CHARACTERIZATION

of

FREQUENCY-DEPENDENT MATERIAL PROPERTIES of HUMAN LIVER and ITS PATHOLOGIES USING an IMPACT HAMMER

by

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This is to certify that I have examined this copy of a master's thesis by

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and have found that it is complete and satisfactory in all respects,
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To them who have a place for me in their hearts

ABSTRACT

The current methods for characterization of frequency-dependent material properties of a whole human liver are very limited. In fact, there is almost no data available in the literature for the dynamic elastic modulus of healthy or diseased of human liver. We show that frequency-dependent dynamic material properties of human liver can be easily and more efficiently characterized by an impact hammer. The procedure only involves a light impact force applied to the organ by a hand-held hammer. The results of our experiments performed on 15 human livers freshly harvested from liver patients show that the proposed approach can successfully differentiate their level of fibrosis. We found that the elastic moduli of healthy and fibrotic livers vary from 10 to 20 kPa and 20 to 50 kPa for the frequency range of 0 to 100 Hz, respectively.

ÖZET

Ne yazık ki, bütün haldeki insan karaciğeri için kullanılan frekansa bağlı malzeme özelliği karakterizasyon yöntemleri henüz yeteri kadar geliştirilememiştir. Aslına bakılırsa, literatürde sağlıklı veya hastalıklı insan karaciğerine ait dinamik elastik modülüs değerleri neredeyse hiç bulunmamaktadır. Biz bu çalışmamızda, darbe çekici cihazının bütün haldeki insan karaciğerinin frekansa bağlı dinamik malzeme özelliklerini kolay ve etkin bir şekilde karakterize edebildiğini gösteriyoruz. Bu yöntem, el ile kontrol edilen darbe çekici cihazı ile organa uygulanan çok hafif bir vurma işleminden ibarettir. On beş karaciğer hastasından alınmış organlar ile vakit kaybetmeden gerçekeştirilen deneylerimiz sonucunda, bu önerilen yöntemin başarılı bir şekilde fibroz seviyelerini ayırt edebildiği gözlenmiştir. Yaptığımız çalışma sonucunda sağlıklı ve fibrotik karaciğerlerin elastik modülüs değerlerinin 0 ile 100 Hz frekans aralığında, sırasıyla 1 0 ile 2 0 k Pa ve 2 0 ile 5 0 k Pa değerleri arasında değiştiğini göstermiş bulunuyoruz

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Last but not least, I am expressing my sincere gratefulness and gratitude to my family for their endless supports and patience against me whatever happens. They were always be there to catch me when I was about to fall. I could not imagine such a beautiful life without them…

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Chapter 1

INTRODUCTION

Accurate characterization of the mechanical properties of soft tissues is important for diagnosing medical pathologies and developing solutions for them. With the recent advances in technologies leading to the development of surgical simulators, medical robots, and computer-assisted surgical planning systems, this topic has gained even more importance. While strain and time-dependent material properties have been investigated extensively, less attention has been paid to frequency-dependent dynamic material properties. However, dynamic response of the soft tissues to periodic or impact loading is important in many areas of biomechanics and biomedical engineering. For example, to understand how an organ gets injured during an accident, its frequency-dependent mechanical properties play a crucial role. Similarly, when designing prosthetic devices for lower amputates, it is important to know how the soft tissue responds to the periodic impacts from the ground. In medical imaging, the dynamic response of the organ to acoustic shear waves propagating with a certain frequency is used to estimate its material properties and diagnose diseases.

The current methods of acquiring the frequency-dependent material properties of soft tissues involve the use of mechanical indenters, rotational rheometers, and medical imaging

techniques. To characterize the frequency-dependent viscoelastic material properties, the most common method is the dynamic loading test: small periodic strains at varying frequencies are applied to the tissue sample and the stress response is recorded. The absolute gain (i.e. the ratio of output stress amplitude to the input strain amplitude) and the phase angle can be plotted in two separate graphs in log scale as functions of driving frequency. Because of the viscoelastic nature of the soft tissues, two stress components, one in-phase with the applied strain and the other out-of-phase, can be obtained from measurements at each frequency. As a result, two moduli, one in-phase and the other out-of-phase, can be calculated by taking the ratio of the stress to strain at each frequency. These two moduli form the so-called complex modulus with the in phase modulus (also known as the storage modulus) being its real part and the out-of-phase modulus (also known as the loss modulus) its imaginary part. The storage and loss moduli define the energy storage and dissipation capacity of the soft tissue, respectively.

In this study, we investigate the frequency dependent viscoelastic material properties of human liver. Most of the existing dynamic data on liver in literature has been obtained from animal tissues using the dynamic test. Liu and Bilston (2000) investigate the linear viscoelastic properties of bovine liver using a generalized Maxwell model and conduct three types of experiments a) shear strain sweep oscillation, b) shear stress relaxation, and c) shear oscillation. The shear stress and strain are calculated based on the torsional load. In

strain sweep oscillation experiments, the liver tissue is subjected to a sinusoidal angular torsion at a fixed frequency of 1, 5, or 20 Hz using a strain controlled rheometer. The strain amplitudes were gradually increased from 0.06% to 1.5% while the storage and loss moduli of the liver are measured. In stress relaxation experiment, sudden torsional shear strain is applied to liver tissue for 0.02 seconds and the shear relaxation modulus is measured over 3000 seconds. Finally, in shear oscillation experiments performed in the range of 0.006 to 20 Hz, the storage and loss moduli are measured again. The results show that the shear relaxation modulus reaches to steady state around 0.6 kPa. The results of the oscillatory shear experiments show that the storage modulus increases from 1 kPa to 6 kPa with increasing frequency and the loss modulus is less than 1 kPa, increases to a peak at about 1 Hz and then decreases to 0.4 kPa as the frequency reaches to 20 Hz. Kiss et al. (2004) perform in vitro experiments with canine liver tissue to characterize its viscoelastic response. They calculate the storage and the loss moduli of the liver tissue from the frequency-dependent complex elastic modulus for the frequencies ranging from 0.1 to 400 Hz by applying cyclic stimuli to the tissue. The resulting moduli spectra are then fitted to a modified Kelvin–Voigt model, which is called as the Kelvin–Voigt fractional derivative model (KVFD) by the authors. They show that there is an excellent agreement between the experimental data and the KVFD model, particularly at frequencies less than 100 Hz. Valtorta and Mazza (2005) developed a torsional resonator to characterize the dynamic material properties of bovine and porcine liver. By controlling the vibration amplitude,

shear strains of less than 0.2% are induced in the tissue so that the material response can be considered as linear viscoelastic. Experiments are performed at different eigenfrequencies of the torsional oscillator and the complex shear moduli of bovine and porcine are characterized in the range 1–10 kHz. The results of the in vitro experiments on porcine liver show that the magnitude of complex shear modulus varies between 5-50 kPa depending on whether the data collected from the external surface or the internal section of the liver (as reported by the authors, the former leads to considerably larger shear stiffness due to the presence of the stiff capsula). The complex shear modulus for bovine liver is shown to vary between 15-30 kPa. Kerdok et al. (2006) investigated the effects of perfusion on the viscoelastic response of pig liver using two indentation devices under 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 small strains up to 5% at frequencies from 0.1 to 200 Hz (Ottensmeyer, 2001) while the other device measured the creep response of the same liver to applied loads for 300 seconds, inducing large strains up to 50%. The results demonstrated that unperfused conditions were stiffer and more viscous than the in vivo state and the responses from the ex vivo perfusion condition closely approximated the in vivo response.

