Relationship between thrombolysis efficiency induced by pulsed focused ultrasound and cavitation bubble size

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Abstract. In this study, the relationship between the efficiency of pulsed focused ultrasound (FUS)-induced thrombolysis and the size distribution of cavitation bubbles has been studied. Firstly, the thrombolysis efficiency, evaluated by degree of mechanical fragmentation was investigated with varying duty cycle. Secondly, the size distribution of cavitation bubbles after the 1st, 10^3th and 10^5th pulse during experiments for various duty cycles was studied. It was revealed that the thrombolysis efficiency was highest when the cavitation bubble size distribution was centred around linear resonance radius of the emission frequency of the FUS transducer. Therefore, in cavitation enhanced therapeutic applications, the essential of using a pulsed FUS may be controlling the size distribution of cavitation nuclei within an active size range so as to increase the treatment efficiency.

1. Introduction

As a completely novel, non-pharmacological approach, focused ultrasound (FUS) thrombolysis has been extensively studied for several decades, which offers the potential to shorten therapy time, increase reperfusion and limit bleeding complications. However, there is still the clinical limit that any clot debris produced by the fragmentation of the original clot may block micro vessels in distal vascular beds. Therefore, it is essential to measure the size distribution of clot debris following FUS-mediated lysis and to evaluate how the size distribution of the clot debris may be related to the acoustic parameters of FUS so that the FUS-induced thrombolytic effect may be optimized. Current research on FUS-induced thrombolysis has been focused on the feasibility of using this technique to break clots [1], while there have only been very limited studies on the size distribution of the resulting clot debris.

Previous studies have shown that thrombolysis is more efficient using pulsed FUS rather than continuous FUS [2], as the degree of acoustic shielding is minimized by the relatively long pauses between pulses, allowing intense cavitation to take place in the focus, which could disintegrate clots mechanically. More specifically, each FUS pulse creates a cluster of microbubbles localized at the transducer focus, whose collapse will cause local stress to remove a portion of the targeted clot. Residual microbubbles may also act as nuclei that can be excited by subsequent pulses, predisposing clot in the focal region to further damage. Therefore, it may be possible to achieve a synergistic effect between residual cavitation nuclei by adjusting the timing between two consecutive pulses. It can be

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hypothesized then that in pulsed FUS-induced thrombolysis, if the size distribution of microbubbles is within an active size range in which the bubbles oscillate strongly in response to the applied acoustic pressure, the cavitation activity will be enhanced and result in optimal thrombolysis. To test this hypothesis, we must know the size of microbubbles generated by the treatment process and how they relate to changes in FUS acoustic parameters.

In this study, the relationship between the efficiency of FUS-induced thrombolysis, the duty cycle and the size distribution of cavitation bubbles will be investigated. The efficiency of thrombolysis was evaluated through the degree of mechanical fragmentation and the size distribution of cavitation bubbles was calculated through the method previously proposed by our group [3].

2. Materials and methods

A schematic representation of the experimental setup is shown in figure 1(a), including US generation, clot sample, passive cavitation detection (PCD), and cavitation bubble size estimation. Exposures were performed in degassed water maintained at 37℃ within a tank. Experimental sample was fresh non-heparinized blood obtained from domestic swine, which will be coagulated at room temperature for 3 h and maintained at 4℃for up to 3 days to allow for maximal clot retraction, lytic resistance, and stability before experiment.

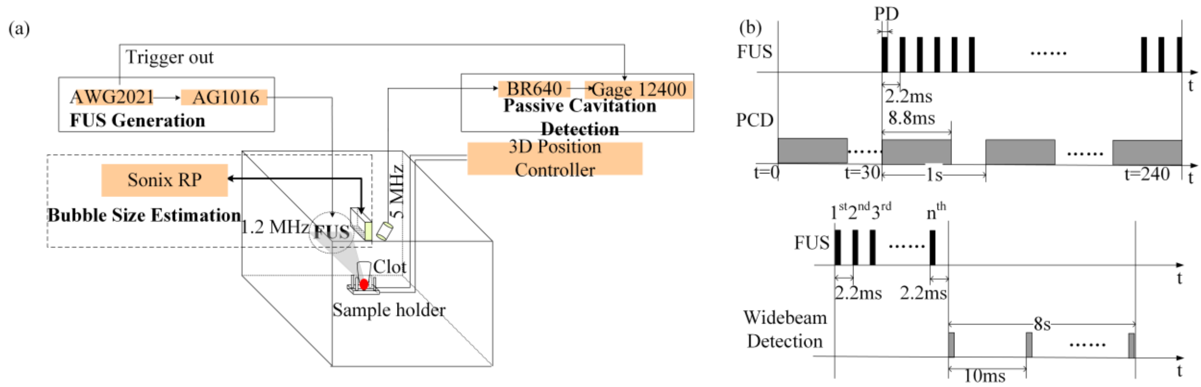

Figure 1. (a) The experimental setup includes FUS generation, sample clot, and PCD. (b) Time sequence between FUS generation and PCD.

