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**Effect of Pulse Duration and Pulse Repetition Frequency of Cavitation
Histotripsy on Erosion at the Surface of Soft Material**

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Abstract

Cavitation histotripsy with the short pulse duration (PD) but high pulse repetition frequency (PRF) disintegrates the tissue at a fluid interface. However, longer PD and lower PRF are used in the other focused ultrasound applications, where the acoustic radiation force, streaming, and cavitation are different, and their effects on erosion are unknown. In this study, the erosion at the surface of phantom/*ex vivo* tissue and the characteristics of induced bubble cloud captured by high-speed photography, passive cavitation detection, and light transmission during histotripsy exposure at varied PDs and PRFs but the same duty cycle were compared. The peak negative pressure of 6.6 MPa at the PD of 20 ms and PRF of 1 Hz began to erode the phantom, which becomes more significant with the increase of peak negative pressure, PD, and interval time between bursts. The increase of the PRF from 1 Hz to 1000 Hz, while the decrease of the PD from 20 ms to 20 μ s (duty cycle of 2%) at the same energy was delivered to the gel phantom immersed in the degassed water led to the decrease of erosion volume but a slight increase of the erosion area and smoother surface. Low PRF and long PD produce the significant tissue deformation, acoustic wave refocusing, confinement of bubbles in a conical region, and more bubble dissolution after the collapse for the high acoustic scattering and light transmission signals. In comparison, high PRF and low PD produce a wide distribution of bubbles with only little wave refocusing at the beginning of cavitation histotripsy and high inertial cavitation. Acoustic emission dose has a good correlation with the erosion volume. The erosion on the porcine kidney at the varied PRFs and PDs with the same energy output showed similar trends as those in the phantom but at a slow rate. In summary, the PRF and PD are important parameters for the cavitation histotripsy-induced erosion at the interface of fluid and soft material, and they should be optimized for the best outcome.

- 1 **Keywords:** cavitation histotripsy, focused ultrasound (FUS), erosion, bubble cavitation, surface
- 2 deformation, acoustic radiation force
- 3
- 4

1. Introduction

Focused ultrasound (FUS) has become more and more popular in clinical therapy, such as high-intensity focused ultrasound (HIFU) in the treatment of cancer and tumor by the temperature elevation-induced coagulation since the middle of the 1990s because of its advantages of noninvasiveness, possible operation in the doctor's office or outpatient clinic with no requirement of a sterile operating room, few complications, less in-hospital cost and risk of metastasis [1]. At such high intensity at the focus (i.e., $> 1000 \text{ W/cm}^2$), mechanical effects of the acoustic wave are significant. Nonlinear acoustic wave propagation results in the formation of a shock front around the focus which creates dramatic mechanical stress in the tissue. The large tensile wave can cause sporadic inertial cavitation or even a cloud of bubbles in the focal region, whose violent collapse produces the tissue destruction. In addition, the production of high order harmonics due to the significant waveform distortion, especially the shock front, enhances the heat deposition and subsequently, the rapid temperature elevation. Such effects could be enhanced by the interaction with bubbles to create the new FUS applications [2]. For example, cavitation histotripsy which used a large number of short (i.e., a few microseconds) but high peak negative pressure at certain pulse repetition frequency (PRF) could physically disintegrate the tissue [3]. To avoid potential cavitation damage to intervening tissue (i.e., vessel and nerve), the peak negative pressure was further increased beyond the intrinsic cavitation threshold (i.e., $> 30 \text{ MPa}$) using very short pulses (< 2 cycles), which was termed as microtriopsy [4]. Whereas the boiling histotripsy utilizes the boiling bubble induced by the shock front of the pressure profile in milliseconds to liquefy the tissue [5, 6]. Both cavitation and boiling histotripsy could be employed as a noninvasive treatment for many diseases, such as malignant tumors, benign prostatic hyperplasia, deep vein thrombosis, and hypoplastic left heart syndrome, with similar

effects achieved by different acoustic pulsing schemes. The liquefied content can be passed out of the natural body orifices or easily reabsorbed by the surrounding tissue [7-9].

The targets of histotripsy could be categorized into two types: inside the soft tissue (i.e., liver and kidney) [10, 11] and at the interface of soft tissue and fluid (i.e., prostate) [12]. In the tissue, the heat diffusion and convection (i.e., by the blood circulation) should be considered, especially for the boiling histotripsy if the interval time between ultrasound pulses is shorter than the thermal diffusion time. Otherwise, the thermal damage will also be produced. With the increase of pulse duration (PD) in boiling histotripsy, the type of emulsified tissue (void with no signs of thermal damage) will be changed to void filled with white paste and surround by the coagulated edge and then to a solid thermal lesion with an evaporated core [6]. In contrast, the application at the interface of soft tissue and fluid (i.e., urine and blood) is different. Because of the much lower acoustic absorption but more significant thermal diffusion of fluid, it's hard to produce the boiling bubbles in the fluid. Therefore, the bubbles at the interface of soft tissue and fluid are mostly induced by acoustic cavitation, and their sizes are smaller than those of the vapors in the tissue by boiling histotripsy. Using cavitation histotripsy, the bubble cloud can mechanically fractionate tissue in a controlled and predictable manner. In addition, the presence of bubbles and tissue disintegration can be visualized in real-time sonography as targeting feedback of the operation.

Erosion at the interface of soft tissue and fluid was found more efficient using certain parameters [3]. With the increase of PD from 3 cycles to 12 cycles, the tendency for the erosion becomes faster when decreasing the PRF from 19.6 kHz to 4.95 kHz. Although the axial erosion rate was slower, the threshold of erosion was found to be $I_{SPPA} \geq 4000 \text{ W/cm}^2$ (the peak negative pressure, $p^- = 8.3 \text{ MPa}$) at the driving frequency of 788 kHz, PD of 3 cycles, and PRF of 20 kHz

for perforation on porcine atrial wall [13]. The treated tissue is fragmented to the subcellular level and surrounded by an almost imperceptibly narrow margin of cellular injury (a few microns between the completely fractionated and intact cells). In most of the cavitation histotripsy experiments, the duty cycle is within the range from 0.1% to 5%, the PRF > 100 Hz, PD < 50 μ s, and p^- > 8 MPa. Furthermore, cavitation histotripsy at the driving frequency of 750 kHz, PD of 5 cycles, PRF of 1 kHz, p^- from 10 MPa to 24 MPa was applied to comminute urinary calculi and can produce only fine fragments as opposed to coarse ones by the conventional shockwave lithotripsy [14]. All natural and artificial stones exposed to ultrasound bursts at $p^- \geq 2.8$ MPa, frequency of 170 kHz, and PRF of 200 Hz were fragmented with a mean treatment duration of 36 s for the uric acid stones and 14.7 min for the cysteine stones [15].