Compared to the animal studies, the number of studies investigating the frequency-dependent characteristics of human liver and pathologies using mechanical loading are

very limited. Most of the earlier studies have focused on the characterization of static and viscoelastic material properties of human liver, but not the frequency dependent properties. Carter et al. (2001) performed static indentation experiments with human subjects in vivo using a hand-held mechanical indenter and estimated the linear elastic modulus of human liver as 270 MPa. Saraf et al. (2007) investigated the dynamic response of human liver in hydrostatic compression and simple shear using the Kolsky bar technique at high strain rates ranging from 300 to 5000 s^{-1}. This technique involves the use of two elastic pistons with a disk-shaped material sample inserted between their ends. A pressure wave travel along one of the pistons is generated by applying an impact at the free end of the piston. By measuring the difference of vibrations at the extremities of the structure, the mechanical properties of the sample can be deduced. They measured the bulk modulus and the shear modulus of human liver under dynamic loading as 280 kPa and 37-340 kPa depending on the strain rate, respectively. Mazza et al. (2007) conducted in vivo and ex vivo experiments with ten human subjects having some liver pathology. Static mechanical properties of human liver were measured at multiple locations using an intra-operative aspiration device. Most of the tests were performed on diseased liver segments undergoing subsequent resection. Measurements were performed by the surgeon on the normally perfused liver in vivo and on the resected specimen ex vivo. The relationship between mechanical parameters and various pathologic conditions affecting the tissue samples has been quantified, with fibrosis leading to a response up to three times stiffer as compared with

normal tissue. Later, Nava et al. (2008) performed aspiration experiments on healthy human liver with the same device. They estimated the long term and instantaneous linear elastic modulus of human liver as 20 kPa and 60 kPa respectively.

In summary, there are limited number of studies and data on the frequency-dependent material properties of healthy and diseased human liver measured by either mechanical indenter or a rheometer. The latter is not even practical for collecting data from a whole organ. While medical imaging technologies are available for the characterization of material properties of human liver and its pathologies, which are discussed later in the article, they only allow the measurement of local shear or elastic modulus of the healthy and diseased liver at a certain excitation frequency rather than the range of frequencies. Moreover, most of the earlier studies have utilized the dynamic loading test for the characterization of frequency –dependent material properties. However, one drawback of the dynamic loading test is that the measurements have to be made at each frequency within the range of frequencies of interest, which may not practical for measuring live (in vivo) tissue properties of human liver in place (in situ) since the time interval for data collection is limited. Moreover, the collected data can be erroneous due to the adverse effect of the subject's breathing on the measurements. Hence, faster and more practical methods are required for the dynamic characterization of the live tissue properties in place.

In this study, we use impact test to characterize the frequency-dependent material properties of human liver and pathologies. This approach enables us to collect data faster than the dynamic loading test for a range of frequencies. It is also simple to implement. The technique involves the use of a hand-held hammer to apply a light impact force on a pre-load mass placed on the liver surface. The whole test takes less than a minute and no harm is made to the organ during the measurements (see Figure 1).

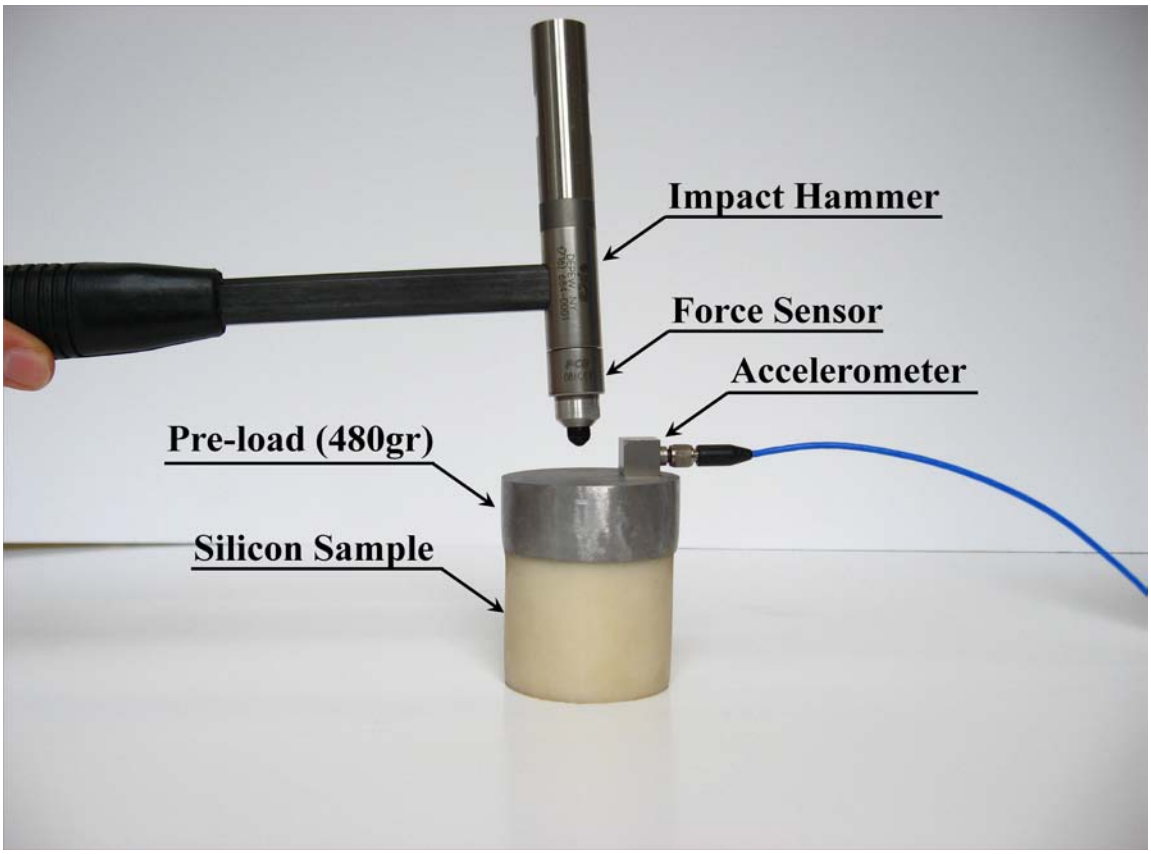


Figure 1. The components of the measurement system.

Chapter 2

METHODS

We model the dynamic response of a test specimen under impact loading using a hysteretic damping model as shown below (Nashif et al., 1985)

$$m_{eff}\ddot{x}(t) + k^{*}(\omega)x(t) = f(t) \tag{1}$$

where m_{eff} is the effective mass of the system including the mass of the pre-load and the effective mass of the specimen, $k^{*}(\omega)$ is the complex stiffness of the specimen, *f(t)* is the excitation force, which results in a displacement *x(t)*. If the excitation force is periodic with a frequency of ω, then the same equation can be written in the frequency domain to obtain the following transfer function (also known as the frequency response function, FRF) as follows

$$T(j\omega) = \frac{X(j\omega)}{F(j\omega)} = \frac{1}{-m_{eff}\omega^{2} + k(\omega)(1+\eta(\omega))} \tag{2}$$

where $k(\omega)$ is the dynamic stiffness and $\eta(\omega)$ is defined as the loss factor. Now, if we define *r* as the ratio of the excitation frequency to the natural frequency, $r = \omega/\omega_n$, then the

complex stiffness and the loss factor of the specimen can be calculated from the measured transfer function and the resonance frequency (close to natural frequency if the loss factor takes small values) as (Lin et al., 2005)

$$k(\omega) = \frac{\mathrm{Re}(T(j\omega))}{|T(j\omega)|(1-r^2)}$$
$$\eta(\omega) = -\frac{\mathrm{Im}(T(j\omega))}{\mathrm{Re}(T(j\omega))}(1-r^2) \quad (3)$$

After obtaining the dynamic stiffness, the dynamic elastic modulus, $E(\omega)$, can be calculated using the following relation which is derived from Hooke's Law

$$E(\omega) = \frac{k(\omega)L_{eff}}{A_{eff}} \quad (4)$$

where, L_{eff} and A_{eff} are the effective length along the direction of the loading and the effective cross sectional area of the specimen. If the pre-load placed on top of the specimen covers its surface and the length of the sample is sufficiently short, then the effective length and the area can be taken as the actual values of the specimen dimensions. Otherwise (if the tested specimen is large and long as in the case of testing a whole liver), it is not straightforward to calculate the effective cross-sectional area and length since the impact

force applied to the large specimen propagates in a complex manner over its surface and also along the direction normal to its surface.

