Multifunctional hard-shelled microbubbles for differentiating imaging, cavitation and drug release by ultrasound

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SUPPORTING INFORMATION

1. Materials

n-Butyl cyanoacrylate (BCA) was purchased from Pansine Chemical Co. (China). Triton X-100, hydrochloric acid, sodium hydroxide and gelatin were acquired from Aladdin (China). Sonovue was purchased from Bracco Suisse SA (Switzerland), and Xueruixin (XRX) was acquired from Runkun Pharmaceutical (China). All commercially available chemicals and reagents were used without further purifications. Ultrapure deionized Milli-Q water was used for all experiments and stock solutions.

2. Methods

2.1 Synthesis of poly n-butyl cyanoacrylate microbubbles (PMBs)

The polymeric microbubbles of PMBs were synthesized with a slightly modified protocol as previously described.[S1] Briefly, ultrapure deionized water (100 mL) was stirred at 3,000 rpm by mechanical agitation using a high-speed dispersion machine (Ultra Turrax T25 digital, IKA, Germany) at room temperature in a 250 mL beaker. Triton X-100 (1.0 mL, 1.7 mmol) was added dropwise when stirring robustly. Hydrochloric acid solution (1.0 M) was added to adjust the pH value to 2.0. After stirring for 5 min, BCA (1.0 mL, 9.4 mmol) was injected dropwise by a syringe. With the agitation speed slowly increased to 10,000 rpm, the mixture was stirred for a further 1.5 h. Afterwards, NaOH aqueous solution (1.0 M) was added to quench the polymerization before the resulted emulsion was transferred into centrifuge tubes and subjected to centrifugation using a high-speed freezing centrifuge (Sartorius Sigma 3-30 KS, Germany). For the size-isolation of PMBs, the centrifugation speed was set to 500 rpm for 3×20 min. The semi-solid layer (PMBs cake) at the top of the solution was carefully collected with a medical ladle, and it was then re-dispersed in Trion X-100 aqueous solution (30 mL, 0.02% (w/v), pH = 7.0) and stored in a refrigerator at 4 °C.

2.2 Characterization of hard-shelled polymeric microbubbles (PMBs)

The bright-field microscopy was performed on an optical microscope (Axio Lab A1, Carl Zeiss GmbH, Jena, Germany). The scanning electron microscope (SEM, Hitachi, Japan) was utilized to acquire the images and visualize the surface morphology and bubble size of PMBs. In addition, the shell thickness of PMBs could be roughly evaluated by SEM images while the PMBs were not stabilized with poly vinyl pyrrolidone (PVP) and disintegrated due to an SEM vacuum environment. The size distribution of PMBs was measured on a Beckmann Coulter Counter (Multi-sizer 4e

Beckmann Coulter Inc., USA). The surface charge and stability of PMBs could be assessed by measuring their zeta potentials using a dynamic light scattering (DLS) system (Zetasizer Nano ZS, Malvern Instrument Inc., Worcestershire, UK). All measurements were performed by three replications, and the results were reported in a standardized format as Mean ± SD.

2.3 Evaluation of cavitation effects at varied US frequency and acoustical power at the presence of PMBs

In a bid to investigate the factors that influence the cavitation effects at the presence of PMBs, US imaging was performed on a phantom prepared by dispersing PMBs in aqueous gelatin solution (2.5% (w/v), [MB] = 1,500 MBs/μL).[S1] US-triggered cavitation was performed using VINNO70, a Color Doppler US system (VINNO, Suzhou, China). Region of interest (ROI) was chosen where the PMBs were uniformly dispersed. In order to acquire US images of ROI pre-cavitation, an US transducer was placed over the phantom with the employment of coupling gels. At the contrast bubble imaging (CBI) mode, the cavitation experiments were performed by modulating US frequency at 1.7, 2.0, 2.5, 3.3 MHz (by a convex transducer S1-8C) and 3.0, 3.7, 4.0, 5.0, 6.3 MHz (by a linear transducer X4-12L). At each frequency, the acoustical power was adjusted from 5% to 100% in 5% increments. Pulse repetition frequency (PRF), pulse length and duration time were maintained at 10 Hz, 26 cycle and 12 s, respectively. As for low frequencies at 1.7 MHz and 2.0 MHz, the pulse length was set to the optional maximum value 17.5 and 21 cycle. The duty cycle value was calculated as follows:

$$\text{Duty cycle} = (1/\text{frequency} \times \text{pulse length}) / (1/\text{PRF}) \qquad \text{(Equation 1)}$$

The US images of ROI post-cavitation were recorded after US-triggered cavitation, and US signal intensity (SI) of pre- and post-cavitation could be read out. Contrast intensity (CI = SI_{pre} - SI_{post}) and %decrease (%decrease = CI/SI_{pre} × 100%) were accordingly calculated to quantitatively evaluate the cavitation effects.

