Effects of perfusion on the viscoelastic characteristics of liver

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Abstract

Accurate characterization of soft tissue material properties is required to enable new computer-aided medical technologies such as surgical training and planning. The current means of acquiring these properties in the in vivo and ex vivo states is fraught with problems, including limited accessibility and unknown boundary conditions in the former, and unnatural behavior in the latter. This paper presents a new testing method where a whole porcine liver is perfused under physiologic conditions and tested in an ex vivo setting. To characterize the effects of perfusion on the viscoelastic response of liver, indentation devices made force and displacement measurements across four conditions: in vivo, ex vivo perfused, ex vivo post perfused, and in vitro on an excised section. One device imposed cyclic perturbations on the liver's surface, inducing nominal strains up to 5% at frequencies from 0.1 to 200 Hz. The other device measured 300 s of the organ's creep response to applied loads, inducing nominal surface stresses of 6.9–34.7 kPa and nominal strains up to 50%. Results from empirical models indicate that the viscoelastic properties of liver change with perfusion and that two time constants on the order of 1.86 and 51.3 s can characterize the liver under large strains typical of surgical manipulation across time periods up to 300 s. Unperfused conditions were stiffer and more viscous than the in vivo state, resulting in permanent strain deformation with repeated indentations. Conversely, the responses from the ex vivo perfusion condition closely approximated the in vivo response.

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1. Introduction

The development of minimally invasive surgical procedures has induced a need for new surgical training and planning methods. Computer simulations of these procedures provide a platform for such training, yet their utility is currently limited by a lack of data on the realistic behavior of soft tissues under deformations typical of surgical manipulations (Delingette, 1998; Fung, 1993; Szekely et al., 2000). Characterizing the heterogeneous, nonlinear viscoelastic behavior of soft nonload-bearing tissues is a difficult challenge. A standard method for testing soft tissues is needed to produce repeatable results that can be mathematically modeled to capture the natural behavior of the tissue.

Realistic tissue characterization via constitutive modeling requires concurrent control of both geometric and physiologic boundary conditions. Typically, force–displacement responses of soft tissues are collected under two conditions: in vivo and ex vivo. In vivo testing maintains the natural state of the tissue, but there are accessibility issues, ill-defined boundary conditions, and ethical issues related to the use of animals and potential risk to human subjects. Researchers have made in vivo mechanical measurements, but the limited data sets, small deformations atypical of surgical manipulations, difficulty in obtaining proper instrument alignment on sizable specimens, complications from physiological noise, and the inability to account for and control the

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internal condition of the organ make interpreting the data difficult (Brouwer et al., 2001; Brown et al., 2002, 2003; Carter et al., 2001; Gefen and Margulies, 2004; Kalanovic et al., 2003; Kauer et al., 2001; Kim et al., 2003; Melvin et al., 1973; Ottensmeyer, 2002; Ottensmeyer and Salisbury, 2001; Rosen et al., 1999; Tay et al., 2002). Ex vivo experiments are good for device, protocol, and model development, as well as for ease of testing, ethical considerations, and boundary condition control. Many attempts have been made to measure the material properties of soft nonload-bearing tissues ex vivo (Dokos et al., 2000; Hu and Desai, 2004; Liu and Bilston, 2002; Miller, 2001; Miller et al., 2000; Nava et al., 2003; Valtorta and Mazza, 2004) despite knowledge that the mechanical properties are altered post mortem (Fung, 1993; Platz et al., 1997; Schon et al., 2001; Yamada, 1970).

These considerations show the importance of understanding the differences in the tissue's mechanical response between the in vivo and ex vivo conditions (Brown et al., 2003; Gefen and Margulies, 2004), and the need for developing a new testing method that incorporates the strengths of both. In this study, we introduce an ex vivo perfusion system that permits carefully controlled mechanical measurements on porcine liver in a nearly in vivo state. Although no ex vivo setup will completely replicate the organ's natural in vivo condition, we hypothesize that maintaining temperature, surface hydration, and vascular pressure to physiologic levels using a physiologic perfusate can closely approximate it. Two indentation devices were used to assess the mechanical properties on freshly harvested whole porcine livers using the perfusion system. One measures small strains across a range of frequencies and the other measures large-strain creep responses over time. Tests at the same locations on the same livers allowed comparisons of the results across four different conditions: in vivo, ex vivo perfused, ex vivo post perfused, and in vitro on an excised section. Fitting the data to empirical models provided a quantitative comparison and time scale estimation of the viscoelastic response of liver across conditions. In addition, a histological comparison is made from biopsies taken during testing.

2. Methods and materials

2.1. Ex vivo liver perfusion system

The liver is a highly vascular organ perfused with 0.5–1 l of blood (Crawford et al., 1998). It contains two inlets on the inferior side: a low-pressure portal vein accounting for 75% of the volume and a high-pressure hepatic artery accounting for the remaining 25%. The low-pressure hepatic vein serves as the outlet on the superior side, draining into the inferior vena cava.