Similar to the complex stiffness, the complex modulus can be written as

$$E^*(\omega) = E(\omega)(1 + \eta(\omega)\, j) \tag{5}$$

Alternatively, the dynamic modulus $E^*(\omega)$ can be written in terms of real and imaginary parts as

$$E^*(\omega) = E_S(\omega) + E_L(\omega)\, j \tag{6}$$

The real part, $E_S(\omega)$, is known as the storage modulus and it is an indicator of energy storage capacity of the viscoelastic material. The imaginary part, $E_L(\omega)$, is known as the loss modulus and it is related the energy dissipation capacity of the material. The ratio of $E_L(\omega)/E_S(\omega)$ is the loss factor $\eta(\omega)$ and equal to the tangent of the phase angle between strain and stress (or between displacement and force in the actual measurements).

Chapter 3

EXPERIMENTS

For the complex manner of the effective area and the effective length phenomenon, first we have conducted experiments on bovine livers to get the material properties. According to the values of these experiments we have prepared silicon samples in different cross-sectional areas (see Figure 2) and in cylindrical shapes according to real liver stiffness and viscosity (see Figure 5). Secondly, we have performed impact experiments with these cylindrical silicon samples of varying sizes to investigate the effect of sample size (cross-sectional area and the length) on its dynamic stiffness. Finally, using the experience and knowledge that we gained from these experiments, we later performed experiments with freshly harvested human livers having some level of fibrosis to characterize their dynamic elastic modulus for a range of frequencies.

Figure 2. The family of silicon samples used in our experiments.

3.1. Impact Test

The impact hammer is a specialized instrument that produces a short duration impulse when the specimen being tested is hit by it. In our experiments, an impulse excitation force is applied to the pre-load (480 gram) placed on top of the specimen by the impact hammer

(PCB Piezotronics Inc., Model 086C03, sensitivity is 2.1 mV/N) equipped with a force sensor (see Figure 1). As suggested by the manufacturer, a soft tip and an extender mass were utilized for better response at low frequencies. The impulse response of the specimen was measured by a piezoelectric accelerometer (PCB Piezotronics Inc., Model 333B30, sensitivity is 101.2 mV/g, where is the gravitational acceleration, range is 0.5-3000 Hz) attached to the pre-load using a thin film of adhesive wax. As suggested by the manufacturer, five measurements are taken from each specimen and the average values are used in the analysis. The accelerometer and the force sensor are connected to a dynamic signal analyzer (Data Physics Corporation, type SignalCalc Mobilyzer) to calculate the frequency response function (FRF). The frequency response function (FRF) of the specimen was obtained by taking the Fourier transform of the impulse response. In dynamic loading test, the same FRF is obtained by applying small periodic strains to the specimen and measuring its force response at different frequencies for a range of frequencies. Compared to the dynamic test, the impact test is more practical and the measurement time is much shorter (see Figure 3).

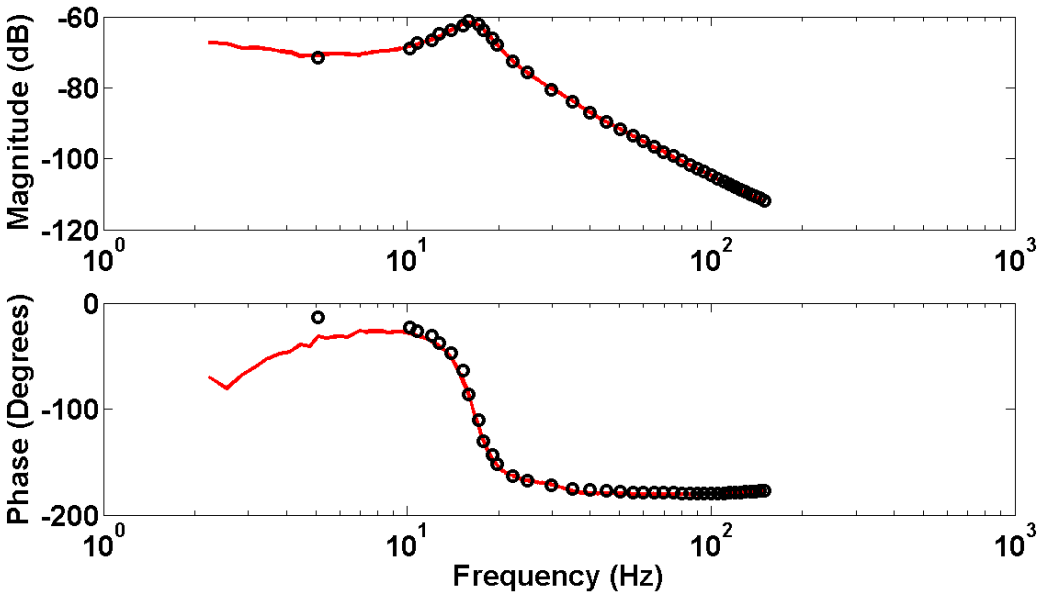


Figure 3. The data (dots) collected by a mechanical shaker from a silicon material via dynamic loading method is compared to the data collected by impact hammer (solid lines).

3.2. Initial Experiments with Bovine Livers and Silicon Samples

In the aim of obtaining material properties, two bovine livers have been obtained from the local slaughter house. The livers have been kept in Lactated Ringer's Solution for preserving the integrity. We have conducted impact hammer experiments on whole bovine livers and obtained dynamic stiffness and loss factor values according to frequency (see Figure 4). But it is obvious that dynamic stiffness and loss factor values depend on physical shape of the specimen rather than the storage and loss moduli. So effective area and

effective lengths of the whole bovine liver is needed to determine to calculate storage and loss moduli.

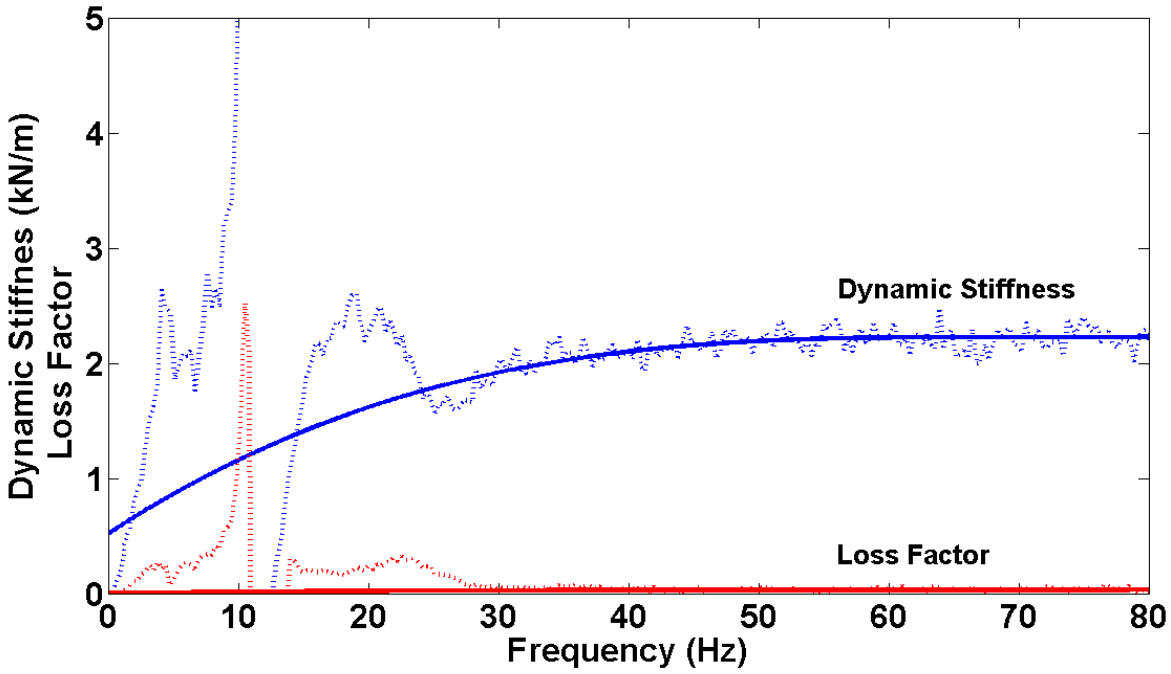
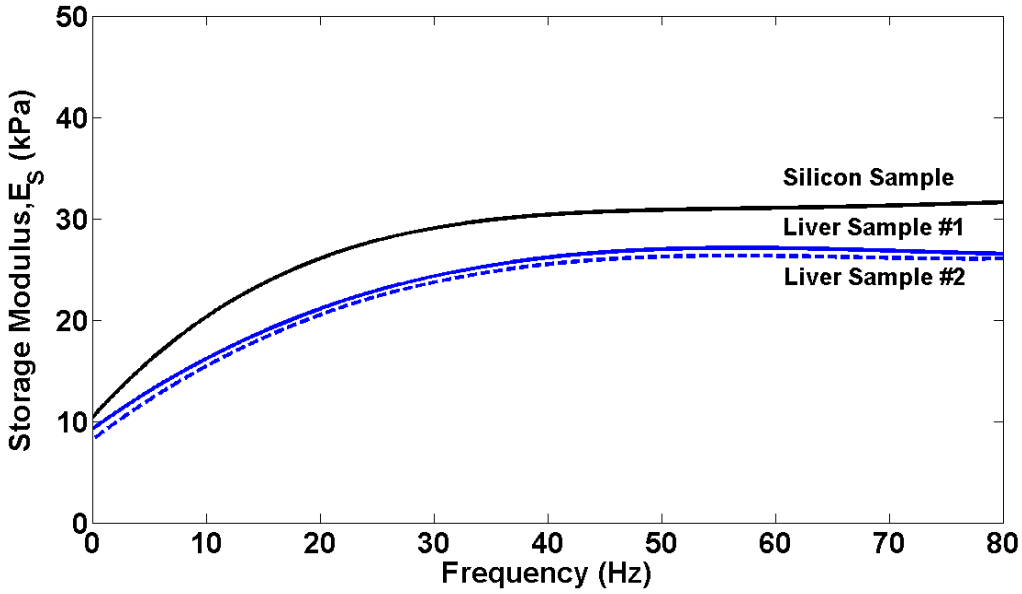