2.4 Evaluation of cavitation effects at varied US pulse duration time at the presence of PMBs

To investigate the time dependency of cavitation effect at the presence of PMBs, the US-triggered cavitations were performed with a convex transducer (S1-8C) at a frequency of 3.3 MHz and an acoustical power of 60% as the optimized conditions. The US imaging phantom was prepared as aforementioned, and ROI was chosen where the PMBs were uniformly dispersed. The transducer was placed vertically above the gelatin phantom in parallel experiments with the US irradiation time varied from 2 to 44 s. The US images of ROI pre- and post-cavitation were acquired, and the time-SI and time-%decrease curves were generated to analyze the time dependency that affects the cavitation effects.

2.5 Comparison of cavitation effects at the presence of PMBs vs. Sonovue and XRX under optimized conditions

To evaluate the cavitation effects at the presence of microbubbles composed of different shell materials, polymeric MBs (PMBs) were selected to compare with commercially available lipid- and protein-based Sonovue and XRX. Two frequencies (3.0 MHz and 5.0 MHz that are commonly used in clinics) and varied acoustical powers (10%, 30%, 60% and 10%, 40%, 80%, respectively) that result in none, partial and complete cavitations were selected. 0.5 mL of MBs with a concentration of 1×10^9 MBs/mL for all cases of PMBs, Sonovue, XRX were injected into deionized water

(500 mL) in a cubic container, and a linear transducer (X4-12L) was placed in the water with a depth of 6.0 cm and a single-point focus of 1.2 cm. ROI was chosen where the PMBs were uniformly dispersed. The US images during the whole cavitation progress before and after a single pulse were recorded, and the average gray-scale values of ROI were calculated by deducting backgroud gray-scale from the gray-scale values pre- and post-US pulses on Image J software. %Decrease was calculated in gray-scale as: %decrease = (gray-scale$_{post}$ - gray-scale$_{pre}$) / gray-scale$_{pre}$ × 100%. All tests of each sample were performed by three replications, and the results were reported in a standardized format as Mean ± SD.

2.6 Statistical analysis

Differences between the experimental groups were analyzed via a standard Student's *t*-test. A p value < 0.05 was considered to be statistically significant.

Reference

S1. Z. Liu, T. Lammers, J. Ehling, S. Fokong, J. Bornemann, F. Kiessling and J. Gaetjens, *Biomaterials* 2011, **32**, 6155-6163.

Table S1. Duty cycles calculated with varied US frequency and pulse length screened in this study.

Transducer	S1-8C				X4-12L				
Frequency(MHz)	1.7	2	2.5	3.3	3	3.7	4	5	6.3
Pulse repetition frequency (Hz)	10								
Pulse length (cycle)	17.5	21	26						
Duty cycle (%)	0.01029	0.01050	0.01040	0.00788	0.00867	0.00703	0.00650	0.00520	0.00413

Table S2. Mechanical index (MI) values varied with US frequency and acoustical power that were screened in this study.

Transducer	S1-8C				X4-12L				
Frequency (MHz) / Acoustical power	1.7	2	2.5	3.3	3	3.7	4	5	6.3
	Mechanical Index (MI)								
100%	1.4	1.4	1.4	1.4	0.91	1.05	1.4	1.4	1.4
95%	1.33	1.34	1.34	1.34	0.88	1.02	1.34	1.35	1.35
90%	1.27	1.27	1.27	1.28	0.81	0.99	1.29	1.29	1.29
80%	1.13	1.13	1.14	1.16	0.78	0.93	1.16	1.19	1.19
70%	0.99	0.99	1.02	1.03	0.75	0.73	1.03	1.07	1.09
60%	0.85	0.87	0.88	0.88	0.65	0.66	0.91	0.94	0.94
50%	0.7	0.72	0.74	0.76	0.53	0.59	0.75	0.82	0.83
40%	0.56	0.6	0.6	0.63	0.43	0.51	0.6	0.67	0.67
30%	0.43	0.43	0.46	0.48	0.41	0.43	0.47	0.5	0.54
20%	0.28	0.29	0.29	0.32	0.27	0.3	0.32	0.36	0.36
10%	0.14	0.14	0.15	0.16	0.14	0.18	0.17	0.17	0.23
5%	0.07	0.07	0.08	0.08	0.08	0.1	0.08	0.09	0.12

