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Integrated Histotripsy and Bubble Coalescence Transducer for Rapid Tissue Ablation

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Abstract

Residual bubbles produced after collapse of a cavitation cloud provide cavitation nuclei for subsequent cavitation events, causing cavitation to occur repeatedly at the same discrete set of sites. This effect, referred to as cavitation memory, limits the efficiency of histotripsy soft tissue fractionation. Besides passively mitigating cavitation memory by using a low pulse repetition frequency (~ 1 Hz), an active strategy was developed by our group. In this strategy, low-amplitude ultrasound sequences were used to stimulate coalescence of residual bubbles. The goal of this work is to remove cavitation memory and achieve rapid, homogeneous lesion formation using a single phased array transducer. A 1 MHz integrated histotripsy and bubble coalescing (HBC) transducer system with a specialized electronic driving system was built in house. High amplitude ($P_- \geq 30$ MPa) histotripsy pulses and subsequent low amplitude (~ 1 -2 MPa) bubble coalescing (BC) sequences were applied to a red blood cell (RBC) tissue-mimicking phantom at a single focal site. Significant reduction of the cavitation memory effect and increase in the fractionation rate were observed by introducing BC sequence. Effects of BC pulsing parameters were further studied. The optimal BC parameters were then utilized to homogenize a 10×10 mm region at high rate.

Keywords

Acoustics; Histotripsy; Bubble coalescence; Cavitation memory effect; Transducers

I. Introduction

HISTOTRIPSY homogenizes soft tissue through the action of acoustic cavitation produced by short, highamplitude ultrasound pulses at low duty cycle ($\leq 1\%$) [1-3]. Recent studies have demonstrated that histotripsy has the potential to be used for a number of clinical applications including the treatment of prostate diseases, liver cancer ablation, kidney stone erosion, and thrombolysis [4-13].

During histotripsy treatment, cavitation bubbles are generated from endogenous nuclei in the target tissue. The rapid expansion and collapse of these bubbles impose high strain conditions on the target tissue. After collapse of a cavitation cloud, residual microbubbles may persist for up to seconds before dissolving completely. These residual microbubbles may then function as cavitation seeds for subsequent cavitation events in the same locations

[14, 15]. When subsequent pulses arrive before the residual cavitation nuclei dissolve, a cavitation cloud with a similar morphology as the previous cavitation cloud is generated by re-exciting the residual nuclei. Pre-focal cavitation or peripheral cavitation surrounding the focal center zone are also typically observed. The cavitation memory effect has been shown to significantly reduce treatment efficacy and may increase peripheral damage outside of the intended target [15, 16].

Wang *et al.* [15] demonstrated that the cavitation memory effect can be removed passively by increasing the pulse separation time. If a histotripsy pulse repetition frequency (PRF) of 1 Hz is used, the cavitation memory effect is approximate to the minimum. The per pulse damage efficiency is maximized and the lesion shape matches well with the intended focus. However, the overall treatment rate is very low.

In previous work by Duryea *et al.* [16, 17], the cavitation memory effect at high rate was mitigated by using 1000-cycle 1-MPa bursts transmitted by a secondary transducer confo-cal with the histotripsy therapy transducer. The mechanism responsible for BC was hypothesized to be the secondary Bjerknes forces associated with the low-amplitude bursts [18,19]. The residual nuclei were actively coalesced to a large bubble by the low-amplitude bursts, effectively reducing or removing the cavitation memory effect. This method allowed the high per-pulse efficiency associated with low pulse rates (1 Hz) to be maintained at high PRF (100 Hz) and produced homogeneous, reproducible lesion shapes.

This paper presents an integrated HBC transducer system to deliver both high amplitude ($P > = 30$ MPa) histotripsy pulses and low amplitude (~ 1 -2 MPa) BC sequences to achieve rapid, homogenous ablation. The integrated transducer design dispenses with the need for a second BC transducer and driving system, minimizing the aperture of histotripsy transducer, and allowing in-line ultrasound imaging for *in vivo* applications. To accomplish this, the BC sequence was generated by a rapid series of bursts from the array modules of the histotripsy therapy transducer. This study presents the design of the integrated HBC transducer system and explores the effects of BC pulsing parameters on treatment efficiency.

