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# Microbubble Generation by Piezotransducer for Biological Studies

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# **[Microbubble generation by piezotransducer for biological studies](http://dx.doi.org/10.1063/1.4936555)**

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Bubbles induced by blast waves or shocks are speculated to be the major cause of damages in biological cells in mild traumatic brain injuries. Microbubble collapse was found to induce noticeable cell detachment from the cell substrate, changes in focal adhesion and biomechanics. To better understand the bubble mechanism, we would like to construct a system, which allows us to clearly differentiate the impact of bubbles from that of shocks. Such a generator needs to be low profile in order to place under a microscope. A piezoelectric transducer system was designed to meet the need. The system uses either a flat or a spherical focusing piezoelectric transducer to produce microbubbles in a cuvette loaded with cell-culture medium. The transducer is placed on the side of the cuvette with its axis lining horizontally. A cover slip is placed on the top of the cuvette. The impact of the waves to the cells is minimized as the cover slip is parallel to the direction of the wave. Only bubbles from the medium reach the cover slip and interact with cells. The effect of bubbles therefore can be separated that of pressure waves. The bubbles collected on a cover slip range in size from 100  $\mu$ m to 10  $\mu$ m in radius, but the dominant size is 20-30  $\mu$ m. © 2015 *AIP Publishing*<br>*IIC* [bttp://dx.doi.org/10.1063/1.4936555] *LLC.* [\[http:](http://dx.doi.org/10.1063/1.4936555)//[dx.doi.org](http://dx.doi.org/10.1063/1.4936555)/[10.1063](http://dx.doi.org/10.1063/1.4936555)/[1.4936555\]](http://dx.doi.org/10.1063/1.4936555)

## **I. INTRODUCTION**

Soldiers who experience the repeated impact of blast waves may develop mild traumatic brain injuries (TBIs), for example, in the form of a concussion.<sup>[1](#page-7-0)</sup> While the cause of TBI is still not well understood, a leading theory is that microbubbles are generated due to the pressure impacts, which subsequently collapse, causing microstreams.<sup>[2](#page-7-1)</sup> The force created by the microstreams could reach a level sufficient to disrupt cell-cell connections and even create membrane pores.<sup>[3,](#page-7-2)[4](#page-7-3)</sup> This theory has been proved in astrocytes,<sup>[5](#page-7-4)</sup> which provides insights for 3-D tissue culture or animal studies. In a tissue subjected to pulsed high-intensity focused ultrasound (pHIFU), acoustic cavitation threshold varies broadly and depends on the tissue composition. $6$  Experimental shock and blast systems that can be repeatedly applied include electrically driven spark gaps, focused laser, shock tubes, and piezotransducers. Each approach has merits and disadvantages. For example, a laser focused in water can create a single shock and bubble precisely on the micrometer scale, therefore becoming highly useful for studying biological responses of a single cell. In the scenario of multiple bubbles, however, several lasers are needed and therefore increase the complexity of the system.<sup>[3](#page-7-2)</sup> Shock tubes can easily reproduce the blast waves that mimic the actual blast waves in a bombshell detonation, but due to the large scale, their use on an *in vitro* microscope study becomes impossible. Spark gaps are easy to construct, especially in water to produce bubbles. In the system reported in our previous work,<sup>[5](#page-7-4)[,7](#page-7-6)</sup> the impact of bubbles on astrocytes was clearly demonstrated and shock alone was shown to be ineffective to induce cell damage. However, one caveat for that system is the presence of an electric field before the breakdown of the spark gap, which can be a source of artifacts and may contaminate the biological results. Although we have not observed the effect directly caused by the electric fields in astrocytes, some other sensitive cell lines may be more susceptible to activation by the electric fields in addition to the bubble effect, making the interpretation of results difficult. It would be necessary to have a cleaner system in which the bubbles are the only stimuli to the cells. In this paper, we report such a system with the use of piezotransducers immersed in water to produce microbubbles. The piezotransducers are driven by RF signals with ultra-low voltages (peak voltage ∼40 V) and the electric fields are no longer a concern. In the liquid environment, the negative pressure in the tensile phase allows bubble creation and growth. Because the transducer is placed on the side of the cuvette and the cover slip on the top of the cuvette, the pressure wave's impact with the cells is minimized. All that interacts with the cells are the bubbles rising to the cover slip on which biological cells are seeded. The biological response to the bubbles can therefore be observed provided that the microscope is focused on the cover slip.

