Destruction Threshold Parameters Estimation for Ultrasonic Contrast Agents

Chih-Kuang Yeh1,*  Shin-Yuan Su2  Wen-Shiang Chen3

1 Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu, Taiwan, 300 ROC
2 Department of Biomedical Engineering, Chung Yuan Christian University, Chung-Li, Taiwan, 320 ROC
3 Department of Physical Medicine and Rehabilitation, National Taiwan University Hospital, Taipei, Taiwan, 100 ROC

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Abstract

Ultrasonic contrast agents (UCAs) have been used to enhance the acoustic backscattered intensity of blood and thereby assist the assessment of blood perfusion. A unique characteristic of UCAs is that they can be fragmented after suitable acoustic excitation. Characterization of contrast agent destruction provides important information for the design of UCAs destruction/replenishment perfusion imaging. In this paper, we proposed an acoustic measurement procedure to obtain relevant threshold parameters including transmission acoustic pressures, pulse lengths and pulse frequencies for bubble destruction. The procedure not only provides a simple and convenient method to determine the bubble destruction threshold, but observes the destruction phenomenon of a group of microbubbles. The condition is more close to clinical usage of UCAs. Two agents with lipid- and polymer-shelled UCAs are introduced in this study. The results show that lipid-shell UCAs can be destroyed in response to a five-cycle pulse at a pressure approaching 0.6 MPa. The polymer-shelled UCAs are hard to be destroyed due to not significant oscillation in response to ultrasound pulse. Potential applications of this technique include UCAs high resolution destruction/replenishment imaging model, drug delivery and gene therapy.

Keywords: Ultrasonic contrast agents, Destruction/replenishment perfusion imaging, Destruction threshold

Introduction

Ultrasound contrast agents (UCAs) have been shown to be useful as a diagnostic tool in radiology. The UCAs are shell-encapsulated microbubbles and used to enhance backscattered echoes from blood. The most unique characteristic of UCAs is their nonlinear oscillation, which has been the subject of many experimental and theoretical investigations [1-4]. Nonlinear oscillations provide the opportunity to distinguish tissue and microbubble echoes based on their center frequency or response to the phase of the transmitted pulse [1-2].

Another unique characteristic of UCAs is that they can be fragmented after suitable acoustic excitation. Following a bubble radius expansion on the order of 300%, a microbubble will rapidly collapse and can divide into small fragments [5]. It is clear that contrast agents can be reliably destroyed using ultrasound pulses with a center frequency below 5 MHz [5-7]. Chomas et al. also found that decreasing center frequency reduces the threshold pressure require for microbubble destruction [5].

Many researches applied the UCAs fragmentation property estimation blood perfusion and as well as new techniques for drug delivery and targeted imaging [8-10]. The famous perfusion estimation model, UCAs destruction/replenishment method, was proposed by Wei et al [8]. Following destruction of the contrast agent, the flow of agents into the sample volume can be monitored over time to produce a local estimate of the velocity of flow in a region of the microvasculature. This replenishment of the contrast agent results in a gradual increase in the echo amplitude that is typically shown as a time-intensity curve (TIC). Therefore, characterization of contrast agent destruction provides important information for the design of this technique.

High-speed optical observation of an ultrasound contrast agent destruction during acoustic insonation was performed [5-7], [11-12]. Images captured by high-speed optical system show destruction of one microbubble during compression. The experiments results identified that pressure, center frequency, and transmission phase have a significant effect on the fragmentation threshold. The optical system provides a tool to observe destruction threshold of one bubble within the sample volume. Actually, commercial UCAs are designed to be delivered to a patient intravenously. A group of bubbles pass through the sample volume simultaneously and thus the bubbles destruction threshold should be discussed under this...
An acoustical method was designed to determine the threshold pressure for bubble destruction. A block diagram of the experimental system is shown in Figure 1. The setup consists of two single element transducers, a 25 MHz spherically focused transducer with a 0.25” element for imaging (model V324, Panametrics, Waltham, MA), and a 1 MHz spherical focused transducer (model V303, Panametrics, Waltham, MA) with a 0.5” element in charge of bubble destruction. Relevant specifications of the transducers are summarized in Table I.

The 25 MHz transducer is fixed at a 45-degree angle with respect to a 200-µm diameter cellulose tube (Spectrum Medical, Laguna Hills, CA) near the outlet of a tube, with the focal region aligned with the tube. The 1 MHz transducer is fixed approximately 7 cm upstream from the 25 MHz transducer on the inlet side of the tube, perpendicular to the tube, and with the tube perpendicular to the line defining the focus. Acoustically-absorbent rubber is placed at the bottom of the tank to minimize reflections.