The acoustic radiation force exerted on an absorbing target is due to the transfer of wave momentum by the beam [16], and its amplitude is proportional to the PD in the ultrasound exposure. In addition, the oscillatory force of bubble dynamics, shear stress related to the streaming of fluid around the bubbles, and the fluid velocity during streaming are dependent on the PD of ultrasound pulses. However, saturation on these mechanisms will occur for a longer pulse. It was found that sonication with very short pulses (PD of 10 μ s, PRF of 1 kHz, and duty cycle of 1%) produce the blood-brain barrier (BBB) disruption at the threshold of 6.3 MPa [17]. However, the 10 ms-pulses at the PRF of 1 Hz (duty cycle of 1%) and p^- of 1 MPa produced about 13% signal intensity change as extravasation of MRI contrast agent [18]. Therefore, the PD is also an important ultrasound parameter in producing the beneficial and effective bioeffects of FUS. However, the long PD of cavitation histotripsy has not been investigated.

In this study, the effects of PD and PRF of cavitation histotripsy pulses on the erosion of gel phantom and soft tissue at the fluid interface were evaluated in order to optimize the

operation parameters. The erosion area and volume produced by pulses at varied acoustic pressure, pulse duration, interval time with long PD but low PRF were investigated first. In addition, pulses with the same acoustic pressure and duty cycle (the same output power) but varied PRFs from 1 Hz to 1000 Hz and corresponding PD from 20 ms to 20 μ s were tested. The acoustic emission signals were measured using passive cavitation detection (PCD) to calculate both inertial cavitation-induced broadband noise and acoustic scattering during the exposure, and bubble dynamics in the focal region were captured by both high-speed photography and light transmission approach. It is found that significantly different characteristics of bubble cavitation and subsequently, the erosion efficiency, are present at the various PRFs and PDs. Acoustic emission dose has a good correlation with the erosion volume and can be used as a monitoring and evaluation technique.

2. Materials and Methods

2.1. Experiment setup

A HIFU transducer (H-102, outer diameter = 69.94 mm, inner diameter = 22.0 mm, $F = 62.64$ mm, $f_0 = 1.1$ MHz, Sonic Concepts, Woodinville, WA) was immersed in the degassed and deionized water ($O_2 < 4$ mg/L, $T = 25$ °C, measured by DO700, Extech Instrument, Waltham, MA) of a Lucite tank ($L \times W \times H = 70 \times 50 \times 30$ cm) and driven by sinusoidal bursts produced by a function generator (AF3021B, Tektronix, Beaverton, OR) together with a power amplifier (BT00250-AlphaA, Tomco Technologies, Adelaide, Australia). An acoustic absorber was placed on the opposite wall of the testing tank to prevent the ultrasound reflection. The transducer was attached to a three-axis positioning system (PT3/M, Thorlabs, Newton, NJ) to align its focus to the surface of the gel phantom (see Fig. 1). The alignment was made by obtaining the maximum echo signal using a pulser/receiver (5072PR, Olympus-IMS, Waltham, MA). A LabView program (National Instruments, Austin, TX) was written to control the ultrasound exposure.

2.2. Gel phantom

Alginate (Jeltrate, Dentsply International, York, PA) at the concentration of 5% was used as the gel phantoms because of its easy fabrication, low cost, biocompatibility, and close properties to those of soft biological tissue [19]. Alginate powder was weighed by a digital analytical balance (ML54, Mettler Toledo, Columbus, OH) and then mixed with deionized and degassed water and 0.3 ml of gas relief drop (Pedia Care, Tarrytown, NY), which can reduce the surface tension, by a blender (Libre, Sharp, Osaka, Japan) for 1 min. The liquid mixture of gel constituents was poured into a mold for gelation at room temperature and then degassed for about 1–2 min in a desiccant chamber (420100000, Scienceware, Pequannock, NJ) with a vacuum pump (VTE8, Thomas, Sheboygan, WI) at a pressure of 150 mbars.

2.3. *Erosion analysis*

After the histotripsy exposure, the gel phantom was recorded photographically by a digital camera (PowerShot SX230 HS, Canon, Tokyo, Japan) and then the area of erosion on the surface was quantitatively determined in digital image processing software (Photoshop, Adobe System, San Jose, CA). The erosion cavities were filled with BegoStone (Bego USA, Lincoln, RI) mixture with a powder to water ratio of 5:1 carefully without trapping bubbles inside. The gel phantom was then put into an incubator (Symphony, VWR, Radnor, PA) at the temperature of 37°C for about 1 hr for the solidification of BegoStone material. Solidified stones were taken out from the cavities, dried in the incubator for another 1 hr, weighted by the digital analytical balance, and then their volumes were determined using the measured density of BegoStone materials (about 2027 kg/m³) as shown in Fig. 2.