### **II. MICROBUBBLE GENERATOR**

# **A. The electrical circuit**

We adopted a commonly used ultrasonic water atomizer circuit to drive the piezotransducer at a resonance frequency

<span id="page-1-0"></span>a)This research was performed while W. Zhu was visiting the Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, Virginia 23508, USA.

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<span id="page-2-0"></span>

FIG. 1. A schematic diagram of the electrical circuit of the microbubble generator. A tank circuit (L1, L3, and C3) determines the oscillation frequency. A power BJT Q1 allows the replenishment of the energy stored in the inductors (L1 and L3) and sustains the oscillation.

of ∼2.1 MHz. A schematic diagram of the driving circuit is shown in Fig. [1.](#page-2-0) The critical component is a power BJT Q1. Two inductors in series (L1 and L3) and a capacitor (C3) in parallel constitute the tank circuit, which determines the frequency of the oscillation. When Q1 turns on as a result of the switching-on of Q2 and charging of C3, L3 and L1 become charged. The voltage at L1 feeds back through C4 to the base of Q1, calling for more current for a fast charging of L1. The oscillation rises from the discharging and charging between C3 and L1, which in turn switches Q1 on and off. In short, L1, L3, and C3 form the oscillation and C5 and C6 provide an AC short path to the transducer. Meanwhile, the energy is replenished by the turning-on of Q1.

The standard household voltage (120 Vrms) is stepped down to ∼28 Vrms to serve as the power source of the circuit. We note that higher voltages, for example, 100 Vrms, can cause visible electrolysis and are therefore not suitable for generating vibrations. A relay (Tyco CNS-35-72) is connected to the primary side of the transformer to control the "ontime" of the circuit, thereby controlling the on-time of the mechanical vibration of the piezotransducer. The on-time in this study was limited to  $~2$  s.

#### **B. Microbubble chamber design**

The size of the microbubbles is typically between singledigit micrometers to hundreds of micrometers. When they are resting on a glass surface, a conventional upright microscope is sufficient to observe these bubbles. However, in order to incorporate the chamber under a microscope for biological studies, a few criteria have to be met: (1) there has to be a light pathway so that the cover slip can be illuminated; (2) the overall chamber size (in particular its height) should be small enough so that it can be placed on the microscope stage; (3) there should be a fairly easy way to place and remove the cover slips; and (4) microbubbles can reach the cover slip with minimal impact from the mechanical vibration generated by the piezotransducer.

Acrylic sheet was initially considered as the material for the chamber since they are easy to machine. However, considerable heat damage to the acrylic sheet on the air side at the line-of-sight of the piezotransducer was observed after repeated operation. This is due to a mismatch between the acoustic impedance of acrylic and the air interface: The acoustic impedance of acrylic is  $3.26 \times 10^{-6}$  kg s<sup>-1</sup> m<sup>-2[8](#page-7-7)</sup> while that of air is 400 kg s<sup>-1</sup> m<sup>-2</sup>. Therefore, the transition of the low impedance to high impedance causes an increased magnitude of the mechanical wave at the interface and excessive heat, leading to the damage of the acrylic sheet (melting point:  $~\sim$ 160 °C).

We therefore chose glass (melting temperature  $1400 °C$ ) as the material to construct the main body of the chamber. The piezotransducer was arranged at 90° to the cover slip to avoid the direct transfer of vibration to the cover slip. A separate compartment for the piezotransducer was constructed with a circular opening ( $\Phi = 1.50$  cm) to the primary chamber. Edge steps were constructed along the top of the chamber walls to support a transparent top cover, where a stepped hole was drilled for the placement of the cover slip. A thin trench of ∼2 mm in width connecting to the stepped hole was made for easy mounting and dismounting of the cover slips with tweezers. The pre-seeded cells on the cover slip face the water bulk in the water so the risen bubbles interact with them as soon as they make contact. Fig.  $2(a)$  shows a schematic diagram of the chamber design with primary dimensions annotated. Fig. [2\(b\)](#page-3-0) shows a picture of the chamber placed on the stage of an upright microscope.

