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Size Measurement of Tissue Debris Particles Generated from Pulsed Ultrasound Cavitation Therapy – Histotripsy

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Abstract

Extensive mechanical tissue fractionation can be achieved using successive high intensity ultrasound pulses (“histotripsy”). Histotripsy has many potential medical applications where non-invasive tissue removal is needed (e.g., tumor ablation). There is a concern that debris generated by histotripsy-induced tissue fractionation might be an embolization hazard. The aim of this study is to measure the size distribution of these tissue debris particles. Histotripsy pulses were produced by a 513-element 1MHz array transducer, an 18-element 750kHz array transducer, and a 788kHz single element transducer. Peak negative pressures of 11-25 MPa, pulse durations of 3 – 50 cycles, pulse repetition frequencies of 100 Hz – 2 kHz were used. Tissue debris particles created by histotripsy were collected and measured with a particle sizing system. In the resulting samples, debris < 6 μm in diameter constituted >99% of the total number of tissue particles. The largest particle generated by one of the parameter sets tested was 54 μm in diameter, which is smaller than the clinic filter size (100 μm) used to prevent embolization. The largest particles generated using other parameter sets were larger than 60 μm, but the value could not be specified using our current setup. Exposures with shorter pulses produced lower percentages of large tissue debris (>30 μm) in comparison to longer pulses. These results suggest that the tissue debris particle size distribution is adjustable by altering acoustic parameters if necessary.

Keywords

histotripsy; therapeutic ultrasound; cavitation; pulsed ultrasound; tissue fractionation; embolization

INTRODUCTION

Previous research has shown that tissue disruption can be generated by high intensity ultrasound induced cavitation (Fry 1970;Dunn and Fry 1971;Frizzell 1983;ter Haar 1986;Fowlkes 1991;Hynynen 1991;Chapelon 1992;Smith and Hynynen 1998;Tran 2003) and shockwave (Debus 1991;Coleman 1995). Our recent investigations have demonstrated that controlled tissue fractionation can be achieved using successive, short (≤50μs), high intensity

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ultrasound pulses delivered at low duty cycles (<5%) (Xu 2004;Parsons 2006a;Roberts 2006). This technique can be viewed as soft tissue lithotripsy, which we have therefore termed “histotripsy”. The acoustic pressures effective for histotripsy are similar to those used in lithotripter shockwave pulses. In comparison to the one cycle pulses commonly used in lithotripsy, the histotripsy pulses are several acoustic cycles in duration. In bulk tissue, histotripsy produces extensive fractionation of tissue structure, resulting in a cavity of liquid-like homogenate (Parsons 2006a;Roberts 2006). At a tissue-fluid interface, histotripsy produces tissue erosion or removal (Xu 2004). The boundaries of histotripsy lesions are very sharp with only a few cell widths between the fractionated cells and intact cells (Parsons 2006a).

The primary mechanism for histotripsy is thought to be acoustic cavitation. The initiation and maintenance of a dynamically changing cavitating bubble cloud (detected by optical and acoustic monitoring) is required to produce tissue fractionation (Xu 2005;Parsons 2007;Xu 2007a). We believe it is the energetic activity of cavitation bubbles that mechanically fragment and subdivide tissue. While histotripsy does cause temperature increases during the high amplitude histotripsy pulse, due to the very short pulse length and the low duty cycle (0.1% - 5%), the thermal effects induced by histotripsy are not significant (Kieran 2007).

Real-time imaging feedback for treatment monitoring and lesion assessment is essential for non-invasive and minimally invasive therapy but is not available for most current techniques. As cavitating bubbles are excellent sound reflectors and contrast agents for ultrasound imaging, these bubbles, in conjunction with ultrasound imaging, provide an inherent real-time imaging feedback for a histotripsy treatment (Hall and Cain 2005; Roberts 2006). Moreover, as cavitation mechanically fractionates tissue resulting in physical and molecular changes, both ultrasound and magnetic resonant imaging (MRI) can easily visualize the developing lesion effects (Hall 2007a; Hall 2007b). For example, a histotripsy lesion with sufficient tissue fractionation is identified as a hypoechoic zone or speckle amplitude reduction within the treated region on an ultrasound image (Hall 2007a). The speckle amplitude reduction may be due to extensive tissue fractionation resulting in a decreasing number and size of effective sound scatters.

With improved imaging feedback and treatment precision, histotripsy has many potential medical applications where non-invasive tissue removal is desired. We are presently investigating the potential of histotripsy to perforate the atrial septum (thin tissue between the two atria in the heart) in the treatment of a congenital heart disease called hypoplastic left heart syndrome (Xu 2004). In this application, there is a concern that the debris produced at the atrial septum and blood interface (i.e. tissue-fluid interface) might become embolization hazard and potentially occlude blood vessels. Therefore, it is essential to measure the size distribution of the debris particles created at a tissue-fluid interface using histotripsy. In comparison, in bulk tissue, histotripsy produces mechanical fractionation or homogenization of tissue structures (Parsons 2006a;Roberts 2006), which could be useful for tissue ablation to treat benign and malignant diseases (Roberts 2006;Lake 2008a;Lake 2008b), (e.g., tumor ablation). For bulk tissue homogenization, the fractionated tissue debris is contained in a sealed volume, and therefore unlikely to cause embolization. However, the size measurement of the tissue debris can reveal the degree of mechanical fractionation and the surviving cell fraction in a histotripsy lesion, which is critical for the cancer treatment.

