However, a pathologist viewing the histological samples of the liver found no significant cell damage. This results suggests that the viscous modulus provides a greater change than the elastic modulus in following thermal effects.

Experimental results suggest that the Zener model represents the data better than the Kelvin-Voigt model, however, the quality of representation has not been subjected to a statistical analysis. The Zener model suggests that the most favorable bandwidth for imaging thermal changes in the conducted experiment is between 100 and 150 Hz.

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