An added benefit of an all-glass chamber is that one can observe and photograph the generation of microbubbles from the side of the chamber while illuminating the chamber with a strong light source. During our experiments, tap water was used to fill the chamber up to the cover slip. No specific degassing procedure was carried out. It should also be noted that bubbles generated in previous cycles sometimes attached to the surface of the piezotransducer. Ultrasound energy in the following cycle is absorbed by these bubbles, preventing further microbubble formation in water. One can either extend the duration of the operation, i.e., use the ultrasonic vibration of the piezotranducer to "shake off" the attached bubbles, or to clean the surface of the piezotransducer before each cycle.

#### **C. Piezotransducers**

Two kinds of piezotransducer (STEMiNC) were used in this study. The spherical transducer has a diameter of 20 mm and a focal point at 30 mm from the transducer surface. It is operated at the resonant frequency of 2.1 MHz. The idea was to generate bubbles preferably at the focal plane of the transducer so that the maximum amount of bubbles rises to the cover slip. The flat transducer has a diameter of 19 mm and a thickness of 1 mm, which has a resonant frequency of  $2.07 \pm 0.05$  MHz. Both transducers were made with similar materials and operate in the thickness vibration mode with the electro-mechanical coupling coefficient,  $k_p \approx 0.59$ .







FIG. 2. A schematic diagram of the microbubble generator chamber (a) and a picture of the chamber under the microscope (b). The electrical wires that connect to the piezotransducer are omitted here. A cover slip that has cells pre-seeded is placed in the top cover, leaving the side with cells facing the chamber water. The bubbles risen from the glass chamber interact with the cells as soon as they reach the cover slip.

# **III. CHARACTERIZATION**

#### **A. Current and voltage characterization**

The voltage supplied to the piezotransducer was monitored via two voltage probes (Tek P6139A). The current behavior was monitored through a Pearson current monitor (Model: 2877). Signals were processed through a digital oscilloscope (Tektronix TDS3054C).

Fig. [3](#page-3-1) shows a typical voltage waveform measured across the piezotransducer, as well as the current through the circuit. The peak-to-peak voltage is measured to be about 70 V with the magnitude of the negative polarity slightly higher than that of the positive. The peak-to-peak current is roughly 5 A, with the positive current slightly higher than that of the negative. Although the driver circuit is of LC resonance in nature, but the phase difference of current and voltage is not 90◦ due to extra stray components of the transducer and connection leads. The electrical power deposition onto the transducer was evaluated to be about 17 W. Considering the electromechanical coupling coefficient of 0.59, only about 10 W is channeled into the mechanical vibration of the piezotransducer.

#### **B. Pressure analysis**

The acoustic pressure generated in water was monitored in a separate chamber by a fiber optic hydrophone (ONDA HFO-

<span id="page-3-0"></span>

<span id="page-3-1"></span>FIG. 3. Current and voltage waveforms of the circuit. The current is not in the same phase as the voltage. The phase relationship is neither as that in a resistive circuit nor a typical resonant circuit.

690) in an upright position, where the piezotransducer was arranged vertically in water (at about 6 cm below water surface) and the fiber tip ( $\Phi = 150 \ \mu m$ ) was placed directly above its surface at various distances. The signal from the hydrophone controller was processed through the digital oscilloscope.

A pressure waveform measured by the fiber optic hydrophone at a distance of 30 mm from the surface of the spherical piezotransducer is shown in Fig. [4.](#page-4-0) The positive pressure reaches about 9.5 MPa while the negative pressure is about −4 MPa.

The pressure of the acoustic wave in water at various distances from the piezotransducer was monitored for both the spherical focusing transducer and the flat transducer. The results are shown in Fig. [4\(b\).](#page-4-0) The spherical piezotransducer was found to have a focal distance at 30 mm, which agrees with the datasheet provided by the manufacturer. The pressure ranges from ∼2 MPa to ∼10 MPa between a fiber-to-transducer distance of 20 mm and 40 mm. The pressure produced by the flat piezotransducer is, however, considerably lower: ∼1.0 MPa at all distances measured (8-40 mm). Nevertheless, both piezotransducers were able to generate microbubbles in water effectively.