This paper presents measurements of the size distribution of the tissue debris particles generated by histotripsy in two environments: at a tissue-fluid interface and inside bulk tissue. The histotripsy treatments were applied using four different sets of acoustic parameters and by three transducers with different frequencies and geometries. By using various acoustic parameters and transducers, we intend to cover an extended range of parameters effective for

histotripsy treatment. The chosen parameters have achieved effective and efficient tissue erosion and homogenization shown in our previous studies (Xu 2004; Hall 2007a; Kieran 2007; Xu 2007b; Lake 2008a; Lake 2008b). The size measurements of tissue debris generated by different histotripsy parameters and in different environments will help address potential clinical concerns for histotripsy applications.

MATERIALS AND METHODS

Tissue Preparation

Tissue erosion at a tissue-fluid interface was produced in porcine atrial walls *in vitro*. Bulk tissue homogenization was generated in porcine livers and kidneys *in vitro*. Fresh porcine atrial walls, livers, and kidneys were obtained from a local abattoir. Porcine atrial walls were preserved in a 0.9% saline at 4°C and used within 24 hrs of harvesting. Porcine atrial walls were submerged in room temperature (~22°C) saline for 1 hr prior to experimentation. During the experiment, atrial wall samples were clamped between two cylinder-shaped chambers filled with saline. Porcine livers and kidneys were preserved in 0.9% saline at room temperature and used within 6 hours of harvesting. Liver and kidney tissues were placed in degassed saline (Kaiser et al. 1996) for 30 minutes to purge surface bubbles prior to experimentation. During the experiment, liver and kidney tissues were sealed in a plastic bag filled with saline.

Ultrasound Generation and Histotripsy Acoustic Parameters

Three transducers were used for histotripsy treatments. A 788 kHz focused single-element transducer was used to generate erosion at a tissue-saline interface. A 1 MHz 513 element 2-D phased array transducer and a 750 kHz 18 element annular array transducer were used to produce homogenization inside bulk liver and kidney tissues, respectively.

The 788 kHz transducer (f-number = 1, Etalon Inc., Lebanon, IN, USA) has an 88 mm focal length and a 37 mm inner diameter hole for a monitoring transducer. The transducer was positioned by a 3-D positioning system (Model A-25, Velmex Inc., Bloomfield, NY, USA). Tissue erosion was produced using 3-cycle pulses and 6-cycle pulses delivered at a pulse repetition frequency (PRF) of 2 kHz.

The 1 MHz 513-element 2-D phased array and the 750 kHz 18-element annular array transducers were designed at the University of Michigan and built by Imasonic (Besançon, France). The 1 MHz array is a 150 mm diameter section of a spherical shell. A central 50 mm diameter hole allows for an inline imaging probe to target and monitor histotripsy treatments. The shell has a geometric radius of 150 mm (natural focal length). Histotripsy treatment of porcine liver tissue consisted of scanning the 1 MHz array focus electronically over a 9×9 (lateral) $\times$ 4 (axially) grid with 1 mm separating adjacent locations to define a $\sim 1 \text{ cm}^3$ volume in liver. In each location, one high amplitude (25 MPa peak rarefactional pressure), 50 cycle ultrasound pulse was applied before moving to the next location. Each location received 300 pulses over the entire treatment, and the delay time between pulses was 20 msec. This yielded an effective duty cycle of 0.25%, a PRF of 50 Hz, a 15 ms ultrasound on-time and a 6 sec treatment time per location, and a 4.86 sec total ultrasound on-time and a 32.4 min total treatment time for the $\sim 1 \text{ cm}^3$ volume.

The 750 kHz 18-element annular array is a 145 mm diameter piezocomposite spherical shell, with a geometric focal length of 100 mm. A central 68 mm diameter hole allows for an inline imaging probe. Homogenization of a $\sim 1 \text{ cm}^3$ volume in kidney cortical tissue was achieved by mechanically scanning a 3×3 (lateral) $\times$ 4 (axial) grid with 2 mm separating adjacent lateral locations and 3 mm separating adjacent axial locations. Mechanical scanning was achieved by moving the kidney using a 3-D motorized positioning system (Parker Hannifin, Rohnert Park,

CA, USA). All the array elements were excited in phase for kidney homogenization. Each location was treated by a total of 2000 high amplitude (22 MPa peak rarefactional pressure), 15 cycle pulses delivered at a PRF of 100 Hz. This resulted in a 0.2% duty cycle, a 40 msec ultrasound on-time and a 20 sec treatment time per location, and a 1.44 sec total ultrasound on-time and a 12 min total treatment time for the $\sim 1\text{cm}^3$ volume.

Both array driving systems were designed and constructed at the University of Michigan (Hall and Cain 2005). The array driving systems were maintained under PC control and consisted of channel-driving circuitry, associated power supplies (HP 6030A, Palo Alto, CA, USA), and a software platform to synthesize driving patterns. The design of arrays and driving systems are described in Hall and Cain's paper (2005).

The pressure waveforms at the focus of all three transducers in the acoustic field (Fig. 1) were measured in degassed water (i.e., free-field conditions) using a fiber optic probe hydrophone developed in-house (Parsons 2006b). The acoustic parameters and tissue types used for histotripsy treatments are listed in Table 1.