2.4. *Cavitation detection*

A focused ultrasound probe (A319S, $f = 15$ MHz, $D = 12.7$ mm, $F = 65$ mm, Olympus-IMS) was aligned confocally and coaxially with the HIFU transducer and worked as a PCD sensor. The whole acoustical emission signals of each exposure were recorded by a digital oscilloscope (Wavesurfer MXs-B, LeCroy, Chestnut Ridge, NY) at a sampling frequency of 50 MHz in the sequence acquisition mode and then transferred to a personal computer (PC) for data analysis using the established method [20]. Acquisition started 85 μ s (acoustic wave propagation time from the HIFU transducer to the focus and then back to the PCD transducer) after triggering the histotripsy exposure, and its duration was set exactly same as the pulse duration. Short-time Fourier transforms (STFT) were performed on the PCD data in Matlab (The Mathworks, Natick, MA) for the corresponding spectrogram, $F(t, f)$. Two specific frequency windows, 2.5–3 MHz (between 2nd and 3rd harmonics) and 3.6–4.1 MHz (between 3rd and 4th harmonics), was chosen

to evaluate the amount of inertial cavitation-induced broadband noise during the histotripsy exposure by calculating the temporal-average frequency-average amplitude

$$\bar{F} = \frac{\int_0^T \int_{f_1}^{f_2} F(t, f) df dt}{T \cdot (f_2 - f_1)} \quad (1)$$

where T is the duration time of each pulse, f_2 and f_1 are the upper and lower bounds of the integral, respectively. In the STFT spectra, few sub-harmonics ($f_0/2$) or ultra-harmonics ($nf_0/2$) were found (see Fig. 7a), which suggests that the stable cavitation does not dominate the histotripsy exposure and is similar to the findings in the previous study at the high peak negative acoustic pressure [21]. The fundamental and harmonics in the spectra are the scattering signals of the histotripsy pulses from the bubble in the focal region so that the amplitude of the 3rd harmonic frequency ($f_1 = 3.2$ MHz and $f_2 = 3.4$ MHz) was used to quantify the scattering effect. Variations of the peak-to-peak PCD signals present the amplitudes of bubble cavitation during ultrasound exposure [22]. Thus, the acoustic emission dose is calculated as

$$\int [A(t)]^2 dt \quad (2)$$

where $A(t)$ is the PCD data, and the integration is through the whole histotripsy exposure.

2.5. High-speed photography

In order to observe the deformation and erosion of phantom surface, optically transparent polyacrylamide gel phantoms was prepared. The mixture of 40% w/v acrylamide solution (ICN Biomedicals, Aurora, OH) with a 19:1 ratio of acrylamide:bis and 10% w/v ammonium persulfate solution (Sigma-Aldrich, St. Louis, MO) was degassed for about 1 hr in a desiccant chamber with a vacuum pump at a pressure of 150 mbars, then poured into a mold for polymerization at room temperature within 1 min of adding N,N,N',N'-tetramethylethylene/diamine (Sigma-Aldrich). Surface of the phantom was aligned to the focal plane

of the HIFU transducer before each experiment. The bubble dynamics induced by the histotripsy pulses at different PRFs and PDs were captured by a high-speed camera (fastcam SA5, Photron, Tokyo, Japan) which was triggered by a transistor-transistor logic (TTL) pulse from a digital delay generator (DG535, Stanford Research Systems Inc, Sunnyvale, CA) to synchronize the photography with histotripsy exposure [23]. By adjusting the delay time, a series of high-speed photographs could be recorded after delivering a certain number of histotripsy pulses. The frame rate of 5000 frames/s at a resolution of 512×320 pixels and shutter time of 1 μs was applied. The extracted images were further analyzed in ImageJ (National Institute of Health, Bethesda, MD). The distance between the phantom interface and tip of wave refocusing, D , and the distance between the bottom of deformed phantom and tip of wave refocusing, F , were determined (see Fig. 3). In addition, a parabolic curve was fitted to the deformed phantom surface by a custom-written code. The focal length, F' , was calculated as

$$F' = R^2 / 4d \quad (3)$$

where R and d are the radius of the rim and the depth of the dish, respectively.

2.6. Light transmission

In order to observe the bubble formation around the surface of the alginate phantom induced by the histotripsy exposure, light transmission was applied [24, 25]. An illumination Helium-Neon laser light (1507-2, $\lambda = 632.8$ nm, 0.95 mW, JDSU, Milpitas, CA) was expanded by a combination of lenses ($F_1 = -12$ mm, $F_2 = 60$ mm, Thorlabs) to a parallel beam in a diameter of 15 mm, transmitted through the focal region, and subsequently focused onto a fast photo-detector (30 dB gain, 3.6 mm sensor size, bandwidth from 0 Hz to 10 MHz, PDA36A-EC, Thorlabs) through a concave lens ($F_3 = 150$ mm, Thorlabs) as shown in Fig. 1. Light transmission signals were recorded by the digital oscilloscope at the sampling rate of 50 MHz

1 and then transferred to the PC for further data processing in Matlab. The recorded signals were
2 first gone through a median filter to reduce the background noise, inverted for easy reading, and
3 then the average signals of every 20 μ s (pulse duration at the PRF of 1000 Hz and duty cycle of
4 2%) were used to represent the down-sampled data. The integration of their absolute values was
5 used for quantitative comparison between different experimental settings.

6 2.7. *Ex Vivo* study

7 The fresh porcine kidney was purchased from a local slaughterhouse (Primary Industries
8 Pte Ltd, Singapore) with the approval of Agri-Food & Veterinary Authority, Singapore and
9 immersed in phosphate-buffered saline (PBS) solution, degassed for at least 30 minutes, and
10 used within 4 hours of harvest. Kidney sample was fixed in a custom-built holder, and ultrasound
11 focus was aligned to its surface. The operation parameters were same as those in the phantom
12 study except elongating the exposure time from 20 s to 200 s. After the exposure, erosion of
13 porcine kidney samples was quantified using the same protocols for the alginate phantom.

14 2.8. *Statistical analysis*

15 To determine the statistical difference between the test groups, one-way Analysis of
16 variance (ANOVA) and then Tukey's multiple comparison tests were performed in SigmaPlot
17 (Systat Software, San Jose, CA). The level of statistical significance was fixed at $p < 0.05$. At
18 least five samples were used in each experimental setting.