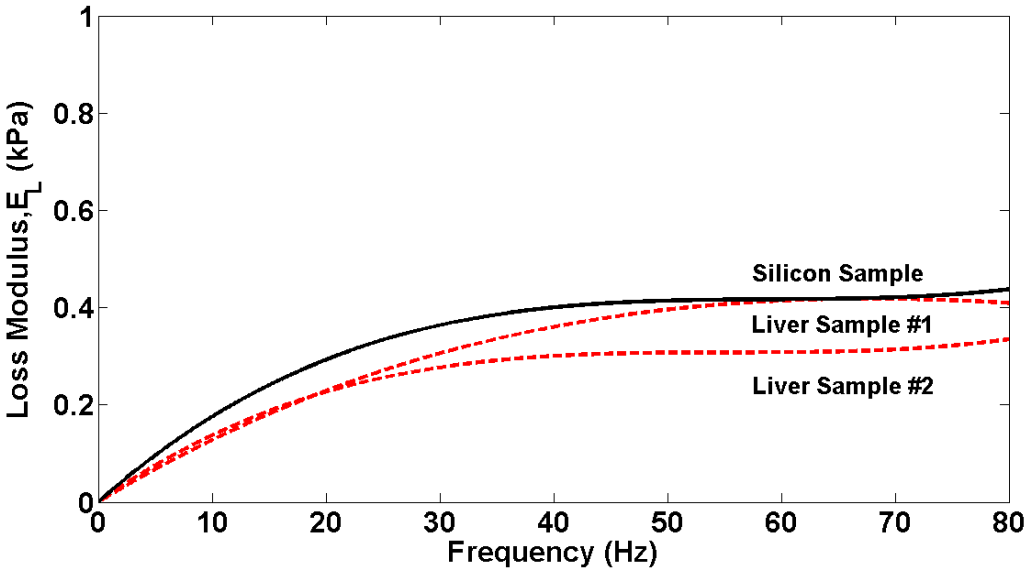
Figure 4. The dynamic stiffness and loss factor of a whole liver (Liver #1).

In the same time, we have prepared cylindrical samples from our two bovine livers with using a custom made cylindrical blade developed for this purpose with bewaring the physical conditions of the livers and preventing the fluid loss and dehydration via covering the samples with Vaseline. Since the cylindrical samples are in defined cross-sectional area and in defined length, calculating storage and loss moduli from dynamic stiffness and loss

factor would be straightforward. Several impact hammer measurements have been conducted with cylindrical liver samples to obtain storage and loss moduli values according to frequency. Storage and loss moduli values relatively represent the stiffness and viscosity of the specimen respectively. According to these knowledge special silicon mixtures has been prepared to simulate real bovine liver characteristics. Figure 5 exhibits the storage and loss modulus values of two bovine liver samples and the custom prepared silicon sample. As the figure mentions, all three storage and loss moduli values are almost identical.



(a)



(b)

Figure 5. The storage (a) and loss moduli (b) of two livers and the silicon sample in cylindrical shape.

Cylindrical silicon samples were prepared using Smooth-Sil 910. The material properties of this silicon are the most appropriate for modeling human organs. In fact, Smooth-Sil 910 is frequently used in movie industry for replicating the human parts like arm, hand, and even face, modeling aliens, or making masks. Moreover, it can be easily obtained via shopping from internet or from a local distributor in many countries. Smooth-Sil 910 is a two-component silicone rubber: Part A is the base material that forms the product and Part B is the catalyst that hardens the silicon. For the adjustment softness, Silicon Oil was used.

Silicon Oil does not change the structure of the silicon material but softens it. To prepare the samples, Parts A, B and oil were mixed in different ratios of mass. Frequent stirring was necessary for obtaining a homogenous mixture. Otherwise, air bubbles occurred in the mixture causing a difference in material properties locally. In order to minimize the stress concentration effects, the samples were molded in cylinder. As our molds, we used cylindrical glass pots for small size samples and PVC pipes for large size samples. The glass pots were preferred for the small samples since it was easy to get hardened silicon from it by breaking the glass. Several samples in different sizes were prepared for our experiments (see Figure 2).

Further impact experiments with larger size samples were performed to investigate the effect of cross-sectional area and length on the sample's dynamic stiffness. In the case of samples with larger cross-sectional area than that of the pre-load mass, the impact load propagates over the contact surface of the sample, but it is only effective within a radius of influence. Figure 6 shows the variation in the dynamic stiffness of a silicon material for different cross-sectional areas of the samples (the length of the samples is fixed to L = 60 mm). As the sample diameter is increased, its dynamic stiffness increases slowly, reaching to a steady state behavior when the area ratio is approximately AR = 5 (observe that the stiffness data for the samples of AR = 5.1 and AR = 6.7 almost overlap in Figure 6).

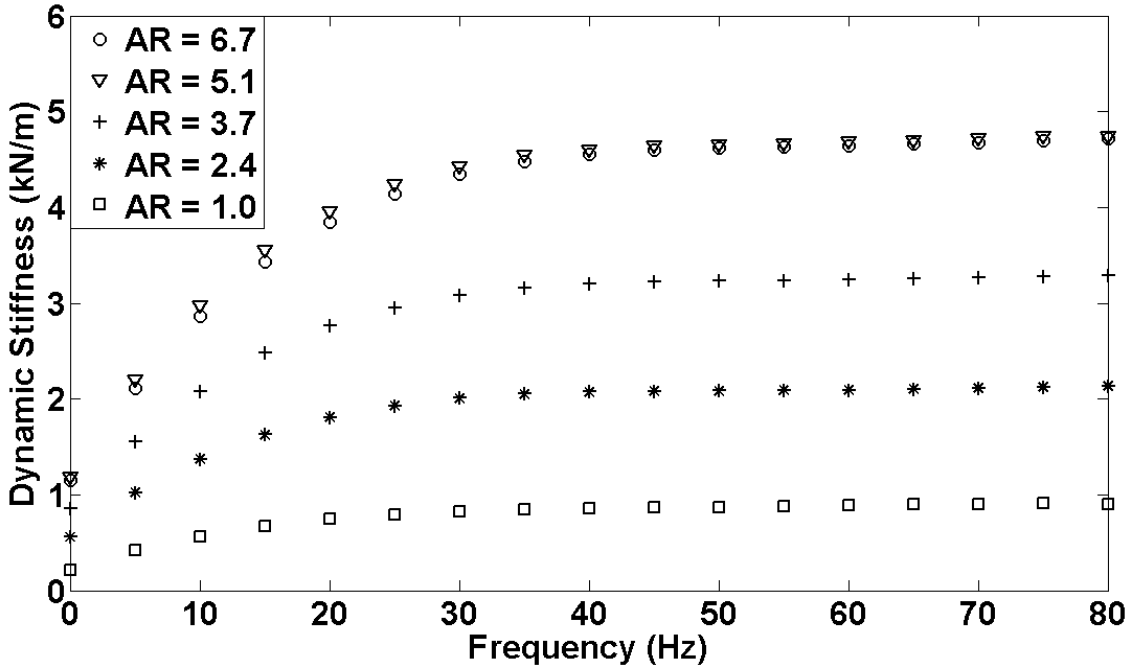


Figure 6. The variation in the dynamic stiffness of "Sample 1" as a function of the cross-sectional area (note that the length of the samples being tested is fixed to L = 60 mm).