Histotripsy Treatment Monitoring using Acoustic and Imaging Feedback

In the histotripsy tissue erosion experiments, treatment targeting and monitoring was achieved using the A-line acoustic backscatter received by a single element focused monitoring transducer. The monitoring transducer ($f_c = 4.5$ MHz, 101 mm focal length, 25 mm diameter, Valpey Fisher, Hopkinton, MA, USA) was mounted confocally with the 788 kHz therapeutic transducer, fixed in its central hole. For targeting, the monitoring transducer passively listened to the acoustic backscatter of the therapy pulses (at lower amplitude) to localize the transducer focus on the tissue surface based on the time-of-flight (Fig. 2a). During the treatment, the monitoring transducer passively received the acoustic backscatter of the therapeutic pulses from the cavitating bubble cloud on the tissue surface, monitoring the erosion process in real time (Fig. 2b). High amplitude, temporally changing acoustic backscatter indicated that the erosion process was progressing normally (Xu 2005).

Histotripsy bulk tissue treatment was guided by B-mode ultrasound imaging. The inline ultrasound imaging was conducted by an ultrasound imaging probe fixed in the central hole of the therapeutic arrays. A commercially available 8 MHz linear array imaging probe (Elegra, Siemens Medical Systems, Inc., Issaquah, WA) was used with the 1 MHz 513-element therapeutic array. A 5 MHz imaging phased array (System FiVe, General Electrics, Milwaukee, WI) was used with the 750 kHz 18-element therapeutic array. For target localization, histotripsy pulses were first applied to a water bath, producing a hyperechoic zone on a B-mode ultrasound image that was marked as the transducer focus. The hyperechoic zone was the cavitation bubble cloud generated by histotripsy. When the liver or kidney tissue was introduced, the target tissue was aligned with the focus marker on the ultrasound image. A small number of histotripsy pulses producing no or minimal tissue damage were then applied to create a hyperechoic zone in the liver or kidney to verify the target location (Fig. 3 a and b). During the treatment, the active cavitation bubbles were moving and changing in size and density. This activity produced a temporally changing hyperechoic zone on an ultrasound image (Fig. 3c), which indicated that the treatment was progressing normally. Cavitation at every individual focal point was clearly visible on the ultrasound image (Fig. 3c). Real-time B-mode ultrasound imaging was only occasionally interfered by scan lines produced by large echoes from the therapeutic ultrasound field, as the histotripsy treatment was performed with low duty cycle. After treatment, the histotripsy lesion appeared as a hypoechoic zone (i.e. speckle amplitude reduction) within the treated region on the ultrasound image (Fig. 3 d-j). The size and shape of the hypoechoic zone matched well with the size and shape of the lesion evaluated by gross morphology and histology (Fig. 3 d-j).

Tissue Debris Particle Collection

To collect the erosion debris, a two-chamber tissue holder was designed and built. During the erosion treatment, the porcine atrial wall was clamped and sealed between two plastic cylinder-shaped chambers with a 40 mm inner diameter, 50 mm outer diameter and a 15 mm thickness. Thin (0.25mm) translucent polycarbonate films (McMaster, Cleveland, OH, USA) sealed the chambers and provided an acoustic window for sonication. Both chambers were filled with air-saturated saline (0.9%) at room temperature (~22°C). Debris generated during erosion was diluted in the saline and collected for size measurement within 2 hrs after the experiment. For each set of erosion parameters, four measurements were taken for each of three pieces of tissue. Three erosions were created on each piece of tissue.

For bulk tissue homogenization, treated liver and kidney tissues were dissected and homogenate debris inside the lesion was carefully removed and diluted in saline for size measurement. Four measurements were taken for each of three homogenized lesions in porcine liver and ten homogenized lesions in porcine kidney cortical tissue.

Tissue Debris Particle Size Measurement

We used a Coulter Counter (Multisizer 3, Beckman Coulter, CA USA) to measure the size distribution of tissue debris particles. The device measures the impedance change due to the displacement of the particle volume of a conducting liquid in which the particles are suspended. The impedance change is proportional to the particle volume. The volume of debris particle is calculated, and the particle size, measured by the diameter, is estimated assuming a spherical shape for each particle. The measurement size range is 2-60% of the aperture tube size which is part of the Coulter Counter. We combined results from 30 µm and 100 µm diameter aperture tubes to achieve a dynamic range of 0.6 – 60 µm in diameter. Debris larger than 60 µm would block the aperture tube and interrupt the measurement, and would be noted. The sizing resolution is approximately 1% of the particle diameter.

Number and volume percentage of tissue debris particles are used to summarize the Coulter Counter measurement. Equations (1) and (2) demonstrate the calculations of the number and volume percentage of tissue debris smaller than or equal to a certain diameter (Dt), respectively.

$$P_n(D \leq D_t) = \frac{\sum_{D_i=0.6}^{D_i=D_t} N_i}{\sum_{D_i=0.6}^{D_i=60} N_i} \times 100\% \quad (1)$$

$$P_v(D \leq D_t) = \frac{\sum_{D_i=0.6}^{D_i=D_t} N_i \times V_i}{\sum_{D_i=0.6}^{D_i=60} N_i \times V_i} \times 100\% \quad (2)$$

Where Pn and Pv are the number percentage and volume percentage, respectively, of the tissue debris particles smaller than or equal to a certain diameter (Dt) in relation to all the tissue debris particles measured by the Coulter counter. D is the tissue debris particle diameter. Ni is the number of particles with diameter between Di-1 and Di. Vi is the volume of particles with diameter between Di-1 and Di. Di, Ni and Vi are measurement outputs from the Coulter counter. Again, Di had a range from 0.6 µm to 60 µm due to the measurement limitation of the Coulter Counter.