3. Results

3.1. Histotripsy-induced erosion

The HIFU transducer has -6 dB beam size of 12×1.7 mm (axial×lateral) as measured by a needle hydrophone (HNA-0400, Onda, Sunnyvale, CA) using the established protocol [26]. The peak positive and negative pressures and acoustic intensities at the focus are listed in Table 1, and the typical waveform could be found in [27]. The rigidity of alginate and polyacrylamide gel phantoms was measured by an electromechanical system (5566, Instron, Norwood, MA), and the mechanical properties of gel phantoms and biological tissues [28, 29] are listed in Table 2 for comparison. The effects of ultrasound parameters of 20 histotripsy pulses with the PD in the order of ms on the erosion were investigated (see Fig. 4). First, if the peak negative pressure, $|p^-|$, is 2.9 MPa at the PRF of 1 Hz and PD of 20 ms, no erosion was found. The surface area and volume of erosion increased from 0.05 ± 0.01 cm² to 0.32 ± 0.07 cm² and from 0.02 ± 0.008 cm³ to 0.1 ± 0.02 cm³ with the increase of peak negative pressure from 6.6 MPa to 9.9 MPa, respectively. The peak negative acoustic pressure of 9.1 MPa was selected in the following comparison because of the much stable performance of the HIFU transducer. At the PRF of 1 Hz, the erosion area quickly reached the saturation at the PD of 10 ms (0.23 ± 0.02 cm²), and the volumes increased almost linearly with the PD from 5 ms to 50 ms. It is also found that the interval time had little effect on the erosion area, but longer interval time (i.e., 980 ms) would increase the erosion volume slightly, which may be due to the bubble dissolution and subsequently, the reduced bubble memory effect [30].

If keeping the output energy the same, for example, the same $|p^-|$, duty cycle, and exposure time (9.1 MPa, 2%, and 20 s, respectively), varying the PRFs and PDs would lead to a significant difference in the erosion area and volumes (see Figs. 2 and 5). Increasing PRF from 1

Hz to 10 Hz while decreasing PD from 20 ms to 2 ms resulted in similar erosion area (0.2 ± 0.01 vs. $0.21 \pm 0.02 \text{ cm}^2$, $p > 0.05$) but decreased erosion volume (0.11 ± 0.03 vs. $0.06 \pm 0.01 \text{ cm}^3$, $p < 0.05$). Further increasing PRF to 100 Hz while decreasing PD to 200 μs resulted in smooth erosion boundaries both on the surface and at the shallower bottom (see Fig. 2), larger erosion area ($0.24 \pm 0.01 \text{ cm}^2$) but smaller volume ($0.05 \pm 0.004 \text{ cm}^3$). PRF of 1000 Hz and PD of 20 μs had similar erosion volume but a smaller area than that at the PRF of 100 Hz and PD of 200 μs . Similar trends and conclusions were also found at the $|p^-|$ of 9.9 MPa (data not included). Therefore, it suggests that delivered acoustic energy and $|p^-|$ may not be the only contributing factors for the soft tissue lysis.

3.2. Bubble cavitation

In order to understand the role of bubble cavitation, bubble activities were monitored by PCD during the histotripsy exposure (20 s at the duty cycle of 2% so that the effective exposure duration is 0.4 s) and then processed using STFT (see Figs. 6 and 7). **Presentative variations of peak-to-peak and room-mean-square (RMS) for every 20-40 μs during the histotripsy exposure at different PRFs are shown in Fig. 6a. It is found that the variations of PCD signals are much less at high PRF.** Then the maximum and average peak-to-peak and RMS amplitudes **of PCD signal for each pulse** were calculated to evaluate the stability of **histotripsy** exposure. At the low PRF and long PD (i.e., 1 Hz and 20 ms) PCD signals had a large variation, which is illustrated by the great differences between the maximum and average peak-to-peak acoustic emission (i.e., 12.7 and 6.8 mV at the PRF of 1 Hz and 1000 Hz, respectively, see Fig. 6b).

Spectrograms of PCD signals show that with the increase of PRF there are much stronger and denser white noises, corresponding to the violent bubble collapse (inertial cavitation). Although some presented between harmonics at the low PRF and long PD (i.e., 1 Hz and 20 ms

in Fig. 7a), their extension toward very high frequency (i.e., > 4 MHz) was highly attenuated through the bubbly focal region, which is similar to our previous study of HIFU-induced both thermal necrosis and mechanical erosion in the gel phantom [20]. The distribution of acoustic scattering during histotripsy exposure had a peak around 10 s except for more stable distribution at the PRF of 1000 Hz and PD of 20 μ s (see Fig. 7b). Such a peak distribution may be due to the presence of bubble nuclei for the subsequent histotripsy pulse after the cavitation-induced by the previous pulse and then their accumulation in the prefocal region as the bubble shielding effect [24, 31]. The PRF of 1 Hz and PD of 20 ms had stronger acoustic scattering than the others despite higher variations and similar levels at the end of histotripsy exposure. In comparison, broadband noise between the 2nd and 3rd harmonics at varied PRFs and PDs was similar (see Fig. 7c). However, that between the 3rd and 4th harmonics was quite different. High PRF and short PD had high inertial cavitation-induced broadband noise at the high frequency than those at the low PRFs and long PDs, which correlates well with our observations of the wide distribution of strong white noise in the spectrograms, and their variations were smaller than those at the low frequency. One of the reasons may be the more attenuation of the high-frequency component through the region with bubble confinement in a conical shape by the wave refocusing (see high-speed photography later). Another reason may be the phantom surface deformation by the acoustic radiation force. Although the -6 dB beam size of PCD transducer is about 19.4 $\times$ 0.5 mm (axial $\times$ lateral), the echoes or collapses occurs away from the focus of PCD transducer will have the reduced receiving sensitivity.

In addition, the acoustic emission dose during the ultrasound exposure using Eq. (2) at all experimental settings (data in Figs. 4 and 5) was found to have a good correlation with the erosion volume generated in this study ($R^2 = 0.904$) as shown in Fig. 8. If the erosion volume is

small (i.e., $< 0.02 \text{ cm}^3$), the prediction error using the regression line is high ($>100\%$). Otherwise, the average and standard deviation of the prediction error are -1.8% and 24.2% , respectively. It suggests that PCD measurement is possible to estimate the erosion outcome in real time for the future *in vivo* study where optical monitoring is infeasible.

