Effect of pulse repetition frequency of high-intensity focused ultrasound on in vitro thrombolysis

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A B S T R A C T

Vascular occlusion by the thrombi is the main reason for ischemic stroke and deep vein thrombosis. High-intensity focused ultrasound (HIFU) and histotripsy or microtripsy pulses can effectively dissolve the blood clot with no use of thrombolytic agent and ultrasound contrast agent (microbubbles). In this study, HIFU bursts at the same duty cycle (2%) but varied pulse repetition frequency (PRF) from 1 Hz to 1000 Hz were delivered to in vitro porcine blood clot for 30 s. Thrombolysis efficiency initially increases slightly with the PRF, 86.4 ± 10.3%, 89.9 ± 11.9, and 92.9 ± 12.8% at the PRF of 1 Hz, 10 Hz, and 100 Hz, respectively, without significant difference (p > 0.05), but then drops dramatically to 37.9 ± 6.9% at the PRF of 1000 Hz (p < 0.05). The particle size in the supernatant of dissolution is 547.1 ± 129.5 nm, which suggests the disruption of thrombi into the subcellular level. Thrombi motion during HIFU exposure shows violent motion and significant curling at the low PRF, rotation about its axis with occasional curling at the moderate PRF, and localized vibration at the high PRF due to the generation of acoustic radiation force and streaming. Quantitative analysis of recorded motion shows the axial displacement decreases with the PRF of delivered HIFU bursts, from 3.9 ± 1.5 mm at 1 Hz to 0.7 ± 0.4 mm at 1000 Hz. Bubble cavitation during HIFU exposure to the blood clot was also monitored. The increase of PRF led to the increase of inertial cavitation but the decrease of stable cavitation. In summary, the PRF of delivered HIFU bursts at the same output energy has a significant effect on the thrombi motion, bubble cavitation activities, and subsequently thrombolysis efficiencies.

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1. Introduction

The occlusion or leakage in the cerebral blood supply causes rapid and maybe permanent neurological injury, such as inability in limb motion, speech formulation and understanding, and visualization [1]. Stroke death worldwide increased dramatically from 9.7% in 2004 to 10.8% in 2008 due to the high prevalence of stroke risk factors in the rapidly aging population and modern society, such as high blood pressure, diabetes, and smoking [2]. Ischemic strokes, occlusion by the blood clot mostly in the middle cerebral artery (MCA) that is the longest blood supplying artery to the brain [3], account for 87% of all strokes [4]. Meanwhile, thrombosis is also the primary cause of myocardial infarction, pulmonary embolism, and deep vein thrombosis (DVT). DVT may begin without symptoms but occurs usually in the legs and then leads to pulmonary emboli, which is widely prevalent with complex pathophysiology and high economic burden. Its affects over 300,000 patients annually in the USA and causes the death of 60,000–100,000 [5,6]. The risk factors include age, obesity, cancer, surgery, trauma, and drugs, and are 8-fold and 22-fold higher for those in hospitals and undergoing surgery, respectively [7].

Symptom occurrence requires thrombolytic treatments instead of anticoagulant medicines so that several strategies have been applied in clinics. Thrombolytic drugs, such as tissue plasminogen activator (tPA), urokinase or streptokinase, or alteplase are used to break up clots [8,9]. Administration of non-site-specific thrombolytic drugs systematically is only effective in the occurrence of the first 2–3 h and is associated with a substantial risk of fatal major bleeding [10]. If the drug delivery is close to the clot occlusion site, such as through a catheter, the thrombolysis efficiency is much higher than by systemic drug infusions. Meanwhile, vein segment isolation, mechanical disruption and aspiration of the clot (rheolytic thrombectomy) are also feasible via the catheter [11]. Catheter-based endovascular procedures have the advantage of localized treatment, but its invasiveness increases the risk of hemorrhage, vascular damage, and infection...
Sonothrombolysis, the use of low-intensity ultrasound for thrombolysis to the obstructive intracranial blood flow, has been applied for the treatment of acute ischaemic stroke and DVT alone or in conjunction with thrombolytic drugs or contrast agents (microbubbles). The introduction of microbubbles can enhance the acoustic cavitation and subsequently, the delivery and penetration of tPA inside the clot and higher rates of recanalization but with the decreased complications, such as symptomatic intracranial hemorrhage [13–15]. In transcranial ultrasound in clinical sonothrombolysis (TUCSON) trial, sonothrombolysis with microbubbles and tPAs showed a trend toward higher early recanalization and clinical recovery rates compared to standard intravenous tPA therapy [16]. In addition, it is found that a variety of commercial ultrasound devices approved by the United States Food and Drug Administration (USFDA) for diagnosis could achieve satisfactory and comparable in vitro sonothrombolysis efficiencies if the acoustic output is sufficiently strong despite significant variations in their specifications (i.e., pulse duration and profile) [17].

