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A Viscoelastic Property Study in Canine Liver Before and After HIFU Ablation *In Vitro*

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Abstract— **Elasticity imaging techniques have shown great potential in detecting High Intensity Focused Ultrasound (HIFU) lesions based on their distinct biomechanical properties. However, quantitative tissue viscoelastic properties and the optimal power to obtain the best contrast parameters remain scarce. In the present study, fresh canine livers were ablated *ex vivo* using six different acoustic powers and time durations, covering an energy range of 80-330 *J*. Biopsy samples were then extracted and examined, using rheometry, to obtain the viscoelastic properties post-ablation *in vitro*. All mechanical parameters were found to be frequency dependent. Both the shear complex modulus and viscosity exhibited monotonic increase for the first 4 groups (80-240 *J*), relatively lower HIFU powers. Similar parameters from groups 5-6 (300-330 *J*) showed relative decrease, still higher than unablated group 0. The tangent of the stress-strain phase shift was found to vary from unablated group 0 to ablated groups 1-6. However, no measurable difference amongst the ablated groups was found. Decreased stiffening at high powers compared to the baseline could likely be due to compromised structural integrity in the pulverized tissue well beyond the boiling point. The findings here can be used to optimize the efficient monitoring and treatment of tumors using any thermally-based methods where strong tissue damage is expected and/or warranted, respectively.**

I. Introduction

Pathological tissues have been shown to exhibit distinct mechanical properties from the normal tissues, *e.g.*, breast tumors exhibiting higher stiffness compared to normal breast tissues, nonalcoholic fatty liver disease undergoing stiffening under fibrosis, stiffening of the calcific aortic nodes and abdominal aortic aneurysmal lesions exhibiting local softening and stiffening [1-5]. Another emerging application for the ultrasound–based elasticity imaging techniques has been the assessment and monitoring of change in tissue mechanical properties during minimally and non-invasive treatment procedures and following therapies such as microwave and Radio Frequency (RF) ablation [6], High Intensity Focused Ultrasound (HIFU) ablation [7], hyperthermia [8], histotripsy [9], and cryoablation [10]. Different studies have been conducted over the past decades on clinical implementation of HIFU-based methods [11]. Harmonic Motion Imaging for Focused Ultrasound (HMIFU) is an acoustic radiation force-based HIFU treatment with previous feasibility studies in tissue elasticity assessment as well as real-time HIFU treatment monitoring *in silico*, *in vitro*, *ex vivo*, and *in vivo* [12-16]. In HMIFU, a focused transducer is used to induce focal ablation while a confocal pulse-echo transducer is simultaneously used to image the tissue and estimate the Harmonic Motion Imaging (HMI) displacements and phase shift. The HMI measurements are used to monitor and assess the contrast in underlying viscoelastic properties between lesion and medium. In order to further enhance the monitoring and assessment capabilities of the method, independent estimation of the change in the tissue viscoelastic properties under different HIFU powers is warranted. Several studies have been shown to examine the various thermal effects on tissue mechanical properties [17], where different soft tissues were found to show different thermal behaviors. It has been shown that different soft tissues undergo (reversible) softening followed by a (irreversible) stiffening phase beyond certain temperatures [15,17]. In addition, few studies have also compared the results of elasticity imaging of HIFU–induced thermal lesions to those from conventional mechanical testing and have reported a lesion stiffness contrast ranging between four to twelve times compared to unablated tissue [12,18]. In addition, HIFU treatment at high levels of intensity may reach boiling, *i.e.* acoustic intensity of few to tens of kW/cm^2, is capable of effectively inducing necrosis in cancerous tissues due to their resistive nature to treatment and angiogenesis facilitating their aggressive growth [11,19]. Nevertheless, a very limited amount of literature exists on the tissue mechanical properties under such high ablation powers. In the present study, we investigated the HIFU boiling effects on the viscoelastic mechanical properties of HIFU lesions on freshly excised canine liver tissue before and after HIFU treatment at high powers beyond the coagulation level, denoting ablation with boiling in this case. We hypothesize that those tissue thermal effects under high ablation powers –including changes in stiffness– will be different from those seen under low ablation powers, given the chaotic mechanisms involved during boiling.

