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Tissue Atomization by High Intensity Focused Ultrasound

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***Abstract* — Liquid atomization and fountain formation by focused ultrasound was first published by Wood and Loomis [1]. Since then, the cavitation-wave hypothesis emerged to explain atomization in a fountain, which states atomization arises from a combination of surface capillary waves and the collapse of cavitation bubbles. More recently, high intensity focused ultrasound (HIFU) has been shown to fractionate tissue through either pulsed-cavitation or millisecond boiling histotripsy therapies; however it is unclear how millimeter-size boiling bubbles or cavitation bubble clouds fractionate tissue into submicron-size fragments. The objective of this work is to test the hypothesis experimentally that atomization and fountain formation occurs similarly in liquids and tissues and results in tissue erosion. A 2-MHz HIFU transducer operating at peak *in situ* pressures of 50 MPa and -11 MPa (intensity = 14 kW/cm^2) was focused at the interface between a liquid or tissue and air. A high-speed camera was used to monitor atomization and fountain formation in water, ethanol, glycerol, bovine liver, and porcine blood clots. The *in situ* intensity threshold for consistent atomization in one 10-ms pulse increased in the order: ethanol (180 W/cm^2) < blood clot (250 W/cm^2) < water (350 W/cm^2) < liver (6200 W/cm^2); glycerol did not atomize. Average jet velocities for the initial spray at the maximum acoustic intensity were similar for all materials and on the order of 20 m/s. The tissue erosion rate of liver approached saturation at around 300 10-ms pulses repeated at 1 Hz, which had an average erosion volume of 25.7±10.9 mm^3. While tissue atomization and fountain formation does not completely mimic what is observed in liquids, atomization provides a plausible explanation of how tissue is fractionated in millisecond boiling and possibly even cavitation cloud histotrispy therapies.**

Keywords— Atomization; fountain; histotripsy; HIFU

I. INTRODUCTION

Ultrasonic atomization, or the ejection of a fine spray from a liquid surface exposed to air, is a well-known phenomenon that has been used industrially in air humidifiers and medical nebulizers [1]. It has also been proposed as a possible explanation for injury to the lungs in diagnostic ultrasound [2]. The objective of this work is to determine (a) whether atomization and fountain formation occur similarly in liquids and tissues, and (b) if atomization of tissue results in erosion.

The cavitation-wave hypothesis, a combination of cavitation and capillary waves, is the most accepted mechanism of ultrasonic atomization in liquids [3]. Several iterations of this hypothesis exist based on the results of Antonevich and Boguslavskii and Éknadiosyants [3-5]. One interpretation of the cavitation-wave hypothesis is that radiation force from the focused transducer causes the liquid surface to bulge, which focuses the waves inverted by the pressure release surface and causes numerous cavitation bubbles to form in the bulged volume of liquid. Oscillation and collapse of the cavitation bubbles enhance microscale surface perturbations (capillary waves), causing droplets to pinch off in atomization.

High intensity focused ultrasound (HIFU) has been used clinically to thermally coagulate tissue and there are several techniques in various stages of development to mechanically fractionate tissue [6-10]. Pulsed cavitation histotripsy and shockwave heating and millisecond boiling (hereafter denoted as boiling histotripsy) are two techniques that have been shown to fractionate tissue into submicron-size pieces with sharp boundaries between treated and untreated tissues [7-10]. In pulsed cavitation histotripsy, microsecond pulses at high pulse repetition frequencies (PRFs) and acoustic pressures (peak positive pressure (p_+) > 80 MPa and peak negative pressure (p_-) < -20 MPa) create and maintain a cavitation bubble cloud several millimeters in size composed of bubbles on the order of hundreds of microns in diameter at the transducer focus [8, 9]. On the other hand, boiling histotripsy uses millisecond pulses at lower PRFs and acoustic pressures as compared to cavitation cloud histotripsy (p_+ > 40 MPa and p_- < -10 MPa) to explosively expand a millimeter-size boiling bubble at the transducer focus. As these techniques seem so disparate yet produce the same effect, it is important to understand the mechanism by which millimeter-size boiling bubbles or cavitation bubble clouds fractionate tissue into subcellular components.