Histological Evaluation and Transmission Electron Microscope (TEM) Examination

All treated tissue erosion samples and bulk tissue homogenization samples (two livers and two kidneys) were processed for histological evaluation and TEM examination. These tissue homogenization samples were not used for the debris measurements. The tissue samples were fixed in formalin for 1-2 weeks after treatment. Each fixed lesion was sliced into two sections. For histological evaluation, one lesion section was paraffin embedded, mounted and stained with Hematoxylin and Eosin (H&E). H&E slides were examined under microscope and representative sections through the center of the lesions were selected and imaged. For TEM evaluation, the other lesion slice was fixed in glutaraldehyde for 3-7 days. The tissues were then embedded in Epon polymer and 70 nm sub-sections were obtained with a diamond knife on a Reichert Jung ultramicrotome (Vienna, Austria), stained with uranyl acetate-lead citrate, and observed with a Philips CM-100 TEM (FEI instruments, Hillsborough, OR., USA) at a much higher magnification.

RESULTS

Tissue-fluid Interface - Erosion Debris Size Distribution

No visible debris particles were observed in any of the histotripsy-induced erosion debris samples. In a total of twelve Coulter Counter measurements of the erosion debris generated using 3-cycle pulses, the 100 μm tube was never blocked. This result indicates that all particles were smaller than 60 μm in diameter, which is the upper limit of detection for the Coulter Counter. Based on the measurements, the largest erosion particle produced by 3-cycle pulses was 54 μm . For the erosion debris generated by 6-cycle pulses, the 100 μm tube was blocked in one of twelve measurements (Table 2). This one blockage suggests that at least one debris particle or debris cluster larger than 60 μm was produced using 6-cycle pulses.

The Coulter Counter measurements of the histotripsy-produced tissue debris are summarized, respectively, as the number (Table 3) and volume (Table 4) percentages of the debris particles in four size bins (< 6 μm , 6-10 μm , 10-30 μm , 30-60 μm) over a range from 0.6 – 60 μm . For conciseness, we only report mean values of the measurements in the text. Corresponding standard deviations are listed in the tables. Size distribution histograms of the erosion debris are shown in Fig. 4. The results show that more than 99% of the total number of the tissue erosion debris particles was less than 6 μm in diameter, and 80% of the total volume of the tissue erosion debris was smaller than 12 μm . In comparison, a red blood cell is 6-8 μm in diameter. The number of particles larger than 6 μm consists of <0.2% of the total number of erosion debris particles. The number percentage decreased with increasing particle size. For example, the number percentage of particles of 6-10 μm , 10-30 μm , and 30-60 μm were 0.03%, 0.009%, and 0.0002%, respectively.

We also compared the erosion debris results to control samples, where the tissue was submerged in saline without ultrasound exposure for the same amount of time. The 100 μm tube was never blocked measuring the control samples, ruling out any particles larger than 60 μm . The number of particles smaller than 2 μm in the erosion sample was two orders of magnitude higher than that in the control sample (Fig. 5), supporting the contention that most small debris (< 2 μm) resulted from erosion. Particles larger than 10 μm were detected in both erosion and control sample measurements. The number of debris >10 μm from erosion was only slightly higher than the control sample (Fig. 5). This comparison suggests that the majority of particles larger than 10 μm may not be the result of the erosion process.

Bulk Tissue – Homogenization Debris Size Distribution

We also measured the homogenate debris produced by histotripsy bulk tissue treatment. The 100 μm tube blockage was observed in 3 of 40 kidney homogenate measurements and 1 of 12

liver homogenate measurements. These results suggest that particles or particle clusters larger than 60 μm were produced in both kidney and liver treatments. The measurements show that more than 99.6% of the total number of the tissue homogenization debris was less than 6 μm (Table 3), with 80% less than 2 μm . The size distribution histograms of the liver and kidney homogenization debris are shown in Fig. 6.

The number percentage of particles < 6 μm was similar in both the liver and kidney homogenization debris. However, particles < 6 μm constituted 80.24% of the total volume of liver homogenization debris but only 55.48% of the total volume of kidney homogenization debris (Table 4). This difference resulted from the difference in the number of particles between 10 μm and 60 μm , which was observed more often in kidney homogenization debris. Even though the number of these larger particles was very few (<0.01% of the total debris particle number), they still constituted a considerable percentage of the total volume due to the large volume of individual particles.

Debris Size vs. Pulse Duration

The largest debris particle size and the debris size distribution depended on applied pulse duration. The largest particle size was smaller with shorter pulses, e.g., 54 μm using 3 cycle pulses and > 60 μm using 6 cycle pulses. Shorter pulses also produced a lower percentage of large particles (>30 μm). Holding all other parameters constant, 3 cycle pulses generated 0.0002% of the total number of erosion debris particles between 30-60 μm , while 6 cycle pulses generated 0.007% (T-test $p < 0.001$). Moreover, shorter pulse duration produced higher percentages of small (< 6 μm) tissue debris particles. The number percentages of erosion debris particles < 6 μm were 99.96% and 99.82% using 3 and 6 cycle pulses, respectively (T-test $p < 0.001$). Correspondingly, the volume percentage of erosion debris particles < 6 μm was 81.89% using 3 cycle pulses and 68.11% using 6 cycle pulses (T-test $p < 0.001$).

Histology and TEM Evaluations

H&E and TEM slides of a histotripsy lesion in bulk tissue both show extensive fractionation of cellular structures in the lesion (Fig. 7). Within the treated region, no distinctive cellular structures remained. Most of the homogenized debris within the region was below 2 μm under TEM examination, which was consistent with the Coulter counter measurements. Tissue erosion debris was not present for H&E and TEM evaluations, because the erosion debris was removed from the tissue and dissolved into saline during the treatment.