3.3. High-speed photography

Bubble cloud on the surface of polyacrylamide phantom at the PRF up to 100 Hz was captured by the high-speed photography (see Fig. 9). It is found that significant differences were found at varied PRFs and PDs. At the PRF of 1 Hz and PD of 20 ms, the acoustic radiation force generated from the histotripsy pulses caused the deformation of the phantom surface in a parabolic shape. Some bubbles may also be pushed and attached to the deformed surface, which will work as an effectively acoustic reflector. As a result, the following ultrasonic wave can be refocused and the induced bubbles will be concentrated in a conical region. With the ongoing of histotripsy exposure, the deformation became wider and deeper, and the acoustic wave refocusing gradually elongated toward the transducer. Permanent damage to the gel phantom may be produced due to the interaction of acoustic pressure and cavitation bubbles as shown by the arrow-head in Fig. 9a. After the termination of histotripsy exposure, wave refocusing would disappear immediately, but the surface deformation would take a while to recover due to the slow response of elastic gel phantom in the relaxation. In comparison, a large area of bubbles was generated by the histotripsy pulses at the PRF of 10 Hz and PD of 2 ms (see Fig. 9b), and less significant wave refocusing was observed (except that at 0.2 ms). More bubble clouds show up immediately after the histotripsy exposure (i.e., $t = 0.2 \text{ ms}$ and 0.6 ms), which suggests the incomplete bubble dissolution between bursts. Bubble cloud would gradually move toward the surface of gel phantom, and its collapse at the termination of ultrasound exposure would produce

1 the significant surface deformation, but with little damage to the phantom. Because of the limited
2 frame rate (5000 frames/s), the motion of bubble cloud during histotripsy exposure could not be
3 observed clearly at the PRF of 100 Hz and PD of 200 μ s. However, it is found that bubbles were
4 produced more widely on the phantom surface, and the damage gradually became large (see Fig.
5 9c). No bubble coalescence into a large single one, strong bubble collapse, and generation of
6 high-speed microjet were not found, which may be due to the low frame rate, low optical
7 magnification, and long shutter time of photography.

8 The formation of wave refocusing is found to be affected significantly by the bubble
9 cloud in the acoustic field. Two typical cases are shown in Fig. 10. If bubble nuclei don't
10 completely dissolve between the pulses, they will expand immediately after the histotripsy
11 exposure and move towards the phantom surface. The wave refocusing was not formed until the
12 disappearance of the bubble cloud in the focal region of the HIFU transducer or attachment to the
13 deformed surface (see Fig. 10a). In addition, if the residual bubbles move toward the gel
14 phantom and interact with the wave refocusing, strong acoustic scattering will result in the
15 disappearance of the conical bubble cloud (see Fig. 10b). So the cause of the few and short
16 refocusing tips formed at the PRF of 10 Hz and PD of 2 ms may be the incomplete bubble
17 dissolution in the prefocal region during the burst interval. The tip of wave refocusing extends
18 toward the transducer immediately after the histotripsy exposure and is about 2.7 mm from the
19 phantom surface at 2.8 ms (see Fig. 11a). After that, the tip will not extend further, but oscillate
20 locally, which may be due to the presence of residual bubbles in the focal region of the HIFU
21 transducer as shown before. The deformed surface could be fitted in a parabolic shape, and the
22 focal length was calculated by Eq. (3) and found to correlate quite well with the measurement
23 results ($R^2 = 0.788$ see Fig. 11b), which may confirm our hypothesis of wave refocusing.

3.4. Light transmission

In order to capture the bubble cloud in the acoustic field at a much higher frequency, light beam transmission through the focal region of the HIFU transducer was measured by the photo-detector. Briefly, the low-frequency (i.e., < 100 Hz) increase in the light transmission signals means the larger bubble area or higher bubble density whereas the high-frequency oscillations (i.e., >10 kHz) mean the inertial bubble collapse. It is found that the low PRF and long PD produce high light transmission amplitudes (see Fig. 12a). Negative and positive spikes may be due to sonoluminescence induced by the inertial cavitation and bubble collapse, respectively. Deformed phantom surface becomes darker in the high-speed photography (see Fig. 9a) and could also contribute to the light transmission signal. Although consistent bubble collapses were found at the high PRF and short PD (i.e., 1000 Hz and 20 μ s), their amplitudes gradually decreased with the ongoing of histotripsy exposure, which may be due to the of bubble nuclei depletion. Integration of light transmission signals at the PRF of 1 Hz and PD of 20 ms (0.198 ± 0.088 mV·s) is significantly larger than the other groups while that of at the PRF of 1000 Hz and PD of 20 μ s is the lowest (0.008 ± 0.002 mV·s in Fig. 12b). The characteristics of bubble cloud in the light transmission correlate quite well with the observation in the high-speed photography. However, the strength of bubble collapse cannot be quantified well by this method, and a little correlation was found with the erosion area or volume (data not included).

3.5. Ex vivo study

In order to further evaluate the effects of PRF and PD on tissue erosion, *ex vivo* experiments were carried out (see Fig. 13). Because of the higher rigidity of porcine kidney in comparison to that of alginate phantom, histotripsy exposure was extended to 200 s. Significant deformation and displacement of tissue surface due to the acoustic radiation force were found at

the PRF of 1 Hz and PD of 20 ms. As a result, the tissue surface was pushed to the post-focal region by a few mm, where the cavitation is usually less than that in the pre-focal region. The initial erosion usually occurred after 30 pulses, and the final erosion surface was quite irregular. In contrast, the deformation and displacement were significantly less at the higher PRF and shorter PD. Erosion initiated after only a few seconds and then gradually grew in both size and depth. However, with the increase of PRF and decrease of PD the erosion depth decreased, and the erosion surface became smoother. The effect of PRF and PD on *ex vivo* porcine kidney erosion shows quite similar trends as that on alginate phantom, but with smaller erosion volume and area even after a long exposure (see Figs. 5 and 13). PRF of 1000 Hz and PD of 20 μ s produced the least erosion, smaller in size and shallow in the depth.