High-intensity focused ultrasound (HIFU) has also been investigated as a stand-alone thrombolytic approach [18,19]. The percentages of reperfusion in the MCA of New Zealand white rabbits after 20-s HIFU exposure (1.5 MHz, 1 ms bursts, 1 Hz PRF) are 0%, 50%, and 70% at the acoustic power of 275 W, 415 W, and 550 W, respectively. Histological analysis confirmed that the sonicated vessels remain intact and detected hemorrhage was outside the focal region of HIFU and close to the base of the rabbit skull. In addition, histotripsy (<50 cycles, duty cycle of 0.1–5%) alone can also achieve effective thrombolysis by initiating a cavitation bubble at the peak negative pressure > 6 MPa. The blood clot was fractionated to debris with >96% weight smaller than 5 μm in size in 1.5–5 min using 5-cycle 1 MHz burst at the PRF of 1 kHz [20]. To avoid potential cavitational damage to the vessel wall but recanalize within the clot, peak negative pressure was further increased beyond the intrinsic threshold (27 MPa for the blood clots) using very short pulses (<2 cycles), which was termed as microtremor [21,22]. At the PRF of 50 Hz, channels in a diameter up to 60% of the vessel size with restored flow up to 500 mL/min and 99.9% debris smaller than 10 μm in a 2 cm clot were achieved within 7 min by scanning the cavitation focus through the clot. The effects of PRF (5, 50, and 100 Hz) on microtremor thrombolysis were investigated, and the use of 50 or 100 Hz PRF was preferred due to the larger resulting flow channel, shorter treatment time, and smaller debris particles [22]. Although HIFU exposure has shown promising in thrombolysis, the underlying mechanisms have not been fully understood. Optimizing the HIFU parameters, observing the process of thrombolysis during HIFU exposure, and measuring the HIFU-induced cavitation may result in the technical development and improved thrombolysis efficiency in a shorter time.

In this study, HIFU bursts at the same duty cycle (2%) but varied PRF of 1 Hz, 10 Hz, 100 Hz, and 1000 Hz were delivered to in vitro porcine thrombi positioned in a polyethylene pipette for 30 s so that the same acoustic energy was exposed. Undissolved thrombi were carefully collected after the treatment to determine the thrombolysis efficiency, and the particle size in the supernatant of dissolution was assessed quantitatively. The motion of thrombi during HIFU exposure was video recorded, and different characteristics of thrombi motion were found at different PRFs. In addition, bubble cavitation signals during the HIFU exposure were measured by passive cavitation detection (PCD), from which the stable and inertial cavitation could be quantified for comparison among the testing groups.