An arbitrary waveform generator (model HP 33120A, Hewlett-Packard Company, Palo Alto, CA) is used to generate 1 MHz tone bursts with 1-10 cycles, and an RF power amplifier (model 3200L, ENI, Rochester, NY) amplifies the pulses to produce the corresponding acoustic pressures ranging from 0 to 0.6 MPa. The commercial contrast agent Definity® (Bristol-Myers Squibb Medical Imaging Inc., N. Billerica, MA) is used at a concentration of 6 µL Definity®/10 mL water (3 mL/5 L blood volume). Definity® is a lipid-shelled microbubbles with the size range from 1.6 to 2.4 µm. The bubble concentration in the 200 micron tube is no greater than 2400 bubbles per microliter. This concentration converts to no more than 7.5 bubbles per 100 microns of linear tube length. Since the beam width of the 25 MHz transducer is 120 microns, there are fewer than 9 bubbles in the sample volume. At the pressure and frequency used in this study, the backscatter is predominantly linear. Therefore, it is safe to assume that with the low concentration of 9 bubbles, the backscattered Doppler power is proportional to the number of bubbles within the sample volume. The destruction threshold does depend on concentration, and the concentration used in the following destruction threshold experiments is conservatively on the higher side than is expected for the microcirculation. A syringe pump regulates the flow rate of contrast agent solution through the tube at 1 mL/hr (8.9 mm/s average). The 1 MHz pulses are transmitted with a pulse repetition frequency (PRF) of 50 Hz, where primary and secondary radiation forces are insignificant [14].

Simultaneous with transmission at 1 MHz, the 25 MHz transducer operates in a pulse-echo mode to detect the
presence of bubbles in the tube. Transmission by the 25 MHz transducer is accomplished using a pulser/receiver (model 5900PR, Panametrics, Waltham, MA) through a transformer diplexer/diode limiter circuit at a PRF of 1 kHz. The received echoes are amplified by 31 dB with a low-noise pre-amplifier (model AU-1114-BNC, Miteq, Hauppauge, NY) and then further amplified or attenuated by the pulser/receiver. The RF echoes are digitized at 125 MSPS using a high-speed 12-bit A/D board (model PDA12A, Signatec, Corona, CA) and stored in M-mode format on a PC for offline processing in Matlab® (Mathworks, Natick, MA).

**Results for the Threshold of Destruction**

The effect of pulse length and acoustic pressure at 1 MHz on microbubble destruction were tested using 1, 3, 5, and 10 cycles and 0-0.6 MPa, respectively. One M-mode recording of 256 axial samples (1.5 mm) for each pulse, and 2048 pulses (2.048 seconds) was acquired for each pressure and pulse length setting, and divided into 8 groups of 256 pulses for statistical analysis. A second order IIR high-pass filter was used to remove stationary echoes from the wall of the tube, as well as wall reverberation echoes that appeared directly below the tube. The filter is a 2nd order Butterworth high-pass with a cutoff frequency of 20 Hz. Figures 2(a) and (b) show the RF and wall-filtered baseband M-mode images, respectively. In Fig. 2(a), the wall reverberation signal appears at a depth of 0.35 mm. The pulsatile modulation over time evident in the echo amplitudes in Fig. 2(b) was produced by the syringe pump.

Post-wallfiltered Doppler power, defined as the square of the baseband signal amplitude, was used to assess microbubble destruction in this experiment. The bubbles were considered destroyed when the echo Doppler power received with the imaging pulses was less than 1% of that recorded before microbubble destruction. The determination of 1% as threshold of complete bubbles destruction is according to the assessment the Doppler power of background noise. Since microbubble echoes are detected far above their linear resonance frequencies, and the microbubbles are small compared to a wavelength at 25 MHz, we assume that they behave as Rayleigh scatterers. Thus, backscattered Doppler power is proportional to the number of intact microbubbles within the tube. Post-wallfiltered Doppler power is first integrated over time, as shown in Fig. 2(c), and the resulting curve is integrated over depth. The integration was performed over a region windowed in depth to reject duplicate echoes due to reverberation. For consistency, the same windowed region was used for all data.

Figures 3(a)-(d) show the post-wallfiltered Doppler power integrated over the pulse index (time in Figure 2) as the 1 MHz pulse length is varied between 1, 3, 5, and 10 cycles, respectively, and the acoustic pressure is varied from 0 to 0.6 MPa. The error bars show one standard deviation estimated from 8 M-modes recorded at each setting. The destruction thresholds of Figs. 3(a)-(d) correspond to 0.63, 0.53, 0.52, and 0.43 MPa for the 1, 3, 5, and 10 cycle pulses, respectively. The results indicate that the threshold of bubble destruction decreases with increasing pulse length.