4. Discussion

In this study, it is found that using low peak negative acoustic pressure could also successfully generate erosion on the surface of the phantom and porcine kidney at long PD and low PRF. The erosion efficiency is highly dependent on the operation parameters, increasing with peak negative acoustic pressure, pulse duration, and interval time between pulses. Increasing the PRF from 1 Hz to 1000 Hz while decreasing the PD from 20 ms to 20 μ s at the same duty cycle of 2% (the same energy delivered) leads to the decrease of erosion volume but the increase of erosion area and more smooth erosion surface. Bubble cloud at the various PRFs and PDs was captured by high-speed photography and passive cavitation detection. Overall, low PRF and long PD produce the acoustic radiation force for significant phantom deformation and more gas dissolution after the bubble collapse due to the long interval time. Consistent refocusing of acoustic waves and confinement of bubbles in a conical region were observed, which corresponds to high acoustic scattering and light transmission signals. High PRF and short PD result in a wide distribution of bubbles with only little wave refocusing and short conical tip at the beginning of histotripsy exposure and high inertial cavitation. Acoustic emission dose has a good correlation with the erosion volume for the future *in vivo* real-time monitoring. The high rigidity of biological tissue makes the erosion more difficult, but in the similar erosion patterns to those on the gel phantom. Therefore, it suggests that the PRF and PD of histotripsy exposure for tissue erosion at the interface of fluid should be optimized for the better outcome, which should be considered in the medical practice for lower energy and shorter treatment time to patients. Different strategies, such as using the moderate PRF and PD (i.e., 10 and 100 Hz, 2 ms and 200 μ s) to quickly initiate the erosion followed by the low PRF and long PD (i.e., 1 Hz and 20 ms) to increase the erosion volume, may be able to enhance the erosion efficiency and will be tested.

Two types of bubble cloud structures (cone-shape and crown-shape) near a tissue-fluid boundary in the acoustic field (frequency of 1.2 MHz, pulse duration up to 100 ms, and spatial average intensity of 6289 W/cm²) were observed early using the high-speed photography with the side illumination and frame interval time of 200 μ s [32]. The cone-shape bubble cloud was characterized by a nearly fixed tip in front of the tissue boundary initiating 600 μ s after the insonation and attaining a stable state after 1.8 ms whereas the initial cavitation bubbles away from the tissue boundary grew larger and were evolved into a crown shape. The percentage of cone-shape bubble cloud, crown-shape bubble cloud, and interchanging of them in 171 images is 85%, 11%, and 4%, respectively. Both types of bubble clouds were also observed in this study, and their occurrence may be dependent on the distribution of bubble nuclei in the focal region and the reflected wave from the deformed surface. Every pulse at all PDs and PRFs can initiate the bubble cavitation at the peak negative pressure of 9.1 MPa which is higher than the cavitation threshold in water [33]. The bubble cloud moves by the primary Bjerknes force due to the pressure gradient. Within the bubble cloud, the second Bjerknes force arising from the pressure field radiated from the pulsating bubbles attracts them to merge. Therefore, the confined bubble in the conical region may have a high possibility of coalescence. Owing to the transparency of polyacrylamide phantom, the deformation of the phantom surface by the acoustic radiation and the damage by the inertial cavitation was clearly shown. However, high rigidity of polyacrylamide phantom and tissue in comparison to that of alginate phantom leads to more surface deformation but less damage, only some cracks and small erosion instead of the crater-shape erosion as shown in Fig. 2a. The tissue boundary restricted the movement and expansion of the bubbles and reduced the effects of acoustic streaming and micro-streaming caused by the bubbles. However, the presence of large and deep erosion in the phantom/tissue may benefit the

1 formation of the wave refocusing and trap more bubble nuclei for acoustic cavitation by the
2 following histotripsy pulses. In addition, the PRF and PD of histotripsy pulses with the same
3 duty cycle result in significant discrepancies in the bubble cloud. Cavitation threshold in water
4 decreases with the pulse duration [33]. Low PRF (i.e., 1 Hz) with long PD and interval time
5 leads to the formation of the cone-shape bubble cloud in the region determined by the parabolic
6 interface deformation and acoustic wave refocusing, and almost complete bubble dissolution
7 during the pulse interval time. In contrast, the residual bubbles after collapse may not dissolve
8 even at the moderate PRF and PD (i.e., 10 Hz and 2 ms) and will serve as nuclei for the coming
9 histotripsy pulses. Therefore, more and wide distribution of bubbles and few cone-shape bubble
10 clouds are found in Fig. 9b. Immediately after the termination of histotripsy exposure, more
11 residual bubbles are also found ($t = 2.2$ ms in Fig. 9b vs. $t = 20.2$ ms in Fig. 9a). Therefore,
12 bubble shielding effect may occur wherein the acoustic energy is scattered or absorbed before it
13 reaches the target. Although erosion rate at the central portion of the acoustic beam may slow
14 down, the enhanced local scattering by a large number of residual bubbles may increase
15 peripheral erosion beyond the beam cross-sectional area [13]. However, great bubble shielding
16 effect would reduce the effective energy arrived at the tissue surface both at the center and in the
17 peripheral region, such as at the PRF of 1000 Hz and PD of 20 μ s. Such bubble shielding also
18 occurs at the HIFU thermal ablation and results in the gradual change of the lesion from the cigar
19 shape to the tadpole one with the large head moving toward the transducer [34]. The tadpole
20 liquefied lesion in boiling histotripsy is also due to it [6]. Overall, the bubble dissolution between
21 ultrasound bursts is important for the consistent bubble cavitation for a predictable outcome. In
22 our previous study, a low-intensity pulsed ultrasound was delivered between lithotripter shock
23 waves to push the residual bubbles away from the focal region in order to suppress the bubble

shielding effect [24]. Subsequently, there is a 2.6-fold increase in stone fragmentation efficiency after 1000 shock waves. The mechanism of refocusing needs further investigation, but seems different from shock scattering from bubbles during histotripsy in free field [35]. Cavitation clouds initiated from single bubbles during the first a few cycles of the histotripsy pulse, and grew along the acoustic axis. The compressive wave inverts upon scattering from them and superimposed on the incident rarefaction wave to create large negative pressure and enhance the cavitation. Thus, the formed bubble cloud is in the shape of a tadpole with the large head gradually moving toward the source as the lesion shape in HIFU ablation only in a few μ s. In contrast, the conical tip of acoustic refocusing found in this study is gradually toward the HIFU transducer, and acoustic reflection from the deformed surface of soft tissue/phantom may have a great influence on the formation. The first refocusing tip at the PRF of 1 Hz usually appears after a few hundred μ s.