2. Materials and methods

2.1. Clot preparation

Fresh porcine blood was collected from a local slaughterhouse (Primary Industries Pte Ltd, Singapore) after the approval of Agri-Food & Veterinary Authority, Singapore, and mixed immediately with an anticoagulant (citrate-dextrose solution, C3821, Sigma-Aldrich, Singapore) at a ratio of 10 mL of anticoagulant per 90 mL of blood. The blood sample was maintained at 4 °C in a refrigerator and could be used for about 2–3 weeks. To prepare the in vitro blood clot, 0.25 ml of calcium chloride (100 mM, Sigma-Aldrich) was added to 1 ml of the blood sample and then mixed gently. After 1 min, the mixture was injected into plastic tubing (WS150 9IN, NBS Group, Singapore), whose inner diameter of 4.7 mm matches the size of adult human’s MCA. Plastic tubing was then stored in an incubator (Symphony, VWR, Radnor, PA) at 37°C for 1 h for a stable clot formation. Afterwards, the blood clots (~20 mm in length) were carefully removed from the plastic tube, weighted by a digital analytical balance (ML54, Mettler Toledo, Columbus, OH) after absorbing the surrounding plasma, and then inserted into the stem of a 3 mL polyethylene disposable transfer pipette (outer diameter of 7.8 mm, inner diameter of 7 mm, Z350796, Sigma-Aldrich) which has an excellent acoustic impedance match to water without attenuating the ultrasound signal significantly [23] filled with phosphate buffered saline (PBS) solution and sealed with paraffilm (Pechiney Plastic Packaging, Chicago, IL). Bubbles in the pipette were avoided by the careful paraffilm seal.

2.2. Thrombolysis

The thrombolytic capability of HIFU pulses was evaluated using our established protocol and experimental setup as shown in Fig. 1 [17]. The sealed pipette stem was fixed by a custom-built holder connected with a three-axis translational stage (PT3/M, Thorlabs, Newton, NJ). An annular focused HIFU transducer (H-102, outer diameter = 69.94 mm, inner diameter = 22.0 mm, F = 62.64 mm, Sonic Concepts, Woodinville, WA) working at its fundamental frequency (1.1 MHz) was used in this study. The HIFU transducer was immersed in the degassed and deionized water (O2 < 4 mg/L, T = 25 °C) of a Lucite tank (L × W × H = 70 × 50 × 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). A silicone elastomer acoustic absorber made in the lab was put on the opposite wall of the testing tank to prevent the ultrasound reflection. A mechanical point attached to the HIFU transducer...
was used to align the HIFU focus to the middle of the blood clot and then removed before the exposure. HIFU focus was not scanned along the blood clot during the treatment. Any visible bubbles attached to the pipette were removed before the HIFU exposure. A LabView program (National Instruments, Austin, TX) was written to control the pulse delivery. In this study, the duty cycle and the total exposure was kept as 2% and 30 s, respectively, while varying the PRF from 1 Hz, 10 Hz, 100 Hz, to 1000 Hz (pulse duration of 20 ms, 2 ms, 200 µs, and 20 µs, respectively) so that the total acoustic energy delivered was the same.

After the HIFU exposure, all the materials in the pipette stem were transferred to a cuvette and sedimented for about 1 h. The large debris and remained blood clot in the sediment passed through Whatman filter paper (Grade 2, the pore size of 8 µm, Sigma-Aldrich), and then were collected carefully, washed with PBS solution thrice, dried with the paper tissue, and weighted as the remaining parts. The percentage of weight loss of the blood clot in comparison to its initial weight is defined as the thrombolysis efficiency. Meanwhile, a control group (sham exposure for 30 s) was also included. Furthermore, the supernatant of the dissolution after being sedimented was diluted for at least 100 times in a 1.5 ml cuvette (Kartell S.P.A., Noviglio, Italy), and the particle size distribution was measured by laser diffraction method at the wavelength of 633 nm (0.3 nm–10 µm, Nano-ZA, Malvern, Worcester, UK).

2.3. Clot motion

The thrombolysis process by the HIFU exposure was recorded by a digital camera (PowerShot SX230 HS, Canon, Tokyo, Japan), and the recorded video was then extracted for analysis in digital image processing software (Photoshop, Adobe System, San Jose, CA) from which the displacement of blood clot under the HIFU exposure was determined quantitatively. Both ends of the blood clots were identified in the extracted images (using the function of Filter/Stylize/FindEdges in the Photoshop). Their maximum displacements during each HIFU pulse exposure (the PRF of 1 Hz and 100 Hz) or the maximum displacements detected between two neighboring harmonics, were used to represent the inertial cavitation-induced broadband noises [26] (see Fig. 2). T was set as 20 ms, which is the effective pulse exposure every second for all the PRFs and includes 1, 10, 100, 1000 pulses at the pulse duration of 20 ms, 2 ms, 200 µs, 20 µs at the varied PRF from 1 Hz to 1000 Hz, respectively in calculating the average cavitation dose each second during the HIFU exposure for easy comparison between the testing groups.