II. Materials and Methods

A. Integrated HBC Transducer System

The integrated transducer used to generate the HBC pulses is shown in Fig. 1(a). The transducer consisted of an array of 15 modules (2-cm diameter each) operating at 1 MHz made in house as previously described [20]. The overall aperture was 9 cm in diameter with a 6.5-cm focal distance. The emission of the 15 modules could be controlled at any permutation and combination. Moreover, the transmitted ultrasound intensity and time delay of each module on the integrated transducer array could be individually controlled by a field-programmable gate array (FPGA) and amplified by an amplifier constructed by our group.

For histotripsy pulses, all 15 modules were excited simultaneously. Histotripsy pulses at the geometric focus had a peak negative pressure (P_-) of 32 MPa. Pressure waveforms were directly measured at the focus (spatial maximum of the peak negative pressure) in degassed

water using a fiber-optic hydrophone as described previously [21]. P - greater than 20 MPa could not be directly measured due to instantaneous cavitation at the fiber tip and therefore was estimated by linear summation of the outputs from three separate clusters of transducer elements (five elements per cluster). The distribution of pressure field (Fig. 1c) under linear focusing and free-field condition was simulated using MATLAB (R2013b, MathWorks, Natick, MA, USA) and FOCUS software package [22, 23]. For BC pulses, each module was excited individually in sequence to produce a high PRF burst with peak amplitudes of 1, 1.5, or 2.2 MPa. The five elements in the inner ring of the transducer (Fig. 1a) were fired one by one at first. Then the ten elements in the outer ring were subsequently fired individually. The BC sequences were applied at a delay of 0.5 ms following the histotripsy pulse, which allowed the histotripsy bubble cloud to collapse and produce residual nuclei in an unperturbed manner [24]. The pressure amplitudes were measured at the fundamental frequency for individual modules directly at the geometric focus. The pressure fields of BC pulses were simulated assuming linear focusing conditions. The distribution of simulated pressure field of one BC pulse is displayed in Fig. 1d. Notably, the focus of histotripsy pulse with all elements fired together was much smaller spatially than the focus of an individual element. This more uniform field was hypothesized to be effective for promoting the secondary Bjerknes force to drive BC.

B. Tissue Phantom Preparation

An RBC tissue-mimicking phantom that allowed direct visualization of cavitation and resulting damage was used to study lesion development process and cavitation patterns [25]. Fresh bovine blood was obtained from a local abattoir (Dunbar Meats, Milan, MI, USA). An anticoagulant solution of citrate phosphate-dextrose (CPD) (C7165, Sigma-Aldrich, St. Louis, MO, USA) was added to the blood with a CPD-to-blood ratio of 1:9 (v:v), and kept at 4 °C before usage. Blood was used within two weeks after collection. The RBC tissue-mimicking phantoms were prepared with 1% agarose powder (DSA20070, DOT Scientific, Burton, MI, USA) and 5% v/v RBCs mixed in normal saline. This RBC tissue-mimicking phantom had a three-layer structure with one very thin ($\sim 500 \mu\text{m}$) RBC- agarose-saline hydrogel layer suspended between two thick (7 mm) transparent gel layers as previously described [26]. The RBC-agarose mixture changed from translucent red to transparent and colorless as a result of RBC lysis, providing real-time visual feedback for histotripsy lesion development [15]. The cavitation bubbles induced in the RBC phantom were easily detected because they blocked the light transmission and appeared as dark shadows on backlit optical images. During the treatment, both the cavitation bubbles and the lesion were imaged with a high-speed optical camera.

