

MRI MONITORING OF ULTRASOUND-TARGETED MICROBUBBLES DESTRUCTION

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INTRODUCTION

Gas-filled microbubbles were originally developed as intravascular contrast agents for ultrasound imaging. Ultrasound-targeted Microbubble Destruction (UTMD) has been used to deliver genes or drugs to specific tissues¹⁻⁵ utilizing microbubble cavitation and sonoporation effect through guidance of ultrasound imaging, including skeletal muscles, myocardium, kidney, neural tissues, vessels, and tumors. However, ultrasound imaging cannot adequately visualize various soft tissues for the specific site and regions behind lung and bone cannot be easily imaged; and application of any imaging ultrasound can cause the microbubbles within imaging view to oscillate in volume or cavitate, leading to global sonoporation or cavitation and non-site-specific therapeutic effect. In comparison, MRI is capable of imaging anatomical structures with high spatial resolution, and it does not cause microbubble sonoporation and cavitation. Gas-filled microbubbles have been shown as an MR susceptibility contrast agent *in vivo*⁶. We hypothesize that the cavitation process can be visualized by MRI because microbubble disappearance will diminish its susceptibility effects. Such MRI visualization may provide the most effective imaging guidance for UTMD-based genes or drugs delivery. In this study, we aim to demonstrate that microbubble ultrasound cavitation can be monitored using MRI through an *in vitro* phantom study.

METHODS

Synthesis of albumin coated microbubbles: Albumin coated microbubbles were produced by sonication, in principle as described by Cerny et al.⁷. Briefly, a 5% solution of bovine serum albumin (B4287, Sigma, St Louis, MO) was preheated to about 70°C and sonicated under aseptic conditions using an ultrasound frequency of 20kHz. Concentration of the microbubbles suspension was carried out by draining off the excess albumin solution from below the floated microbubbles. The microbubble suspension was filled into a plastic phantom tube of 12 mm in diameter, with 2-5% in volume fraction.

Ultrasound Targeted Microbubble Destruction: UTMD was performed using ultrasound transducer of 5 MHz center frequency (A309S-SU, Panametrics, Waltham, NY). An arbitrary waveform generator (33120A, Hewlett Packard, Palo Alto, CA) was used to generate the ultrasound waveform that was amplified by an RF amplifier (320L, ENI, Rochester, NY). The ultrasound transducer was mounted at one end of the plastic phantom tube for ultrasound irradiation inside the magnet. Ultrasound cavitation of albumin coated microbubbles was carried out by applying 5 MHz 10 V peak-to-peak input to the transducer for 5 minutes.

MRI: MRI was performed on a 7 Tesla scanner (Bruker PharmaScan®). The microbubble phantom was gently mixed for 2 minutes outside the magnet prior to MR measurement. The phantom was then continuously stirred by rotation inside the magnet to ensure uniform suspension of microbubbles, and mounted firmly immediately before the imaging sequence started. T₂*-weighted images were acquired before and during the ultrasound irradiation with 2D FLASH sequences (TR/TE = 20/15 ms and 35/30 ms, 64 x 64 matrix, slice thickness = 1 mm, FOV = 3.00 cm, temporal resolution = 960 ms and 1680 ms, NEX = 1).

Data Analysis: Images were analyzed using ImageJ. The signal intensities were measured by drawing circular region-of-interest (ROI) in the images before and after ultrasound irradiation. Percentage changes were calculated for the ultrasound cavitation process.

RESULTS AND DISCUSSIONS

The MR images of the microbubble phantom before and after UTMD were depicted in Figure 1. Signal intensities were observed to increase after UTMD. The low signal intensities in Figure 1(a) and (c) were caused by the induction of local magnetic perturbation and microscopic susceptibility effect of the gas-filled albumin coated microbubbles. Note that the lower parts of the phantom were of higher signal intensities, likely due to the upward migration of microbubbles with the buoyancy force after the phantom was positioned firmly; thus resulting in lower concentration of microbubbles and hence higher signal intensities. After the UTMD with ultrasound irradiation, signal intensities shown in Figure 1(b) and (d) were increased due to microbubble destruction and diminished susceptibility effect. Intact albumin coated microbubbles were observed before ultrasound irradiation (Figure 2(a)) and no microbubbles were observed after ultrasound irradiation (Figure 2(b)) under microscope, confirming UTMD was successfully carried out by ultrasound irradiation. The signal intensities before UTMD, after UTMD and its percentage changes were tabulated in Table 1. Percentage changes were 147.69% and 317.83% for TR/TE = 20/15 ms and 35/30 ms respectively, suggesting UTMD can be captured using MRI sensitively via signal intensities measurements.

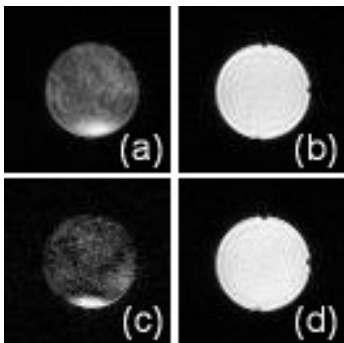


Figure 1 T₂*-weighted MR images of the plastic phantom tube of TR/TE = 20/15 ms (a) before and (b) after Ultrasound-targeted Microbubble Destruction; TR/TE = 35/30 ms (c) before and (d) after Ultrasound-targeted Microbubble Destruction.

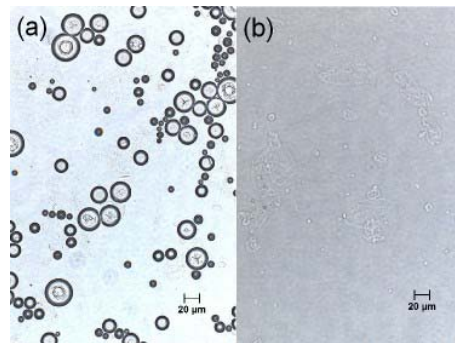


Figure 2 Micrographs show (a) intact albumin coated microbubbles before ultrasound irradiation; and (b) no microbubbles after ultrasound irradiation.

Table 1 Signal intensities before Ultrasound-targeted Microbubble Destruction, after Ultrasound-targeted Microbubble Destruction for TR/TE = 20/15 ms and 35/30 ms in mean ± standard deviation and their percentage changes.

	Signal Intensities (Arbitrary Units)		
	Before Cavitation	After Cavitation	Percentage Changes
TR/TE = 20/15 ms	11615.60 ± 2024.91	28770.34 ± 702.70	147.69%
TR/TE = 35/30 ms	7167.25 ± 2420.99	29947.21 ± 674.49	317.83%

CONCLUSIONS

In this study, we demonstrated for the first time that MRI monitoring of microbubble cavitations and UTMD is feasible. With such approach, real-time monitoring of UTMD *in vivo* can be potentially achieved through dynamic imaging, leading to a better guidance technology in UTMD-based therapeutic applications without influencing microbubble sonoporation and cavitation. Work is currently underway to characterize the microbubble MR properties and to improve the temporal resolution of dynamic imaging. In addition, by designing gas-filled microbubbles with contrast-embedded albumin shells, microbubble susceptibility can be improved⁸ so that microbubbles and their cavitations can be monitored with high sensitivity and low concentrations.

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