2.4. Cavitation detection

A focused ultrasound probe (A319S, f = 15 MHz, D = 12.7 mm, F = 65 mm, Olympus-IMS, Waltham, MA) was aligned confocally and coaxially with the HIFU transducer and worked as a PCD sensor [24]. PCD signals of each burst were recorded by a digital oscilloscope (Wavesurfer MXs-B, LeCroy, Chestnut Ridge, NY) at a sampling frequency of 50 MHz and then transferred to a personal computer for data analysis using the established method [25]. Sequence mode was used in the data acquisition. However, because of the limited memory in the digital oscilloscope only PCD signals in the first 25 s were collected. Small-time Fourier transforms (STFT) were then performed on the collected PCD data in Matlab (The Mathworks, Natick, MA) for the corresponding spectrogram, F(t, f). The small time window was set as 40 µs, and Hanning curve and zero padding were applied to minimize the spectral leakage. Three specific frequency windows (2.15,2.25], [2.4,3.35], and [3.5,4.45] MHz), which are between the two neighboring harmonics, were used to represent the inertial cavitation-induced broadband noises [26] (see Fig. 2). T was set as 20 ms, which is the effective pulse exposure every second for all the PRFs and includes 1, 10, 100, 1000 pulses at the pulse duration of 20 ms, 2 ms, 200 µs, 20 µs at the varied PRF from 1 Hz to 1000 Hz, respectively in calculating the average cavitation dose each second during the HIFU exposure for easy comparison between the testing groups.

2.5. Statistical analysis

The sample size for each testing group was at least five. ANOVA and Mann-Whitney rank sum test was carried out in SPSS® Statistics (IBM Software, Somers, NY) to calculate the statistical difference between the testing groups that was fixed at p < 0.05.
3. Results

3.1. Thrombolysis efficiency

The acoustic pressure waveform at the focus of the HIFU transducer was measured by a needle hydrophone (HNA-0400, Onda, Sunnyvale, CA) which has a broad bandwidth and is robust to cavitation damage. The peak positive and negative pressure was 25.2 ± 0.9 MPa and 8.5 ± 0.2 MPa, respectively, and -6 dB beam size was 12 × 1.7 mm (axial × lateral). This power level was selected because the high percentage of initiating a bubble cloud (>99.6%) and subsequently greater thrombolysis were found with the peak negative pressure larger than 8 MPa in the histotripsy-induced thrombolysis [20]. Although the delivered energy is the same (same duty cycle and total exposure time), varying the delivered PRF results in different thrombolysis efficiencies.

![Image](image_url)

Fig. 3. (a) Representative photos of the blood clots after the treatment placed in the 6-well cell culture plate (one sample treated at the PRF of 10 Hz was diluted in order to illustrate the small debris shown as the arrow, and no visual debris was found at the bottom row of cell culture plate) and (b) comparison of the thrombolysis efficiency after 30-s exposure of high-intensity focused ultrasound at the varied pulse repetition frequency of 1 Hz, 10 Hz, 100 Hz, and 1000 Hz with duty cycle of 2% (n = 5). *: statistically different from the others, p < 0.05.
efficiencies (see Fig. 3). The blood clot exposed to HIFU bursts at the PRF of 1 Hz, 10 Hz, and 100 Hz had similar thrombolysis efficiency from 86.4 ± 10.5 to 92.9 ± 12.8% (p > 0.05). However, the thrombolysis at the PRF of 1000 Hz was only 37.9 ± 6.9%, which is significantly lower than the other groups (p < 0.05), while the corresponding value of the control group was 16.8 ± 7.7%. In some cases, only a few debris (~2–4 mm) in much lighter color than the whole blood clot were found (shown by the arrow in Fig. 3a). The mean particle size (number weighted) in the supernatant of the dissolved clot solution was 547.1 ± 129.5 nm and much smaller than that of fresh porcine blood, 2796 ± 953.8 nm (the size of red blood cell [27]), as shown in Fig. 4. It illustrates that HIFU exposure could disrupt the most blood clot into subcellular fragments though a few large debris were remained in some settings.