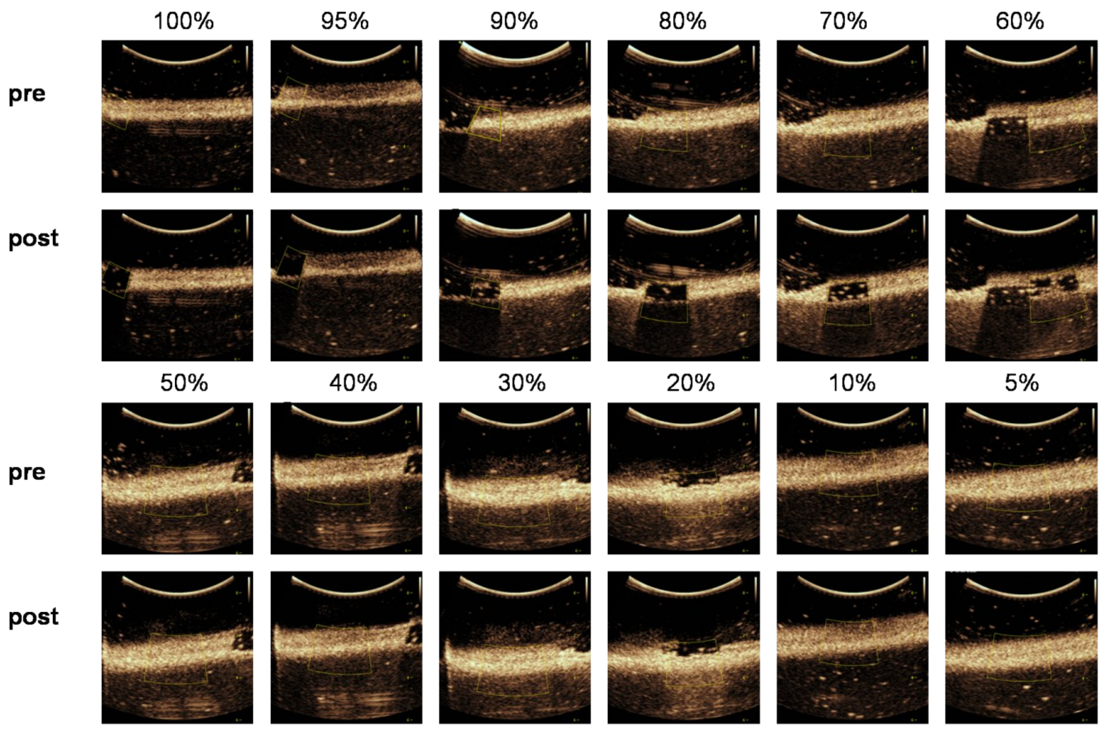

Fig. S1 The US-triggered cavitation effect and US images at the presence of PMBs at a frequency of 1.7 MHz using a convex transducer of S1-8C (yellow line-confined area indicates ROI).

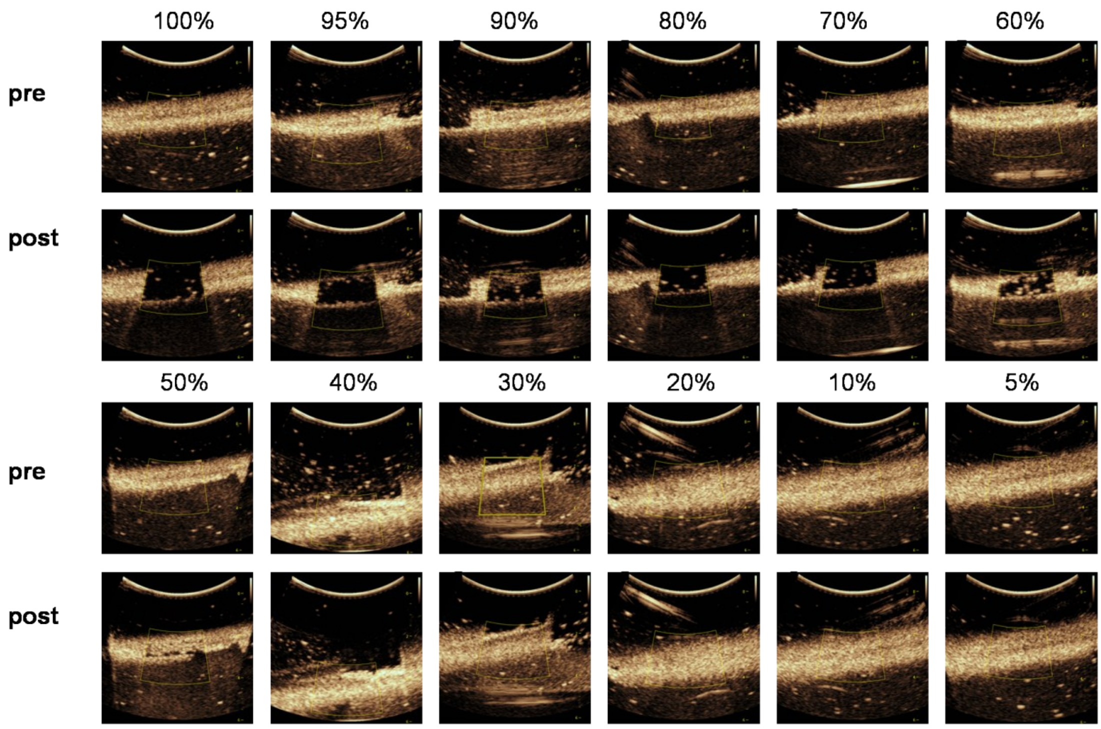

Fig. S2 The US-triggered cavitation effect and US images at the presence of PMBs at a frequency of 2.0 MHz using a convex transducer of S1-8C (yellow line-confined area indicates ROI).