The goal of this study is to evaluate the hypothesis that atomization may be the mechanism by which boiling histotripsy fractionates tissue. Through high-speed photography, observations of atomization will be compared between liquids and tissue, specifically looking at the thresholds for atomization, the temporal progression of atomization, and the erosion of tissue. The experiments mentioned in the current paper are partly described in [11].

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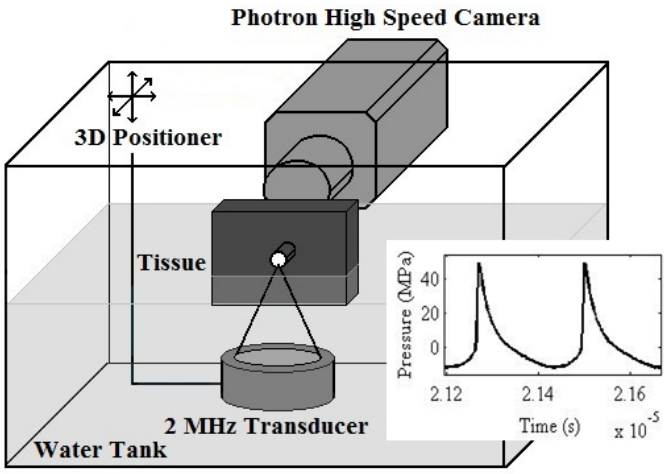

Fig. 1. The experimental setup for the "bubble-like" interface (a manmade cylindrical hole of approximately 1-mm diameter bored through tissue). The inset shows the waveform of the 2-MHz transducer at the *in situ* pressures used in these experiments.

II. Methods

An air-backed, spherically focused piezoceramic crystal (PZ 26, Ferroperm Piezoceramics, Kvistgaard, Denmark) with an operational frequency of 2.165 MHz was used. The diameter and radius of curvature of the transducer was 45 mm. For these experiments, a function generator (Model 33250A, Agilent, Palo Alto, CA, USA) and a linear radiofrequency amplifier (55 dB gain, Model A300, ENI, Rochester, NY, USA) were used to drive the transducer at a maximum linearly estimated *in situ* intensity [12] of 14 kW/cm^2 (p_+ = 50 MPa and p_- = -11 MPa) as shown in the Fig. 1 inset. The waveform was measured in water before the experiments with a 100-µm active diameter fiber-optic probe hydrophone (FOPH 2000, RP Acoustics, Leutenbach, Germany). In tissue the waveforms were then derated using a previously developed derating method [13], with the attenuation coefficient (α) in bovine liver of α=0.7 dB/cm/MHz [9] and the attenuation coefficient in porcine blood clots of α=0.93 dB/cm/MHz [14]. All measurements and experiments were conducted in filtered and degassed room temperature (~20^{o}C) water.

A. Experimental Arrangements

Previously, researchers considered the role of cavitation through the manipulation of ambient pressure, ultrasonic frequency, liquid viscosity, temperature, and surface tension [3]. A reprise of these studies was conducted, focusing on the manipulation of shear viscosity through the use of: water (shear viscosity 0.0009 Pa·s), ethanol (0.001 Pa·s), and glycerol (1.2 Pa·s). The experimental setup was similar to that shown in Fig. 1; however the liquid, with a thickness of 2-25 mm, was held in a custom-designed container with an acoustically transparent thin plastic film bottom. Bovine liver and porcine blood clots were cut into pieces of approximately 5.5 cm x 5.5 cm with a depth varying between 1 cm and 1.5 cm and were held in a similar container without the thin plastic film bottom. The transducer was focused at the liquid or tissue top surface using pulse echo with the delay recorded on a digital oscilloscope (Model LT432, Lecroy, Chestnut Ridge, NY, USA). A Photron APX-RS high speed camera (monochrome, Photron, San Diego, CA, USA) was used to film the liquid surface at 20,000 frames per second with a resolution of 256x512 pixels. A Carl Zeiss lens (Makro-Planar T*2/100, Thornwood, NY, USA) with a bellows extension was used to allow for a resolution on the order of 40 µm/pixel. A continuous disperse light source (Photogenic PowerLight 2500DR, Bartlett, IL, USA) was used to backlight the liquid or tissue to air interface.