3.2. Clot motion

The motion of blood clot under HIFU exposure was captured graphically and characterized (see Figs. 5 and 6). It is found that the blood clot moved violently inside the stem of polyethylene pipette at the PRF of 1 Hz, not only translating along the pipette but also curling up inside it (i.e., T = 10 s in Fig. 5a). The corresponding displacement is in the range from 1.3 mm to 9.1 mm. However, the blood clot was always trapped in the focal region of the HIFU field, not being pushed to one end of the pipette and never coming back. In comparison, the increase of PRF led to less blood motion along its axial direction. For example, at the PRF of 10 Hz and 100 Hz the blood clots rotated along their axes and sometimes curled slightly during the rotation. At the PRF of 1000 Hz the axial motion of the blood clot was much less (see Fig. 5b), and its range was only from 0.2 mm to 1.7 mm (see Fig. 6).

3.3. Bubble cavitation

Bubble cavitation during the HIFU-induced thrombolysis process was monitored by the PCD approach, and the stable and inertial cavitation levels were quantified using the STFT for comparison as shown in Fig. 7. It is found that the increase of PRF resulted in smaller stable cavitation, but higher inertial cavitation. Although the variations were high (~3–6 dB) and statistical difference might not be significant between some neighboring groups (i.e., between the PRF of 10 Hz and 100 Hz, p > 0.05), the trends were quite clear and there were significant differences between the PRF of 1 Hz and 1000 Hz in both temporal-average stable and inertial cavitation every second during the exposure (p < 0.05).
percentage of clot mass loss in the control group is similar to our previous in vitro sonothrombolysis studies using the same clot model for 1 h [17,30], which suggests that the clot mass loss may be mainly due to the plasma effusion from the formed blood clot and systematic error of measuring clot weight instead of its structure disruption. The thrombolytic efficacy of tPA for 1 h using the same clot model was about 30% [17,30]. Acoustic radiation force and acoustic cavitation may be the contributory factors for the different phenomena. Duty cycle (2%) and high PRF (1000 Hz) used here are within the parameter range of histotripsy for the soft tissue disintegration [28]. The duty cycle is higher than the histotripsy-induced thrombolysis (0.5%) [20,29], and the peak negative pressure is lower than that of microtripsy (15 MPa) [21,22]. The thrombolysis efficiency measured here at the PRF from 1 Hz to 100 Hz is comparable to those by histotripsy at the peak negative pressure of 8 MPa after 300 s (clot weight pre- and post-treatment of 354 ± 25 mg and 64 ± 52 mg, respectively) [20]. Therefore, it suggests that even at shorter exposure time satisfactory thrombolysis could also be achieved by optimizing the ultrasound parameters. However, whether this conclusion is valid in practice needs further animal investigation.