### **C. Imaging**

Images of the bubbles inside the chamber were captured via a digital SLR camera (Nikon D300S) with a macro lens coupled with a 2× teleconverter (Nikon TC-20EIII). The CCD of the camera has a pixel resolution of 181 pixels/mm. With the  $2\times$  lens, the camera should in principle be able to capture microbubbles of a diameter of roughly  $3 \mu m$  (1 pixel). Bubbles grown large enough to rise to the surface of the cover slip due to buoyancy were photographed by a CCD camera (Olympus DP70) through an upright microscope (Olympus BX51) with a 10× magnification. The size of the bubbles in the photos was analyzed using *ImageJ*. [9](#page-7-8)

# **IV. BUBBLE GENERATION**

## **A. Bubbles in water**

Microbubbles nucleate in the presence of impurities or pre-existing bubbles in water and oscillate in phase with the

<span id="page-4-0"></span>

FIG. 4. Pressure waveform measured by fiber optic hydrophone (a) and axial pressure distribution of the spherical and the flat transducer (b).

applied sonic wave. The bubbles expand during rarefactions (negative pressure) and contract during compressions (positive pressure). A typical still image with microbubbles in water is shown in Fig.  $5(a)$ . The image was taken at an f-stop of 90 with an exposure time of 40 ms and a synchronized flashlight. A stainless steel needle of thickness 190  $\mu$ m was included as a reference to infer the size of the bubbles. The radius of the bubbles captured in the images ranges from about 10  $\mu$ m to about 100  $\mu$ m. By analyzing a series of images taken at a rate of 24 frames/s, we observed that when the piezotransducer was initially turned on, bubbles generated in water were extremely small (only a few pixels in the images). However, more visible, larger bubbles quickly appear, either due to the direct growth of smaller bubbles or due to the collision and merging of multiple bubbles. Bubbles may also get close to each other and form bubble clusters instead of merging into one single bubble. A few examples of bubble clusters are shown in Fig. [5\(b\).](#page-4-1) Interestingly, no bubbles with a radius greater than 100  $\mu$ m were observed, which may indicate the upper limit of the bubble size in the present system.

#### **B. Bubbles on cover slip**

Bubbles that grow large enough rise to the surface of the cover slip and were monitored under the microscope with a  $10\times$  objective lens. Before each run, bubbles on the cover slip from the previous cycle were cleaned with *KimWipes*®

<span id="page-4-1"></span>

FIG. 5. A still image of the bubbles generated in water. A needle of 190  $\mu$ m is included as a reference to infer the size of the bubbles (a) and a few examples of bubble clusters (b). The images were taken with an exposure time of 40 ms with a synchronized flash light, at an f-stop of 90. Bubbles tend to form bubble clusters instead of merging into one single bubble.

delicate task wipers. Figure  $6(a)$  is a superimposed image of 17 images taken under the microscope at the same location of the cover slip. The field of view is roughly  $1.2 \times 1.2$  mm<sup>2</sup>.<br>A total of 237 bubbles were analyzed through *Image I*. The A total of 237 bubbles were analyzed through *ImageJ*. The relay control was set to 2 s on-time and the images were taken after a 3 s delay to allow enough time for the bubbles to rise to the surface of the cover slip. It should be noted that some small bubbles (single digit microns in diameter) do rise to the surface of the cover slip but collapse rather quickly before other bubbles settle on the cover slip. The histogram of the bubble radii is shown in Fig.  $6(b)$ . The radii distribute between 5 and 92  $\mu$ m, with the highest percent frequencies appearing between 10 and 30  $\mu$ m. Bubbles with larger radii appear less frequently. This result is consistent with the size of the bubbles observed in water and that reported in Ref. [5,](#page-7-4) where the microbubbles were produced by electrical discharges in water.

Bubbles of similar sizes may collapse at different speeds on the cover slip. We analyzed a series of 896 images of two bubbles captured in a time span of 60 s. Thirty images with the same time spacing were chosen and are illustrated in Fig. [7.](#page-5-1) The two bubbles are noted as bubble 1 and bubble 2, respectively, for easy reference. The initial radius of bubble 1 (14  $\mu$ m) is bigger than that of bubble 2 (12.5  $\mu$ m). This rather small difference in radii, however, leads to a 40% difference in the bubble volumes. The time evolution of the radii and the calculated volumes of the bubbles are plotted in Figs.  $8(a)$ and  $8(b)$ . The radius of bubble 2 decays much faster than that of bubble 1. The volumes of bubbles 1 and 2 decay steadily

<span id="page-5-0"></span>

FIG. 6. A superimposed image of 17 images taken at the same location of the cover slip (a) and the histogram of a total of 237 bubbles (b). The bubbles whose radii distribute between 10 and 30  $\mu$ m appear with the highest percent frequencies.

with time and can be described with a 3rd order polynomial function.