We have built a system that attempts to maintain the mechanical integrity of a whole liver while mimicking physiologic conditions in an ex vivo setting. The perfusion system (Fig. 1) maintains temperature, surface hydration, and pressure for a whole porcine liver ex vivo. Veterinary lactated Ringer's solution (Henry Schein, Melville, NY) hydrostatically maintains physiologic pressures of 97 ± 5 mmHg to the hepatic artery and 18 ± 2 mmHg to the portal vein. The perfusate is allowed to drain via the intrahepatic vena cava into a bath where it is heated to 39 °C (porcine core temperature) and circulated to the arterial reservoir via a pump. The arterial reservoir overflow feeds the portal venous reservoir whose overflow provides hydration to the organ's surface without submerging the organ. To ensure consistency in our measurements, the organ rests on a sturdy plate covered with fine grit sandpaper to stabilize the area of tissue under study. The perfusion pressure is held constant, rather than mimicking physiologic pulsatile pressure to enable accurate force–displacement measurements.
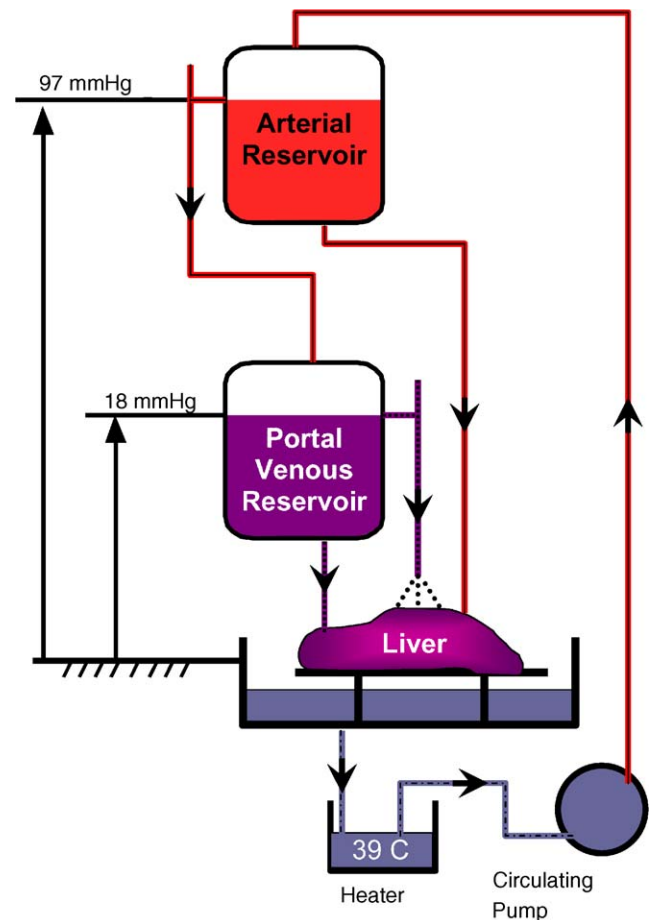

Fig. 1. The ex vivo liver perfusion system.

2.2. Indentation test instruments

Two indentation instruments were used to acquire viscoelastic mechanical measurements on the surface of intact livers across conditions. The Tissue Material Property Sampling Tool (TeMPeST) examined the small-strain (0–5%) frequency response (0.1–200 Hz) (Ottensmeyer and Salisbury, 2001), while a creep indentation device captured the large-strain (10–50%) time domain response (over 300 s).

The TeMPeST measures the small-strain compliance of solid organs (Fig. 2A). A 5 mm circular punch vibrates the tissue while recording applied load (LPM 562 force sensor, Cooper Instruments, Warrenton, VA) and relative displacement (099 XS-B LVDT position sensor, Schaevitz, Hampton, VA). A voice coil motor is controlled in open loop mode, using commanded current as a proxy for applied force. The sampling frequency of 2 kHz enables measurements to approximately 200 Hz. The range of motion is 1 mm (RMS 0.18 μm) and forces can be exerted up to 300 mN (RMS 0.15 mN) (Ottensmeyer, 2001).

For this study, the TeMPeST applied a sinusoidal indentation force with monotonically increasing or decreasing frequency (chirp) to the tissue between 0.1 and 200 Hz, under either a 45 or 90 mN nominal preload force (actual mean loads varied between 10 and 70 mN due to tissue relaxation) and a nominal amplitude of 30 mN (measured between 4 and 10 mN below instrument resonance (80 Hz), and up to 30 mN at resonance) to avoid loss of tissue contact. In these tests, only relative displacement from the preloaded depth could be

measured. A minimum of five tests was done for each condition at each location with roughly 11 indentations per test. One data set was collected every 2–3 min, with the actual time of testing taking 16.4 s in vivo and 32.8 s ex vivo.

The creep indenter performed normal indentation tests with large strains typical of surgical manipulations (Fig. 2B). The device was rigidly mounted to the same platen on which the liver rests to avoid relative motion artifacts. A 6 mm diameter flat cylindrical punch rests on the tissue surface with only 3 g load due to counterweights. A standard laboratory brass weight was placed at near-zero velocity onto a platform mounted coaxially with the indenter tip. An 11.5 cm lever arm with 4 cm of vertical travel connected the indenter to the base, allowing measurements nearly anywhere on the organ surface. Applied loads of 20 and 100 g generated nominal surface stresses of 6.9 and 34.7 kPa, respectively, which are lower than the liver's reported failure stress of 232 kPa (Melvin et al., 1973) and breaking stress 451 kPa (Seki and Iwamoto, 1998). The angular position of the measurement arm was sampled at 1 kHz using a miniature contactless rotary position sensor (Midori America Corporation, Fullerton, CA) (resolution 11 μm, RMS 20 μm) over 5 min. Organ thickness measurements were taken prior to every indentation measurement with a dial indicator for purposes of reporting nominal strain (displacement/preindented thickness).

2.3. Experimental protocol and environmental conditions

Livers from four pigs 27–37 kg (mean 32.5 kg) were used in this study. All were tested in vivo, ex vivo perfused, and ex vivo post perfused, while three had sections removed for in vitro testing. The total duration of testing varied based on number of locations and indentations, but on average, the total test time was between 5 and 8 h. The experimental protocol was approved by the Harvard Medical School Center for Animal Resources & Comparative Medicine Institutional Review Board. An additional pig was used for an independent study on the unperfused condition.