Although low-intensity ultrasound with alteplase and microbubbles, such as the 1–2 h CLOTBUST (design of a randomized trial of ultrasound-enhanced thrombolysis for acute ischemic stroke) and TUCSON, has performed phase II trials with promising results of vascular recanalization rates and high-intensity ultrasound (HIFU, histotripsy, and microtripsy) has successfully dissolved the thrombi in animal models, mechanisms of sonothrombolysis are not fully understood [30,31]. In the acoustic field, microstreaming patterns can be formed by the oscillation bubbles close to the blood clot. Consequent shear stress would accelerate the diffusion of the thrombolytic drug into the fibrin matrix [32]. Stable cavitation is evidenced by the presence of ultraharmonic (UH), and significant correlation was found between UH emissions and thrombolysis efficiency [33]. The violent inertial cavitation can produce intense localized stress forces and a high-speed microjet towards the blood clot to disintegrate its structure and increase the permeability [34]. Broadband noise emissions due to inertial cavitation were much lower (∼31 dB) than UH levels at low-intensity ultrasound with the domination of stable cavitation [33]. To initiate inertial cavitation high-intensity ultrasound bursts were applied. No significant UH signals were found in this study. Low PRF may form large bubbles for oscillation in the focal region of the HIFU transducer, but acoustic scattering and attenuation from them reduce the effective energy delivered to them for violent collapse. Acoustic scattering may enhance the stable cavitation. In addition, long pulse duration results in strong acoustic radiation force to push the bubbles away from the focus and generate streaming inside the tube. Therefore, the inertial cavitation was weak at the focus as that at the low PRF. Varying the PRF seems to have opposite trends in the induced stable and inertial cavitation as shown in this study. The effects of HIFU-induced translational motion, curling up, and spinning of the clot, which may affect the strength of thrombi, on the thrombolysis are unknown. Low and moderate PRFs have similar thrombolysis but different characteristics of clot motion. If the motion-induced stress in the clot is larger than a threshold, fatigue in the fibrin network will occur and result in the clot dissolution. Such stress threshold is dependent on the clot structure and material properties. However, such motion in the partially constrained media here are within the parameter range of histotripsy for the soft tissue disintegration [28]. The duty cycle is higher than the histotripsy-induced thrombolysis (0.5%) [20,29], and the peak negative pressure is lower than that of microtripsy (15 MPa) [21,22]. The thrombolysis efficiency measured here at the PRF from 1 Hz to 100 Hz is comparable to those by histotripsy at the peak negative pressure of 8 MPa after 300 s (clot weight pre- and post-treatment of 354 ± 25 mg and 64 ± 52 mg, respectively) [20]. Therefore, it suggests that even at shorter exposure time satisfactory thrombolysis could also be achieved by optimizing the ultrasound parameters. However, whether this conclusion is valid in practice needs further animal investigation.

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radiation force. The amplitude of the acoustic radiation force is proportional to the attenuation and temporal-average acoustic intensity. The clot translated synchronously with the initiation and cessation of the ultrasound exposure [33]. Its room-mean-square velocity correlates with the lytic rate. However, whether such correlation is valid for more violent motion observed in this study needs further investigation. Since the acoustic absorption in the human thrombi is two orders larger than that in plasma [35], the clot motion by the acoustic radiation force should dominate over acoustic streaming [33]. The mismatch of the HIFU focus with the mass center of the clot even slightly may cause the thrombi to spin. The spinning velocity is found to increase with the PRF. In the constrained media, the magnitude and pattern of acoustic radiation force will be affected by the presence of the vessel wall. Vortical flow will be generated inside it, which may be the reason of translation motion of the clot. In addition, the acoustic radiation force combined with the bubble cloud confine the fluid flow to the boundaries of the vessel wall and produce recirculation streaming, which would trap the embolus in the lower pressure locations [36]. If the clot is adjacent to the bubble cloud (i.e., <10 mm), it would spontaneously move towards the bubble cloud [20]. Furthermore, residual bubbles may also recycle by the vortices for continuous cavitation.

High positive peak pressure scattered from bubbles has the phase inversion, and its superimposition on the negative cycles in the incident wave results in much higher negative pressure to generate dense bubbles. In short-pulse ultrasound exposure at the high PRF, cavitation memory effects where residual bubble nuclei from the collapse of inertial cavities from previous pulses may become cavitation sites for the subsequent cavitation events [37]. Residual bubbles persist on the vessel wall due to the high surface tension, making it more susceptible to cavitation-induced damage by the subsequent bursts. However, the pre-focal cavitation did not significantly affect the focal cavitation which was

Fig. 7. Comparison of the (a) stable and (b) inertial cavitation levels during the high-intensity focused ultrasound exposure in the first 25 s at the varied pulse repetition frequency from 1 Hz to 1000 Hz with the duty cycle of 2%.
always well confined in the vessel lumen at all PRFs [22]. Only minor cavitation-induced hemorrhage confined to the vessel lumen and in contact with the vessel wall was found in in vivo histotripsy study [29]. Mapping of cavitation during HIFU exposure could be passively measured by a diagnostic array, which provides more spatial information than PCD [38]. Bubble-induced color Doppler (BCD) in histotripsy-induced tissue fractionation and liquefaction was found to agree well with particle image velocimetry (PIV) and have a strong linear correlation with the fractionation progress in ex vivo porcine liver, which is another way of real-time monitoring [39]. The characteristics of bubble formation, dynamics, and motion for long-pulse HIFU exposure needs further investigation for the mechanisms of thrombolysis and potential of vascular injury.

