Real-time Monitoring of Focused Ultrasound Inertial Cavitationon on Microbubbles by Gradient Echo MRI

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Introduction: Gas-filled microbubbles (MBs) were originally developed as an intravascular contrast agent to enhance backscattering signals in ultrasound imaging. Applying focused ultrasound (FUS) with MBs is well-known to be able to increase the permeability of the blood-brain barrier (BBB). Thus, the efficiency of drug delivering to targeting location can be improved and the problem of systemic toxicity can be reduced [1]. While MBs can be locally cavitated by FUS, MRI may provide useful imaging information to real-time monitor the process of FUS cavitation on MBs. In previous study, we have investigated the inertial cavitation effect on MBs by a spin-echo based HASTE sequence [2]. In this study, a gradient echo based sequence was used to observe the signal changes during FUS cavitation for *in vitro* gel phantom experiments. MBs were diluted to around 1000 times (0.001X) and be applied by burst ultrasound to approach the conditions of *in vivo* BBB opening experiments [3]. In addition, images were acquired under different conditions of MBs concentrations, FUS powers, and FUS modes to comprehend the reason of signal drops. Preliminary results concluded from *in vitro* studies shall provide useful information for the *in vivo* drug delivery or BBB opening experiments in the future.

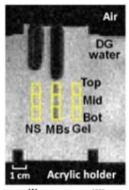

Fig.1. The experimental setup. The solutions of NS and MBs were injected into a gel phantom with two hollow chambers. Yellow rectangles: ROIs for computing SI.

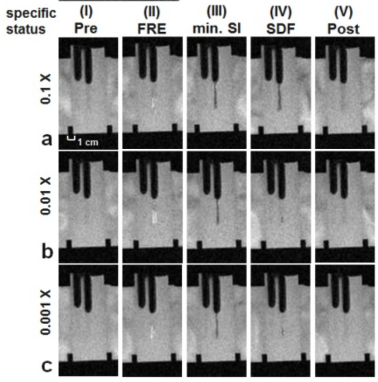

Fig. 2. Magnitude images acquired with 0.1X (a), 0.01X (b), and 0.001X (c) MBs at status I-V of the experimental process.

Methods and Materials: A single-element focused piezoelectric transducer (central frequency 1.85 MHz, 10 cm diameter, 12.5 cm curvature, Imasonic, Besancon, France) was used as the source of FUS sonication. FUS pulses with powers of 8, 5, 2 watt (8 watt of acoustic pressure is measured as 2650 kPa) were applied. The experimental setup was shown in Fig. 1. The solutions of normal saline (NS) and MBs (lipid shell with C_3F_8, mean diameters as Number % was 1.25 µm (range: 0.7-18µm), concentration = (4.36±0.32)x10^{10}droplets/mL) [4] were injected into a gel phantom (2% agarose) with two hollow chambers (diameter=6 mm). MBs were diluted to the concentrations of 0.1X (90% NS+ 10%MBs), 0.01X, and 0.001X. The Fast Low Angle Shot (FLASH) sequence (TR/TE= 8/3.61 ms, pixel size = 1.56 x 1.56 mm², flip angle=20°) was performed for real-time monitoring of MBs cavitation in a 3.0 Tesla MR scanner (Trio, Siemens, Erlangen, Germany). To clarify the effect of signal drops and mimic the condition for *in vivo* experiments where slice thickness may be larger than vessels, experiments were acquired with slice thicknesses of 3, 6, 8 mm. All images were acquired at the focal plane and were perpendicular to the direction of ultrasound beams. Temporal resolution was 0.8 s and 270 measurements (216 s) were acquired. In this study, three designs of FUS were performed to disrupt MBs: (1) consecutive FUS mode applied continuous FUS pulses for consecutive 94s (ON: t=30s, OFF: t=124s); (2) intermittent FUS mode repeated 4 times of continuous FUS pulses in a manner of interleaved ON-OFF(ON: 3.2s, OFF: 25.6s); (3) burst mode performed FUS pulses for consecutive 94s (ON: t=30s, OFF: t=124s) with 10% duty cycle, 18500 cycles, and burst period: 0.1 s so that to approach the conditions of *in vivo* experiments. To evaluate changes of signal intensity (SI), regions of interesting (ROIs) were selected manually at top, mid (at focal point), and bottom (Bot) parts in chambers of MBs, NS, and gel (Fig. 1). The SI_{MB} within ROI was normalized to SI of mean SI before turning-on of FUS pulses (pre-FUS): normalized SI_{MB} = $(SI_{MB}/SI_{Pre-FUS\,MB})$ x100%.