The H&E and TEM slides also demonstrated sharp boundaries of histotripsy lesions both in bulk tissue and at a tissue-fluid interface (Fig. 7). Dissections of individual cells or functional units were observed at the lesion boundaries. In the H&E atrial wall slide, part of a myocyte was dissected at the erosion boundary, while the rest of the myocyte was still intact (Fig. 7b). The TEM kidney slide shows extensive cellular fractionation 3-5 μm from the boundary towards the treated region, while cellular structures remained intact 3-5 μm from the boundary towards the untreated zone (Fig. 7d).

DISCUSSION

Histotripsy produces tissue erosion at a tissue-fluid interface, breaking down tissue into small debris particles. As we are investigating histotripsy's effectiveness in perforating the atrial septum in the heart to treat hypoplastic left heart syndrome, there is a concern that the resulting erosion debris might cause an embolization hazard. As 100- μm mechanical filters have been successful at preventing embolization in catheter-based cardiovascular procedures, our current goal is to limit all the debris particles to below 100 μm in diameter. This paper measures the size distribution of the tissue debris particles generated by histotripsy using a Coulter counter.

Our measurement results suggest that the largest particle produced by histotripsy erosion was 54 μm using 3 cycle pulses and > 60 μm using 6 cycle pulses with the other acoustic parameters listed in Table 1. This result suggests that 3 cycle pulses may not produce hazardous emboli. We could not identify the largest particle size for 6 cycle pulses due to the limitation of our current setup. We plan to further evaluate the embolization risk from histotripsy-induced erosion debris by improving our setup for the *in vitro* experiments and measuring the debris sizes *in vivo* using clinic mechanical filters.

In bulk tissue, histotripsy produces mechanical fractionation of tissue structures, resulting in homogenized tissue debris within the treated volume. We also measured the tissue homogenization particle sizes using the Coulter Counter. The measurements demonstrate that more than 99% of the total number of homogenization debris particles is less than 6 μm . This result holds true for the liver volume homogenization as well as the kidney volume homogenization created using different frequency transducers, acoustic parameters and treatment scanning schemes. The small debris sizes agree with the histological and TEM evaluations of the homogenized tissue samples, showing histotripsy fractionates tissue and cell structures to sub-cellular levels, with almost no recognizable structures larger than 10 μm within the treatment region. However, the Coulter Counter measurements indicated that the largest debris particle was larger than 60 μm for both kidney and liver homogenate, and large particles (>30 μm) were detected at a discernable rate. It is possible that the large particles are clusters of multiple smaller sub-cellular structures.

We also found that the tissue debris size distribution depends on pulse duration. Shorter pulses produce a lower percentage of large particles (> 30 μm) and a higher percentage of small (< 6 μm) debris particles. This result is likely due to smaller cavitating bubbles generated by shorter pulses, which results in smaller tissue particles. This hypothesis is consistent with our high speed photography of the bubble cloud generated by histotripsy, which shows smaller bubbles produced by shorter pulses (Fig. 8). Based on this hypothesis, it may be possible to change the tissue debris size distribution by altering histotripsy acoustic parameters that can affect bubble sizes, including pulse duration and other pulse parameter such as pulse pressure and PRF (Xu 2007c; Xu 2008).

In this paper, tissue erosion and homogenization were generated by three transducers with different frequencies and geometries and using different acoustic parameter combinations. Our earlier studies show that the key to controlling the tissue fractionation process is to use specific pulse sequences informed by cavitation feedback monitoring. The histotripsy pulse sequences consist of successive high pressure ($P > 8 \text{ Mpa}$), short pulses (3-50 cycles) delivered at low duty cycles (<5%). This provides a wide range for parameter selection. The parameters used in this study were chosen because they achieved effective and efficient tissue erosion and homogenization and also cover an extended range of histotripsy parameters. As we hypothesize that acoustic parameters may affect cavitation bubble sizes and, in turn the debris sizes, our selected parameters should produce a spectrum of debris sizes that may be generated by histotripsy.

Moreover, the histological and TEM evaluations of histotripsy lesions show remarkably sharp boundaries, dissecting individual cells or functional units at the boundary. This narrow margin may allow histotripsy to improve the tissue ablation accuracy and reduce the collateral tissue damage in comparison to currently available tissue ablation techniques, including radiofrequency ablation, cryoablation, and ultrasound thermal therapy. The sharp margin of histotripsy lesions most likely results from a steep spatial threshold gradient. Our previous high speed imaging study of a cavitating bubble cloud suggested a lower cavitation intensity threshold at a tissue-fluid interface than inside bulk tissue (Xu 2007c). At a tissue-fluid interface, the erosion expands until it reaches a place where the pulse pressure is right below

the cavitation threshold. In bulk tissue, part of the tissue is first fractionated to very small debris particles to form a homogeneous liquid, resulting in a tissue-fluid interface. Then, this process becomes internal erosion in bulk tissue. Tissue homogenization can continue to expand to where the pulse pressure is just below the cavitation threshold at a tissue-fluid interface, resulting in a sharp boundary. In surrounding bulk tissue, the cavitation pressure threshold is much higher than that at a tissue-fluid interface. Therefore, the surrounding tissue is effectively isolated from the cavitation damage.

The small observed particle sizes are also consistent with the very sharp boundaries. The controlled cavitation of histotripsy results in many very small energetic microbubble “scalpels”, producing debris on the same size scale as the observed boundary between homogenized and normal tissue. This makes it easier to understand how boundary feature sizes an order of magnitude less than a wavelength of the incident ultrasound can be achieved.

