Real-time Monitoring of Inertial Cavitation Effect on Diluted Microbubbles by MRI

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Introduction: The technique of transmitting focus ultrasound (FUS) with usage of microbubbles (MBs) is well-known as a strategy of increasing blood-brain barrier (BBB) permeability and therefore is able to improve the efficiency of drug delivery [1]. The mechanical force caused by MBs inertial cavitation provides a non-invasive, transient, and reversible BBB disruption [2]. In addition to use a second US probe to monitor the process of cavitation, MRI can also provide helpful imaging guidance not only to localize the targeting region but also to observe the course of FUS transmission. Consequently, the safety and the efficiency of BBB opening can be improved. Nevertheless, the technique of real-time monitoring of MBs cavitation is rarely reported. In our previous study, the Half Fourier Acquisition Single Shot Turbo Spin Echo (HASTE) sequence was performed for a real-time monitoring of high concentrations of MBs for preliminary *in vitro* experiments [3]. The reduced signals were observed while applying FUS and the signal drops endured about 20 seconds. However, the cavitation effect for MBs shall be in a time scale of only µsec to msec. Thus, the reason of several seconds of signal drops is still not clear. In this study, MBs were diluted to around 1000 times to approach the conditions of *in vivo* BBB opening experiments. In addition, images were acquired with different slice thicknesses to comprehend the impact of slice thickness on signal drops.

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 power of 8 watt (acoustic pressure=~2650 kPa) were applied. The experimental set-up was shown in Fig. 1. The solutions of normal saline (NS) and homemade MBs (lipid shell with C_3F_8, mean diameters = 0.92 µm, concentration = $(4.36\pm0.32)\times10^{10}$ droplets/mL) [4] were injected into a gel phantom (2% argarose) with two hollow chambers (diameter=6 mm). MBs were diluted to the concentrations of 0.001X (99.9% NS+ 0.1% MBs), 0.00075X, and 0.0005X. The HASTE imaging (TR/TE= 1440/49, pixel size = 0.8 x 0.8 mm^2, slice thickness = 3, 6, 8 mm) was performed on a 3T clinical scanner (Trio, Siemens, Erlangen, Germany) for real-time monitoring of MBs cavitation. To mimic the condition for future *in vivo* experiments whose 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 1.44s and 150 measurements (216s) were acquired. In this study, two designs of FUS were performed to disrupt MBs. One is the continuous FUS transmission which was applied for consecutive 94s (ON: t=30s, OFF: t=124s), and the other one is the intermittent FUS transmission which repeated 10 times of FUS transmission in a manner of interleaved ON-OFF (ON: 2.88s, OFF: 7.20s). To evaluate changes of signal intensity (SI), a region of interesting (ROI) were selected manually at chambers of MBs and NS. The SI of MBs within ROI (yellow rectangle in Fig. 1) was normalized to SI of NS ($SI_{MB-n} = (SI_{MB}/SI_{NS})$ x100%) for comparing SI of different experiments.

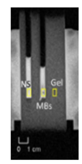

Fig. 1 The solutions of normal saline (NS) and homemade MBs were injected into a gel phantom with two hollow chambers. The SI of MBs within ROI was normalized to SI of NS for comparing SI of different experiments.

Results: Figure 2 indicated signal changes of one of experiments with 0.001X MBs. Before and after FUS transmission (Pre-FUS and Post-FUS), SI_{MB-n} was around 100% of NS (Fig.2, periods A and D). During applying FUS, SI_{MB-n} dropped to ~8% at first 2.8~4.3 sec (point B in Fig.2). The substantial signal void was resulted from the onset of inertial cavitation which caused strong turbulence in solutions. Therefore, with performing HASTE sequence, it was difficult to refocus signals and thus signal drops were revealed. After the occurrence of cavitation, SI_{MB-n} recovered to ~85% quickly and stayed consistently until turning off FUS pulses (period C in Fig. 2). The signal drops in period C, namely Signal Drop of Focal point (SDF) period, were resulted from the restricted regional turbulence caused by FUS at focal point. Figure 3 showed the influence of slice thickness on signal changes. The SI_{MB-n} at the onset of cavitation rised from 8% for 3 mm to 26% and 40% for 6 and 8 mm, respectively. Figure 4 and Table 1 illustrated the mean SI_{MB-n} of four experiments at different periods with highly reproducibility, reflected by low standard deviations.

