Experimental investigation of microbubble response to ultrasonic pulses used in therapeutic applications

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Introduction

Research interest in the area of ultrasound contrast agents is shifting towards therapeutic applications such as targeted drug delivery, gene and DNA transfection [1], sonothrombolysis [2], and sonoporation [3]. To date however, the microbubble response to therapeutic-type excitations has not been studied in detail and it is not well understood. Often the conditions used in published works are simply a case of trial and error during which the microbubbles are subjected to pulses ranging from a few cycles [1] up to several thousand cycles [4].

In the present work, we present an in-vitro experimental method developed to examine the response of microbubbles to ultrasonic pulses of various amplitudes, pulse duration and pulse repetition frequency (PRF) in an attempt to find the optimal conditions to use in therapeutic applications. Two in-vitro setups are considered, a) with the microbubbles freely suspended in deionized water and b) with the microbubbles enclosed in a capillary.

Materials and methods

A schematic of the ultrasonic enclosure designed to accommodate two single element circular transducers (Panametrics-NDT, Waltham, Massachusetts, USA) is shown in figure 1. A focused 1.0 MHz transducer was used as the transmitter and another 2.25 MHz as the receiver. Care was taken so that the foci of the two transducers are placed in the same spot. An acoustic absorbing material lined the walls to minimize reflections and contamination of the scattered signal from microbubbles. In the setup with freely suspended microbubbles, a dilute concentration (36 bubbles/μL) of SonoVue (Bracco, Geneva, Switzerland) was used in order to achieve response from single microbubbles. Before each excitation, the solution was stirred and allowed to settle, enough for the bubbles to stop moving but not float. In the capillary setup, the microbubbles were allowed to flow in a 200 μm acoustically transparent cellulose capillary which was placed at the overlapping foci of the two transducers. This setup was designed to better imitate in-vivo conditions of microbubbles flowing in the microcirculation. Before each excitation, the flow was stopped for the bubbles to remain still inside the capillary. The pulse settings studied are: number of cycles (10-2000), MI (0.05-1.1) and PRF (0.05-1.0 KHz). For all experiments, an iU-22 scanner (Phillips, Bothell, WA, USA) was used to verify the uniformity of the solution. An example of the microbubble-filled capillary moments after a therapy pulse is fired is shown in fig.2. The area where the bubbles were destroyed is marked with a circle.

Figure 1: Schematic of enclosure

Figure 2: Microbubble disruption in capillary due to therapy pulse firing
Data analysis and results

The response of freely suspended microbubbles to a series of 1 MHz, 10-cycle tone bursts spaced 20 ms apart (PRF = 50 Hz) is shown in figure 3. MI 0.1, 0.2 and 0.4 are shown in fig.3 (a)-(c), respectively. The horizontal axis is time in milliseconds and the vertical axis is the scattered intensity in mV. At MI 0.1, the amplitude of response remains unaltered suggesting that the microbubble remained intact for the duration of the experiment. At MI 0.2, there’s a gradual decrease in amplitude of the response, suggesting disruption of the microbubble and gradual diffusion of the gas. Finally at MI 0.4, there is no response from the 2nd burst (detected signal is a reflection from the walls) suggesting that the microbubble is destroyed by the 1st pulse and completely diffuses away within 10 ms.

The response of microbubbles to such short tone bursts is of course very well known and previously published. The response to much longer pulses similar to those used in therapy, however, is not yet clearly understood. Figure 4(a) shows the response to a series of 500-cycle bursts at MI 0.2. At this pressure, bubble destruction and gradual diffusion is expected. Instead the signal seems to increase with time. Microbubbles are probably moving from the perimeter to the center of the detection area. Similar results that suggest motion of the microbubbles were seen even at lower pressures. The bubble response to 500-cycle bursts of MI 0.1 and 2000-cycle bursts of MI 0.05 are shown in figs. 4 (b) and (c), respectively. At such low pressures, the scattered signal intensity was expected to stay constant throughout the experiment instead of fluctuating considerably as seen in the figures. Microbubbles that move in and out of the center of the detection area can explain the fluctuations in both these cases. The motion of microbubbles during these therapy-type excitations can be attributed to acoustic streaming, the motion of the fluid in the direction of propagation of the sound [5]. Streaming was verified with ultrasound imaging where video loops of moving microbubbles during the excitations have been recorded. Since any motion of the microbubbles during an excitation affects the results, longer bursts using the freely suspended microbubbles setup were not investigated.

Streaming is successfully eliminated in the capillary setup and thus longer tone burst may be investigated. The capillary set-up better resembles in-vivo conditions where streaming does not occur in the microcirculation and microbubbles flowing in capillaries are not allowed to be “pushed” by the ultrasound. Figure 5 shows the response of microbubbles in the capillary to a series of 200-cycle tone bursts spaced 10 ms apart (PRF=100 Hz). MI 0.1, 0.2 and 0.4 are shown in fig. 5 (a)-(c) respectively. In a similar fashion to the freely suspended bubbles, we observe that MI 0.1 is non-destructive (intensity remains constant with time), MI 0.2 is semi-destructive (intensity gradually decreases), and MI 0.4 is highly destructive (bubble disappears by
the 2\textsuperscript{nd} pulse). At MI 0.4 the bubble scattered signal disappears completely half-way through the 1\textsuperscript{st} pulse (i.e. within 100 cycles or 100 $\mu$s). Two regions are selected within the 1\textsuperscript{st} pulse, one at the beginning and one at the end. A Blackman-Harris window is applied to the selected regions and their frequency content is plotted. The time domain and frequency spectrum of the first region is shown in figs. 5(d) and (e), respectively whereas the spectrum of the 2\textsuperscript{nd} region is shown in fig. 5(f). The absence of higher harmonics in fig. 5(f) verifies that the bubbles in the detection area have been destroyed.

Figure 5: 200 cycles, PRF=100 Hz, MI = 0.1(a), 0.2(b), 0.4(c). (d)-(e) time domain and spectrum of 1\textsuperscript{st} selected region of pulse at MI 0.4, (f) spectra of 2\textsuperscript{nd} selected region

Discussion

The response of microbubbles to a large range of pulse settings has been studied in two different experimental setups: (a) with the microbubbles freely suspended in deionized water and b) with the microbubbles enclosed in a capillary. We have shown that acoustic streaming occurs in the freely suspended microbubbles setup (during long, high energy pulses) and induces motion of the microbubbles that affects the results. Experimental setups similar to the one described here where the microbubbles are freely suspended in a medium (e.g. microbubbles in opti-cell) probably suffer from the similar problems.

Enclosing the microbubbles in a cellulose capillary eliminates acoustic streaming and allows a more accurate observation and measurement of the bubble response, while at the same time closely resembles the in-vivo scenario of microbubbles in the microcirculation. An MI less than 0.2 was found to be non-destructive while destruction begins at an MI 0.2. At MIs greater than 0.4, the microbubbles were destroyed and diffused within 100 cycles or 100 $\mu$s irrespective of the pulse length (number of cycles). Hence a question arises whether the use of longer ultrasound pulses at such high pressures is beneficial or not. The results shown in the present work refer to Sonovue. Experiments with polymer shelled and drug loaded microbubbles are underway.

References