SUMMARY

Histotripsy produces mechanical fractionation of tissue structure, which may be used for non-invasive tissue removal in many clinical applications. We measured the size distribution of fractionated tissue debris particles produced by histotripsy. Size measurement results show that >99% of the total number of tissue debris particles was smaller than 6 μm . The largest particle size was 54 μm for erosion debris generated by 3 cycle pulses and > 60 μm for homogenate debris and erosion debris generated by 6 cycle pulses. Histology and TEM evaluations demonstrated that histotripsy fractionated tissue into acellular debris, with no distinctive cellular structures remaining within the treated region. Further, the debris size distribution depends on histotripsy acoustic parameters. Erosion debris particles generated by shorter pulses consists of a lower percentage of large particles (>30 μm) than longer pulses. This result suggests that it is possible to adjust the tissue debris sizes by adjusting acoustic parameters if needed.

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REFERENCE LIST

- Chapelon JY, Margonari J, Vernier F, Gorry F, Ecochard R, Gelet A. In vivo effects of high-intensity ultrasound on prostatic adenocarcinoma Dunning R3327. *Cancer Research* 1992;52:6353–7. [PubMed: 1423282]
- Coleman AJ, Kodama T, Choi MJ, Adams T, Saunders JE. The cavitation threshold of human tissue exposed to 0.2-MHz pulsed ultrasound: preliminary measurements based on a study of clinical lithotripsy. *Ultrasound Med Biol* 1995;21:405–17. [PubMed: 7645132]
- Debus J, Peschke P, Hahn EW, Lorenz WJ, Lorenz A, Ifflaender H, Zabel HJ, Van Kaick G, Pfeiler M. Treatment of the Dunning prostate rat tumor R3327-AT1 with pulsed high energy ultrasound shock waves (PHEUS): growth delay and histomorphologic changes. *Journal of Urology* 1991;146:1143–6. [PubMed: 1895442]
- Dunn F, Fry FJ. Ultrasonic threshold dosages for the mammalian central nervous system. *IEEE Transactions on Biomedical Engineering* 1971;18:253–6. [PubMed: 4997992]
- Fowlkes JB, Carson PL, Chiang EH, Rubin JM. Acoustic generation of bubbles in excised canine urinary bladders. *J Acoust Soc Am* 1991;89:2740–44. [PubMed: 1918622]
- Frizzell LA, Lee CS, Aschenbach PD, Borrelli MJ, Morimoto RS, Dunn F. Involvement of ultrasonically induced cavitation in hind limb paralysis of the mouse neonate. *J Acoust Soc Am* 1983;74:1062–65. [PubMed: 6630721]