The area ratio (AR) is defined as the ratio of the cross-sectional area of the cylindrical samples to the cross-sectional area of the cylindrical pre-load mass. Figure 7 shows the changes in the resonance frequency of the same silicon material as a function of AR. The solid line is obtained by curve-fitting an exponential function to the experimental data. As shown in the figure, the resonance frequency increases slowly and reaches to a steady state value around AR = 5.

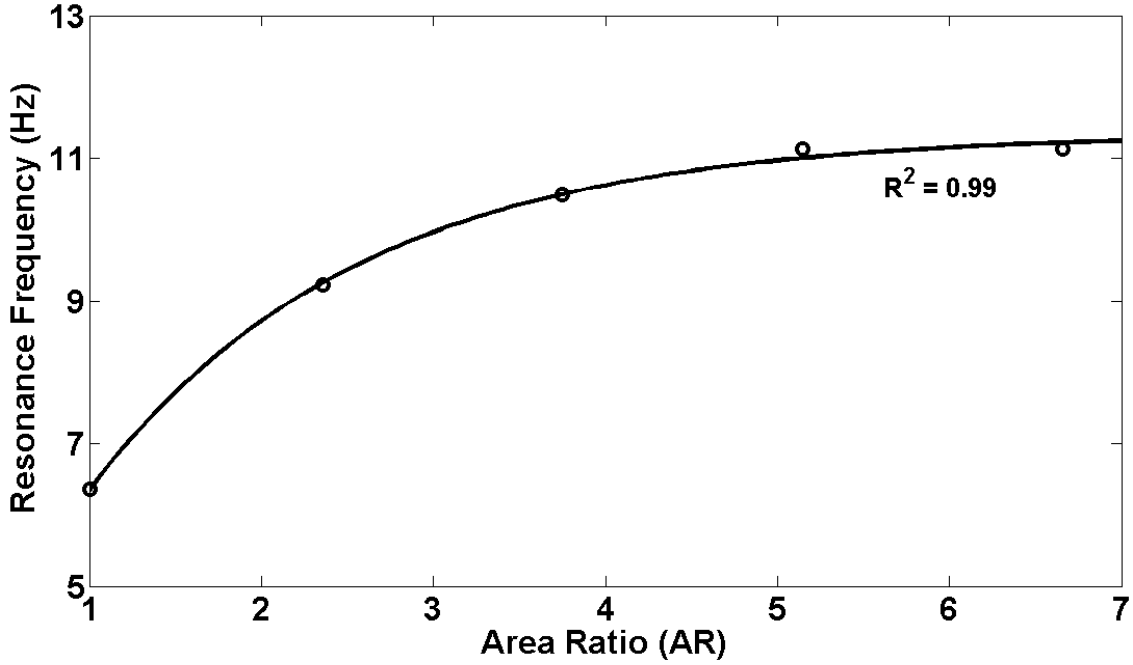


Figure 7. The variation in the resonance frequency of "Sample 1" as a function of AR (i.e. the ratio of the cross-sectional area of the sample to the cross-sectional area of the pre-load).

The similar reasoning can be followed for the length of the sample being tested. Figure 8 shows the variation in the dynamic stiffness of the same silicon material for different sample lengths (the area ratio of the samples is fixed to AR = 5.1). As the length increases, the dynamic stiffness decreases slowly reaching to a steady state behavior when L > 200 mm. A similar comment can be obtained from Figure 9 with resonance frequencies as the sample length increases.

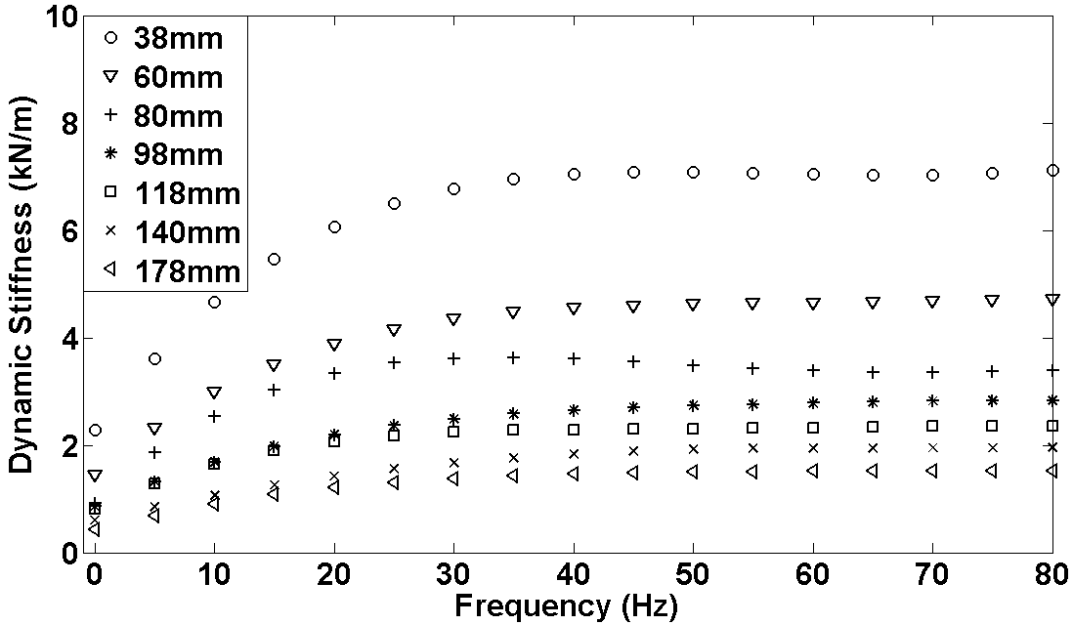


Figure 8. The variation in the dynamic stiffness of "Sample 1" as a function of the length (note that the area ratio of the samples being tested is fixed to AR = 5).

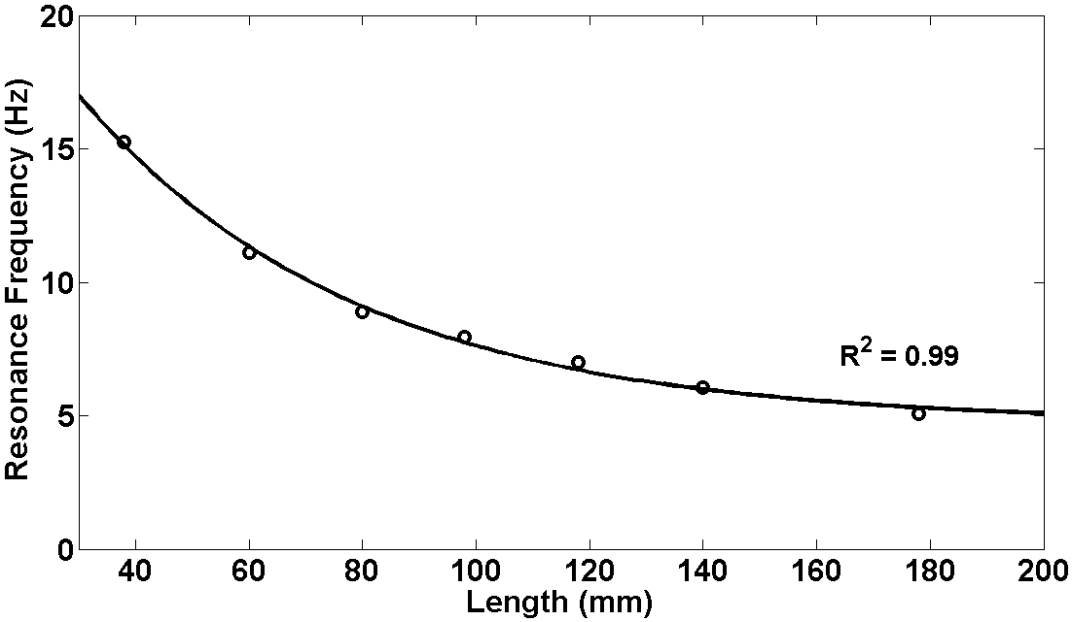


Figure 9. The variation in the resonance frequency of "Sample 1" as a function of length.

