Real-time Monitoring of Cavitation Effect of Microbubbles by MRI: *In Vitro* Experiments

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Introduction: Within the central nervous system, the blood–brain barrier (BBB) excludes larger (>400 Da) molecules from entering the brain parenchyma [1], and it prohibits delivery of many potentially effective diagnostic or therapeutic agents. To increase the permeability of the BBB, using focused ultrasound (FUS) in the presence of microbubbles (MBs) is a well-known strategy for local, non-invasive, transient, and reversible BBB disruption [2]. In addition to utilize another single-element transducer for monitoring of BBB-opening process [3], using MRI-guided system [4] has a potential to guide the process as well. Gas-filled MBs itself can potentially be used as a unique MR contrast agent applied on animal model because of their magnetic susceptibility effect and localized manipulation via FUS cavitation [5]. Nevertheless, the technology of real-time monitoring of MBs cavitation is not established. In this study, we aim to investigate how MR signal intensity (SI) of MBs changes before, during, and after MBs cavitation. Different signal changes with varied conditions of MBs concentration and FUS power were demonstrated.

Methods and Materials: The mean diameter of homemade MBs was 0.92 um and the concentration of MBs was $(4.36\pm0.32)\times10^{10}$ droplets/mL. To prepare MBs with different concentration, MBs were diluted with normal saline (NS) to 0.2 X (20%MBs+80%NS) and 0.05 X. A single-element focused piezoelectric transducer (central frequency 1.85 MHz, 10 cm diameter, 12.5 cm curvature, Imasonic, Besancon, France) was immersed in 37 ^{o}C degassed water and was used as the source of sonication. All MR images were acquired on a 3T clinical imager (Siemens Trio, Erlangen, Germany). The pulse sequence of Half Fourier Acquisition Single Shot Turbo Spin Echo (HASTE) [6] was used to real-time monitoring of signal changes of MBs during performing FUS. Imaging parameters were TR=3000 msec, TE=69 msec, flip angle=180^{o}, FOV=159*200 mm^2, matrix size=256*204, slice thickness=5 mm. All images were acquired at the focal plane and were perpendicular to the direction of ultrasound beams. MBs and NS were injected into chambers in a gel phantom, which was mounted on an acrylic holder immersed in degassed water (Fig.1). The FUS beams were focused on the center of MBs. Multislice T2-weighted images were acquired before and after monitoring processes, to localized the chamber of MBs. During real-time monitoring processes, FUS transmitted pulses at t=21~114 sec with a series of HASTE acquisition simultaneously. . Table 1 summarized six experimental configurations with different conditions of MBs and FUS pulses. The yellow lines shown in Fig. 1 denoted the selections of region of interest (ROI) for MBs, NS, and Gel, respectively. To compare signal intensity (SI) of different experiments, the SI of MBs was normalized to the SI of NS.

TABLE 1. Experimental configurations. .

Experiment number	Microbubbles concentration	FUS power	Applied FUS time
1	0.2x	1w	93s
2	0.2x	2w	93s
3	0.2x	5w	93s
4	0.05x	1w	93s
5	0.05x	2w	93s
6	0.05x	5w	93s

Note：”0.2X“: MBs was diluted to 20% concentration.

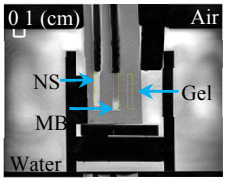

Fig.1. The experimental set-up shown on T2-weighted images acquired before applying FUS. The signal intensity of MBs was much lower than that of normal saline (NS).

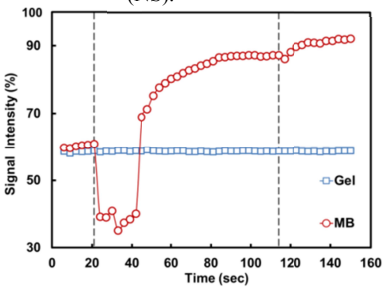

Fig.2. The time courses of SI (normalized to SI of NS) of HASTE images for MBs and Gel (Exp. 3). The two vertical lines denoted the duration of performing FUS. Before applying FUS, the SI of MBs was about 60% of NS.