To monitor the cavitating bubble cloud at the tissue-fluid interface, acoustic scattering from bubbles and optical attenuation method were used previously [25]. The acoustic backscattering was more sensitive for bubble detection, especially for the bubbles trapped on the uneven tissue surface and not actively cavitating [25]. Although the light only transmitted through a portion of the bubble cloud because of its small width ($0.48 \text{ mm} @ 1/e^2$), the light attenuation signals correlated well with temporal and spatial variation in the acoustic backscattering which was required for producing mechanical breakdown of bulk tissue [36] and served as an excellent indicator of tissue erosion [37]. However, the optical approach cannot be used *in vivo* because of the non-transparency of intervening tissue. Both acoustic and optical monitoring was used in this study, but there are some discrepancies between previous methods. Because the thickness of porcine atrial wall was only 1-2 mm, acoustic scattering signals

measured were mostly from the histotripsy-induced bubbles, whose variations in the size cause the changes in the amplitude of acoustic backscattering [37]. The alginate phantom is much thicker, and the received signal is mainly due to the backscattering from the bubble and reflection from the surface. Although the significant deformation of the phantom (~2.5 mm) interface produced by the long PD is much smaller than the axial beam length of PCD transducer (19.4 mm), the shifted position of cone-shaped bubble cloud and the change of acoustic field would make the detected PCD signals more complicated. Passive cavitation mapping may provide more spatial information of cavitation [38]. Secondly, a broad laser beam was used here to cover the whole exposure region. So the problem of cavitating the bubble cloud moving in or out of a small laser beam, such as by the second Bjerknes force towards the tissue surface, can be avoided.

Tissue disintegration at the tissue-fluid interface and inside the tissue has significant differences. The availability of free gas nuclei and the gas replenishing rate in the fluid is much higher to facilitate the bubble formation and growth, which depends on the vascular transport and diffusion. So the threshold to initiate cavitation is lower at the tissue-fluid interface, and the erosion volume is larger. In addition, the viscosity and stiffness of tissue restrict the bubble growth. In this study, no large boiling bubbles were found in either water or the polyacrylamide phantom, and there were no sudden increases of PCD signals due to the occurrence of boiling bubble [5]. The reason may be due to the low acoustic attenuation of water and gel phantom, the large heat capacity of water, and quick thermal diffusion into the water. So the erosion is mainly due to the cavitation effect. For highly attenuating media at the high frequency, appreciable force is mainly generated in the near field, exciting a larger volume of tissue with a more evenly distributed force [39]. In contrast, for moderately attenuating media at the low frequency, the

1 near field losses are negligible, and only a smaller volume of tissue within the focal region is
2 excited [39]. The different characteristics of bubble dynamics during the histotripsy exposure in
3 the first 2 ms (i.e., at the PDF and PD of 1 Hz and 20 ms and 10 Hz and 2 ms, respectively) may
4 be due to the bubble nuclei distribution for the subsequent acoustic wave propagation and
5 radiation force. The effects of PRF on the bubble dynamics and the production of erosion inside
6 the gel phantom and tissue need further investigation.

7 There are several limitations in the current study. The limited frame rate and low spatial
8 resolution of high-speed photography do not allow us to investigate the dynamics of an
9 individual bubble, obtaining the radius-time curve and measuring the bubble collapse time.
10 Characterization of bubble dynamics would further our understanding of the underlying
11 mechanisms (i.e., cavitation initiation, growth, and collapse). Although the numerical simulation
12 could provide some insight, extending from a single bubble to bubble cloud is still technically
13 challenging due to the complexity in coupling multiple phenomena. It is also possible to simulate
14 the deformation of the tissue surface according to the acoustic radiation force and wave
15 refocusing. The inherent wave divergence in the post-focal region of the HIFU transducer, which
16 makes the wave refocusing from the parabolic surface more complicated, can be considered in
17 the simulation and then compared with experimental results. Another limitation of this study is
18 the small range of parameters tested. The peak negative pressure threshold for erosion initiation
19 at different PD and PRF and the dependence of the threshold on the material properties of tissues
20 are useful for practice. Investigating the correlation between the percentage of wave refocusing
21 and erosion volume would help to illustrate the erosion phenomenon. Our future research will
22 focus on the mechanisms and bubble dynamics of histotripsy exposure.

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Table 1. Peak pressures and acoustic intensities at the focus of HIFU transducer

Peak-to-peak voltage of function generator (V)	p^+ (MPa)	$ p^- $ (MPa)	I_{SPPA} (W/cm ²)
0.1	3.9±0.04	2.9±0.2	294
0.3	11.8±0.7	6.6±0.4	2498
0.5	24.5±0.6	9.1±0.2	5817
0.7	36.7±0.8	9.9±0.3	7864

p^+ : peak positive pressure, $|p^-|$: peak negative pressure, I_{SPPA} : spatial-peak pulse-average acoustic intensity.