II. Materials and Methods

A. Sample Preparation

Eight mongrel canine liver specimens were freshly excised immediately after sacrifice and kept immersed in de-ionized and degassed PBS in room temperature during the *in vitro* experiment, completed within 24 *h* post-mortem.

B. HIFU Ablation

The samples were placed over an acoustic absorber submerged in a de-ionized and degassed PBS bath. A customized Focused Ultrasound (FUS) system (*Riverside Research Institute, NY*) with a focused transducer (focal

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depth of 9 *cm*, central frequency of 4.755 *MHz*) was used to induce HIFU lesions in the liver samples. In order to provide a sufficiently large and homogenously ablated region for subsequent extraction of mechanical testing samples, a raster scan was performed to generate a conglomerate set of thermal lesions spanning across an area varying between 1.5×1.5 cm^2 and 2.5×2.5 cm^2. The system consisted also of a confocally-aligned 7.5 *MHz* pulse-echo imaging transducer (*V320-SU-PTF, Olympus NDT, MA*) that was used for raster scan targeting. A portion of each liver was examined unablated as the control group, group 0, and the rest was ablated under different ablation powers of 8, 10 & 11 *W*, and time durations of 10 & 30 *s*, generating increasing energies of 80, 100, 110, 240, 300 & 330 *J*, in groups 1-6, respectively.

C. Rheometry Mechanical Testing

After completion of the ablation, both unablated and ablated tissues were sectioned for mechanical testing using shear rheometry *(ARES-G2, TA Instrument, DE)*. A 6 *mm* biopsy punch was used to extract a total of fifty (n=50) cylindrical samples from the unablated and ablated tissue samples. First, a 5% compressional strain was applied on the samples to increase the shear surface grip between the tissue-fixture interfaces. The oscillatory shear test was performed by applying a periodic shear strain, $\gamma(t)$, as follows:

$$\gamma(t) = \gamma_0 sin(\omega t) = \gamma_0 sin(2\pi f t) \ , \quad (1)$$

where $\gamma_0$is the magnitude of the shear strain, t is time, and ω and f is the radial and linear frequency, respectively; and measuring the resultant shear stress, $\tau(t)$, in the form of:

$$\tau(t) = \tau_0 sin(\omega t + \delta) = \tau_0 sin(2\pi f t + \delta) \ . \quad (2)$$

where τ_0 is the magnitude of the shear stress and δ is the phase shift between stress and strain. The applied shear strain was set at $\gamma_0 = 0.01$ within a sweeping frequency range of $f = 0.1 - 10\ Hz$. The strain and stress magnitudes were used to calculate the complex shear modulus, G^*, as follows: $G^* = {}^{\tau_0}/_{\gamma_0}$. (3)

The complex shear modulus and phase shift were also used to compute the shear loss (viscous) modulus, $G^{"}$, and storage (elastic) modulus, G', as follows, respectively:

$$G^{"} = G^* sin\delta = {}^{\tau_0}/_{\gamma_0} sin\delta \ , \quad (4)$$

$$G' = G^* cos\delta = {}^{\tau_0}/_{\gamma_0} cos\delta \ . \quad (5)$$

Additionally, the ratio of the viscosity to elasticity as represented by $tan\delta$, and the dynamic shear viscosity coefficient, $\eta^{"}$, were calculated as follows, respectively:

$$tan\delta = {}^{G^{"}}/_{G'} \ , \quad (6)$$

$$\eta^{"} = {}^{G^{"}}/_{\omega} = {}^{\tau_0}/_{\gamma_0 \omega} \ sin\delta \ . \quad (7)$$

