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Dynamic Perfusion Assessment by Contrast-Enhanced Ultrasound in Blood-Brain Barrier Disruption

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***Abstract*—Recently, blood-brain barrier disruption (BBBD) has been performed by focused ultrasound (FUS) combining with microbubbles (MBs). The outcome of BBBD enhances local drug or gene delivery for improving the treatment efficiency of brain diseases. However, over-excitation of FUS may cause brain damage such as shutdown blood flow, intracerebral hemorrhage and brain edema. Therefore, it is essential to develop a an imaging system to assess dynamic perfusion changes during FUS-induced BBBD process. Here, we used the high-frequency destruction/reperfusion contrast-enhanced imaging technique to observe the cerebral perfusion under the cases of with/without hemorrhage in BBBD procedure. The BBB was disrupted by a 2.25 MHz FUS combining with MBs at 0.5-0.7 MPa (pulse repetition frequency: 1 Hz, pulse length: 1 ms, sonication time: 60 s). The results showed that the velocity of blood flow decreased after BBBD induced by FUS sonication. Particularly, the plateau of time-intensity curve was higher than prior to MBs destruction at 20 s after sonication and the blood flow would be obstructed due to the blood coagulates at 60s after sonication. The pattern of hemorrhagic damage caused by FUS can be monitored by the TIC. In addition, the location of blood flow velocity decrease was consistent with the areas of BBBD and the variation of blood flow depends on the applied acoustic pressure. In conclusion, the blood flow velocity changes have potential as an *in vivo* tool for quantifying the extent of the FUS-induced BBBD and detecting intracerebral hemorrhage occurrence.**

Keywords: Blood-brain barrier, microbubbles, focused ultrasound, destruction/reperfusion

I. INTRODUCTION

Several reports have confirmed that focused ultrasound (FUS) and microbubbles (MBs) can induce temporal and local Blood-Brain Barrier disruption (BBBD), thus providing a window of opportunity for local delivery of therapeutic agents into the brain [1, 2]. However, unsuitable FUS sonication may induce brain tissue damage like erythrocyte extravasation, cell necrosis and intracerebral hemorrhage within sonicated area. Furthermore, some studies also indicated that FUS exposure can induce local arteriolar vasoconstriction and shutdown cerebral blood flow [3].

The magnetic resonance imaging has been widely used to indicate BBBD location via Gd-DTPA leakage into brain tissue by T1-weighted sequence. Moreover, the intracerebral hemorrhage caused by FUS sonication could be recognized in T2*-weighted MRI. However, the low temporal resolution of MRI restricts the dynamic observation the physiological changes within brain. Thus, it is essential to develop a higher temporal and spatial resolution imaging technique for real-time detecting the brain damage occurrence during BBBD process.

The contrast-enhanced destruction/reperfusion (D/R) method has been proven the potential of observing the blood flow and quantifying vascular flow-rate-constant parameter. The MBs were destroyed by a high pressure FUS and subsequently MBs replenished by means of bloodstream into the sample volume of microcirculation. Thus, the flow-rate-constant parameter proportional to flow rate was computed from time-intensity curves (TICs) obtained from the B-mode images. Note that the plateau of TIC indicated the information of blood volume [4].

In this paper, we proposed a an high-resolution ultrasound imaging system with D/R technique, which can be used to identify BBBD region even brain tissue damage according to cerebral blood flow variation in the rat model.

II. MATERIAL & METHODS

A. Animal preparation

The experimental protocol of this study was approved by National Tsing Hua University animal experiment committee. The total of 26 adult male Sprague-Dawley rats were used in this study (300-350 g). Before starting experiments, the rat was performed craniotomy and cannula of venous. The cranial window (nearly 1 cm by 0.4 cm) was administered with a high-speed drill to reduce the attenuation and distortion of ultrasound beam. The catheter was placed into jugular venous for injection MBs and Evans Blue (EB) dye during experiments.