To determine whether ultrasound could cause atomization in a tissue-vapor interface similar to the millimeter-diameter bubble in boiling histotripsy, the setup shown in Fig. 1 was used. A millimeter-diameter cylindrical hole was bored in tissue approximately 1 cm from the lower tissue surface, with the cylindrical hole being a 2-dimensional representation of the 3-dimensional boiling bubble. The idea was to determine whether a millimeter-diameter boiling bubble is of sufficient size to act as a pressure-release interface, a necessity for atomization. To reiterate, these experiments began in an existing void that simulates the bubble created in boiling histotripsy. Then, as with the boiling histotripsy case, the insonification of the void continued and the interaction between the HIFU waves and the void was studied to see if the result was fractionated tissue. The camera, lens, light, function generator, and amplifier are the same as was described previously.

B. Tissue Preparation and Analysis

Bovine liver was purchased from a local abattoir (Schenk Packing, Stanwood, WA, USA) and used within 8 hours of harvesting. The liver was sectioned, immediately submerged in PBS, and placed in a desiccant chamber on ice for at least 1 hour. Porcine blood was collected from a terminal porcine study, allowed to clot for approximately 1 hour at room temperature, and used immediately. The volume of eroded tissue was calculated as a spherical cap from caliper measurements of the eroded hole that were rounded to the nearest half-millimeter. Tissue projectiles were also collected by placing a microscope slide below the fountain and stained with Hematoxylin and Eosin (H&E) for visualization of cellular components.

III. Results

Atomization and fountain formation were compared between water and bovine liver using high speed photography for the maximum *in situ* intensity of 14 kW/cm^2. Fig. 2 shows a series of frames taken in (a) water and (b) bovine liver. In water, significant atomization occurred by the second video frame, taken 70 µs after the ultrasound wave reached the surface. As the sonication continued, a bulge formed in the water surface and the number and size of the droplets emitted from the water surface increased until the camera field of view was almost completely obscured. However, in bovine liver a small spray of atomization was ejected by the third video