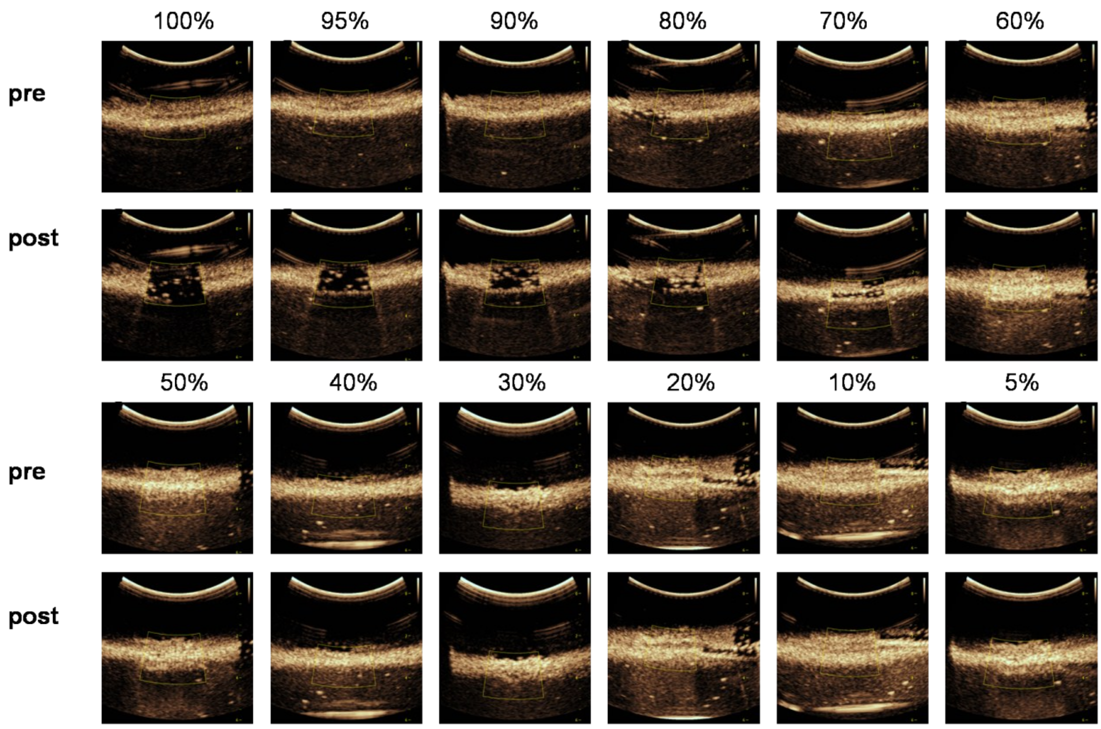

Fig. S3 The US-triggered cavitation effect and US images at the presence of PMBs at a frequency of 2.5 MHz using a convex transducer of S1-8C (yellow line-confined area indicates ROI).