The results of these experiments proved that dynamic stiffness depends on the sample geometry. However, dynamic elastic modulus is a material property and must be independent of the sample size and shape. Hence, effective cross-sectional area and length must be considered for large size samples to estimate their dynamic elastic modulus from the measured dynamic stiffness (refer to Eq. 4). To better understand the concept, imagine a very long cylindrical sample with an extremely large cross-sectional area. The impact load applied to this sample will be effective only within a small semi-spherical volume under the pre-load (see figure 10). If the actual cross-sectional area and length are used in the calculations instead of the effective ones, the dynamic elastic modulus will be estimated incorrectly smaller than the expected value.

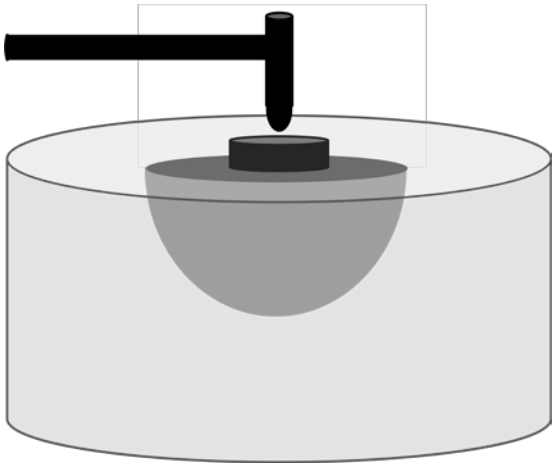
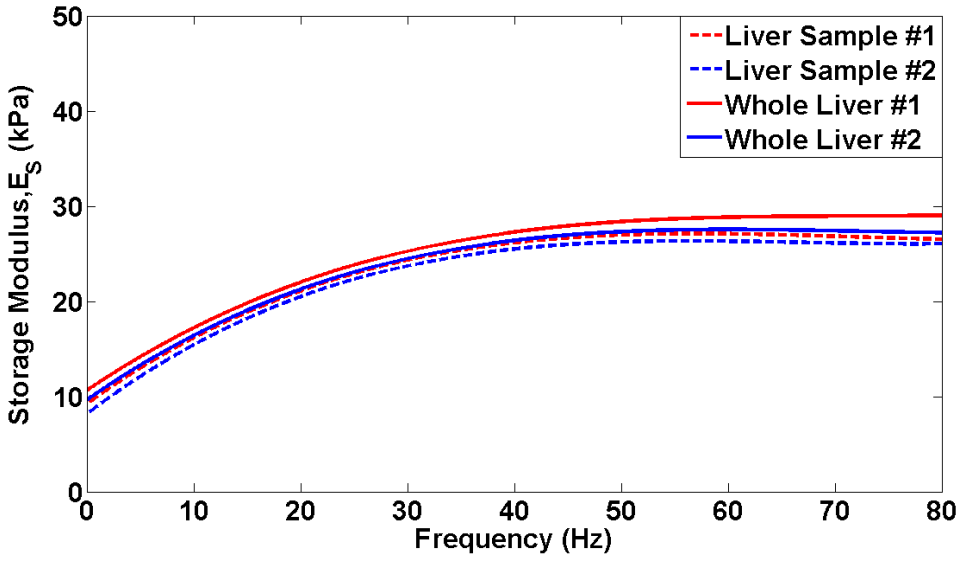
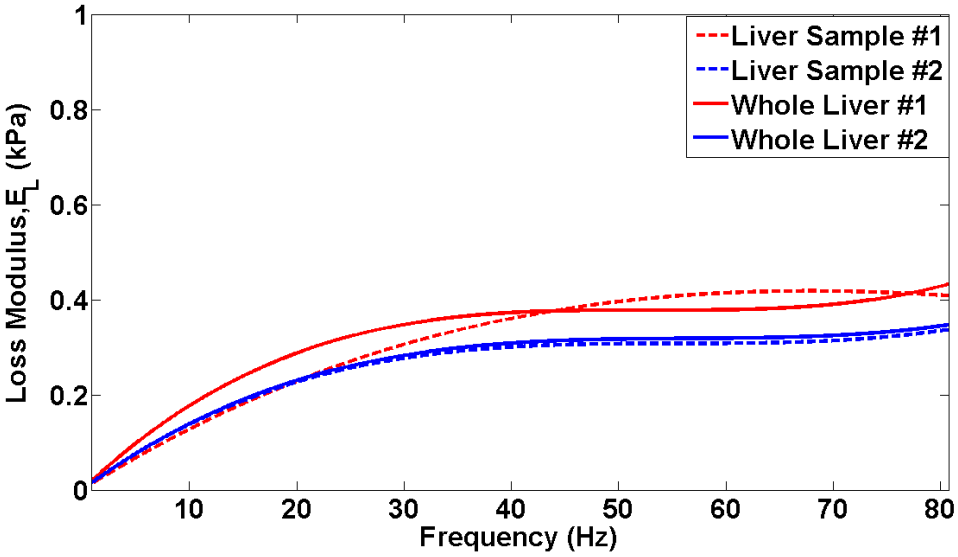


Figure 10. Schematic of effective cross sectional area and effective length phenomenon.

Storage and loss moduli of the cylindrical and whole liver samples have been compared in Figure 11. The effective area for the whole bovine liver has been chosen as AR=5. We have defined this value where the slope in Figure 7 is decreased to 2%. The effective length of the whole livers has been used as the original values since the whole liver lengths are smaller than 200mm. (138mm for liver #1 and 142mm for liver #2) The storage and loss moduli values are almost identical in Figure 11. This similarity proves not just the effective area and effective length approach but also the correct values that we have invented.



(a)



(b)

Figure 11. Comparison of storage (a) and loss moduli (b) obtained from the whole livers using the effective area and length with the ones obtained from the cylindrical liver samples.

3.3. Human Experiments

A total of 15 liver samples were collected from patients having a liver disease (age = 51 ± 10, gender = 12 male, 3 female). The study was approved by the ethical commission of the Florence Nightingale Hospital at Istanbul. All the tests were performed on the excised livers. The area ratio (the ratio of visible surface area where the test is applied to the area of the pre-load) was AR = 23.52 ± 7.06. The length of the livers along the direction of loading

was L = 81 $\pm$ 30 mm. For each subject, the experimental data was collected at the operating room within 30 minutes after the liver was removed from the body. The surface area of each excised liver was estimated from the digital picture taken by a camera using a calibration grid. The height of the liver was measured using a digital micrometer at the location where the data was collected. After the impact measurements, each liver was sent to the Pathology Department of Istanbul University for histological examination. All specimens were analyzed independently by an experienced pathologist blinded to the clinical data and the results of the liver stiffness measurements. Fibrosis scores were assigned to each liver according to the METAVIR scoring system ranging from F0 to F4 (F0: no fibrosis, F1: portal fibrosis without septa, F2: septa fibrosis, F3: numerous septa without cirrhosis, and F4: cirrhosis). There were 2 patients in F0, 4 patients in F2, 4 patients in F3, and 5 patients in F4.