There are several limitations of this study. First, the PBS solution in the sealed pipette is stationary. The disrupted particles from the thrombi, most of the red blood cells, during the HIFU exposure would darken the PBS solution gradually with the progress of thrombolysis. As a result, the remained thrombi cannot be observed clearly. In order to mimic the in vivo environment, pulsatile flow model is preferred. As bubble dynamics is highly sensitive to their environment, high flow velocities may hinder the bubble cavitation and sweep out the bubble cloud [40]. The fractionated debris may be flushed towards downstream and then collected to measure the numbers and sizes using the filter weight and Coulter counter combination method and to observe its morphology and structure, evaluating the potential of embolism [20]. Furthermore, the remained thrombi could be visualized clearly during the whole thrombolysis. In the histotripsy studies, it was found that debris particles no larger than 100 μm were unlikely to cause the hazardous emboli [29]. Whether large debris fractionated from the thrombi could still be trapped in the focal region of the HIFU field, especially the violent motion of thrombi at the low PRF, is critical in vivo concern. Otherwise, the focus of another HIFU transducer will be aligned to the downstream vessel to trap large debris for further thrombolysis at the high PRF [36]. However, debris up to 1000 μm produced by mechanical thrombectomy showed no severe embolism in human treatments [41]. Only the weight of remained blood clot and large debris was measured here. Their size distribution will be determined later for better characterization of sonothrombolysis performance, and the risk of embolism is required. Second, the pipette is larger than the diameter of thrombi so that the motion of the partially constrained thrombi is significantly different from the that in the practical occlusion. A smaller silicon tube with multiple small through holes in a stopper could be used to simulate the occlusion [17], and the clot can be formed directly in a vessel-mimic tube, such as clamping the tube after mixing the blood with calcium chloride and injecting into the tube. Vessel recanalization and restoration of blood flow, which are of importance in the treatment of ischemic stroke, should also be measured. Third, the blood clot was only incubated for 1 h. In clinics, a thrombus could be formed around fresh atherosclerotic plaques, or an aged thrombus could travel in the arterial blood stream. The strength of the cross-linked fibrin fiber depends on the age of the blood clot and determines the subsequent thrombolysis efficiency [42]. Fourth, besides the thrombolysis the potential of induced collateral damage (e.g. vascular injury) is another critical concern in the clinical practice and should be evaluated in the future animal experiment. Finally, the acoustic radiation force and streaming at different PRFs could be measured quantitatively using laser Doppler anemometry (LDA) and particle image velocimetry (PIV) [43]. In addition, acoustic streaming in an enclosure could be simulated numerically using an explicit time-marching algorithm [44] and then compared with the experimental results.

5. Conclusion

HIFU bursts could successfully dissolve the blood clot aligned at the focus of the transducer. The motion of the blood clot, bubble cavitation, and subsequent thrombolysis efficiency strongly depend on the pulse repetition frequency used if the duty cycle, total exposure time, and the electrical driving power are kept the same. At the low PRF (1 Hz), the blood clot has significant deformation and violent motion along the tube axis; whereas the deformation and axial motion become less at the moderate PRFs (10 Hz and 100 Hz). High PRF (i.e., 1000 Hz) had only localized displacement of thrombi and achieved the lowest thrombolysis efficiency. With the increase of PRF the inertial cavitation and rotation speed of blood clot on its axis increase, but the stable cavitation decreases. Although thrombolysis efficiencies using both low and moderate PRFs are statistically similar, moderate PRFs are preferred due to the stable clot position during the HIFU exposure for the easy monitoring and high control of the outcome. PRF in HIFU-induced thrombolysis is an important operational parameter and should be optimized for the best outcome.

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