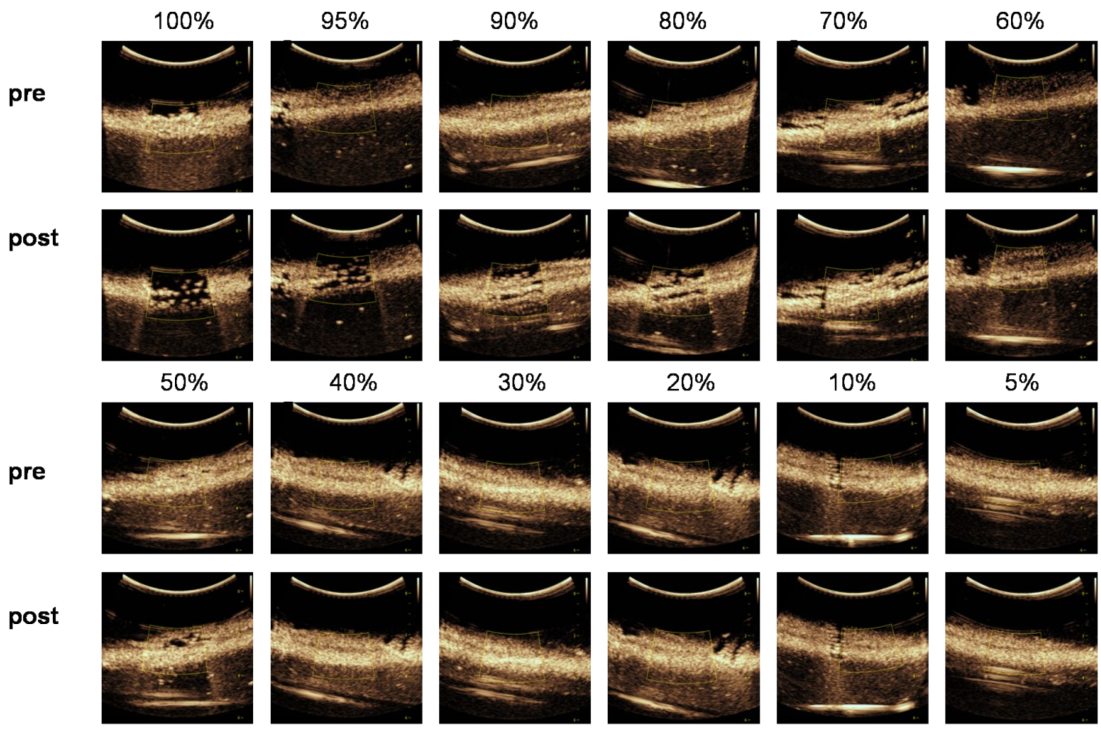

Fig. S4 The US-triggered cavitation effect and US images at the presence of PMBs at a frequency of 3.3 MHz using a convex transducer of S1-8C (yellow line-confined area indicates ROI).

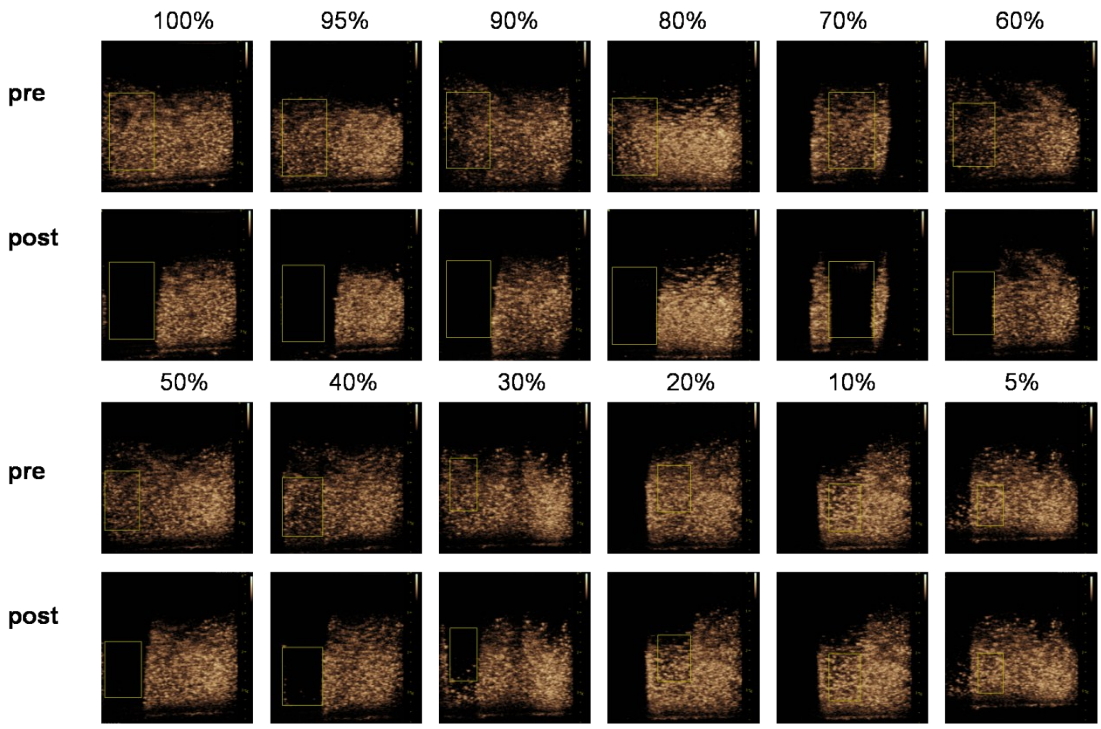

Fig. S5 The US-triggered cavitation effect and US images at the presence of PMBs at a frequency of 3.0 MHz using a linear transducer of X4-12L (yellow line-confined area indicates ROI).