Samples for histological analysis were taken in three of the livers using a 15-gauge core biopsy needle (Meditech Boston Scientific, Watertown, MA) on a lobe of the liver that was not tested. A control sample was taken immediately post sacrifice and again post harvest after flushing with the perfusate. Samples were then taken every hour across all conditions tested, including one upon test completion (24 samples in total), stored in 10% buffered formalin for 36 h and then transferred to 70% alcohol for transport to a histology laboratory (Mass Histology Service, Warwick, RI). A grid report of the hematoxylin and eosin stains was obtained from a pathologist to determine cellular damage and structural integrity across conditions.
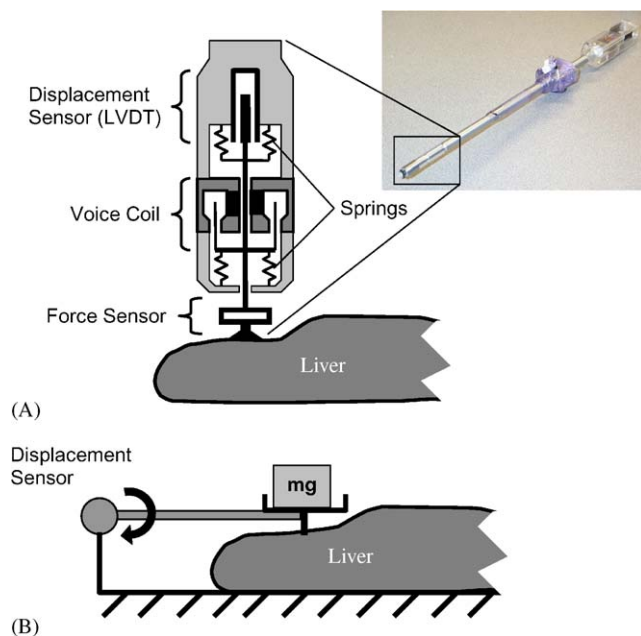

Fig. 2. (A) Small-strain frequency response device. Detail shows the voice coil driven indenter with flextures and sensors. (B) Large-strain creep indentation device.

2.4. In vivo whole organ testing

In vivo tests were performed on deeply anesthetized animals on assisted ventilation with 100% oxygen. The abdomen of the pig was exposed and a lobe of the liver was situated on the platen of the creep device. Locations where the thickness was between 20 and 37 mm (mean 24.8 mm) were chosen and marked with a tissue-marking pen. The TeMPeST acquired compliance data on the liver for periods of 20 s. Ventilation was suspended to prevent pulmonary motions from saturating the position sensor measurements. Indentations using the creep device were made at the same locations, but without the necessity for suspending ventilation. Two to four indentations using both the 20 and 100 g loads were made at each location. Initial position sensor values were noted and the load was applied in pseudo-random order for 300 s, with repetition of the first load at the end. The organ was allowed to recover to within 1 mm of its preindented state (typically 200 s) before applying the next load. The total time for in vivo testing was between 1.5 and 3 h, depending on the number of tests performed.

2.5. In vitro excised section testing

Following in vivo testing, heparin was injected systemically to minimize clotting (1 ml/10 kg of 1000 units/ml) and the animal was sacrificed with an injection of KCl. The liver was harvested, and a lobe was removed and tested immediately with the TeMPeST (in vitro excised section testing). The cut surface of the remainder of the organ was cauterized to prevent leakage and the organ was flushed with 1 l of heparinized (5000 units) cold lactated Ringer's solution. The samples were packed on ice and transported to the laboratory. The excised section was tested again with the TeMPeST in the same locations at room temperature approximately every 20 min. This condition most closely represents many previous ex vivo tests: blood-filled cut sections tested at room temperature several hours post mortem (Dokos et al., 2000; Hu and Desai, 2004; Liu and Bilston, 2002; Miller, 2001; Miller et al., 2000; Nava et al., 2003; Valtorta and Mazza, 2004). Long data collection periods for the creep device precluded timely collection of both ex vivo perfused and in vitro excised measurements. The number of in vitro indentations was therefore limited in favor of gathering more complete ex vivo perfused data. One of the excised lobes was tested with both instruments post mortem and again at the laboratory using the 20 g load for the creep device.

2.6. Ex vivo perfused whole organ testing

Upon arrival at the laboratory (114 ± 22 min of cold ischemic time post mortem), the hepatic artery and portal veins of the liver were sutured to the arterial and portal venous perfusate reservoir tubing. Perfusion was begun and the organ was allowed to come to physiological temperature before testing was resumed within 20 min. A cannula was sutured to the hepatic inferior vena cava to keep the outflow patent. TeMPeST and creep indentation tests were performed in the same manner and at the same locations as the in vivo tests to adequately compare across conditions and minimize variation in the measurements due to unknown locations of large vessels or connective tissues within the organ. The total time for ex vivo perfused testing was 183 ± 47 min depending on the number of locations tested.

2.7. Ex vivo post-perfused whole organ testing

Following the completion of testing on the perfused organ, the inlet tubes were clamped to stop the flow, while the outlet remained patent. The organ was tested again with both instruments in the same locations to observe any further changes in the response, typically 30–60 min. The creep device applied 3–4 sequential indentations, one every 10–20 min. Since initial trials had revealed that the tissue's response changed with indentation in the unperfused states and because we were interested in the large-strain response, only the 100 g load was used.

2.8. Ex vivo whole organ independent post-perfused test over time

To ensure that the differences in the responses seen between the ex vivo whole organ perfused test and the ex vivo whole organ post-perfused test was not due to the latter occurring after the perfusion test, we conducted an independent ex vivo post-perfused test. A 30 kg pig was systemically heparinized and sacrificed, and the liver was harvested and flushed as described above. The liver was brought to the laboratory on ice, flushed again with perfusate at 39 °C, and the inlets were clamped as in the post-perfused condition. Creep measurements using 100 g were made on the same location (22.8 mm thick) beginning 100 min post sacrifice. Measurements were taken every 20 min for 100 min.