C. Experimental Setup

Before treatment, the RBC tissue-mimicking phantom was mounted to a three-axis mechanical positioning system and positioned above the transducer such that the RBC layer was oriented vertically within the water tank (i.e., the RBC layer was parallel to the transducer axis as shown in Fig. 2). Deionized water contained in the water tank was degassed to a desired level (22-25%) of oxygen saturation prior to the experiment at room temperature ($\sim 22^\circ\text{C}$). The focus of the transducer was aligned with the RBC layer in the phantom using the following approach. A bubble cloud was first generated in the water

using the histotripsy pulses. The location of the bubble cloud was indicated using two 1-mm-wide 5-mW laser beams perpendicular to the ultrasound beam and crossed at the middle of the bubble cloud. Then the phantom was placed in the tank with the two laser beams intersecting at the RBC layer.

During histotripsy treatment, histotripsy lesion development and cavitation bubble clouds in RBC tissue-mimicking phantoms were evaluated by optical images captured by a high-speed camera (Phantom 210, Vision Research, Wayne, NJ, USA). A 135 mm Tominon macro lens (Kyocera Optics, Nagano, Japan) and bellows were attached to the camera. The resolution of these captured images was approximately 17-18 μm per pixel. This image size ensured imaging of the overall bubble cloud and the entire lesion. Two images were acquired for each histotripsy pulse, each with an exposure time of 10 μs . The first image was taken 1 ms before histotripsy pulse firing to capture the RBC phantom without cavitation bubbles. This timing allowed for imaging of the lesions without interference from the bubbles. The second image was acquired at 45 μs after histotripsy pulse fired, which was empirically determined to capture the histotripsy bubble cloud near the point of maximum expansion. The RBC phantoms were back-lit with an in-house made LED strobe lamp, thus the captured optical images would appear as “shadow graphs” in which the cavitation bubble clouds appeared dark black, the RBCs appeared gray, and the histotripsy-induced lesions appeared white.

The histotripsy-induced lesion areas were significantly brighter than the intact areas (Fig. 3a), and could be detected by a threshold approach [27]. A pixel brightness threshold value for lesion was set at 5 standard deviations above the average pixel brightness in a 1×4-mm region within the intact background area. Pixels with brightness higher than this threshold were considered “damaged.” Using this threshold, the regions with brightness higher than the threshold were assigned “1 (white)” in the binary image and considered as “damaged”. In contrast, the regions with brightness less than the threshold were assigned “0 (black)” and considered as “intact” (Fig. 3b).

To study the lesion development and treatment efficiency, the lesion area was plotted as a function of the number of the histotripsy pulses applied. In addition, per-sequence acoustic energy efficiency was calculated as the lesion volume produced by one histotripsy pulse being divided by the total energy of one HBC sequence as shown in Eq. 1.

$$\text{Efficiency}_n = \frac{A_n - A_{n-1}}{E_{Hn} + \sum_i E_{BCn}(i)} \quad (1)$$

In Eq. 1, Efficiency_n is the acoustic energy efficiency of the n-th HBC sequence, A_n is the lesion area produced by n histotripsy pulses, $A_n - A_{n-1}$ is lesion area produced by the n-th histotripsy pulse, E_{Hn} is the acoustic energy applied at the -6 dB area of focal plane by the n-th histotripsy pulse, $E_{BCn}(i)$ is the acoustic energy applied at the focus by the i-th BC pulses following the n-th histotripsy pulse, $\sum_i E_{BCn}(i)$ is the acoustic energy applied by sequence of BC pulses following the n-th histotripsy pulse, and $E_{Hn} + \sum_i E_{BCn}(i)$ is the

total energy of the n -th HBC sequence. The energy of one pulse is defined as the product of pulse duration and integration of the standard spatial peak pulse average acoustic intensity over the histotripsy focal plane of -6 dB area.

The cavitation memory effect could be identified by the highly correlated bubble patterns generated by consecutive pulses. Cavitation bubbles appeared as dark shadows on the backlit images (Fig. 3c). Bubble cloud images were quantified in a similar way with lesion qualification, except that pixel brightness threshold was set at 5 standard deviations below the average pixel brightness in a 1×4 -mm region in the intact background area (light gray area on the images). The pixels with intensities lower than this threshold were considered in the areas of the cavitation bubbles. Using this threshold, the grayscale image was converted to a binary bubble image where 1 (white) represented the presence of bubbles and 0 (black) represented the absence of bubbles (Fig. 3d). The correlation coefficient between bubbles patterns generated by consecutive pulses was calculated using the cross correlation described in previous papers [15].