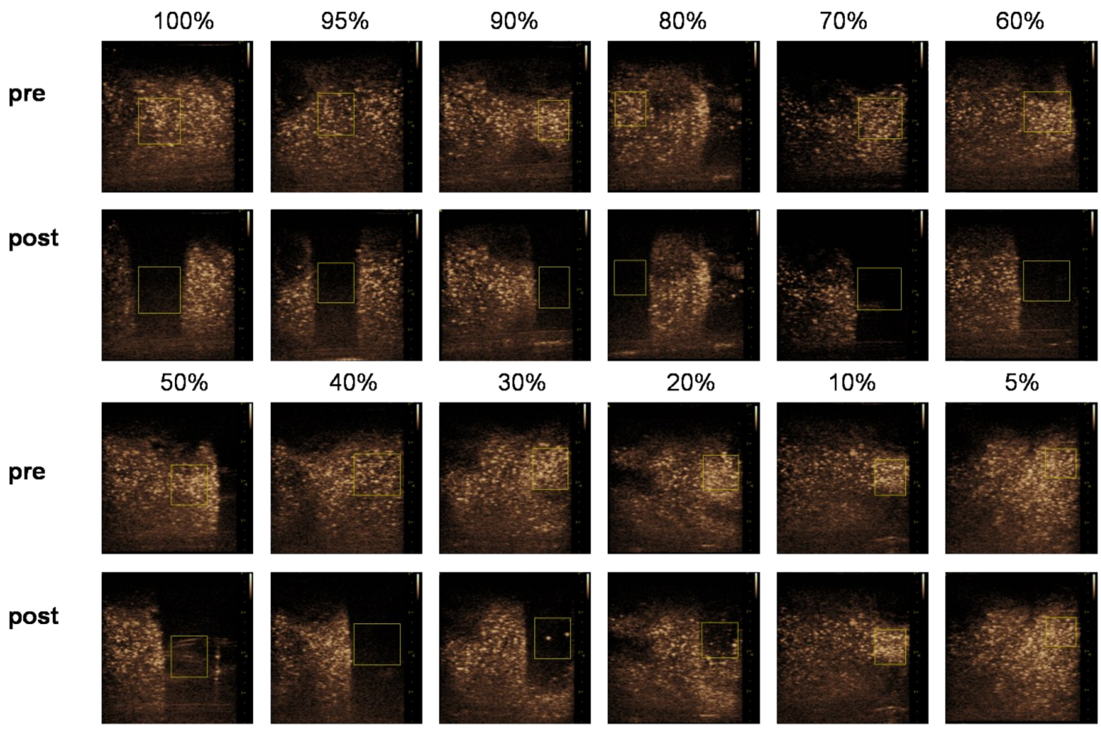

Fig. S6 The US-triggered cavitation effect and US images at the presence of PMBs at a frequency of 3.7 MHz using a linear transducer of X4-12L (yellow line-confined area indicates ROI).