2.9. Data analysis—lumped element modeling

To compare the effects of environmental conditions on the viscoelastic properties of porcine livers, the data were fit to empirical first-order (TeMPeST) and second-order (creep device) lumped element models (Fig. 3). These models are not material constitutive laws, but rather a means of quantifying the differences seen across conditions and to determine the time scales of the tissue's viscoelasticity.
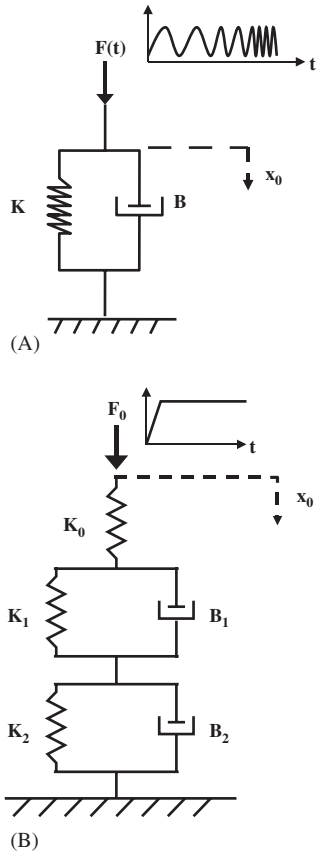
Fig. 3. Lumped element models used to quantitatively describe the tissue response. (A) First-order Voigt model for small-strain response to a force input of a sinusoidal frequency chirp $F(t)$. (B) Second-order model for large-strain response to a step load F_0.

The simplest model that captures small-deformation viscoelastic behavior is the first-order parallel spring-dashpot (Voigt) model (Fig. 3A). Fitting this model to the TeMPeST data involved calculating the frequency-dependent complex compliance of the tissue by taking the ratio of the fast Fourier transforms of the position and force signals over the frequency range of interest. A characteristic Voigt model curve was fit to each of the unfiltered compliance versus frequency data sets by minimizing the sum of squared errors. This yielded the static compliance (inverse stiffness $1/K$, m/N) and the characteristic (break) frequency (f_c, Hz). The damping constant of the Voigt model was then calculated as $B = K/(2\pi f_c)$.

A first-order model showed poor agreement for the creep results and a second-order model was used (Fig. 3B). The position and time data from the creep indentation device were filtered forward and backward using a second-order low-pass Butterworth filter with a cutoff frequency of 50 Hz. Since instantaneous load application is not possible, the creep tests were divided into loading (ramp) and response phases. An independent test measured the time of load application using a

force sensor: the duration of the ramp phase was $t = 0{-}0.163$ and $0{-}0.236$ s for the 20 and 100 g loads, respectively. The response

$$x_0(t) = A_0 - A_1 e^{-t/\tau_1} - A_2 e^{-t/\tau_2}$$

was fit from the end of the ramp phase to 290 s, where A_0 is the amplitude of the steady-state displacement defined by $A_0 = F_0(1/K_0 + 1/K_1 + 1/K_2)$, and $A_1 = F_0/K_1$ and $A_2 = F_0/K_2$ are the amplitude contributions from the creep time constants $\tau_1 = B_1/K_1$ and $\tau_2 = B_2/K_2$. A gradient decent search was performed to minimize the normalized mean square error (MSE) between the model response and data.

3. Results

3.1. Frequency response indentation tests

Typical compliance and phase versus frequency plots with the model fit for the ex vivo perfused condition under a low preload (mean = 24.7 mN) are shown in Fig. 4. The first-order Voigt model captures the nearly in-phase variation between force and displacement at low frequencies and the 90° phase shift at the upper end of the measured frequency range. Below ∼40 Hz the tissue behaves like a linear elastic spring with compliance ∼0.02 m/N, while above 40 Hz the viscous behavior is evident. The observed low-frequency noise is a result
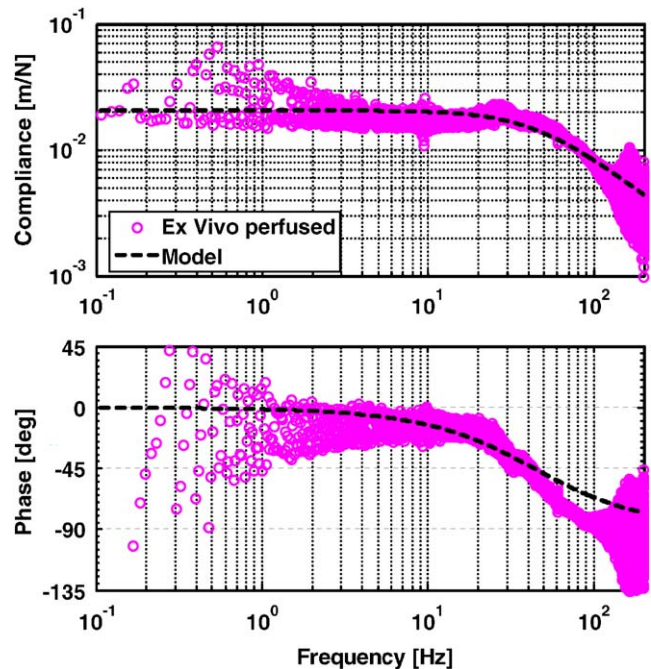

Fig. 4. Typical Bode plot of compliance (top) and phase (bottom) versus frequency. The circles are data from an ex vivo perfused test under a low preload (24.7 mN) and the dashed line is the Voigt model fit.

of the tissue's inherent nonlinearity; the nonlinear stiffness results in a distorted sinusoidal response, leading to oscillations in the Fourier transform of the measured position and force signals. This oscillation is greatly reduced when testing linearly elastic materials.

The mean and standard deviation of compliance and damping constant versus mean preloaded force for all conditions are shown in Fig. 5. The variation in preload force is due to stress relaxation. These results illustrate that both tissue stiffness (1/compliance) and damping coefficient increase with applied load in all cases.