B. Experimental setup

The imaging system consisted of a single-element circular-aperture FUS transducer with a hole in its center for an imaging transducer. The outer transducer was a spherical-focused annulus with a central frequency of 2.25 MHz (model V3966, Panametrics, OLYMPUS; diameter = 28 mm, focal length = 26.16 mm), providing a high power energy to destroyed MBs and to generated the BBBD excitation pulse. It was driven by a function generator (HP 33120, Hewlerr-Packard, Houston, TX, USA) through a power amplifier (A150, E&I, NY, USA). The electric impedance within the transducer was matched to output

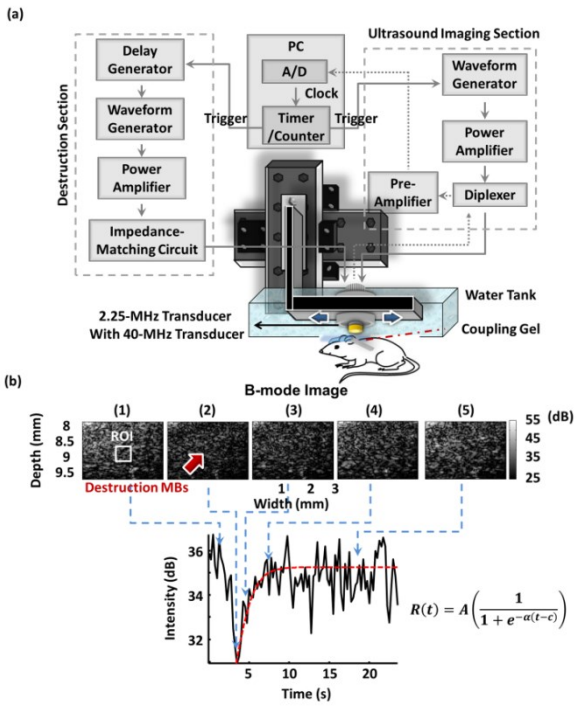

Figure 1. (a) Experimental setup and (b) the ultrasound contrast-enhanced destruction/reperfusion model.

impedance of the power amplifier by an external impedance-matching circuit.

The inner one was a piston transducer with a central frequency of 40-MHz for acquiring B-mode images. The arbitrary waveform generator (AWG-2040, Tektronix, CA, USA) provided impulse excitation through the power amplifier (325LA, Electronic Navigation Inc., NY, USA) to the imaging transducer. To avoid the imaging transducer receiving the echoes from the destruction pulses, a delay generator (DG-535, Stanford Research system, CA, USA) was used for separating imaging and destruction two backscattered echo signals. The radio-frequency (RF) echoes were received using the same transducer through a diplexer circuit (model DIP-3, Matec Instruments NDT, MA, USA). The received RF echoes were magnified by the preamplifier (model AU-1114, MITEQ Inc., NY, USA) and then digitized at the sampling rate of 400 Msamples/s by the PC-based 12-bit analog-to-digital board (model CS12400, Gage Applied Tech Inc., IL, USA) and stored in a PC.

Both the transducers were affixed to the two-dimension motion stage for obtaining ultrasound B-mode images. During the experiment, the animals were fixed on the stereotaxic apparatus for avoiding undesirable motion (Fig. 1a). A syringe pump was used for injecting MBs (the dosage of MBs: 11.43 mL/kg) into the brain of rat with the flow rate of 2.63 mm/s.

C. Preparation of MBs

MBs were manufactured through the thin-film hydration method. DSPC and DSPE-PEG-2000 at 10:2 (mg) ratios were mixed in 1 mL chloroform and were dried to lipid-film

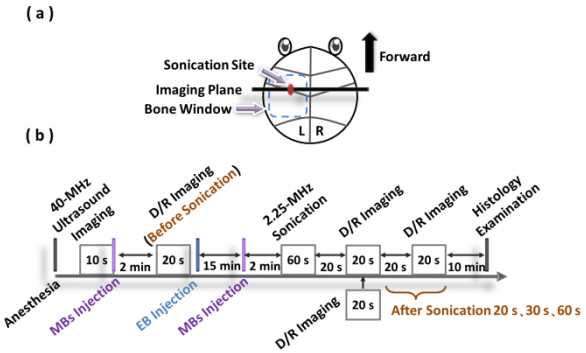

Figure 2. (a) FUS sonication site (b) experimental procedure

under reduced pressure with rotary evaporator for over 24 h. Then, the film was hydrated by 5 μL/mL glycerol PBS and the perfluoropropane gas was substitute for air. Finally, the solution was shaken by an in-house agitator for 45 s to obtain MBs suspensions. The MBs were analyzed by multisizer 3 (Beckman Coulter, Brea, CA, USA), the results show that the mean size and concentration of MBs were 1.18 ± 0.01 μm and (25.74 ± 0.49) × 10^9 MB/mL, respectively.