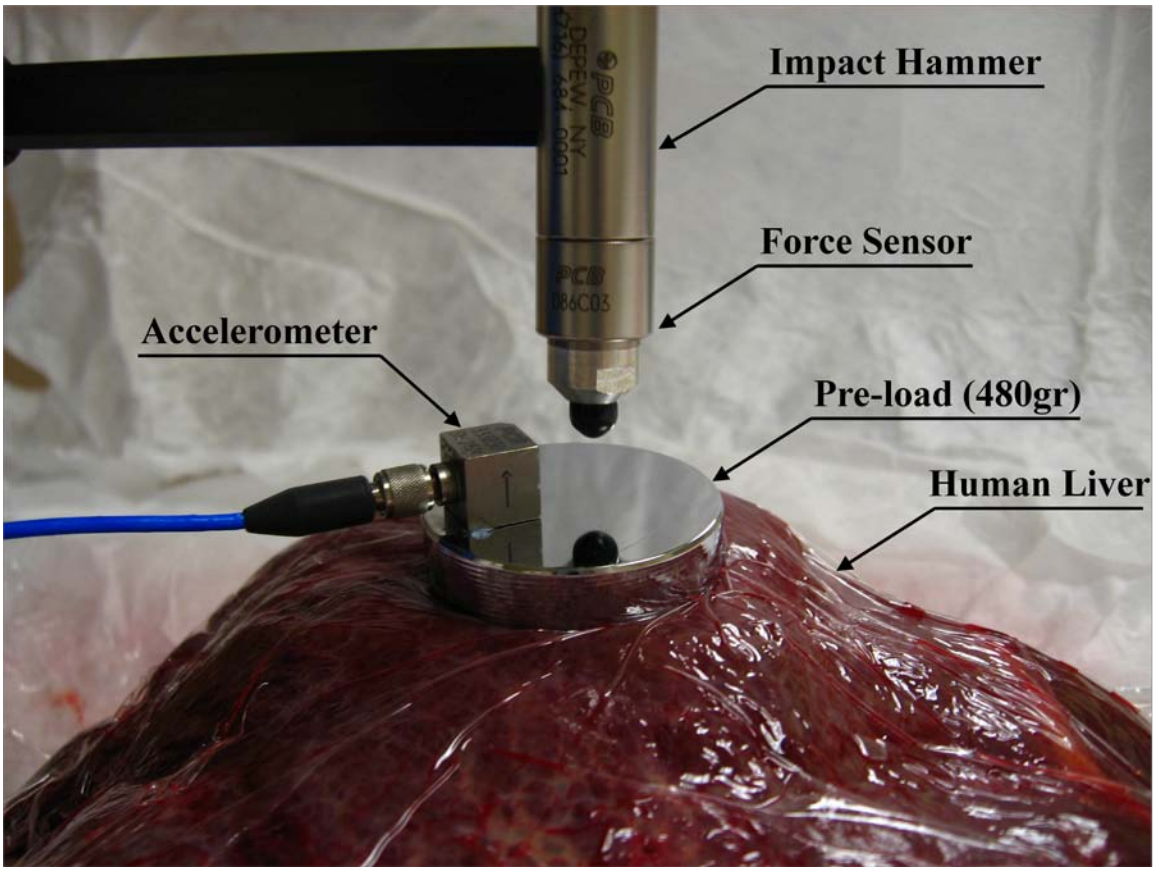


Figure 12. Dynamical material properties of human liver were measured as a function of frequency by an impact hammer.

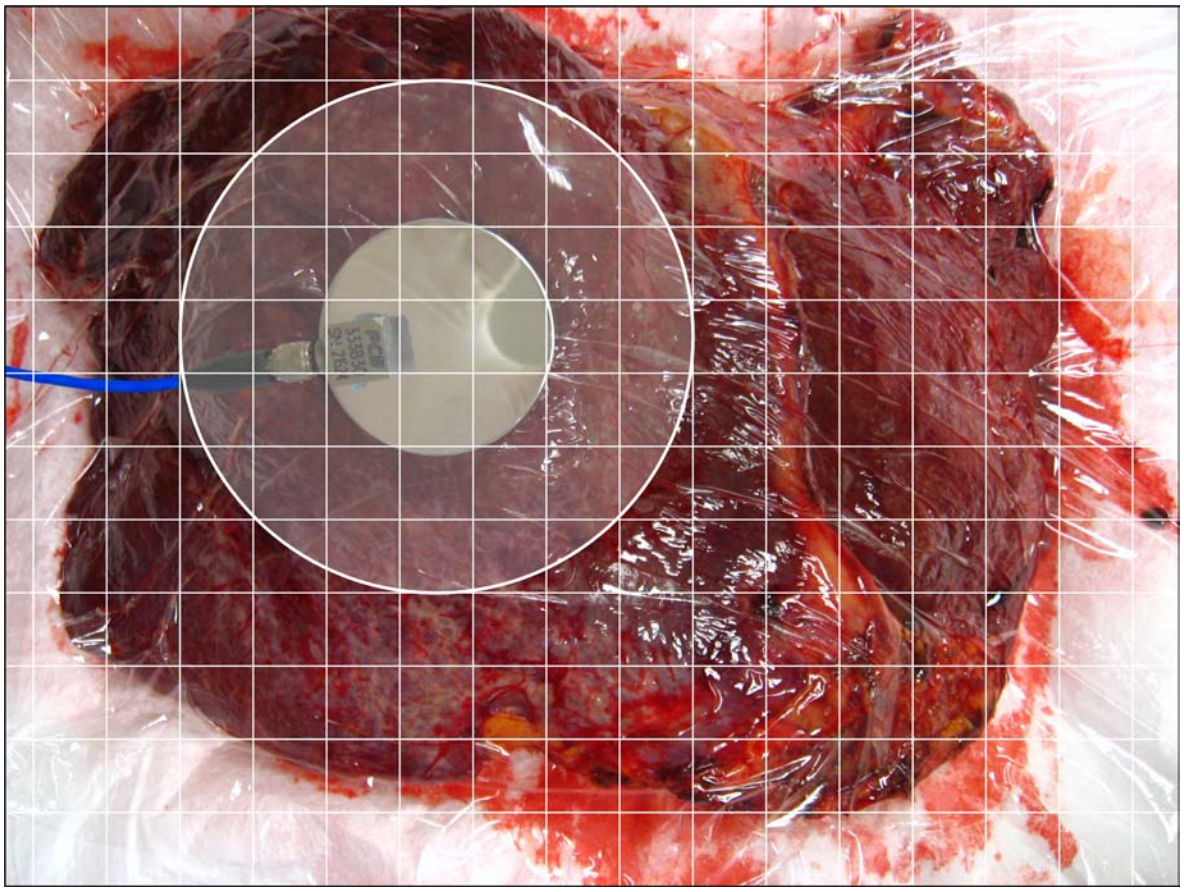


Figure 13. The impact load in our set-up is affective only within the area of liver (large circle) that is 5 times greater than the cross-sectional area of the pre-load (small circle) placed on the liver.

Chapter 4

RESULTS

Since the cross-sectional areas of all human livers tested in our study were significantly bigger than that of the pre-load used in our experiments, an effective value for the cross-sectional area was utilized in Eq. 4 to estimate the dynamic elastic modulus of human liver from the measured dynamic stiffness. This value was set based on the results of the experiments performed on liver and silicon samples before. Hence, the effective cross-sectional area of the harvested livers tested in our study was set to five times the cross-sectional area of the pre-load in the calculation of dynamic elastic modulus. For the length, no adjustment was necessary. As shown in Figure 8 and Figure 9, there is also a tendency in dynamic stiffness and resonance frequency data to converge to a steady state response as the length is increased, but occurs after $L > 200$ mm. The lengths of the harvested livers tested in this study were much lower than this threshold value. Hence, the effective length of each liver was taken as its original length. Figure 14 shows that impact test can successfully differentiate the levels of fibrosis in terms of storage modulus; higher the fibrosis level the stiffer the liver. The storage modulus varied from 10 to 20 kPa with the excitation frequency for the livers with no fibrosis (F0), from 20 to 50 kPa for the livers with some level of fibrosis. The cirrhotic livers (F4) were almost three times stiffer than the livers with no fibrosis (F0) ($p < 0.001$). The comparison of fibrosis levels by means of

ANCOVA test showed significant differences between the storage modulus of fibrosis levels (F0-F2; $p < 0.001$, F0-F3; $p < 0.001$, F0-F4; $p< 0.001$, F2-F3; $p < 0.001$; F2-F4; $p < 0.001$; F3-F4; $p < 0.001$).