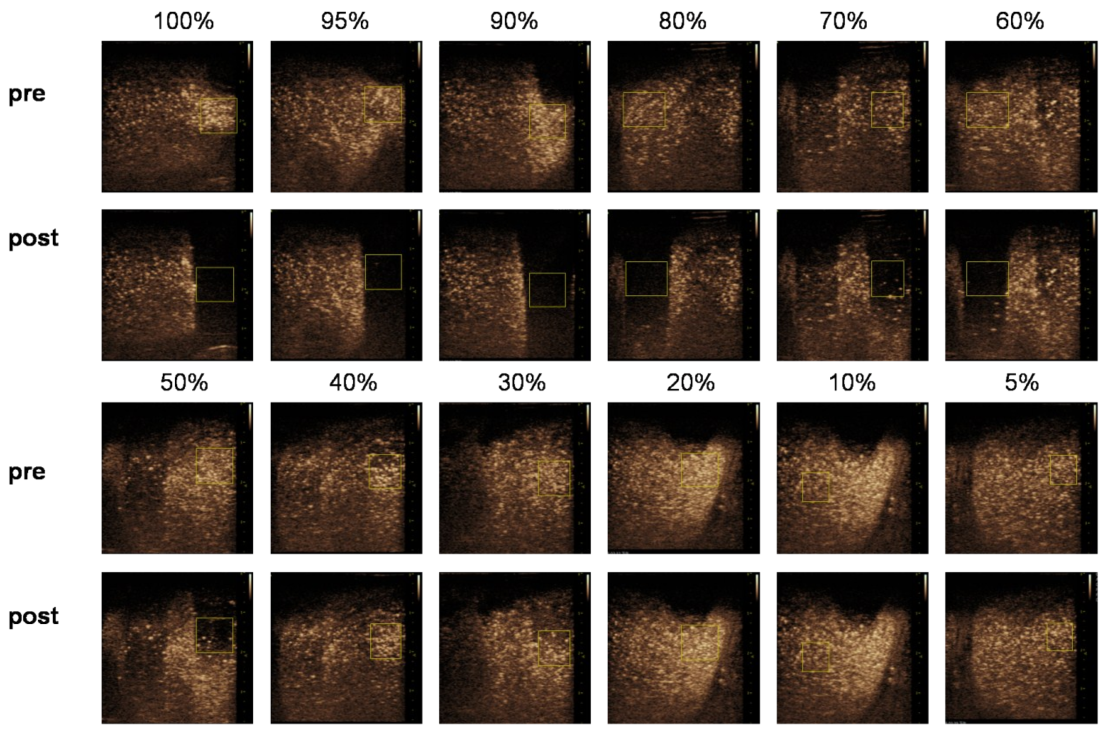

Fig. S7 The US-triggered cavitation effect and US images at the presence of PMBs at a frequency of 4.0 MHz using a linear transducer of X4-12L (yellow line-confined area indicates ROI).

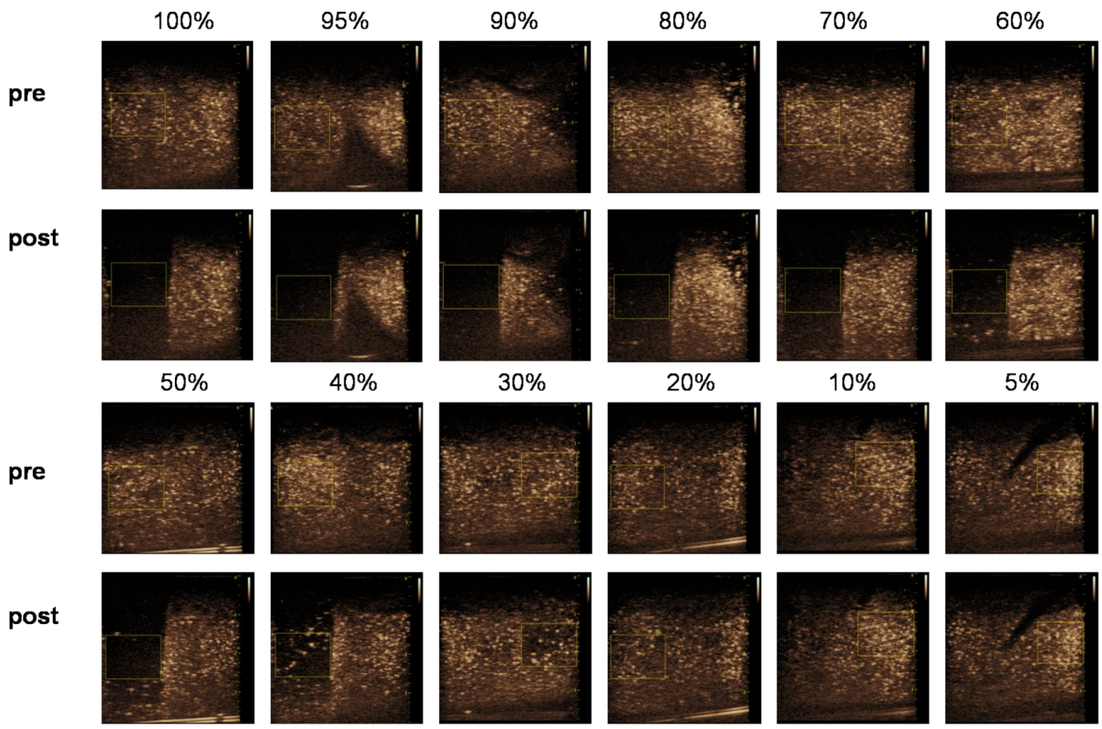

Fig. S8 The US-triggered cavitation effect and US images at the presence of PMBs at a frequency of 5.0 MHz using a linear transducer of X4-12L (yellow line-confined area indicates ROI).