D. FUS sonications

Three types of BBBD experiments were performed (1) control group without FUS sonication (n = 7); (2) BBBD without hemorrhagic damage (acoustic pressure = 0.5 MPa, MI = 0.354, PRF = 1 Hz, burst length = 2,000 cycles [1 ms], sonication time = 60 s, n = 9) and (3) BBBD with hemorrhagic damage (0.7 MPa, MI = 0.495, n = 9) (Fig. 2b). Delivery of the 2.25 MHz pulses to the sonication site was ensured with guidance by the 40-MHz ultrasound B-mode image. Then, the 2.25 MHz transducer was shifted to the sonication location by the motion stage. Immediately, the 40-MHz transducer returned back the BBBD region within few seconds and then B-mode imaging commenced. A sequence of D/R B-mode images were obtained at a frame rate of 8 for 20 s. The imaging field of view was 1.8-mm in depth by 3-mm in width which included the cortices of sonicated and unsonicated left hemisphere brain. The D/R B-mode images were acquired at three time points for dynamically investigating the relationship between BBBD and brain perfusion/damage with respect to initial FUS sonication conditions: (1) 15 min before; (2) 20 s after; (3) 60 s after (Fig. 2b).

E. D/R model

MBs can be destroyed by a high amplitude FUS and subsequently replenished by the following blood flow into the sample volume. This method had been proposed to estimate blood flow velocity of myocardium by Wei et al [4]. The rising rate of echo intensity caused by MBs flowing into the region-of-interest (ROI) could present the flow velocity and could be calculated from the time-intensity curve (TIC) (Fig. 1b). The TIC was modeled by a sigmoidal function in previous research [5]. The equation of received signal intensity after MBs destruction was given by,

$$R(t) = A\left(\frac{1}{1+e^{-\alpha(t-c)}}\right)$$

where R (t) is the received intensity following time; A is the echo intensity of steady state by MBs refilled; α is the rate constant of the monoexponential and proportional to the mean blood flow velocity; and c is the time interval from the start to the reflection point in the sigmoid curve.

MBs were destroyed by the 2.25 MHz with a 10-cycle pulse and the acoustic pressure of 0.6 MPa at focus spot. The level of acoustic pressure could destroy over 95 % of MBs and no erythrocyte extravasation in previous experiment. The 40-MHz transducer was driven with a 3-cycle pulse and the acoustic pressure of 2.4 MPa (MI = 0.38).

F. Data processing

The acquired D/R B-mode images were offline analysed by the MATLAB™ (The MathWorks, Natick, MA, USA) software. In order to identify the change of contrast replenishment, the TIC of MBs was obtained by averaging all of the pixels within the ROI in D/R B-mode images and fitted by the nonlinear least algorithm. The local TIC was used to construct the α parametric maps by means of a sliding window of size 345 × 345 μm^2. Then, the α variation map in scanning region was performed by subtracting the α parametric map after sonication from that of before sonication.

G. Histology

The EB dye was injected by a bolus injection to verify BBBD region. The animals were sacrificed after completing experiment procedure at 10 min and perfused by 0.9 % saline through the left ventricle while colorless perfusion fluid appeared from the right atrium. Then, the rat brain was removed and embedded by optimal-cutting-temperature compound and preserved in -50 ^{o}C. The histological section of brain was obtained by Cryostat Microtome at a slice thickness of 10μm in the same direction of ultrasound imaging.

III. RESULTS

A. Time-intensity curve

The typical TICs were performed in a size of 345 × 345 μm^2 within FUS sonication site (as shown in Fig. 3). The echo intensity increases follow the reperfusion time and reaching a plateau when the MBs refilled the sample volume prior to FUS sonication. Note that the slope of TIC had been altered and the reperfusion time increased in groups 2 and 3. Particularly, the plateau of TIC was higher than prior to MBs destruction at 20 s after sonication in hemorrhagic group and it became noisy at 60 s. We conjectured that MBs and erythrocyte outflowed through the sonicated site on blood vessel and led to the plateau higher than that of prior to MBs destruction. Then, the blood coagulates blocked the blood flow and led to the TIC to be noisy at 60 s after sonication. However, the MB could not reperfusion within sample volume after destruction. The results show that the TIC nonlinear fitting failed to converge on a reasonable estimate of α parameter. We considered that the occurrence of hemorrhagic damage needs a short duration after sonication.