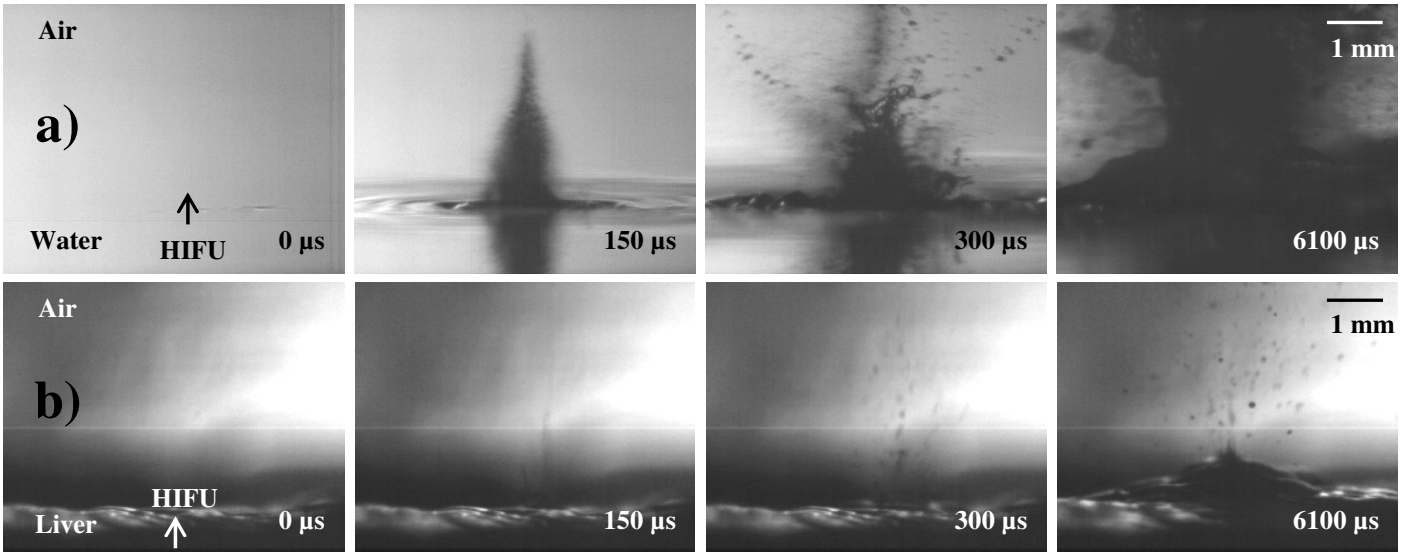

Fig. 2. A high-speed photographic comparison between a) a water-air interface and b) a bovine liver-air interface for a 10-ms pulse at the maximum *in situ* intensity. The first frame in both instances is taken 20 µs after the ultrasound wave arrives at the surface. At 150 µs, the water shows well-developed atomization and height, while the liver surface shows only a couple of faint sprays. At 300 µs, the water shows even more dramatic atomization with a larger average droplet size, while the bovine liver shows a slightly more developed surface spray. Finally, at 6100 µs into the 10-ms pulse, the amount of water being ejected from the surface has almost completely occluded the camera field of view, while in liver, a surface bulge has formed, which is accompanied by more dramatic atomization.

frame, taken 120 µs after the ultrasound wave arrived at the interface. As in water, once a bulge formed in the liver surface, the number and size of ejected fragments increased significantly. The surface bulge took longer to form on the liver surface as compared to the liquid surface.

When the thresholds for consistent atomization in one 10-ms pulse were compared for water, 70% ethanol, glycerol, bovine liver, and porcine blood clot, it was found that the *in situ* intensity thresholds increased in the order: ethanol (180 W/cm^2) < blood clot (250 W/cm^2) < water (350 W/cm^2) < liver (6200 W/cm^2); glycerol did not atomize. At intensities near the threshold, the liquids formed a drop-chain fountain before atomization occurred (shown in previous studies [15]). A similar phenomenon occurred in tissue near the threshold, with a surface bulge forming before atomization occurred. In both liquids and tissues, as the intensity increased beyond the threshold, atomization commenced earlier in the ultrasound pulse and from smaller surface bulges until atomization began from a flat interface (as shown in Fig. 2). For all species that atomized, the average jet velocity at the maximum *in situ* intensity of 14 kW/cm^2 for the initial spray that develops 70 µs to 170 µs after the ultrasound reaches the interface were similar and on the order of 20 m/s. The velocities of the projectiles were inversely related to the size of the projectiles.

To answer the question of whether the millimeter-diameter bubble in boiling histotripsy is large enough to act as a pressure release interface, a 1.7-mm diameter, cylindrical hole was bored through bovine liver. Fig. 3 shows the results from a 10-ms pulse. From the sequence of photographs, it is obvious that atomization and a miniature acoustic fountain can form in a 1.7-mm diameter cylindrical hole. Interestingly, atomization in the cylindrical hole appears even more dramatic than atomization of the flat interface for similar intensities (see Fig. 2.b.). In the cylindrical hole, the surface bulge occurs much earlier in the 10-ms pulse and continues to grow until the hole is completely occluded. Because of this, it may be possible to cause similar degrees of fractionation with shorter pulses in the hole as compared to the flat tissue surface.

The end result of each ultrasound exposure was a hole on the surface of the bovine liver (Fig. 4 insert). The size of the surface hole was measured with calipers and plotted as volume of eroded tissue versus the number of 10-ms pulses at 1 Hz pulse repetition frequency as shown in Fig. 4. The volume of eroded tissue appears to approach saturation at around 300 10-ms pulses, which has an average erosion volume of