It is interesting to note that the smaller the bubble gets, the faster it collapses on the cover slip. Fig. [9](#page-6-0) plots the radial collapsing speed versus the radii of the bubbles. The radial

<span id="page-5-1"></span>

FIG. 7. Photo montage of two similar bubbles collapsing on the cover slip. The initial radius of bubble 1 (14  $\mu$ m) is bigger than that of bubble 2  $(12.5 \ \mu m).$ 

<span id="page-5-2"></span>

FIG. 8. The time dependence of the cross-sectional area of the bubbles in Fig. [7](#page-5-1) (a) and the time dependence of the radius of the cross-sectional circle (b). These results suggest bubbles of similar sizes collapse at different speeds on the cover slip.

collapsing speed of bubble 1 is similar to that of bubble 2 within its detected radius range (8-14  $\mu$ m), while bubble 2 collapses faster with the decrease of the bubble size.

This phenomenon has been consistently observed on many bubbles captured on the cover slip. We generally summarize our observations as follows: (1) All bubbles collapse on the cover slip given enough time. The smaller the bubble is, the faster it collapses. Bubbles with a radius of  $>12 \mu$ m take more than 60 s to collapse on the cover slip. (2) Bubbles with parting walls tend to coalesce and form bigger bubbles, which in turn collapse at their own pace. (3) Smaller bubbles may exist within larger bubbles given that they arrive at the cover slip first.

Furthermore, not all bubbles have the same wall thickness. Some larger bubbles with thick walls seem to have smaller bubbles embedded in or attached to their walls (shown as voids on the dark walls of bubbles in Fig. [10\)](#page-6-1). These bright spots are not all in perfect circular shape, which mean that they could also be the results of the collapse of smaller bubbles on the wall of bigger bubbles.

# **V. DISCUSSION**

A continuous sinusoidal ultrasonic wave was used in our system. In general, pre-existing gaseous impurities serve as

<span id="page-6-0"></span>

FIG. 9. Radial collapsing speed versus the radius of the cross-sectional circle. As the bubble becomes smaller, the faster it collapses.

the nuclei of bubble formation. During the negative portion of the pressure wave, water is pulled apart at the sites of impurity, forming acoustic microbubbles. During the positive portion of the pressure wave, these bubbles are compressed, albeit not completely. This process repeats in the following acoustic cycles, and the bubbles formed grow until reaching a critical size known as *resonance size*. Beyond this size, the bubbles either become unstable and collapse violently in a few cycles or oscillate near the resonance size for many more cycles. Bubbles may also become fragmented and reduce their sizes, which then serve as nuclei of new cavitation cycles. The relationship of the resonance radius of the bubble with the frequency  $f$  is given by Young<sup>[10](#page-7-9)</sup> as

$$
R_r = \sqrt{\frac{3\gamma p_{\infty}}{\rho \omega^2}},\tag{1}
$$

where  $\gamma$  is the specific heat ratio of the gas inside the bubble, which in our case is 1.33 for steam (water).<sup>[11](#page-7-10)</sup>  $p_{\infty}$  is the ambient liquid pressure, which is approximately considered as 1 atm.  $\rho$  is the liquid density, which is 999.97 kg/m<sup>3</sup> for water and  $\rho$  is the angular frequency of ultrasound, which in our case is ω is the angular frequency of ultrasound, which in our case is

<span id="page-6-1"></span>

FIG. 10. A photogram of bubbles captured on the cover slip with different bubble wall thicknesses and voids inside the bubbles.

 $2\pi f = 1.32 \times 10^7$  Hz. The resonance radius is then evaluated to be about 1.52  $\mu$ m. The resulting diameter of the bubbles is <sup>∼</sup><sup>3</sup> µm, which is beyond the detection range of our CCD camera.