- Fry FJ, Kossoff G, Eggleton RC, Dunn F. Threshold ultrasound dosages for structural changes in the mammalian brain. *J Acoust Soc Am* 1970;48:1413–17. [PubMed: 5489906]
- Hall, TL.; Cain, CA. International symposium on therapeutic ultrasound. Boston, USA: AIP; 2005. A Low cost, compact, 512 channel therapeutic system for transcutaneous ultrasound surgery; p. 445–49.
- Hall TL, Fowlkes JB, Cain CA. A real-time measure of cavitation unduced tissue disruption by ultrasound imaging backscatter reduction. *IEEE Trans Ultrason Ferroelectr Freq Control* 2007a;54:569–75. [PubMed: 17375825]
- Hall TL, Lee GR, Hernandez L, Cain CA. Relaxation Properties of Cavitation Induced Tissue Lesions. Joint Annual Meeting ISMRM (International Society for Magnetic Resonance in Medicine)-ESMRMB (European Society for Magnetic Resonance in Medicine and Biology) 2007b:Poster 1118.
- Hynynen K. Threshold for thermally significant cavitation in dog's thigh muscle in vivo. *Ultrasound Med Biol* 1991;17:157–69. [PubMed: 2053212]
- Kieran K, Hall TL, Parsons JE, Wolf JS, Fowlkes JB, Cain CA, Roberts WW. Refining histotripsy: defining the parameter space for the creation of nonthermal lesions with high intensity, pulsed focused ultrasound of the in vitro kidney. *J Urol* 2007;178:672–6. [PubMed: 17574617]
- Lake AM, Hall TL, Kieran K, Fowlkes JB, Cain CA, Roberts WW. Histotripsy: Minimally Invasive Technology for Prostatic Tissue Ablation in an In. *Urology* 2008a;14:14.
- Lake AM, Xu Z, Wilkinson JE, Cain CA, Roberts WW. Renal Ablation by Histotripsy-Does it Spare the Collecting System? *J Urol* 2008b;179:1150–54. [PubMed: 18206166]
- Parsons JE, Cain CA, Abrams GD, Fowlkes JB. Pulsed cavitationultrasound therapy for controlled tissue homogenization. *Ultrasound Med Biol* 2006a;32:115–29. [PubMed: 16364803]
- Parsons JE, Cain CA, Abrams GD, Fowlkes JB. Spatial variability in acoustic backscatter as an indicator of tissue homogenate production in pulsed cavitationultrasound therapy. *IEEE Trans Ultrason Ferroelectr Freq Control* 2007;54:576–90. [PubMed: 17375826]
- Parsons JE, Cain CA, Fowlkes JB. Cost-effective assembly of a basic fiber-optic hydrophone for measurement of high-amplitude therapeutic ultrasound fields. *J Acoust Soc Am* 2006b;119:1432–40. [PubMed: 16583887]
- Roberts WW, Hall TJ, Ives K, Wolf JJS, Fowlkes JB, Cain CA. Pulsed cavitationultrasound : a noninvasive technology for controlled tissue ablation (histotripsy) in the rabbit kidney. *Journal of Urology* 2006;175:734–8. [PubMed: 16407041]
- Smith NB, Hynynen K. The feasibility of using focused ultrasound for transmyocardial revascularization. *Ultrasound Med Biol* 1998;24:1045–54. [PubMed: 9809638]
- ter Haar GR, Daniels S, Morton K. Evidence for acoustic cavitation in vivo: threshold for bubble formation with 0.75-MHz continuous-wave and pulsed beam. *IEEE Trans Ultrason Ferroelectr Freq Control* 1986;33:162–64. [PubMed: 18291766]
- Tran BC, Seo J, Hall TL, Fowlkes JB, Cain CA. Microbubble-enhanced cavitation for noninvasive ultrasound surgery. *IEEE Trans. Ultrason Ferroelectr Freq Control* 2003;50:1296–304.
- Xu Z, Fowlkes JB, Rothman ED, Levin AM, Cain CA. Controlled ultrasound tissue erosion: the role of dynamic interaction between insonation and microbubble activity. *J Acoust Soc Am* 2005;117:424–35. [PubMed: 15704435]
- Xu Z, Hall TL, Fowlkes JB, Cain CA. Optical and acoustic monitoring of bubble cloud dynamics at a tissue-fluid interface in ultrasound tissue erosion. *J Acoust Soc Am* 2007a;121:2421–30. [PubMed: 17471753]
- Xu Z, Ludomirsky A, Eun LY, Hall TL, Tran BC, Fowlkes JB, Cain CA. Controlled ultrasound tissue erosion. *IEEE Trans Ultrason Ferroelectr Freq Control* 2004;51:726–36. [PubMed: 15244286]
- Xu, Z.; Ludomirsky, A.; Fowlkes, JB.; Cain, CA. International Symposium on Therapeutic Ultrasound. Seoul, Korea: 2007b. In vivo atiral septum perforation using pulsed cavitation ultrasound therapy (histotripsy) for cardiac application; p. OS05_5
- Xu Z, Raghavan M, Hall TL, Chang C-W, Mycek M-A, Fowlkes JB, Cain CA. High Speed Imaging of Bubble Clouds Generated in Pulsed Ultrasound Cavitationul Therapy -Histotripsy. *IEEE Trans Ultrason Ferroelectr Freq Control* 2007c;54:2091–101. [PubMed: 18019247]
- Xu Z, Raghavan M, Hall TL, Mycek M-A, Fowlkes JB, Cain CA. Evolution of bubble clouds produced in pulsed cavitationultrasound therapy - histotripsy. *IEEE Trans Ultrason Ferroelectr Freq Control* 2008;55:1122–32. [PubMed: 18519220]

Fig. 1.

Acoustic pressure waveforms of histotripsy pulses measured by a fiber-optic probe hydrophone in degassed water. The histotripsy pulses were generated by the three transducers (labeled) used in this study. For (c), only the first 4 acoustic cycles of the 50-cycle pulse waveform generated by the 1 MHz therapeutic array are shown. The pressures of the third and fourth cycles should represent the pressures of the following cycles, as the ring-up time for this transducer to reach its stable output is three cycles.

Fig. 2.

A-line acoustic backscatter feedback of the histotripsy treatment at a tissue-fluid interface and gross morphology of tissue erosion produced using histotripsy. a) Acoustic backscatter from the target tissue (A) before erosion for targeting. B and C correspond to the front chamber and back chamber of the tissue holder, respectively. b) Acoustic backscatter from the cavitation bubble cloud (D) was high amplitude and temporally changing during the treatment, which was used for real-time treatment monitoring. c) Perforation of the atrial septum generated by tissue erosion using histotripsy.

Fig. 3.

Ultrasound imaging feedback of the histotripsy treatment in bulk tissue and gross morphology of tissue homogenization produced using histotripsy. a) Ultrasound B-mode image of *in vitro* porcine kidney before treatment. b) A bubble cloud was generated in the kidney (above “x”) using a small number of histotripsy pulses for target localization. c) Temporally changing hyperechoic zones showing the cavitation bubble cloud in the porcine liver tissue (outlined by dashed square) during the treatment. The ablation of the square volume was achieved using electronic scanning of the phased array focus. Each bright spot within the square was a group of bubbles at an individual focal point. d) Ultrasound B-mode image of the liver tissue before treatment. e) A square hypoechoic zone in the liver on B-mode image corresponding to a square histotripsy liver lesion. f) Gross morphology of the 1 cm square histotripsy liver lesion after being fixed in formalin for a week. h) A rectangular hypoechoic zone in the kidney on the B-mode image corresponded to a rectangular histotripsy kidney lesion. i) Gross morphology of a rectangular histotripsy kidney lesion. Liquid-like homogenate was apparent within the lesion and was of the same hue with the surrounding tissue. j) An empty lesion cavity remained after the homogenate was irrigated out.

Erosion Debris Size Histogram (3-cycle pulses)

Erosion Debris Size Histogram (6-cycle pulses)

Fig. 4.

Size distribution histograms of number and volume percentage of the debris particles generated from tissue erosion at a tissue-water interface by a 788 kHz single element transducer. The number and volume percentage of erosion debris were calculated by summing over all the erosion treatments using the same parameter set. Pulse duration of 3 cycles and 6 cycles were used. A total of 60 bins, with a bin size of 0.65 μm , were used for displaying all the histograms in this paper. All other acoustic parameters are listed in Table 1.