In order to observe the relationship between frequency of transmitted pulse and bubble destruction, a 2.25 MHz transducer (model V305, Panametrics, Waltham, MA) was used to substitute for 1 MHz transducer in above mentioned experiments. All experimental parameters and setup are the same with previous one except the destruction pulses...
transmitted with a PRF of 100 Hz. The destruction thresholds of Figs. 4(a)-(d) correspond to 0.65, 0.56, 0.53, and 0.48 MPa for the 1, 3, 5, and 10 cycle pulses, respectively. The pressure threshold of bubble destruction in 2.25 MHz transmitted pulse is higher than that in 1 MHz transmitted pulse when the pulse cycle is the same.

Results for Sub-Micron Ultrasonic Contrast Agents

The sub-micron ultrasonic contrast agents examined in this study are manufactured by POINT Biomedical Corporation and are nitrogen-filled microbubbles with a bilayered bi-Sphere® shell composed of a biodegradable polymer and human serum albumin. Manipulations in the manufacturing process of these agents can generate structures varying in size and wall thickness. Two agents were used in this study (M1134 and M1090) that have identical shell formulations and peak volumetric diameters of 0.74 µm, 0.50 µm. For clarity, these two agents will be referred to in this paper as P-074 and P-050. The size distributions of these agents were measured with a Horiba LA-920 laser diffraction particle size analyzer (Horiba Laboratory Products, Irvine, CA). Contrast agent suspensions were prepared by reconstitution of one vial of lyophilized agent with 2 mL of distilled water. These preparations were further diluted by a factor of 10,000 (1.0 µL in 10 mL water) for use in the acoustic experiments.

In the experiments, a 50 MHz transducer with a 0.25" element (model V358-SU, Panametrics, Waltham, MA) was introduced to replace the 25 MHz transducer in charge of imaging Doppler power. The 50 MHz transducer provides a better spatial resolution of 50-100 µm and higher backscatter sensitivity. The experimental setup was similar with previous one. The 2.25 MHz transducer was responsible for the bubbles destruction. Figures 5 (a)-(d) show the results of P-074 with 1, 3, 5 and 10 cycle transmitted pulses, respectively. In the cases of 1 and 3 cycle pulses as shown in Figs. 5(a) and (b), the wall-filtered echo Doppler power is not down to zero even the transmitted pulse with an acoustic pressure to 2.95 MPa. The destruction thresholds of Figs. 5(c)-(d) correspond to 2.75 and 2.13 MPa for the 5 and 10 cycle pulses, respectively.

Figure 6 shows the results of P-050. The results indicate that the Doppler power decreases with increasing pulse length. As shown in Figs. 6(a)-(d), the P-050 agent can not be completely destroyed by the 2.25 MHz transducer with 1 to 10 cycle transmitted pulses. The possible reasons include the P-050 agent does not oscillate significantly in response to ultrasound and the polymer shell is hard to be destroyed.
Conclusions

In this paper, we proposed an acoustic measurement procedure to obtain a pressure and pulse cycle threshold for the destruction of ultrasonic contrast agents. The first experiments results identify that destruction pulse transmitted in lower frequency is easier to destroy microbubbles. Besides, two agents with lipid- and polymer-shelled were introduced to perform the relationship between bubbles shell property and destruction threshold. The results demonstrate that the lipid-shell contrast agents are easier to be destroyed in response to a five-cycle pulse, at a pulse pressure approaching 0.6 MPa, and at two-cycle excitation pulse with a center frequency of 2.25 MHz [5-6].

On the contrary, the polymer-shelled sub-micron bubbles are not completely destroyed up to an acoustic pressure of 2.8 MPa. Acoustic experiments demonstrate that the bubble destruction threshold of a lipid-shelled microbubble contrast agent in response to an acoustic pulse is quantitatively different from that of a polymer-shelled agent. The polymer-shelled bubbles results with optical-based measurements, the destruction threshold was hard to observe due to the spatial resolution limitation of optical system [13].

Comparison with the optical-based measurements, the acoustic measurement not only provides the simple and convenient method to determine the bubble destruction threshold, but observes the destruction phenomenon of a group of microbubbles. It is more close to the real usage of ultrasonic contrast agents in human body. In addition, the high-speed optical system can not clearly observe the sub-micron bubbles destruction due to the limitation of spatial resolution [13]. Potential applications of this technique include microbubbles destruction/reperfusion blood perfusion model, drug delivery and gene therapy.

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