However, two pathways may lead to further growth of these small bubbles beyond the resonance radius *R*r, namely, rectified diffusion and bubble coalescence. Rectified diffusion involves an unequal mass transfer across the bubble interface in the rarefaction and compression phases of the ultrasound wave. Eller and  $Flynn^{12}$  $Flynn^{12}$  $Flynn^{12}$  suggested that gas diffusion into/out of a bubble depends on the surface area of the bubble. A bubble has a larger surface area during its expansion than its compression, therefore leading to more gas diffused into than out of the bubble. They also suggested that the wall of bubble thins during its expansion, making it easier for gas to diffuse in. Bubble coalescence involves multiple bubbles coming into contact with each other and forming wall partitions. The parting walls rupture when they become sufficiently thin, leading to the formation of bigger bubbles.<sup>[13](#page-7-12)</sup>

Since our configuration has a glass wall directly facing the ultrasonic wave produced by the piezotransducer on the far end of the chamber, reflected wave interfere with incoming waves, leading to nodes and antinodes of standing waves. Microbubbles formed at the antinodes that grow to a radius  $>R_r$  will be pushed toward the nodes by primary Bjerknes forces (acoustic radiation forces on gas bubbles) $14$  and become "inactive bubbles" (bubbles that do not implode violently within the liquid near the site of creation). These bubbles eventually become large enough and rise to the surface of the cover slip due to buoyancy once the acoustic wave is interrupted. A more comprehensive discussion of bubbles in an acoustic field can be found in the overview by Ashokkumar and co-workers.<sup>[13](#page-7-12)</sup>

The radius of the bubbles that arrive at the cover slip is distributed between 5 and 92  $\mu$ m, and >50% of the bubbles have a radius between 10 and 30  $\mu$ m. These bubbles gradually collapse on the cover slip or merge to form larger bubbles. The smaller the bubble is, the faster the collapse is. We believe these bubbles are of similar properties as those produced by the electrical discharges in water as reported in Refs. [5](#page-7-4) and [7.](#page-7-6) The collapsing time of bubbles with initial radius of ~12  $\mu$ m is about 60 s. This long collapse time may be favored for biological studies where the observation of time evolution is of essence.

We purposely designed the chamber in such a way that the direction of the mechanical wave is perpendicular to the cover slip, so as to separate the effect of the pressure wave and the collapsing of bubbles on the cover slip during future biological experiments. The all-transparent chamber allows easy access to imaging of the microbubbles in water through the side walls or observation of the event on the cover slip under the microscope. On a cover-slip that has only monolayer cells, the dynamics of microbubble may differ from the tissue which has different surface properties. Thus, the findings of the results may not be directly translatable to tissues, and therefore, our setup may not be applied to tissue. But one can use 3-D artificial matrices to produce physiologically relevant multicellular structures on the cover slip and allow studies to be conducted closer towards actual tissues.

Finally, 2.1 MHz was chosen as the driving frequency of the piezotransducer in our experiments for three reasons: (1) ultrasound frequency at ∼20 kHz is known to generate large bubbles for mechanical shearing applications such as emulsification;  $15$  (2) frequencies between 100 kHz and 1 MHz are used to generate smaller bubbles for sonochemical purposes; $\frac{16}{16}$  $\frac{16}{16}$  $\frac{16}{16}$  and (3) ultrasound with a frequency above 1 MHz has weaker cavitation effects but is commonly used for medical and imaging purposes.<sup>[17](#page-7-16)</sup> By choosing a frequency of 2.1 MHz, we safely stay away from "power ultrasound," yet still produce microbubbles for biological studies.

# **VI. CONCLUSIONS**

An all-transparent chamber was designed to house a vertically arranged piezotransducer, which was driven by a RF voltage at 2.1 MHz directly in water to produce microbubbles for biological studies under a microscope. Bubbles of radius between 5 and 92  $\mu$ m were observed on the cover slip with the highest percent frequency between 10 and 30  $\mu$ m. These bubbles collapse with time: The smaller the bubble is, the faster it collapses. Bubbles of around  $12 \mu m$  in radius take about 60 s to collapse. The fact that these bubbles take longer to collapse may be beneficial to biological studies as the mechanical waves are clearly separated in time from the impact of microbubbles, allowing easy analysis.

#### **ACKNOWLEDGMENTS**

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