Fig. 5.

Representative histograms of the number of erosion debris particles (left) in comparison to number of particles in the control sample (right). The number of debris particles smaller than 2 μm in the erosion sample was two orders of magnitude higher than that in the control sample. The number of erosion debris particles larger than 10 μm was only slightly higher than the control sample.

Fig. 6.

Size distribution histogram of debris particles generated from tissue homogenization inside porcine liver (upper row) using a 1 MHz 513-element phased array and inside porcine kidney (lower row) using a 750 kHz 18-element annular array. The number and volume percentage of the liver and kidney homogenate debris were calculated by summing over all the liver and kidney treatments, respectively. Acoustic parameters are listed in Table 1.

Fig. 7.

Histological (H&E staining) and TEM views of tissue erosion and tissue homogenization generated by histotripsy. (a) H&E slide of tissue erosion generated in porcine atrial wall at a tissue-water interface. Ultrasound pulses were delivered from the top to the bottom. Tissue was physically removed within the treated region (T) and tissue structures outside the treated region (U) were intact. (b) Magnified view of the tissue erosion boundary from (a) shows bisected myocytes at the erosion boundary. (c) H&E slides of tissue homogenization in porcine kidney cortical tissue. Within the treated region (T), cells were fragmented to acellular debris with no distinct cellular features. Outside the treated region (U), cells were intact. (d) TEM slide (1100x) of the tissue homogenization boundary shows a sharp margin with only a few

microns between the homogenization zone and intact cells outside the zone. (e) H&E slide of homogenization boundary in the porcine liver tissue. (f) TEM slide of liver homogenate showing most homogenate debris smaller than 2 μm .

Fig. 8. Backlit images of bubbles (appear dark against the light background) generated at a tissue-water interface by (a) 3 cycle pulses and (b) 10 cycle pulses. Ultrasound pulses were propagated from the left to the right of the image.

Table 1

Tissue Types and Acoustic Parameters used in the Histotripsy Treatments

Treated Tissue	Treatment Condition	Transducer Frequency (MHz)	Pulse Duration (cycles)	P-/P+ (MPa)	I _{SPPA} (W/cm ²)	I _{SPTA} (W/cm ²)	PRF (kHz)	Duty Cycle	Total On-time (sec)	Total Time (min)
Atrial Wall	Tissue-fluid	0.79	3	11/36	9 k	67.5	2	0.75%	0.9 - 4.5	2-10
Atrial Wall	Tissue-fluid	0.79	6	11/36	9 k	135	2	1.5%	1.8 - 9	2-10
Liver	Bulk Tissue	1.00	50	25/190	47 k	117.5	0.5	0.25%	4.86	32.4
Kidney	Bulk Tissue	0.75	15	22/76	40 k	80	0.1	0.2%	1.44	12

Table 2

Number of Observed Blockages in Coulter Counter Measurement (in 100 μm tube, i.e., number with particles $> 60 \mu\text{m}$)

Tissue Debris Type	# Lesions	# Meas./Lesion	# Blockage/total Meas.
Erosion (3 cycles)	3	4	0/12
Erosion (6 cycles)	3	4	1/12
Homogenate (kidney)	10	4	3/40
Homogenate (liver)	3	4	1/12

Table 3Number Percentage of Histotripsy Generated Debris Particles (diameter range: 0.6 – 60 μm , mean $\pm$ SD)

Tissue Debris Type	Particle size range			
	$\leq 6 \mu\text{m}$	6 – 10 μm	10 – 30 μm	30 – 60 μm
Erosion (3 cycles)	99.96 $\pm$ 0.03%	0.03 $\pm$ 0.02%	0.009 $\pm$ 0.009%	1.7e-4 $\pm$ 1.5e-4%
Erosion (6 cycles)	99.82 $\pm$ 0.12%	0.15 $\pm$ 0.10%	0.03 $\pm$ 0.02%	0.007 $\pm$ 0.006%
Homogenate (kidney)	99.66 $\pm$ 0.10%	0.29 $\pm$ 0.08%	0.05 $\pm$ 0.03%	0.001 $\pm$ 0.001%
Homogenate (liver)	99.95 $\pm$ 0.01%	0.04 $\pm$ 0.006%	0.008 $\pm$ 0.003%	8.3e-5 $\pm$ 9.4e-5%

Table 4

Volume Percentage of Histotripsy Generated Debris Particles (diameter range 0.6 – 60 μm : mean $\pm$ SD)

Tissue Debris Type	Particle size range			
	$\leq 6 \mu\text{m}$	6 – 10 μm	10 – 30 μm	30 – 60 μm
Erosion (3 cycles)	81.89 $\pm$ 11.98%	5.40 $\pm$ 3.69%	9.62 $\pm$ 7.35%	3.01 $\pm$ 2.00%
Erosion (6 cycles)	68.11 $\pm$ 6.76%	10.51 $\pm$ 1.08%	14.24 $\pm$ 2.90%	7.13 $\pm$ 4.49%
Homogenate (kidney)	55.48 $\pm$ 8.56%	16.85 $\pm$ 2.23%	18.36 $\pm$ 5.55%	11.30 $\pm$ 7.66%
Homogenate (liver)	80.24 $\pm$ 4.71%	7.51 $\pm$ 1.13%	10.33 $\pm$ 4.09%	1.92 $\pm$ 1.86%