III. Results

Figure 1 indicates the rheometry measurements on shear complex modulus, G^*, tangent of the phase shift, $tan\delta$, and the viscosity coefficient, $\eta^{"}$, respectively, for the unablated group, group 0, and the ablated groups, groups 1-6, as a function of shear strain frequency, *f* (logarithmic scale). All three parameters were found to be frequency–dependent; direct relationships for the shear modulus and the tangent of the phase shift; similar to the data reported elsewhere [21], and an inverse relationship for the shear viscosity coefficient. It was found that the shear complex modulus for all ablated tissue samples was approximately an order of magnitude higher than that of the unablated samples (Fig. 1.A). Thus, a higher degree of stiffening compared to the unablated tissue was obtained in samples ablated using lower HIFU energies (*i.e.* groups 1-4) compared to the stiffening of the samples ablated with higher energies (*i.e.* groups 5-6). Regarding the tangent of the phase shift, it was found that only slight variations were seen across different ablated groups.

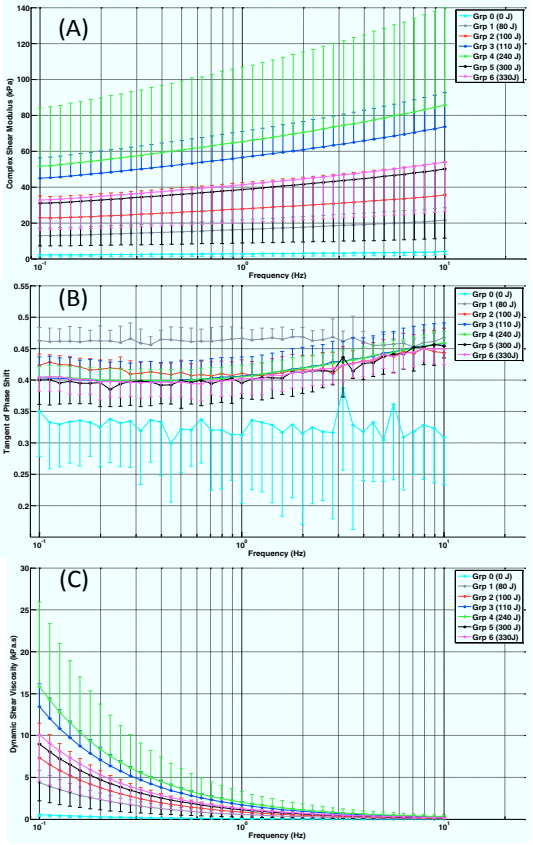

Figure 1. (A)Complex shear modulus, (B)Tangent of the stress-strain phase shift, and (C)Dynamic shear viscosity, *vs*. applied shear strain frequency (logarithmic scale) for unablated, group 0, and ablated, groups 1-6, canine liver tissue samples (*Grp* indicates Group). The error bars (either upward or downward) indicate the standard deviations.

However, they all show an average of 25% increase in tangent of the phase shift compared to the unablated tissues (Fig. 1.B). Similar to the shear complex modulus, the dynamic shear viscosity coefficient of all ablated tissues was also found significantly higher than that of the unablated group (Fig. 1.C); with a relative decrease in groups 5-6 compared to groups 1-4. Figure 2 shows the change in the shear modulus, tangent of the phase shift and shear viscosity of the tissue samples only at the shear frequency of *f*=10 *Hz* against separately change in ablation powers of 8, 10 & 11 *W*, and time duration of 10 & 30 *s*. The frequency 10 *Hz* was the highest frequency examined here and the closest to the HMI frequency of 50 *Hz*. The results indicate that effects that the change in viscoelastic properties could be different at various powers and time durations. For instance, both the shear modulus and viscosity increase over increasing time duration under lower ablation powers of 8 & 10 *W*, but decrease under ablation power of 11 *W* (Figs. 2.A-2.C).

Interpreting the same results under time variable, it is seen that for short ablation period of 10 *s*, both the shear modulus and viscosity increase for increasing powers of 8, 10 & 11 *W*, but the trend becomes reversed at higher ablation period of 30 *s* (Figs. 2.A-2.C).