Destructive US were achieved using a 1.2-MHz single element, high-intensity focused US (HIFU) transducer(15 cm aperture, 12 cm focal length), which was pulsed at an acoustic power of 60 W at a PRF of 454 Hz throughout this study(figure 1 (b)). A 5 MHz single element transducer, adjusted to confocal with HIFU transducer through a needle hydrophone, was used to receive acoustic backscatter signals. Each signal will then be used to calculate the inertial cavitation dose (ICD) through the conventional PCD method [4].

In order to investigate the relationship between the efficiency of thrombolysis and the duty cycle, three pulse durations (PDs) (50µs, 200µs, and 400µs) per pulse period (1/454 Hz≈2.2 ms) were evaluated, corresponding to three duty cycles (2.3%, 9%, and 18%), which were chosen based on preliminary experiments that showed obvious thrombolytic effects at these settings. The total treatment time was 210 s and the PCD was started 30 s prior to treatment time to determine the baseline noise level (upper in figure 1 (b)). Clot debris resulting from the treatment group was also compared to the control group, in which the clot was submerged in saline without FUS exposure for the same period of time.

For each duty cycle, the size distribution of residual cavitation bubbles after the *n*th pulse (n=1, 10^3, 10^5), which can be regarded as the size distribution of cavitation nuclei for the (n+1)th pulse, was estimated using the wide beam ultrasonic method described in our previous paper [3]. The time sequence between the FUS transducer and the wide beam ultrasonic detection using the linear array is illustrated in the bottom in figure 1 (b).

After each treatment, saline samples containing suspended clot debris were removed from the condom and the size distribution of debris particles was measured using the Coulter Counter (Multisizer™ III, Beckman Coulter, USA) particle sizing system.

3. Results and discussion

Figure 2 shows the number and number percentage of clot debris particles induced by FUS at three duty cycles, as well as the control group. These results demonstrate that the number of debris using a 9% duty cycle was higher than that when the lower (2.3%) or higher (18%) duty cycle was used. Furthermore, the mean size of clot debris particles generated using a 9% duty cycle was smaller than that generated at the two other duty cycles.

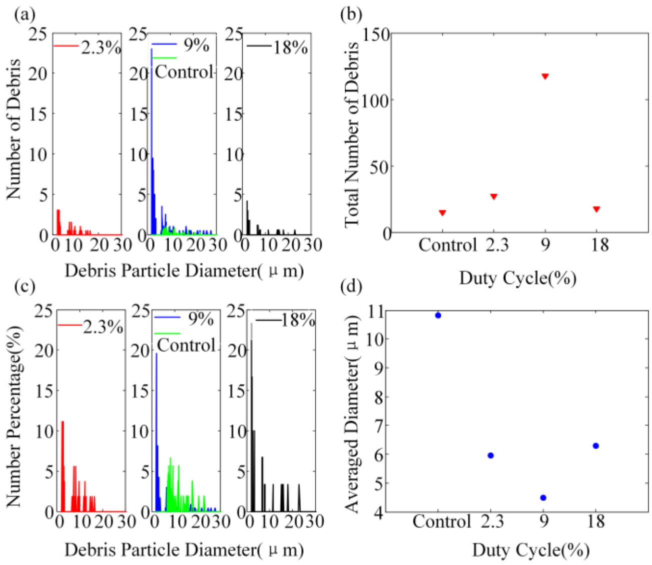

Figure 2. Histograms of the (a) number and (c) number percentage of clot debris particles with sizes ranging from 2 to 30 μm. (b)Total number and (d) average diameter of clot debris particles as a function of duty cycle.

Figure 3(a) shows the inertial cavitation activity for the three tested duty cycles over time. For the shorter duty cycle (2.3%), the amplitude of the broadband noise is low but increases steadily with time. The cavitation activity slowly accumulates over the course of the treatment. For the longer duty cycle, the cavitation activity initially rapidly reaches steady state, but then gradually decays to a steady state that is higher than the baseline noise (18%). The ICD from the 9% duty cycle treatment was greater than that measured using the other two duty cycles (figure 3(b)).