A two-sample, two-tailed Student's t-test assuming unequal variances was conducted on the means of both parameters for each preload and compared to the in vivo condition with a 5% significance level. The compliance and damping coefficient for the ex vivo

perfused condition were statistically the same as those for the in vivo condition under the same preload. Conversely, the other two conditions' parameters were significantly different from the in vivo condition for both preloads. The high preload results indicate that the ex vivo post-perfused and excised section conditions are both stiffer (47% both cases) and more viscous (23% and 87%, respectively) than the in vivo condition and that the ex vivo perfused condition is similar to the in vivo condition.

3.2. Large-strain creep indentation

Typical creep response and model fits for three indentations across all conditions for one representative liver are shown in Fig. 6. The in vivo and ex vivo perfused conditions show a consistent response for repeated indentations. Conversely, both unperfused conditions show permanent deformation as well as stiffening and a decrease in viscosity with repeated indentations. Table 1 reports the mean and standard deviations of changes in indentation characteristics between pairs of successive indentations within 18 min of each other across three conditions including results from the independent unperfused test (Fig. 7). These characteristics are represented in terms of differences in permanent deformation (Δ_i, the difference in the indenter's initial contact with the surface as compared to the first indentation), depth of indentation (Δ_D), and nominal steady-state strain (Δ_{ss}). The results show that the in vivo and ex vivo perfused conditions were statistically indistinguishable from each other for both loads with the exception of steady-state strain in the 100 g case ($\Delta_i p_{20\,g} = 0.30$, $p_{100\,g} = 0.33$; $\Delta_D p_{20\,g} = 0.96$, $p_{100\,g} = 0.20$; $\Delta_{ss} p_{20\,g} = 0.10$, $p_{100\,g} = 0.04$). In contrast, the post-perfused condition showed an inconsistent response with repeated indentations, including 11.9% permanent strain deformation, 7.2% difference in indentation strain, and 4.6% difference in steady-state nominal strain. The results from the independent unperfused test are similar to the post-perfused results with a 15% permanent strain deformation, 5% difference in indentation strain, and 10% difference in steady-state nominal strain.

To compare the variability in the liver time scales and amplitudes between indentations and across conditions, the means and standard deviations of the model parameters (τ_1, τ_2, A_0, A_1, A_2) for pairs of sequential indentations were calculated and are shown in Fig. 8. Again, the parameters for the in vivo and ex vivo perfused conditions were similar to each other and consistent with repeated indentations under both loads. The mean values for τ_1 and τ_2 are 1.86 ± 1.03 and $51.3 \pm 18.0\,\text{s}$, respectively, with similar weights on the order of 0.08 for the 20 g load and 0.026 for the 100 g load. The value of A_0 was 0.239 ± 0.078 and
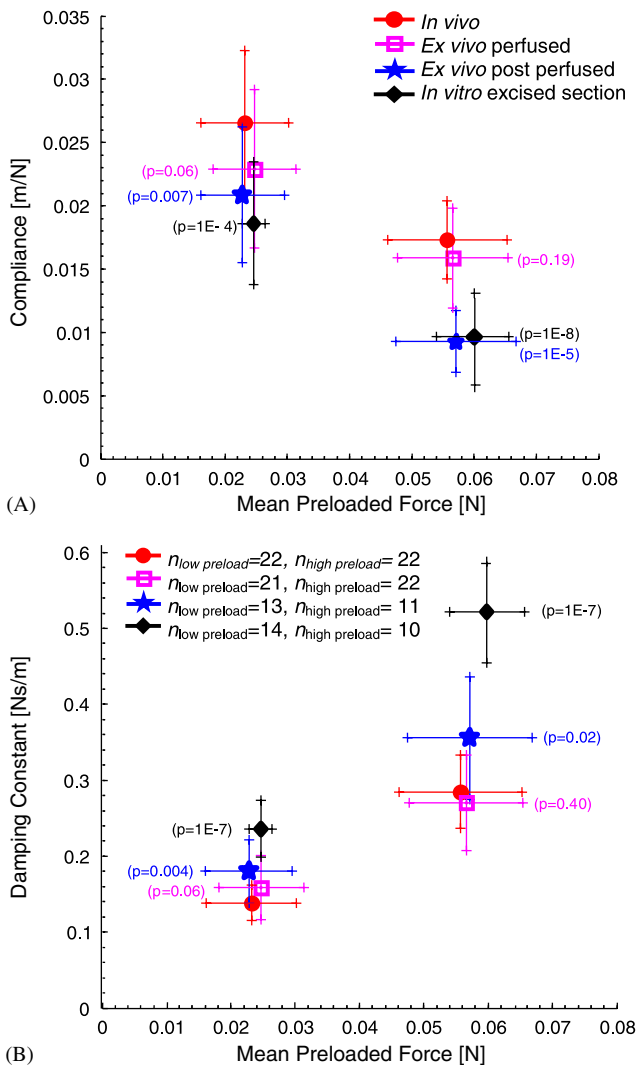