B. Blood flow velocity distribution in rat brain

Figure 4 presents example images of the α parametric map for reflecting the distribution of cerebral flow velocity. The results showed that there was no obvious blood flow velocity change in group 1 (Fig. 4a). In contrast, the local flow velocity was slow down after BBBD (Fig. 4b and 4c) due to the local vasoconstriction induced by FUS and MBs. Moreover, in group 3, the change of flow velocity was larger than that of group 2. At 60 s after FUS sonication, the flow velocity map performed the decreasing tendency in groups 2 and 3.

The maps of flow velocity decrease were obtained by the α parametric map at 20 s after sonication subtracts from reference map (before sonication) (Fig. 5b). The α variation map showed no obvious variations of cerebral blood flow after FUS excitation in group 1. In group 2 and 3, the distribution of flow velocity decreased matched the extent of BBBD validated by the EB distributions. These observations strongly support that the flow velocity maps can reflect the area of BBBD and the occurrence of hemorrhage.

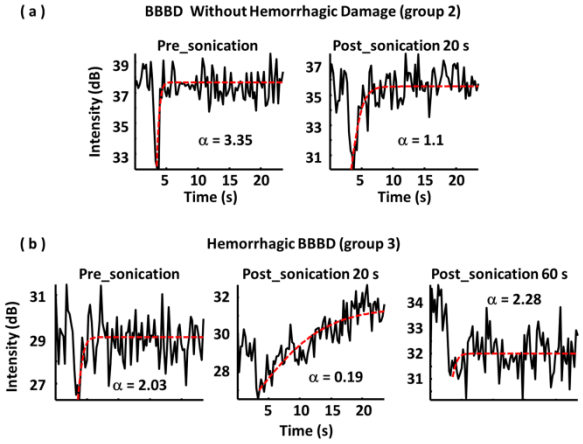

Figure 3. The change of TIC in with/without hemorrhage group after sonication

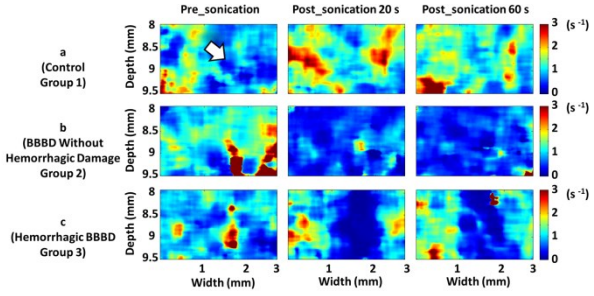

Figure 4. The blood flow velocity distribution in left rat brain before sonication, at 20 s after sonication and 60 s later, the arrow indicate the FUS sonication site.

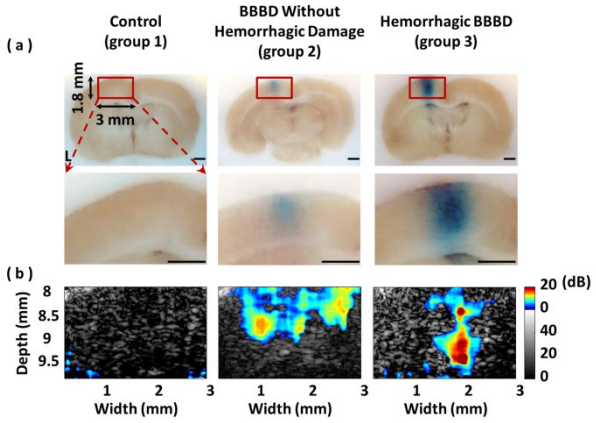

Figure 5. (a) EB dye verification of BBBD and (b) the α variation map at 20 s after sonication. Scale bar is 1 mm

C. Cerebral flow velocity changes

The α variation value was evaluated in three time points after sonication. The results of EB dye verification of BBBD showed the significant difference at 20 s and 30 s time points between the experimental groups (Fig. 6a). However, at 60 s after sonication, the α variation value was less pronounced. The possible reason was that the erythrocyte would extravasate when hemorrhage occurred and the blood coagulates led to the background intensity enhancement to affect α estimate (Fig. 6b)[6].