D. Experimental Design

The effects of parameters of BC pulses on damage development and cavitation memory effect were first studied at a single focal site. As frequency and number of cycles of one BC pulse had already been determined by the HBC system, the parameters of the BC sequence explored here were number of pulses, P -, and separation time between pulses. The optimal BC parameters with the highest damage efficiency were then extended to volume treatments (large lesion generation).

1) Parameter Study: To reduce cavitation memory effect and improve damage efficiency, the impact of BC-sequence parameters on lesion development and cavitation bubble patterns was evaluated. The numbers of pulses in the BC sequences were 15, 60, and 105. The upper limit 105 was set to keep the additional acoustic energy delivered by BC pulses to the target below 50% of the energy delivered by histotripsy pulses. Values of P - of BC pulses were varied from 1.0 to 2.2 MPa. The maximum 2.2 MPa was set to avoid damage to the transducer and excessive energy deposition at the focus. The time delay of one BC pulse following the previous BC pulse was defined as the pulse separation time. The separation time of BC pulses applied was 5, 25, 90, and 150 μ s.

2) Large Lesion Generation: The best BC parameters from the parameter study at the single focal site were then used to produce volume lesion in the RBC tissue-mimicking phantom. A 10×10 mm region was treated within the RBC layer. To follow a scan route shown in Fig. 4, the transducer was kept stationary while the tissue phantom was translated via a motorized positioning system through a series of 10-mm motion paths with 1.43-mm spacing between the paths. The RBC layer in the tissue phantom was aligned parallel to and intersected with the central axis of the transducer (Fig. 2) during the treatment, which kept the cavitation generated in the RBC layer. Histotripsy was applied continuously at a PRF of 100 Hz as the tissue phantom was mechanically scanned through this pattern (Fig. 4). The scanning speed of the positioning system was set to 1, 2, or 3 mm/s. 3 mm/s was the maximum motion speed that could be used without significant lag in the corners of the

trajectory. For each dose, six trials were conducted with and without the incorporation of BC sequences. The resulting images were processed to calculate the lesion area and treatment efficiency.

III. Results

A. Effects of BC Sequence

1) Lesion Development: Lesions generated in the RBC phantom using histotripsy pulses alone and with BC sequence are shown in Fig. 5a. 60 successive BC pulses of 2.2 MPa formed the BC sequence. Pulse separation time of the BC sequence was 90 μ s. 500 histotripsy pulses were applied to the RBC phantom. Lesion development (Fig. 5b) and per-sequence acoustic energy efficiency (Fig. 5c) were calculated as a function of the numbers of the histotripsy pulses.

Lesions generated by histotripsy pulses alone at 1 Hz PRF were used as a passive control, as previous studies showed 1 second time between histotripsy pulses was sufficient for the residual cavitation nuclei to passively dissolve [15]. The shape and location of lesions generated by the histotripsy pulses alone at 1 Hz PRF matched well with the shape of transducer's focal zone. The lesions had complete and uniform damage with smooth and sharp boundaries. However lesions had jagged boundaries and undamaged areas in the center (Fig. 5a) when applying histotripsy pulses alone at 100 Hz PRF. The center of mass of the lesions also had an average 0.80 mm shift towards the transducer compared to the center of mass of histotripsy lesions at 1 Hz PRF. Combining histotripsy pulses at 100 Hz PRF with the BC sequences, the shape and location of the lesion resembled the control lesions generated by histotripsy pulses alone at 1 Hz PRF. Lesions also had uniform damage with smooth boundaries. The lesion generated by the histotripsy pulses with BC had the largest area and highest per-sequence efficiency, while the lesion generated by histotripsy alone at 100 Hz PRF had the smallest area with the lowest per-sequence efficiency (Fig. 5b, c).