Fig. 5. Model parameters from the small-strain frequency response. (A) Mean compliance versus preloaded force±standard deviation (SD) and (B) mean damping constant versus preloaded force±SD for each condition. p-values for all cases compared to the in vivo case are shown next to their corresponding mean values.
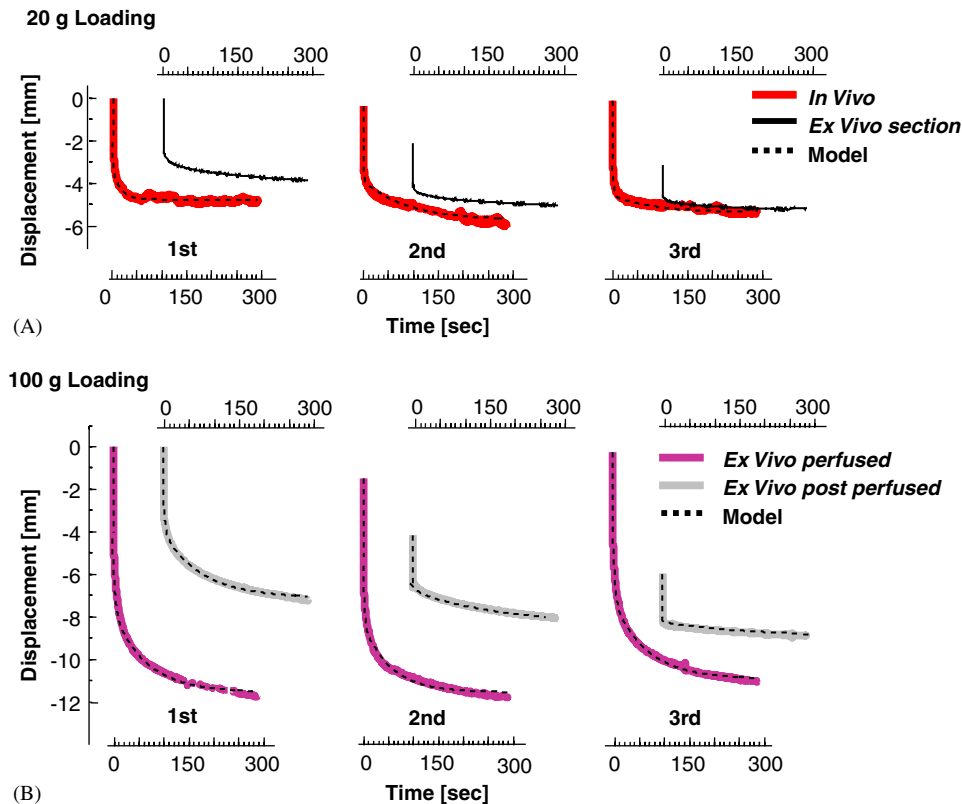
Fig. 6. Typical creep data for three indentations from one porcine liver for all four conditions. (A) The top plot compares indentations between the in vivo response (90 min between the first and last indentation) and the in vitro response (30 min between the first and last indentation) under a 20 g load. (B) The bottom plot compares indentations between the ex vivo perfused (78 min between the first and last indentation) and ex vivo post-perfused (30 min between the first and last indentation) responses under a 100 g load. The initial ramp and second-order model fits to the in vivo, ex vivo perfused, and ex vivo post-perfused indentations are plotted as dotted lines.

0.466 ± 0.083 for the 20 and 100 g loads, respectively. The ex vivo post-perfused condition had a consistent value for τ_1 between indentations ($p = 0.283$) that was also statistically indistinguishable to the in vivo case ($p = 0.603$). The rest of the parameters suggest that this condition is either not repeatable, not comparable to the in vivo case, or both.

The initial displacements of all trials were zeroed, and the means of each condition were compared at every point in time to evaluate the overall shape of the large-strain creep response. Fig. 9 shows means and the standard deviation of the means for the responses for the three conditions tested. The results show that the ex vivo perfused condition closely approximated the in vivo condition with $p = 0.70$ at all times in the 20 g loading condition. Similarly, $p = 0.40$ for times greater than 27 s in the 100 g loading condition. The ex vivo post-perfused response is significantly different from the in vivo case with $p = 0.006$ for times greater than 22 ms. Lastly, strain hardening was observed in the in vivo and ex vivo perfused conditions since increasing the load by a factor of five doubled the mean steady-state response.

3.3. Histology

The analysis of the histology specimens from the three livers indicates that the structural integrity of the tissue was maintained over time in the experimental states compared to the control states. On a scale of 0–4, where 0 indicates no observed changes and 4 indicates severe changes, the mean score was between 0 and 1.7 across all conditions, time (5 h), and features. Some minimal changes were noted in the cytoplasm beyond 2 h into the perfused case and in the nonperfused conditions. It was also noted that the ex vivo perfused samples experienced some cellular contraction, contrasted with the cellular swelling seen in the in vitro excised sections. Minimal cell death was observed.

4. Discussion

This study presents a standard method for testing whole organs that produces repeatable results that can be mathematically modeled to capture the realistic behavior of soft tissues. We built an ex vivo perfusion

Table 1
Means $\pm$ SD of the differences observed in the indentation characteristics for pairs of successive large-strain creep indentations across three conditions including the independent unperfused test

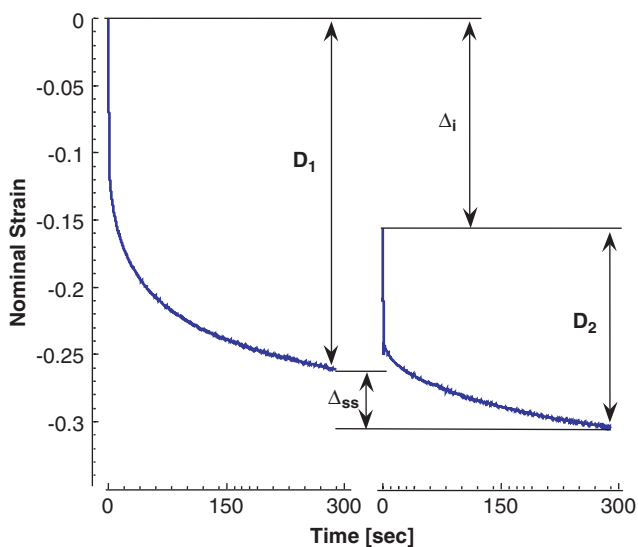|	Condition	No. of pairs	Δ_i, mean initial contact strain ($\pm$ SD)	$\Delta_D = D_1 - D_2$, mean indentation strain ($\pm$ SD)	Δ_{ss}, mean steady-state strain ($\pm$ SD)
20 g	In vivo	4	0.008 (0.008)	0.016 (0.009)	−0.021 (0.013)
	Ex vivo perfused	4	−0.001 (0.023)	0.015 (0.016)	0.015 (0.032)
100 g	In vivo	4	0.042 (0.035)	0.036 (0.020)	−0.043 (0.021)
	Ex vivo perfused	5	0.043 (0.081)	0.014 (0.015)	−0.039 (0.088)
	Ex vivo post perfused	4	0.119 (0.031)	0.072 (0.031)	−0.046 (0.031)
	Independent ex vivo unperfused	1	0.150	0.050	−0.100