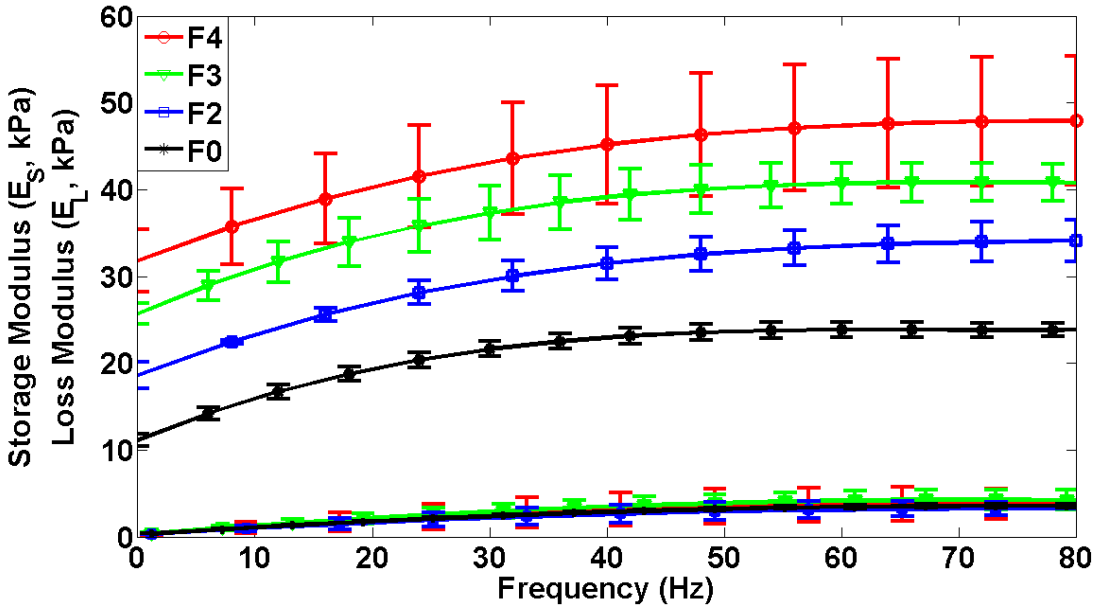


Figure 14. Storage (upper curves) and loss (lower curves) moduli of human liver as a function of frequency for different levels of fibrosis.

Chapter 5

DISCUSSION and CONCLUSION

We presented a new approach for dynamical characterization of a soft organ using an impact hammer. Our approach is the extension of the experimental technique proposed by Lin et al. (2005) to measure hysteretic damping of rubber materials. The original technique enables the measurement of only dynamic stiffness of a viscoelastic material as a function of frequency and is limited to the characterization of small and short samples. The estimation of dynamic elastic modulus of a whole liver from the measured dynamic stiffness is not trivial since its size is significantly larger than that of the pre-load. While the dynamic stiffness of a liver heavily depends on its size and geometry, its dynamic elastic modulus is a material property and must be independent of its size and shape. However, it is highly challenging to develop analytical models of impact wave propagating inside a liver body to estimate its dynamic elastic modulus since the impact force applied to the liver propagates in a complex manner over its surface and along the length. For this reason, we used a general mathematical relation which is derived from Hooke's Law ($E = kL/A$) and investigated the effects of length and cross-sectional area on the dynamic stiffness of a material through a set of controlled experiments performed with tissue-like silicon samples in our laboratory. We then performed experiments in an operating room with freshly harvested human livers having a liver disease. Unfortunately, as cited in the

introduction section, there is very limited number of biomechanical studies (see the next paragraph for a discussion on existing imaging technologies) available in the literature on the dynamic properties of healthy or diseased human liver to compare our results directly. There are commercial rheometers available for the dynamical characterization of soft tissues, but they are mainly designed to work with small samples and are not practical for collecting data from an organ. This also limits their usage in in-vivo studies. The hand-held probes and robotic devices have been developed for in-vivo measurements, but data has been collected mostly from animals. Using these devices, an organ can be loaded dynamically at different frequencies for a range of frequencies (i.e. frequency sweep) during an in-vivo measurement. However, this process is time-consuming and measurement errors may occur due to the pulmonary motion. While artificial ventilation can be used to overcome this problem, it can only be applied for short period of time, limiting the time interval for data collection. The impact hammer can be a remedy for this problem since the impact test is fast and reliable and the impulse response of a soft material is equivalent to its frequency response obtained by dynamic test.

In this study, we showed that the frequency-dependent dynamic material properties of a soft organ can be measured more easily and efficiently using a hand-held impact hammer. Moreover, we characterized the dynamical material properties of human liver having different levels of fibrosis. The measurements in this study were performed on freshly

harvested livers to collect more accurate data for characterization, but the impact hammer can be perhaps used externally to estimate the level of fibrosis non-invasively. The evaluation of liver fibrosis is of great clinical interest. The early detection of liver fibrosis may lead to a more successful treatment. In fact, some cases of early fibrosis may be reversible with the elimination of the cause. Detecting the liver disease at the stage of cirrhosis, which is the end-stage progression of fibrosis, is relatively easy, but not much helpful from a clinical point of view. It is often more difficult to tell by clinical parameters how much fibrosis exists in a liver. Currently, the level of fibrosis in a diseased liver is assessed by biopsy which is an invasive and painful procedure. An alternative technique based on transient ultrasound elastography, called FibroScan (EchoSens S.A., Paris, France), has been developed to quantify hepatic fibrosis non-invasively by Sandrin et al. (2003). This system is equipped with a probe consisting in an ultrasonic transducer mounted on the axis of a vibrator. A vibration of mild amplitude and low frequency (50 Hz) is transmitted from the vibrator to the tissue by the transducer itself. This vibration induces an elastic shear wave that propagates through the tissue. The propagation velocity of the wave within the tissue is directly related to the tissue stiffness (or elastic modulus). The harder the tissue, the faster the shear wave propagates. The results obtained by Sandrin et al. (2003) indicate that liver gets harder as fibrosis spreads out. They found that the elasticity of fibrotic livers varied from 3.35 to 69.1 kPa, where the largest variation was observed for F4 (14.4 to 69.1 kPa). In a more comprehensive study, Ziol et al., (2005) also

correlated the stiffness values of liver samples measured by FibroScan to the fibrosis stage. The elastic modulus of fibrotic liver was found to vary from 4.1 to 7.1 kPa for F0-1, from 4.8 to 9.6 kPa for F2, from 7.6 to 12.9 kPa for F3, and 16.3 to 48 kPa for F4. The cut-off values for deciding on the fibrosis level were determined as 8.8 kPa for $F \geq 2$, 9.6 kPa for $F \geq 3$, and 14.6 kPa for $F = 4$. Magnetic Resonance Elastography (MRE) is another noninvasive imaging technique that can be used to measure the stiffness of liver (Manduca et al., 2001, Rouviere et al., 2006). MRE is performed by transmitting shear waves within the liver and then imaging the waves using Magnetic Resonance techniques. Recent studies show that the MRE technique is also a feasible method to stage liver fibrosis and diagnose cirrhosis (Huwart et al., 2006). The results showed that the liver elasticity estimated by MRE increased with increasing stage of fibrosis. The shear modulus was found to fluctuate between ~2-7 kPa at the excitation frequency of 65 Hz, depending on the fibrosis level.

Our measurements are most compatible with the FibroScan measurements in terms of the range of elasticity values. It is important to note that a direct comparison of our biomechanical approach with these imaging technologies is not feasible due to the nature of measurement technique. For example, the elasticity values reported in FibroScan and MRE studies are typically measured at a certain frequency rather than a range of frequencies. Moreover, there are still open questions about the accuracy and validity of both FibroScan and MRE. First of all, MRE has a potential but it is still in clinical research.

FibroScan based on ultrasound elastography is commercially available and correlated with fibrosis in patients with chronic hepatitis, but this technique works best for separating patients with minimal or no fibrosis from those with significant fibrosis (Lucidarme, et al., 2009). Moreover, Bensamon et al. (2008) demonstrates the sensitivity of the FibroScan technique to local measurements. They compare the liver stiffness measured by FibroScan with MRE and finds higher variability in FibroScan measurements. This variation is attributed to the thickness of subcutaneous tissue and the movements of the probe during the exam, which effects the direction of the wave pressure and consequently the liver stiffness measurement.

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