2) Cavitation Patterns: The cross-correlation coefficients of the cavitation patterns between consecutive pulses were calculated for histotripsy alone at 1 Hz PRF and 100 Hz PRF as well as histotripsy at 100 Hz PRF incorporating with BC pulses (Fig. 6). When applying histotripsy alone at 1 Hz PRF, cross-correlation coefficients of the bubble patterns were relatively low, indicating a low level of cavitation memory effect. Cross-correlation coefficients of the bubble patterns were much larger during histotripsy treatment at 100 Hz PRF, indicating a high level of cavitation memory effect. However, after combining histotripsy pulses at 100 Hz PRF with BC sequence, the cross-correlation coefficients of bubble patterns were reduced significantly, very close to those of histotripsy treatment alone at 1 Hz PRF.

B. Parameter Study

Effects of BC pulse parameters, including pulse number, P , and separation time between pulses, on lesion development and bubble patterns were studied. For each part of the parameter study, value of the studied parameter was varied but values for other parameters

were kept constant. The constant values of pulse number, P -, and separation time between pulses were 60, 2.2 MPa and 90 μs .

1) Pulse Number: To investigate the effect of number of pulses in BC sequence on efficacy of histotripsy therapy, the numbers of BC pulses following each histotripsy pulse were set as 15, 60, and 105 respectively. Lesion shapes resulting from 500-pulse histotripsy treatments are shown in Fig. 7. using 15 pulses, a calabash-shape lesion was produced, and prefocal damage was observed (Fig. 7a). using 60 pulses, the lesions had the cigar-shape with smooth boundaries. using 100 pulses, the lesions still had a cigar shape but with more jagged boundaries. The lesion area was also the largest when number of BC pulses was 60. (Fig. 7b). The per-sequence acoustic energy efficiency was the highest with 60 BC pulses (Fig. 7c). Meanwhile, the centers of mass of the lesion shapes moved away from the transducer with increasing of the pulse numbers. Compared to centers of mass of reference lesions produced by histotripsy alone at 1 Hz PRF, the mean centers of mass shifted -0.32 , 0.19 , and 0.36 m away from the histotripsy transducer respectively with 15, 60, and 105 BC bubble patterns were reduced significantly when the number pulses. Cross-correlation coefficients with different numbers of BC pulses was increased from 15 to 60, but were almost of BC pulses are shown in Fig. 7d. Correlation coefficients of the same when the number of BC pulses was increased from 60 to 105.

2) Peak Pressure: Lesion formations and correlation coefficients of bubble patterns under different P - of BC sequence are shown in Fig. 8. The values of P - were 1.0, 1.5, and 2.2 MPa. At 1 MPa, a calabash-shape lesion was produced, and prefocal damage was observed (Fig. 8a). At 1.5 and 2.2 MPa, the lesions had a cigar shape with smooth boundaries (Fig. 8a). With increasing P - of BC pulses, the lesion area increased (Fig. 8b) and the centers of mass shifted 0.68 , 0.41 , and -0.19 mm compared to the center of mass of 1 Hz reference lesions. The development of lesions (Fig. 8b) and the per-sequence acoustic energy efficiency increased (Fig. 8c) with increasing P -. In addition, cross-correlation coefficients of bubble patterns decreased with increasing P - of BC pulses (Fig. 8d), which indicated the reduction of cavitation memory effect.

3) Pulse Separation Time: The impact of pulse separation time on lesion formation and correlation of bubble patterns was studied (Fig. 9). The separation times of BC pulses were set to be 5, 25, 90, and 150 μs . With 5- μs and 25- μs pulse separation time, lesion shapes had a large head and a thin tail. With increasing pulse separation time, the lesion shapes extended to a cigar shape with smooth boundaries and center of mass of the lesions shifted in direction of ultrasound propagation (Fig. 9a). Lesion area as well as per-sequence efficiency increased by increasing pulse separation time in a range smaller than 90 μs but slightly dropped after increasing pulse separation time to 150 μs (Fig. 9b and 9c). Cavitation memory effect (cross-correlation coefficients of cavitation patterns) was reduced significantly when pulse separation time increased from 5 to 90 μs , while cross-correlation coefficients were very similar when pulse separation time increased from 90 to 150 μs (Fig. 9d).