Table 2. Comparison of material properties of gel phantom and some biological tissues

material	Speed of sound (m/s)	Attenuation (Np/cm/MHz)	Rigidity (MPa)
alginate phantom (5%)	1550	0.07	0.039
polyacrylamide phantom	1545	0.016	0.06
kidney	1561	0.115	0.18
liver	1549	0.058	0.04
brain	1560	0.069	0.012
heart	1546	0.185	0.124
muscle	1547	0.085	0.45

Figure Legends

- Figure 1. Schematic diagram of the experimental setup for the erosion from the surface of gel phantom and porcine kidney induced by histotripsy pulses and high-speed photography of bubble dynamics at the interface.
- Figure 2. (a) Representative photos of erosion at the surface of alginate gel phantom and (b) the Bego stones filled in the erosion cavities induced by cavitation histotripsy pulses with the varied pulse repetition frequency from 1 Hz (top row) to 10 Hz, 100 Hz, and 1000 Hz (bottom row) at the duty cycle of 2% and peak negative pressure of 9.1 MPa for 20 s.
- Figure 3. Representative high-speed photo from which D : the distance between the phantom interface and tip of wave refocusing, F : the focal length or the distance between the bottom of deformed phantom and tip of wave refocusing, R : radius of rim, d : the depth of the dish, and a fitted parabolic curve to the deformed phantom surface are derived.
- Figure 4. Comparison of the induced erosion area and volumes after 20 histotripsy pulses (a) at the peak negative acoustic pressure from 2.9 MPa to 9.9 MPa, duty cycle of 2%, pulse repetition frequency of 1 Hz, (b) at the pulse duration from 5 ms to 50 ms (or duty cycle from 0.5% to 5%), peak negative acoustic pressure of 9.1 MPa, pulse repetition frequency of 1 Hz, and (c) at varied interval time from 180 ms to 1980 ms (or pulse repetition frequency from 5 Hz to 0.5 Hz or duty cycle from 10% to 1%), pulse duration of 20 ms, peak negative acoustic pressure of 9.1 MPa. Same letter means no significant difference ($p > 0.05$) while different letters mean a significant difference ($p < 0.05$).

Figure 5. Comparison of the induced erosion area and volumes after histotripsy pulses exposure for 20 s at varied pulse repetition frequency from 1 Hz to 1000 Hz, duty cycle of 2%, peak negative acoustic pressure of 9.1 MPa. Same letter means no significant difference ($p > 0.05$) while different letters mean a significant difference ($p < 0.05$).

Figure 6. (a) Representative **peak-to-peak (blue line) and RMS (red line)** of acoustic emission signals of histotripsy pulses exposure for 20 s at the duty cycle of 2%, peak negative acoustic pressure of 9.1 MPa, and pulse repetition frequency from 1 Hz to 1000 Hz and pulse duration from 20 ms to 20 μ s measured by passive cavitation detection **every 20-40 μ s** and (b) comparison of their **statistical analysis over every delivered histotripsy pulse**.

Figure 7. (a) Representative spectrograms of the acoustic emission signals of histotripsy pulses exposure for 20 s at the duty cycle of 2%, peak negative acoustic pressure of 9.1 MPa, and pulse repetition frequency from 1 Hz to 1000 Hz, and comparison of (b) scattering levels measured within the frequency range from 3.2 MHz to 3.4 MHz, (c) **broadband noise** levels measured within the frequency range from 2.5 MHz to 3.0 MHz, and (d) **broadband noise levels** measured within the frequency range from 3.6 MHz to 4.1 MHz.

Figure 8. Correlation of acoustic emission doses calculated from the histotripsy pulses exposure for 20 s at various testing conditions (duty cycles, peak negative acoustic pressure, and pulse repetition frequencies) with the produced erosion volumes at the surface of the alginate gel phantom.

Figure 9. High-speed photography (5000 frames/s) of bubble formed by histotripsy pulses at the PRF and PD of (a) 1 Hz and 20 ms (1st pulse), <http://www3.ntu.edu.sg/home/yfzhou/PRF-1.flv>, (b) 10 Hz and 2 ms (5th pulse), <http://www3.ntu.edu.sg/home/yfzhou/PRF-10.flv> (c) 100 Hz and 200 μ s with duty cycle of 2% at the peak negative acoustic pressure of 9.1 MPa. Dashed ellipse: wave refocusing and formation of a conical tip; blue dashed line: confinement of bubble in a conical region; arrow: acoustic radiation force-induced surface deformation; arrow head: damage to the gel phantom; dashed arrow: the collapse of bubble cloud induced surface deformation. Please watch supplementary video 1 and 2 (half frame rate) for the PRF and PD of 1 Hz and 20 ms, and 10 Hz and 2 ms, respectively). The scale bar represents 1 mm.

Figure 10. High-speed photography of (a) the forward motion of the bubble cloud to the phantom surface and then the formation of wave refocusing, and (b) the interaction of acoustic wave refocusing with the coming bubble cloud induced by the histotripsy pulse at the peak negative pressure of 9.1 MPa, duty cycle of 2%, and pulse repetition frequency of 1 Hz. The arrow shows the moving forward bubble cloud. The scale bar represents 1 mm.

Figure 11. (a) The progressive extending of the wave refocusing tip from the phantom interface, D , with the histotripsy exposure (peak negative pressure of 9.1 MPa, pulse duration of 20 ms, $n = 13$), and (b) the correlation between the measured focal length, F , and the corresponding value from the fitted parabolic curve ($n = 277$).

Figure 12. (a) Representative light transmission signals of histotripsy pulses exposure for 20 s at the duty cycle of 2%, peak negative acoustic pressure of 9.1 MPa, and pulse repetition frequency from 1 Hz to 1000 Hz and pulse duration from 20 ms to 20 μ s measured and (b) comparison of their integration values.

Figure 13. (a) Photos of erosion at the surface of porcine kidney induced by histotripsy pulses exposure for 200 s at the duty cycle of 2%, peak negative acoustic pressure of 9.1 MPa, and varied pulse repetition frequency and pulse duration from 1 Hz and 20 ms (bottom row) to 10 Hz, 100 Hz, and 1000 Hz and 20 μ s (top row) and (b) the comparison of erosion area and volumes.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figure 8.

0 ms

0.2 ms

0.4 ms

1.4 ms

2.8 ms

6 ms

(a)

13 ms

20.2ms

22 ms

0 ms

0.2 ms

0.6 ms

1.4 ms

1.8 ms

2 ms

(b)

2.2 ms

3 ms

5 ms

Figure 9.

Figure 10.

Figure 11.

Figure 12.

Figure 13.