Fig. 7. Definitions of measures for characterizing differences observed between successive large-strain creep indentations, shown on a representative plot of data from a pair of ex vivo post-perfused indentations. Δ_i is the change in the initial point of contact with the surface (permanent deformation), Δ_D is the difference in depth of indentation ($D_1 - D_2$), and Δ_{ss} is the difference in nominal steady-state strain.

system that controls the perfusate, perfusion pressure, temperature, and surface hydration. Our results suggest that both geometric and physiologic boundary conditions must be considered when characterizing well-vascularized, solid, whole organs. Specifically, the elastic and viscous properties of liver are affected by perfusion as measured by both small-strain frequency response and large-strain creep indentation tests. The small-strain frequency tests show that both unperfused conditions were stiffer and more viscous than the in vivo condition under high preloads. The large-strain creep device showed permanent strain deformation for successive indentations for the post-perfused ex vivo tissues. The new perfusion system allowed for nearly in vivo results using both instruments in a controlled ex vivo setup.

The ex vivo perfused tissues exhibited similar viscoelastic behavior to the in vivo tissues and showed consistency (less than 4% strain differences) between successive indentations.

The results obtained here enlarge upon previous work on in vivo and ex vivo soft tissue material properties. While direct comparisons are not straightforward, the results of Brown et al. (2002, 2003) qualitatively support our observation that the liver is stiffer ex vivo than in vivo. They also note that the in vivo condition had more recovery between indentations than did the ex vivo. Gefen and Margulies (2004) made indentation measurements (strain $< 20\%$) in three locations in porcine brains in both the in vivo and ex vivo states to compare the effects of perfusion. They reported that perfusion did not make a significant difference in material property identification. Brain tissue is nearly homogeneous, however, and does not hold the same volume fraction of blood as the liver. Thus the effects of perfusion on the viscoelastic properties of tissue are likely organ dependant. Although Dokos et al. (2000) and Gefen and Margulies (2004) tried to provide more realistic conditions for their ex vivo samples by controlling for external hydration and temperature, no one has made mechanical measurements on perfused ex vivo organs.

Many researchers "precondition" their ex vivo tissue samples with cyclic loading to obtain a steady-state response (Brouwer et al., 2001; Dokos et al., 2000; Liu and Bilston, 2002). Although this may make sense to simulate the in vivo state for tissues that undergo cyclic deformations like the heart and tendons, our results suggest that preconditioning soft nonload-bearing unperfused tissues drives fluid from the tissue, changing its viscous characteristics and internal boundary conditions. Our results show both an increase in the stiffness and viscosity of the organ, and a permanent deformation with repeated indentation for the excised section and the ex vivo post-perfused condition. Conversely, the stiffness and viscosity remained consistent with repeated indentations for both the in vivo and perfused ex vivo conditions, demonstrating the need for preserving the
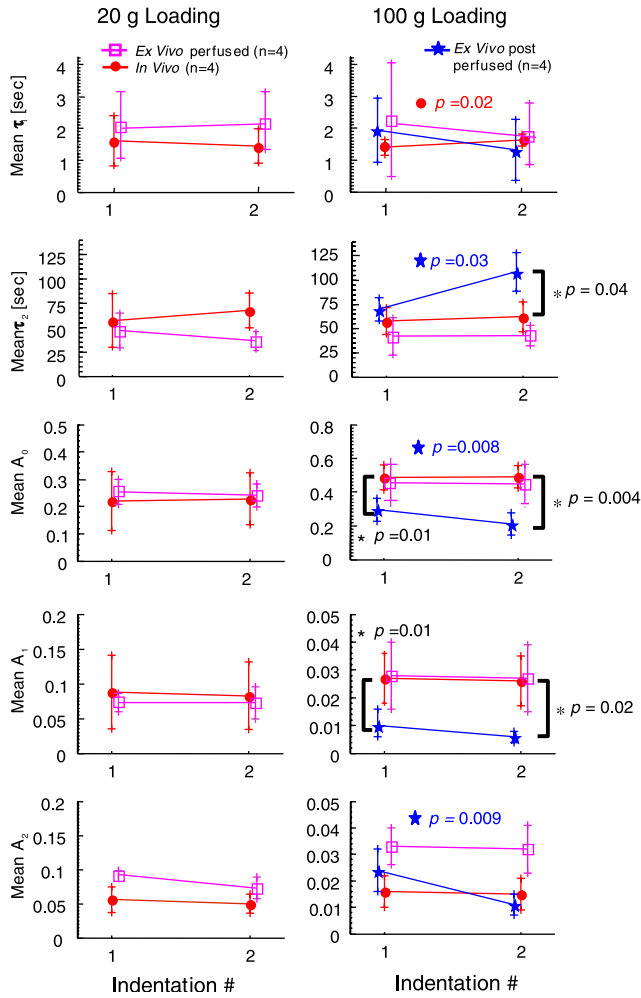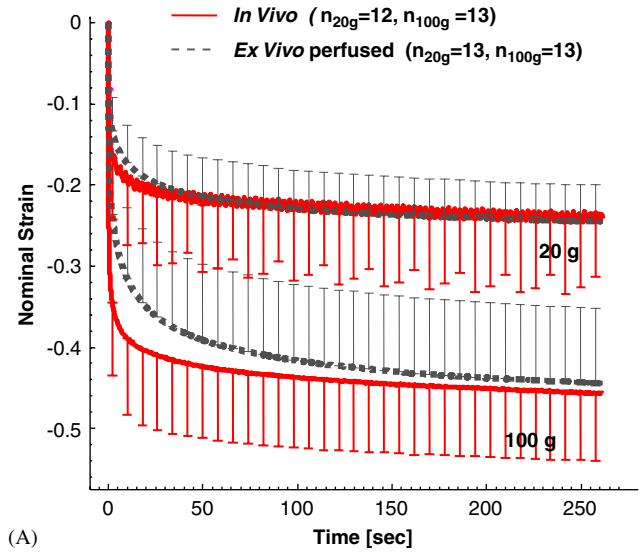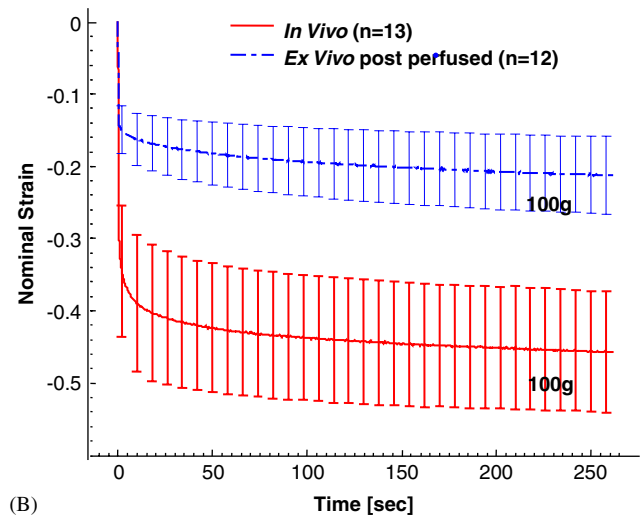
Fig. 8. Mean and SD of the creep model parameters (τ_1, τ_2, A_0, A_1, and A_2) for sequential indentations under both loads across the in vivo, ex vivo perfused, and ex vivo post-perfused conditions. p-values with a symbol denote a significant difference between indentations for that condition and an asterisk (*) denotes a significant difference compared to the in vivo case for a particular indentation.