C. Large Lesion Generation

The best parameters selected from the parameter study for the single point lesion were utilized to produce larger lesions. The BC sequence contained 60 pulses under P- of 2.2 MPa with 90 μ s pulse separation time. The transducer focus was scanned over a 10 $\times$ 10 mm target region at linear scan speeds of 1, 2, and 3 mm/s. Fig 10 shows the large lesions formed by histotripsy at PRF of 100 Hz without (Fig. 10a) and with (Fig. 10b) BC pulses. Using histotripsy only, sparsely damaged lesions were produced. The undamaged area within the 10 $\times$ 10 mm target zone increased with increasing scan speed, i.e., lower histotripsy dose or fewer total number of histotripsy pulses over the target area. The undamaged area within the 10 $\times$ 10 mm target zone was 5.7 ± 4.3 , 47.0 ± 10.7 , and 56.9 ± 16.5 mm² at linear scan speeds of 1, 2, and 3 mm/s, respectively (Fig 10a and 10c). With incorporation of the BC sequence, the undamaged area was 0.2 ± 0.1 , 1.6 ± 1.6 , and 2.0 ± 1.1 mm² at scan speeds of 1, 2, and 3 mm/s, respectively (Fig 10b and 10c). There was an obvious reduction of undamaged areas by using histotripsy pulses combined with the BC sequence in comparison to its histotripsy-only counterpart (t-test, $P < 0.02$).

IV. Discussion

This study demonstrates the feasibility of applying high amplitude histotripsy pulses interleaved with low amplitude BC sequences generated by a single array transducer with specialized electronic driving system. The lower amplitude BC sequences were produced by sequentially emitting pulses from each of the 15 elements in the histotripsy array. The focus of an individual element was much larger than the focus of histotripsy pulse. This more uniform field was hypothesized to be more effective for BC by applying the secondary Bjerknes force uniformly over all the histotripsy focus area. Therefore, this integrated HBC transducer system showed the abilities to significantly reduce the cavitation memory effect and increase the treatment efficiency.

After one histotripsy pulse, thousands of daughter bubbles were generated during the collapse of the cavitation bubble cloud. These daughter bubbles took one second or longer to completely dissolve, providing cavitation nuclei for subsequent histotripsy pulses in the same locations, termed as cavitation memory effect. As a result, sites where remnant nuclei persisted between pulses would experience the repetitive nucle- ation of cavitation activity and became over-treated, whereas those without remnant nuclei remained undertreated. Thus, the cross-correlation coefficients of cavitation patterns generated by consecutive histotripsy pulses were high. For example, the cross-correlation coefficients of cavitation patterns generated by histotripsy alone at 100 Hz PRF were much larger than the cross-correlation coefficients generated by histotripsy at 1 Hz PRF, which indicated a greater extent of the cavitation memory effect. In addition to the cavitation memory effect, attenuation of histotripsy waveforms was also hypothesized to contribute to the reduction of per-sequence efficiency based on recently studies [17, 24]. The presence of pre-focal residual cavitation bubbles persisting from pulse to pulse shielded and attenuated part of the subsequent histotripsy pulse, distorting the waveform and reducing its amplitude at focus. As a result pre-focal lesion surrounding the focal zone could always be generated during histotripsy treatment at high PRF. In general, the residual cavitation nuclei are the key factor

to both cavitation memory and attenuation effects. Besides residual cavitation nuclei, pre-focal endogenous cavitation nuclei in the targeted tissue may also attenuate part of the histotripsy pulse, resulting in the reduction of amplitude of histotripsy pulses at focus. According to Xu *et al.* [28], applying preconditioning pulse before histotripsy treatment could improve histotripsy treatment by removing endogenous cavitation nuclei.