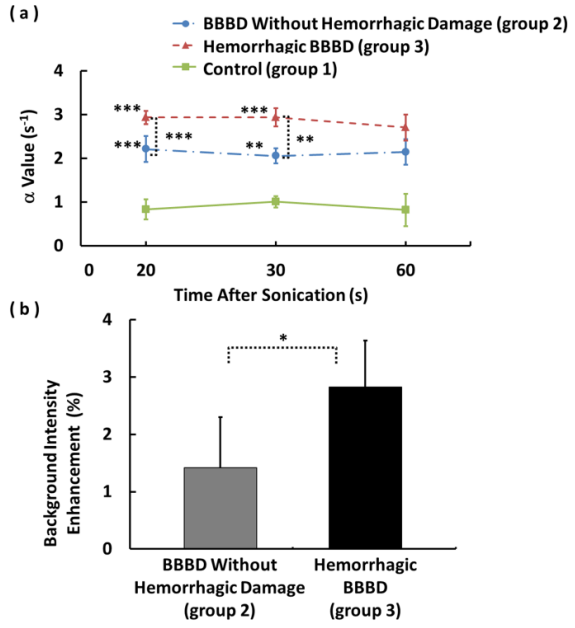

Figure 6. (a) Level of α variation after 60 s sonication and (b) the percentage of background intensity enhancement after BBBD ($^{*}p < 0.05$, $^{**}p < 0.01$, *** p < 0.001)

IV. DISCUSSION

In this study, the variation of cerebral blood flow based on contrast-enhanced ultrasound during BBBD induced by FUS with MBs at different acoustic pressures were mapped and quantified. The results showed that the effect of MBs cavitation would induce the vasoconstriction, thus decrease the blood flow velocity. Furthermore, the extent of vessel constriction was strongly correlated with the acoustic pressure. The effect has been reported by Raymond et al [3].

Particularly, the raised plateau of TIC after sonication caused under the case of blood coagulation in group 3 was observed, suggesting the potential of this proposed technique in hemorrhage detection during BBBD process. However, this technique still has some limitations such as TIC fitting and α parameter calculation which were accurately estimated only within 30s after sonication. The main reason is that the blood clot obstructed of blood flow in hemorrhagic BBBD. Clinical application is to apply this technique with lower frequency ultrasound imaging systems to overcome the hinder of ultrasound beam penetration into the brain caused by human skull.

V. CONCLUSIONS

In this study, our proposed imaging technique provided a useful tool to monitor both the extent of BBBD and presence of hemorrhage by the α variation map and the TIC pattern, individually. The results can be used to establish an immediate-feedback control tool for preventing the induction of intracerebral hemorrhage during FUS treatment.

REFERENCES

[1] S. Meairs and A. Alonso, "Ultrasound, microbubbles and the blood-brain barrier," *Prog Biophys Mol Biol,* vol. 93, pp. 354-62, Jan-Apr 2007.

[2] N. McDannold, N. Vykhodtseva, and K. Hynynen, "Effects of acoustic parameters and ultrasound contrast agent dose on focused-ultrasound induced blood-brain barrier disruption," Ultrasound Med Biol, vol. 34, pp. 930-7, Jun 2008.

[3]S. B. Raymond, J. Skoch, K. Hynynen, and B. J. Bacskai, "Multiphoton imaging of ultrasound/Optison mediated cerebrovascular effects in vivo," *J Cereb Blood Flow Metab,* vol. 27, pp. 393-403, Feb 2007.

[4] K. Wei, A. R. Jayaweera, S. Firoozan, A. Linka, D. M. Skyba, and S. Kaul, "Quantification of myocardial blood flow with ultrasound-induced destruction of microbubbles administered as a constant venous infusion," *Circulation,* vol. 97, pp. 473-83, Feb 10 1998.

[5] C. K. Yeh, S. Y. Lu, and Y. S. Chen, "Microcirculation volumetric flow assessment using high-resolution, contrast-assisted images," *IEEE Trans Ultrason Ferroelectr Freq Control,* vol. 55, pp. 74-83, Jan 2008.

[6] C. H. Fan, H. L. Liu, C. Y. Huang, Y. J. Ma, T. C. Yen, and C. K. Yeh, "Detection of intracerebral hemorrhage and transient blood-supply shortage in focused-ultrasound-induced blood-brain barrier disruption by ultrasound imaging," *Ultrasound Med Biol,* vol. 38, pp. 1372-82, Aug 2012.