Fig. 9. (A) Mean nominal strain ± SD of the means versus time for the in vivo and ex vivo perfused creep data sets. The upper SD is shown for the ex vivo perfused and the lower SD for the in vivo. (B) Mean nominal strain ± SD of the means versus time for the in vivo and ex vivo post-perfused creep data for the 100 g tests.

physiological boundary conditions when making mechanical measurements.

To maintain the internal boundary conditions and mechanical viability of the liver post mortem for the time course of our tests, we designed a perfusion system for ex vivo organs based on organ transplant systems. It has been shown that cellular injury occurs within 60–240 min of warm ischemia time (Platz et al., 1997; Schon et al., 2001). Using normothermic perfusion systems as a bridge to transplant, researchers have been able to maintain the viability of livers for up to 72 h (Butler et al., 2002; Platz et al., 1997; Schon et al., 2001). For this study, we were not interested in maintaining liver function but rather its structural integrity. The consistency seen in this study with repeated indentations, the similarities to the in vivo condition, and the histology results showing minimal changes in cellular

integrity suggest that our cold ischemic time did not cause cell damage and that the perfusion system preserved the mechanical viability of the liver for the 5 hour duration of our ex vivo testing.

Despite the similarities in the viscoelastic response of the liver in the in vivo and ex vivo perfused conditions, small differences were seen that motivate improvements to the system for future measurements. The ex vivo perfused condition was slightly stiffer, thicker, less viscous, and experienced cellular dissociation as compared to the in vivo condition. This suggests that there was a mismatch in perfusion pressures and perfusate concentration. More recent examination of the literature on porcine physiology has shown that the perfusion

pressure for the hepatic portal venous branch of the system is smaller than the value used in this study, which was based on human values (Rasmussen et al., 1999). Optimally, the perfusate pressures would be determined with presacrifice arterial and venous measurements. It may also be useful to include perfusates with plasma proteins or other blood mimicking products to better match the oncotic and viscous properties of blood.

To address the needs of surgical simulation, our goal is to develop a constitutive model of the whole liver. Successful modeling requires data from a known geometry, with known loading conditions and external constraints. This study shows that both geometric and physiologic boundary conditions must be controlled, and that these can be done concurrently. One remaining challenge is accounting for the initial stress state. Placing the organ on a hard surface induces stresses that are not present in the in vivo state, and these must be taken into account when developing a constitutive law. It would also be ideal to model the individual contributions of the various tissue constituents (capsule, parenchyma, vessels) in the context of the whole organ. However, the intimate connections of the vasculature and capsule throughout the parenchyma make this a near-impossible task. It may be possible to make measurements that emphasize one of the constituents over the others; e.g., we are developing internal testing devices to make measurements that do not directly involve the external capsule (Kerdok and Howe, 2003).

Quantitative analysis of the perfused creep data under large deformations typical of surgical manipulation suggests that at least two time scales (1.86 and 51.3 s for the 300 s creep test reported here) are needed to describe the viscous parenchymal response of the liver. Physical reasoning suggests that the two time constants are from movement of fluid in two distinct pathways: one is from the inherent viscosity of the cells and the free exchange of interstitial fluid in the liver parenchyma, and another is due to blood flow in the liver's microvasculature. In future studies, it may be possible to use the perfusion system to vary the perfusate pressure to tease out the viscoelastic effects from those due to the interstitial fluid and extracellular matrix and from those due to the blood and vasculature.

Lastly, although this study used noninvasive surface indentations to allow for quantitative comparisons of mechanical response across conditions, complete mechanical characterization will involve stresses and strain rates that will bring the organ to failure and induce impact injuries. The need for boundary condition control and the potential invasiveness of future tests will mandate the use of ex vivo testing that can nearly approximate the in vivo condition. Once the models are well established using animal ex vivo tests, we can turn to in vivo testing to determine the human parameter values for the models.

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