By introducing BC pulses, a significant reduction of cavitation memory and attenuation effects was observed in this study. This effect could be attributed to the combination of the primary Bjerknes force and the secondary Bjerknes force, which were generated in the acoustic field of BC pulses. Previous studies showed that bubble coalescence by secondary Bjerknes forces seems to be the dominant factor [18]. The coalescence of residual cavitation nuclei significantly reduced the number of residual cavitation nuclei and thus removes the effects of cavitation memory and attenuation. By removing the cavitation memory effect, lesions were much more consistent, uniform, smooth, and displayed less peripheral damages (Fig. 5a). These advantages not only yielded better delimited lesion boundaries but also improved the reproducibility of lesion features. The per-sequence acoustic energy efficiency improved significantly as well. The improved predictability and treatment efficiency should benefit clinical applications in the future.

Lesions produced by histotripsy with BC were larger with a higher per-sequence efficiency compared to the lesions generated by histotripsy only at 1 Hz PRF, which provides sufficient time for the majority of the residual cavitation nuclei to passively dissolve. It is hypothesized that in addition to gathering the residual nuclei to larger bubbles, BC pulses redistributed the coalesced seeding nuclei. These redistributed nuclei may then augment cavitation and enhance the lesion generated by subsequent histotripsy pulses. Some of the nuclei may be transported to peripheral zone of original focus, enhancing the lesion generation in this zone and enlarging the lesion area. As a result, the lesion generated by the histotripsy pulses with BC had the largest area and highest per-sequence efficiency. When combining BC sequence with histotripsy, we hypothesize that the “right” level of consolidation and redistribution of cavitation nuclei are desired and result in uniform and smooth lesions with high per-sequence efficiency. The second hypothesized reason was that the residual bubbles produced by histotripsy pulses may also be stimulated by BC pulses and then contribute to the therapy effect.

BC pulse parameters had an important impact on the cavitation memory effect and lesion formation. In the previous work by Duryea *et al.*, the BC sequence was one low amplitude long pulse, which had up to thousands of cycles. The length of one pulse generated in the HBC sequence was just 1.5 cycles, which was determined by the HBC driving system. Therefore there was a need to study the parameters of BC sequence delivered by HBC system. First, the appropriate number of pulses in a BC sequence was optimized to reduce cavitation memory effect and redistribute the coalesced bubbles. Too few BC pulses were not sufficient to reduce cavitation memory effect, while too many pulses led to scarcely any bubbles left to act as cavitation nuclei to be redistributed sufficiently. second, increasing P- of BC pulse could promote removal of cavitation memory effect and lesion formation. It is hypothesized that there is a P- limit for BC. Based on previous studies both simulation results and experimental validations, secondary Bjerknes force is stronger with stable

oscillations. When amplitude of BC is too high the oscillation tends to be unstable and bubbles go through massive growth and collapse instead of coalescence [25]. Under the pressure of 2.2 MPa inertial cavitation may occur as well, but massive aggregation and subsequent coalescence of residual bubbles still took place rapidly. Third, lesion development is also affected by the pulse separation time of BC sequence. After BC pulses were applied to the target, the residual cavitation nuclei were reactivated. The residual bubbles oscillated and dissolved after the activation. The size distribution and number of residual cavitation bubbles changed with time. It is assumed that the shorter separation times may be too short for the bubbles to finish oscillation and return to their equilibrium size. Therefore, the pulse separation time of BC pulses became one of the important impactors for the per-sequence damage efficiency.

The per-sequence efficiency increased with increasing number of pulses at ~50 pulses and then reduced afterwards according to the curves of per-sequence efficiency. Thus, for treating a larger volume, it is hypothesized that distributing the histotripsy pulses evenly across the target region would be the most efficient approach. In a separate set of large volume generation experiments not included in this manuscript, the results showed it was more uniform and less untreated sites left in a constant scanning manner than a point-by-point scanning manner. During the constant scanning experiment, the focus scanned at 2 mm/s along the treatment path. In the point-by-point scanning experiment, 50 pulses were applied at one location. The focus was then moved with 1-mm interval to the next location along the treatment path. Consequently, in the large lesion study in this paper, the transducer focus was scanned at a constant speed over the targeted volume. In the volume treatment case of previous study, cavitation memory can reduce the damage efficiency as well, leaving structurally intact tissue persisting within the treatment volume [15, 29, 30]. In this study, similar results using histotripsy alone was observed in the volume treatment in RBC tissue-mimicking phantom. However, BC pulses combined with histotripsy showed the ability for faster homogenized fraction. Thus, the HBC system showed the potential usage in the improvement of histotripsy volume treatment.