Results: As shown in Fig.2, the normalized SI of gel phantom was maintained unchanged during the monitoring process (Exp. 3: 0.2X, 5W FUS). As for MBs, significant signal decaying was observed at the beginning of FUS transmission. After minimal signal intensity was achieved, SI increased quickly and a plateau was approached gradually, demonstrating the termination of disruption of MBs. As shown in Fig.3, before applying FUS, SI curves of MBs were about 60%~70%, depending on concentrations of MBs. Figs 3. (a,b) showed signal changes of MBs with concentrations of 0.2X and 0.05X, respectively. While applying FUS with 1W and 2W, SI of MBs first showed maximal decreases of 9.33% and 18.4% for MBs with 0.2X and 0.05X, respectively. The signal intensity increased to 80% gradually and stayed relatively unchanged. An increase of 15%~20% of SI after FUS transmission was observed. As for performing higher FUS power of 5W, SI descended from 60% to around 40% and rose to 90%, which was with 10% lower comparing to SI of NS. With lower power of FUS, the minimal SI was reached with longer time, suggesting that FUS powers might affect the efficiency of cavitation.

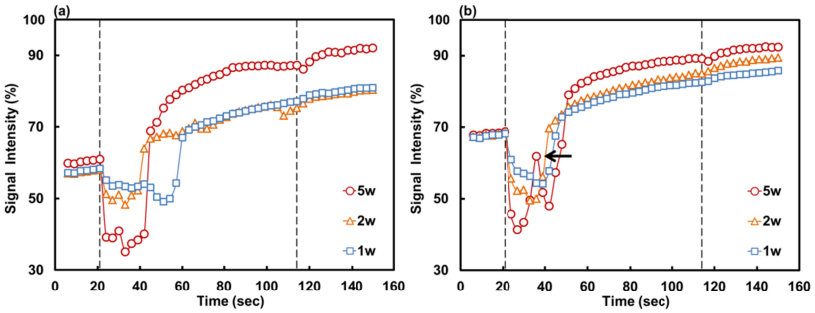

Fig.3. The SI time courses (normalized to SI of NS) of MBs with diluted concentrations of 0.2 X (a) and 0.05 X (b). Two vertical lines denoted the duration of transmitting FUS. While applying FUS, the SI curves were decreased to different levels, depending on FUS power. The minimum values of SI were reached with longer time if lower power of FUS were transmitted. The arrow in (b) denoted the sudden SI increase during the cavitation.

Discussion and Conclusions: Because of the inherent magnetic susceptibility effect of gas-liquid interface, MBs have been used as a unique MR contrast agent to localize targeting tissues and as a US contrast agent to enhance cavitation effect [5]. Nevertheless, the real-time evaluation of cavitation effect by MRI is not investigated. In this study, MR HASTE images were acquired for real-time monitoring of cavitation effect during FUS transmission. Before applying FUS, the SI difference between MBs and NS is perceptible, suggesting that MBs could be utilized to indicate the position of tumors where with higher vascular permeability. . During applying FUS, reduced SI denoted the duration of cavitation effect. Moreover, a positive correlation between SI decreases and powers of FUS suggested that the power of FUS might affect the duration and the efficiency of cavitation. The sudden rise in Fig.3(b) (indicated by arrow) could be observed only with applying high power (ex. 5W) and may result from image slice acquired which excluded the localized cavitation region due to high power FUS. In conclusion, the pulse sequence of HASTE has been proved to be a useful technique for real-time monitoring of signal changes of MBs while performing FUS pulses to induce cavitation effect. The signal changes of different concentrations of MBs sonicated with varied power of FUS have been studied. A systemic study regarding to lower concentrations of MBs and shorter FUS transmitting time should be further investigated. In the future, this technique may provide helpful information for monitoring of cavitation effect for *in vivo* BBB-opening and drug delivery with MBs.

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