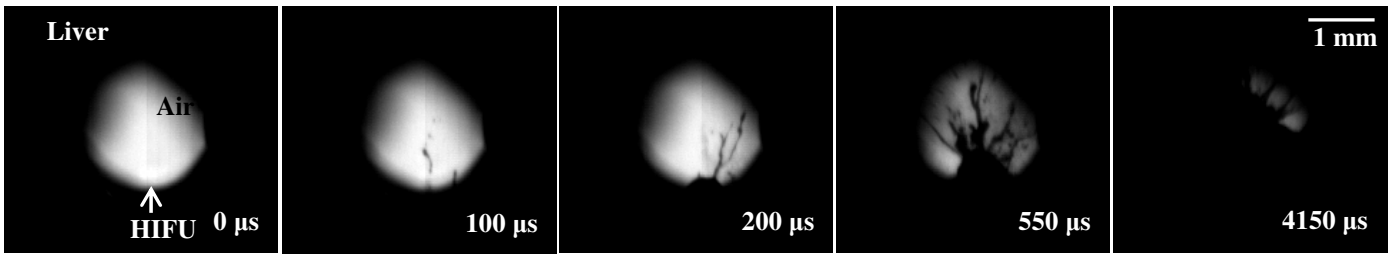

Fig. 3. High-speed photographs showing the temporal progression of a fountain for a 10-ms pulse at the *in situ* intensity of 14 kW/cm^2 in a manmade 1.7-mm diameter cylindrical hole, which is thought to be similar to the vapor bubble created in bulk tissue during boiling histotripsy. The first frame is taken 20 µs after the ultrasound wave arrives at the interface. Fine sprays are detected very quickly, in less than 100 µs after the start of the video. At 200 µs, a small bulge forms within the cylinder. As HIFU continues, the size of the bulge grows with enhanced atomization until the hole becomes completely occluded (4150 µs).

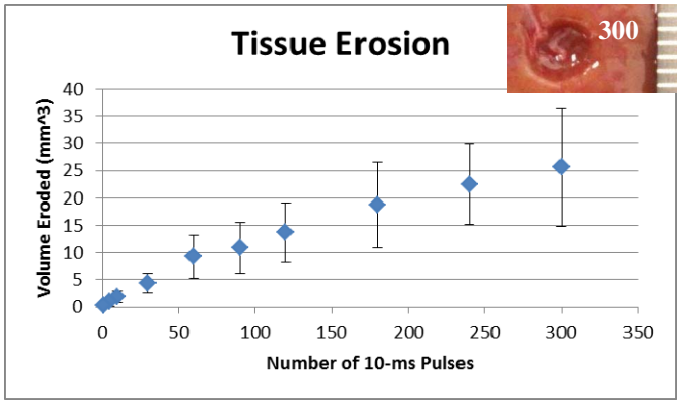

Fig. 4. Plot showing the volume of tissue eroded from the surface of bovine liver plotted *versus* the number of 10-ms pulses repeated at 1 Hz. The inset shows a typical hole from 300 pulses in the liver surface.

25.7±10.9 mm^3 based on 9 samples. The liver fountain projectiles were stained with H&E and microscopically analyzed, where it was determined that the projectiles contained whole and disrupted cells and nuclei. As the contents of the holes created in bulk tissue with cavitation cloud and boiling histotripsy contain only subcellular (submicron) components, the histological differences between the bulk histotripsies (boiling and cavitation cloud) and free-surface atomization need to be reconciled.

IV. Discussion/Conclusions

While tissue atomization and fountain formation does not entirely mimic what is observed in liquids, it provides a plausible mechanism to describe how millimeter-size boiling bubbles or cavitation bubble clouds fractionate tissue into submicron-size fragments. Even though the histological results differ between free surface atomization and bulk boiling and cavitation cloud histotripsies, this difference may be explained by recirculation. In free surface atomization, the tissue fragments are ejected from the surface and collected on a microscope slide, while in bulk histotripsy, atomization is contained in a small tissue volume, which could allow for recirculation of the liquid slurry and cause the tissue fragments to break up further into subcellular components.

Ex vivo tissue preparation techniques were found to greatly influence the efficiency of tissue fractionation from atomization. Tissues that were submersed in a phosphate-buffered saline (PBS) solution for a period of time before experimentation atomized much more efficiently than tissues that were either wetted with PBS or left in its natural state wetted only by blood. From these observations, we hypothesize that tissue "wetness" may help predict the success of boiling histotripsy or surface atomization in various types of tissues. Further research is needed to determine how tissue preparation techniques influence atomization and how to make *ex vivo* tissue as similar as possible to the *in vivo* case.

While all the work presented thus far was conducted in *ex vivo* tissues, preliminary results in *in vivo* porcine liver without a capsule indicate that atomization is even more dramatic *in vivo*, perhaps due to the presence of blood. As in *ex vivo* tissue, the end result of each exposure is a hole in the tissue surface with little to no evidence of thermal injury. Interestingly, tissue fractionation by atomization is very difficult to achieve when trying to breach the liver capsule. Determining the parameters that influence tissue atomization and potentially bulk boiling histotripsy will be useful as the techniques progress to the clinical regime.

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