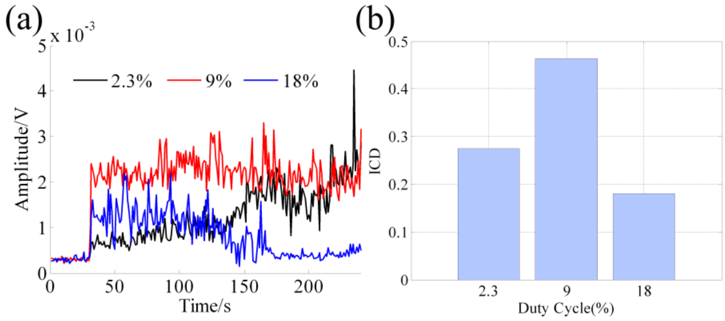

Figure 3. (a) Passively detected broadband noise emission over time and (b) ICD generated during the total treatment time as a function of duty cycle.

Figure 4 shows the evolution of the size distribution of cavitation bubbles for all three tested duty cycles. In figure 4 (a), when the duty cycle was 2.3%, the size distribution of the cavitation bubble from the 1st, 10^3th, 10^5th pulse increased slightly, but appears fairly smaller than the resonance radius of the emission frequency of the FUS transducer ($R_{res}≈3/f_0$, f_0= 1.2 MHz, vertical solid line in figure 4). When the duty cycle was 9%, the size distribution of the cavitation bubble remained nearly unchanged, right around the resonance radius (figure 4(b)). When the duty cycle was increased to 18%, the mean size of the cavitation bubble increased to a size that is substantially larger than the resonance radius (figure 4(c)).

It is known that periodic switching on and off of the sound field is a way to increase the efficiency of these cavitation-enhanced applications, the rationale behind which is to stabilize the bubble size distribution within the "active" radius range, which spans from the Blake-threshold to a radius within an order of magnitude of the linear resonance radius. When the duty cycle was too small, such as 2.3%, cavitation bubbles either dissolve completely or to a size below the threshold required for nucleation. Conversely, when duty cycle is too large, such as 18%, cavitation bubbles may grow too large during US sonication and reach a size beyond the range of "active" radii, resulting in a decrease of subsequent cavitation activity [5]. Therefore, determining whether bubbles induced by the previous round of US sonication are within the ''active'' size interval is helpful in optimizing the sonication scheme.

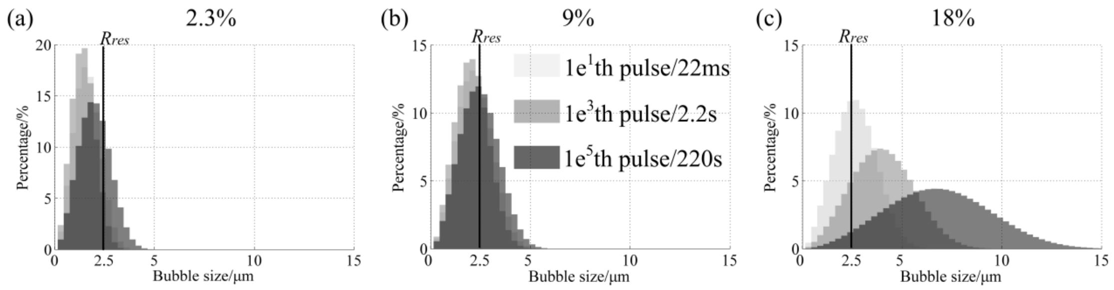

Figure 4. Size distributions of the induced cavitation bubbles after the 1st, 10^3th, 10^5th pulse at a duty cycle of (a) 2.3%, (b) 9% and (c) 18%.

4. Conclusions

It has been known that the number of clot debris particles was largest with a 9% duty cycle, while the mean diameter was smallest. Moreover, the size distribution of cavitation bubbles was mainly centred around the linear resonance radius of the emission frequency of the FUS transducer at 9% duty cycle, while the size distribution was centred around a smaller value for the 2.3% duty cycle, and a larger value for the 18% duty cycle. In addition, the ICD from the 9% duty cycle treatment was much higher than the ICD from the other two duty cycles. The results indicated that pulsing ultrasound enhances cavitation via controlling the size distribution of cavitation bubbles within the active size range, which was found to be near the linear resonance radius of the emission frequency of the FUS transducer.

References

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