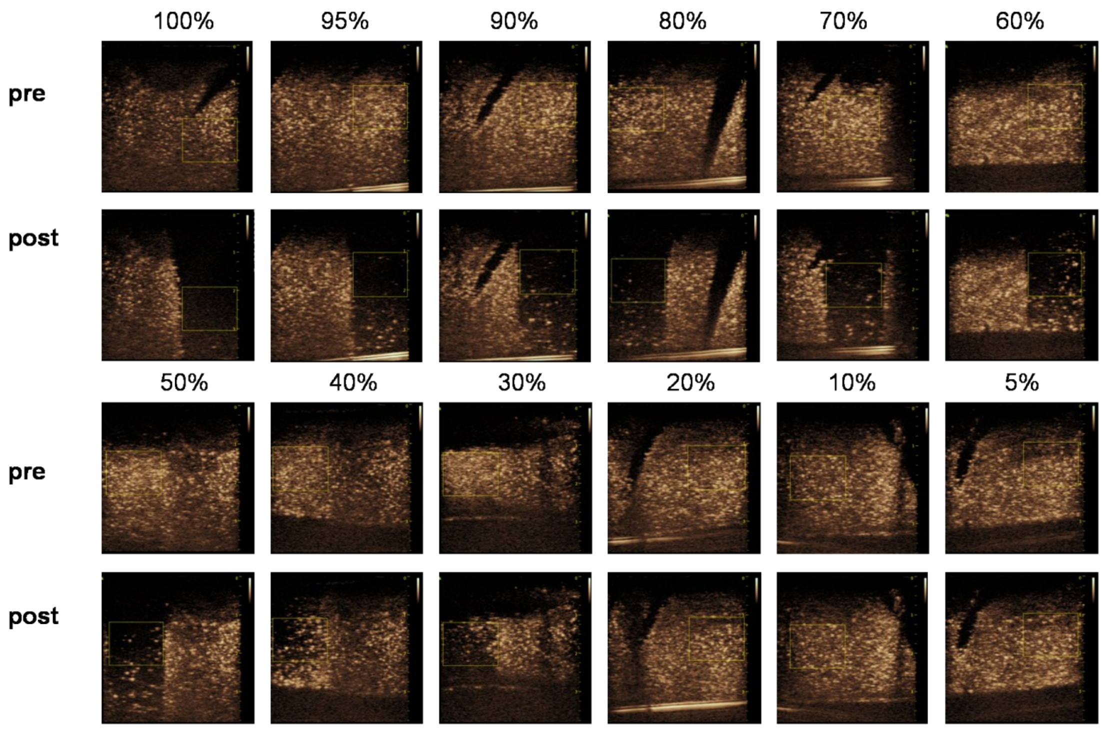

Fig. S9 The US-triggered cavitation effect and US images at the presence of PMBs at a frequency of 6.3 MHz using a linear transducer of X4-12L (yellow line-confined area indicates ROI).

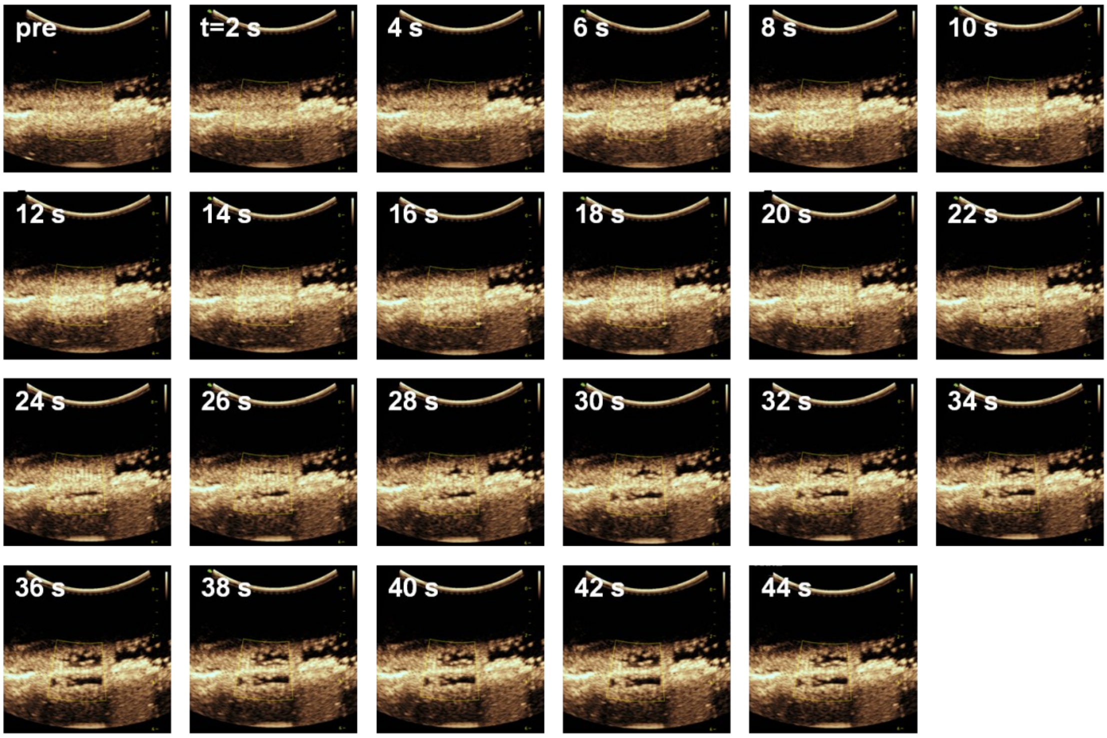

Fig. S10 The US images at varied pulse duration time for the evaluation of cavitation effects by using a convex transducer S1-8C. (US frequency: 3.3 MHz, acoustical power: 60%, US images recorded through a duration time of 44 s, yellow line-confined area indicates ROI)