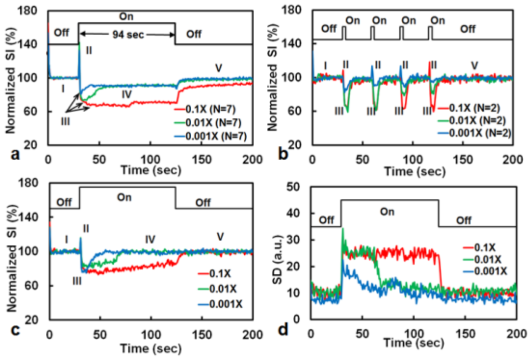

Fig. 3. The time courses of normalized SI of experiments with 0.1X, 0.01X and 0.001X MBs for consecutive FUS (a), intermittent FUS (b) and burst FUS (c) mode. (d) The SD for burst mode showed the signal variation around the focal point.

Results: Figure 2 showed magnitude images at certain specific statuses during the process: (I) Pre-FUS; (II) Flow-related enhancement (FRE) at the beginning of FUS transmission; (III) minimal SI; (IV) signal drop due to focus (SDF); (V) Post-FUS. Each status was indicated in Fig. 3 as well. Figures 3 (a-c) indicated the time courses of normalized SI for consecutive mode, intermittent mode, and burst mode of FUS transmission, respectively. At either mode, the effect of FRE, owing to the replenishment of fresh protons inflowing into imaging slice at status II, can be observed clearly at the beginning of each FUS transmission. The normalized SI dropped from 100% to a minimum of 60~75% at status III. In consecutive mode, with higher concentrations of MBs exhibited longer periods of reduced SI (status of III and IV): 94.4, 15.6, 4.4 sec for 0.1X, 0.01X, and 0.001X of MBs, respectively (Fig.3a). In intermittent mode with 4 times of interleaved On-Off FUS transmission, 0.1X MBs demonstrated significant decreased SI (~60%), as shown in Fig. 3b. In contrast, 0.01X showed SI of 60% in the first two times of FUS transmission and less reduced SI of 80% in the last two. Microbubbles with 0.001X showed much minor reduced SI, particularly in the last three times of transmission. In Fig. 3c, burst mode exhibited a longer period of reduced SI compared to consecutive mode. Since temporal resolution in MRI is much lower compared with that of occurrence of FUS cavitation, a longer period of reduced SI may be beneficial for observing SI changes. Figure 3d showed the standard deviation (SD) changes of consecutive mode experiments with 0.1X, 0.01X, and 0.001X of MBs. Significant changes of SD during transmitting FUS pulses indicated the complex vortical flow attributed to cavitation effect and locally disturbed flow around focus. Figures 4(a-c) showed the mean normalized SI of five statuses with different concentrations of MBs, FUS powers, and slice thicknesses. Acquiring images with slice thickness thicker than chamber diameter, FRE effect cannot be observed, as shown in the status II in Fig. 4c. Nevertheless, the reduced SI can be observed clearly at statuses of III or IV. The SD values of different slice thickness were shown in Fig. 4d.

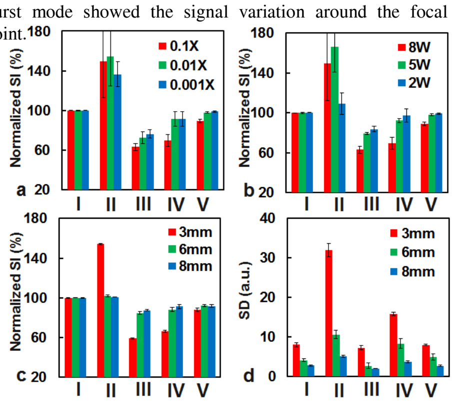

Fig. 4. The normalized SI with different experimental conditions. (a) Consecutive FUS of 8 W with 0.1X, 0.01X and 0.001X MBs. (b) 0.1X MBs with consecutive FUS of variant power (8,5,2W). (c) Images acquired with variant slice thickness of 3, 6, 8 mm. (d) The SD of experiments for slice thickness of 3, 6, 8 mm.

Discussion and Conclusions: Under these diluted concentrations of 0.001X MBs, which was close to *in vivo* experiments, long-lasting reduced SI were able to be observed, demonstrating the possibility of this technique being used for *in vivo* experiments. Whenever consecutive, intermittent, or burst mode of FUS pulses were applied, apparent signal drops displayed significantly (Figs. 2,3). In this study, we investigated SI changes under different concentrations of MBs, FUS powers, and imaging slice thicknesses. Even with conditions of diluted MBs of 0.001X, low FUS power of 2W, or thicker slice of 8 mm, reduced SI still can be observed (Figs.4a-c). As for the FRE effect, it might attribute to the fresh spins inflowing into the imaging slice and exhibited only while imaging slice thickness is thinner than chamber diameter (Fig.4c, status II) In conclusion, the pulse sequence of gradient echo has been proved to be a useful technique for real-time monitoring of SI changes when transmitting FUS to MBs. Further studies shall be performed to clarify the flow effect on SI changes for animal *in vivo* BBB-opening experiments in the future.

References: [1] Liu H.L., PNAS 2010;107:15205–15210 [2] Lin S.C., ISMRM 2013. [3] Ting C.Y., Biomaterials 2012:33:704-712. [4] Fan C.H., Ultrasound in Med. & Biol. 2012:38:1372-1382.