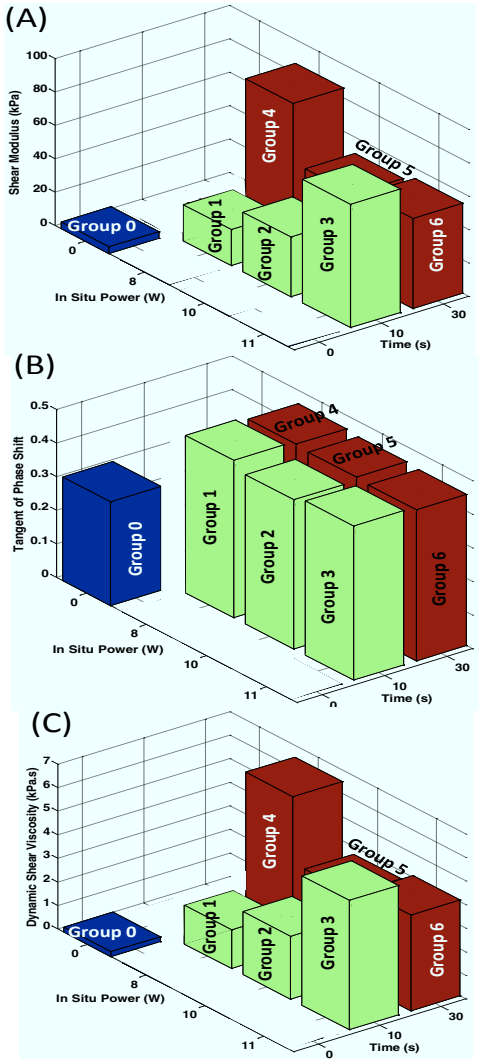

Figure 2. Change in (A)Complex shear modulus, (B)Tangent of the stress-strain phase shift, and (C)Dynamic shear viscosity, all measured at f=10 *Hz*, *vs*. ablation time and power for unablated, group 0, and ablated, groups 1-6, canine liver tissue samples.

IV. Discussion

The changes in the tissue mechanical properties during pathological conditions have been the basis for various diagnostic techniques. Recent improvements in elasticity imaging have made it a promising technique for noninvasive estimation of the tissue focal mechanical properties *in situ*. In order to further enhance the monitoring and assessment capabilities of the elasticity imaging-based techniques, independent estimation of the change in tissue viscoelastic properties is warranted. This study aims at characterizing the effects of HIFU energy on the viscoelastic properties of the ablated canine liver tissues. Shear rheometry measurements were made on ablated tissues under a HIFU energy range of 80-330 *J*, and will be used to improve the understanding of our ongoing HMIFU studies. It has been shown that using very high HIFU energies might be necessary in ablating the tumorous lesions in the tissues in order to achieve a full tumor treatment [19]. It has been widely discussed that phenomena taking place in tissues at high energies could be very different from those taking place at lower energies. The possibility of thermal necrosis by heating, and tissue emulsification by cavitation or by use of repetitive millisecond shock wave-based boiling have all been recently discussed [22]. It has been shown that within a range of low temperatures, *i.e.* 42-46◦*C*, only apoptosis may occur [23]. The mechanical properties of ablated lesions, particularly under high energies, have remained largely understudied. In this study, fresh canine liver tissues were ablated *ex vivo* under HIFU energies of 80-330 *J* and their viscoelastic properties were obtained post-ablation using shear rheometry. An average complex shear modulus of about 3 *kPa* was measured in unablated liver tissues –consistent with measurements reported elsewhere [21]. It was found that the shear modulus was sharply increased after ablation, which shows that a monotonic increase in thermal ablation energy does not correspond to a monotonic increase in the lesion modulus (Figs. 1 & 2). A very similar trend has previously been reported indicating that the complex modulus of porcine liver increases of up to 6-11 times as the temperature rises from 40°*C* to about 75°*C*, beyond which it decreases about 10-20% up to the tested temperature of about 90°*C* [21]. These findings could be explained based on the fact that unlike in thermal ablation at lower energies, which causes cell shrinkage and stiffening of thermally–coagulated tissue, higher ablation energies may induce tissue emulsification, as a result of which the tissue strength is reduced due to compromised structural integrity of gelated collagen [21]. A similar trend to the shear modulus was also found here for the change in viscosity coefficient as a function of HIFU energy. The data on the tangent of the phase shift showed a change from an average of 0.3 for unablated tissues to an average of about 0.4 for all ablated groups 1-6. However, no inter-group significance was found amongst the ablated groups. Given the thermocouple limitations in measuring temperatures beyond boiling, it was not possible to make temperature measurements in groups 5-6. However, based on the thermocouple measurements on the groups 1-4 ablated tissues indicating the temperature to exceed the boiling point [24], it is more strongly expected that tissue pulverization is induced in groups 5-6. The excitation frequency used in the HMI experiments usually falls within higher range, *e.g.* f=50 *Hz* [12,24] than what is examined in the rheometry testing here. Applying shear strain frequencies higher than 10 *Hz* was not possible due to mechanical limitations of the rheometry system. However, we focused on the maximum frequency tested, *i.e.* closest to the HMI frequency, to obtain the contrast plot for all three viscoelastic parameters of shear modulus, tangent of phase shift and viscosity, as a function of HIFU powers and time durations (Fig. 2). It is found that the changes in tissue viscoelastic properties are a function of not only the HIFU ablation energy, but also separate functions of the duration time and powers. It is found that the tangent of the phase shift might be the least sensitive parameter for monitoring the change in tissue properties under increasing ablation energies, whereas the shear modulus and viscosity may be used for such purposes more efficiently. Given this finding as well as the observation that only some particular