V. Conclusions

An integrated HBC transducer system was designed and built to apply high-pressure histotripsy pulses interleaved with low pressure BC pulses using one single ultrasound transducer array. Cavitation memory caused cavitation to occur repeatedly at the same seeded locations which diminished treatment efficiency. The results showed that, using appropriate BC parameters, the integrated HBC system was able to increase the uniformity, consistency, speed, and efficacy of the lesion formation. This increase was achieved due to reduction of the cavitation memory effect and redistribution of the cavitation nuclei. The BC parameters, including the number of pulses, P-, and separation time between pulses in the BC sequence, had significant impact on reducing cavitation memory effect and redistributing cavitation nuclei, and thus lesion formation. The optimal BC sequence obtained in the study was formed by sixty 2.2-MPa pulses with pulse separation time of 90 μ s. Future work is needed to demonstrate the safety and efficacy of HBC system on *ex vivo* and *in vivo* tissues.

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Biography

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Jon Lundt is currently a Ph.D. candidate in the Department of Biomedical Engineering at the University of Michigan, Ann Arbor, MI. He received the B.S. degree in physics from the University of Washington in 2008 and the M.S. degree in Mechanical Engineering from Boston University in 2010 where he was a Dean's Fellow. From 2010 to 2014 he worked professionally as a mechanical engineer for a venture-backed startup and a medical device engineering consultancy, both in Seattle, WA. He is listed as sole or co-inventor on 7 issued and 12 pending patents. His research focuses on the development of histotripsy with electronic focal steering for the noninvasive ablation of large-volume tissue targets. He is the founding president of the IEEE-UFFC student chapter at the University of Michigan.

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Fig. 1.

HBC transducer (a) and general pulse scheme (b) used to study the integration of histotripsy and BC. Simulated pressure field for the histotripsy(c) is much smaller spatially than the field from a single transducer module (d). A train of pulses from alternate firing of the modules formed the BC sequence.

Fig. 2.
A diagram of experimental set up

Fig. 3.

Converting grayscale images ((a) and (c)) to binary images ((b) and (d)) to identify the lesions ((a) and (b)) and cavitation bubble clouds ((c) and (d)). The ultrasound propagation direction is from bottom to top of the image.

Fig. 4. Scan route in the RBC phantom for large lesion generation.

Fig. 5. Lesion patterns in the RBC phantom after treatment of 500 histotripsy pulses (a). (b) Lesion area and (c) per-sequence acoustic energy efficiency as a function of the number of histotripsy pulses applied. $N = 6$.

Fig. 6.
Cross-correlation coefficients of cavitation patterns at a function of histotripsy pulses applied ($N = 6$).

Fig. 7.

Effect of pulse number on lesion generation ($N = 6$). (a) Lesion patterns after treatment of 500 histotripsy pulses. (b) Lesion area, (c) per-sequence acoustic energy efficiency, and (d) cross-correlation coefficients of cavitation patterns as a function of the number of histotripsy pulses applied.

Fig. 8. Effect of peak pressure on lesion. Lesion patterns after treatment of 500 histotripsy pulses (a). (b) Lesion area, (c) per-sequence acoustic energy efficiency, and (d) cross-correlation coefficients of cavitation patterns as a function of the number of histotripsy pulses applied. $N = 6$.

Fig. 9.

Effect of pulse separation time on lesion. Lesion patterns after treatment of 500 histotripsy pulses (a). (b) Lesion area, (c) per-sequence acoustic energy efficiency, and (d) cross-correlation coefficients of cavitation patterns as a function of the number of histotripsy pulses applied. $N = 6$.

Fig. 10.

Representative volume treatments in the RBC phantom at scan speeds of 1 mm/s, 2 mm/s and 3 mm/s without ((a)) and with ((b)) BC. Quantized volume treatments (c). N = 6.