Discussion and Conclusions: Under these diluted concentrations of 10^7 droplets/mL, which was close to *in vivo* experiments [5], substantial SI drops were able to be observed, demonstrating the possibility of this technique being used for *in vivo* experiments. The substantial signal void at the onset of applying FUS pulses could be derived from the strong turbulence cause by inertial cavitation which made difficulty of refocusing signals while performing HASTE sequence. Whenever continuous or intermittent FUS pulses were applied, apparent signal drops were displayed only at the very beginning of transmission (Fig.1). Thereafter, low SI (~80%) shown during SDF periods reflected the minor regional turbulence caused by FUS at focal point. To approach the conditions of *in vivo* experiments, variant slice thicknesses were performed to imitate different vessel volumes in an imaging slice. Figure 4 demonstrated that significant reduced values of SI were shown at cavitation stage whenever with slice thicknesses of 3, 6, or 8 mm, reflecting the strong turbulence resulted from cavitation had a considerable impact on signal intensity. In conclusion, the pulse sequence of HASTE has been proved to be a useful technique for real-time monitoring of SI changes resulted from inertial cavitation while transmitting FUS to MBs. Under the conditions of diluted MBs and imaging acquisitions with different slice thicknesses, substantial SI drops were still observable at the very beginning of FUS transmission, suggesting the feasibility of applying this scheme for *in vivo* experiments. 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] Pardridge W.M, Neuron 2002;36:555-558. [2] Kang J, J Ultrasound Med 2010;29:61-70. [3] Li H.H., ISMRM, 2012. [4] Fan C.H., Ultrasound in Med. & Biol. 2012;38:1372-1382. [5] Skyba M, Circulation 1998;98:290-293.

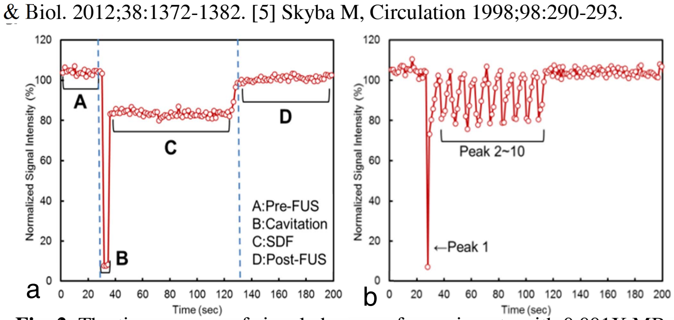

Fig. 2. The time courses of signal changes of experiments with 0.001X MBs for continuous FUS (a) and intermittent FUS (b), respectively. Substantial signal drops were observed at the very beginning of FUS transmission for both designs of experiments, indicating strong turbulence at the onset of inertial cavitation (Point B). The signal drops in period C, namely Signal Drop of Focal point (SDF) period, showed the restricted regional turbulence caused by FUS at focal point. A: SI_{MB-n} before FUS exposure, B: Inertial cavitation, C: SDF period. D: SI_{MB-n} after FUS transmission.

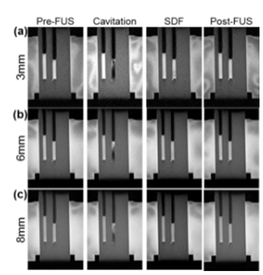

Fig. 3. HASTE images acquired with 3 mm (a), 6 mm (b), and 8 mm (c) slice thickness, respectively. The SI_{MB-n} at the onset of cavitation raised from 8% for 3 mm to 26% and 40% for 6 and 8 mm, respectively.

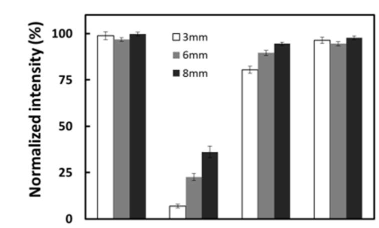

Fig. 4. Significant reduced values of SI were shown at cavitation stage whenever with slice thicknesses of 3, 6, or 8 mm, reflecting the strong turbulence resulted from cavitation had a considerable impact on signal intensity.

Table 1 SI_{MB-n} in different conditions.

Slice Thickness	Pre-FUS	Cavitation	SDF	Post-FUS
3mm	100%	8%	85%	100%
6mm	100%	26%	93%	100%
8mm	100%	40%	96%	100%