data can be of inter-group statistical significance, we believes that the optimal contrast monitoring can be achieved based on all contrast parameters, combined. The HMIFU has also been demonstrated by our group to be capable of detecting lesions based on the lesion-to-background displacement contrast [12,13]. However, the comparison of the modulus contrast between the present study and the HMIFU study requires obtaining the focal radiation force as it could vary given the change in tissue acoustic properties, such as attenuation, as a function of depth and temperature. In particular, focal increase in lesion attenuation can occur [20] because of boiling and thermal effects, resulting in an increase in radiation force and induced displacement [24]. It has been shown that the mechanical properties of the liver tissue could change during the heating and cooling, *e.g.* liver tissue stiffening after cooling down from temperature above $50°C$ [17]. Given our mechanical testing being performed on the liver samples *in vitro* after being stored in room–temperature PBS for 2-3 hours, it is expected that the mechanical properties measured here be different than those *ex vivo*. However, the primary objective of this study was to obtain the tissue mechanical property contrast between the lesion and the background, post-ablation. Indeed, we found the *in vitro* protocol helpful in increasing the repeatability of our mechanical testing measurements by making the entire raster ablated lesion more thermally and mechanically homogeneous. Finally, it should be noted that any mechanical measurements *in vitro* can be affected by factors such as sample size, shape and the boundary conditions [20]. To minimize such confounding effects, all samples in this study were prepared from the homogenous regions of the tissue, under the same shape and size, and were applied the same force and boundary conditions during the testing.

V. Conclusion

Independent mechanical property measurements on HIFU lesions in soft tissues are warranted for enhancement of the monitoring and treatment techniques. This study described the quantitative viscoelastic properties of canine liver tissues *in vitro* following HIFU ablation *ex vivo*. Proper considerations are needed when implications of these results are extended to applications *in vivo*. The results showed an order of magnitude increase in the stiffness and viscosity of the lesions obtained at various levels of HIFU energies. However, HIFU lesions ablated at lower energy range *e.g.* 80-240 *J*, were found to indicate higher shear modulus and viscosity than those ablated at higher energy range, *e.g.* 300-330 *J*. More importantly, it was found that the changes in viscoelastic properties are not only a function of ablation energy, but also separate functions of ablation time and powers. It was concluded that a multi–parametric assessment of tissue properties contrast, including the shear modulus, tangent of phase shift and viscosity, may prove more efficient in enhancing ablation monitoring where potential tissue boiling and pulverization is anticipated or warranted.

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