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Monitoring of Focused Ultrasound-Induced Blood-Brain Barrier Opening in Non-Human Primates Using Transcranial Cavitation Detection In Vivo and the Primate Skull Effect

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***Abstract*—Focused ultrasound (FUS) with microbubbles (MB) is promising for assisting the delivery of drugs across the blood-brain barrier (BBB). To assess the safety and efficacy, the monitoring using passive cavitation detection (PCD) is critical and yet the reliability of transcranial detection in large animals remained questioned. To study the primate skull effect, the PCD through the in-vitro monkey and human skulls and in the in vivo monkeys during the sonication (FUS frequency: 500 kHz) were investigated, with the use of in-house made lipid-shelled, monodisperse MB (median diameter: 4-5 μm) and a flatband hydrophone served as a passive cavitation detector. In the in vitro experiments, the MB were injected to the channel of the phantom under a degassed skull for sonication (peak negative pressure/PNP: 50-450 kPa, pulse length/PL: 0.2 ms, PRF: 10 Hz, duration: 2 s). A diagnostic B-mode imaging system was also used to monitor the cavitation. In the in vivo study, the PCD was realtime monitored during the sonication for PCD calibration (PNP: 50-700 kPa, PL: 0.2 ms and 10 ms, PRF: 2 Hz, duration: 10 s) and BBB opening (PNP: 200-600 kPa, PL: 10 ms, PRF: 2 Hz, duration: 2 min). The stable cavitation dose using harmonics (SCD_h) and ultraharmonics (SCD_u) and the inertial cavitation dose (ICD) were quantified. Results showed that the SCD_h, SCD_u, and ICD were detectable in vitro at 50 kPa and above, and the B-mode imaging showed bubble collapse at 200 kPa and above. The detection thresholds increased with the skulls in place, with the signal reduction of 15.4 dB for the monkey skull and 34.1 dB for the human skull. In the in vivo experiments, the SCD_h and ICD was detectable at and above 100 kPa and 250 kPa, respectively, and the SCD_u was less reliable due to spontaneous occurrence. The BBB was found to be disrupted in 250-600 kPa without edema, hemorrhage, and physiological changes were found. In conclusion, the SCD_h was more detectable and reliable than the SCD_u in assessing stable cavitation in vivo, and the inertial cavitation was detected at 250 kPa and may occur at lower pressures.**

Keywords— blood-brain barrier opening; focused ultrasound; passive cavitation detection; skull effect; primates

I. INTRODUCTION

Focused ultrasound (FUS) with microbubbles (MB) has shown great promise in assisting brain drug delivery by noninvasively and transiently opening the blood-brain barrier (BBB) in primates [1-5]. Previous studies have shown that the strength of harmonics, ultraharmonics and broadband signals in the passive cavitation detection (PCD) are related to the opening and brain damage [4-5], indicating that the treatment could be accessed as well as controlled through the PCD. For the future treatment to be safe and effective in clinics, realtime monitoring with transcranial PCD is critical without requiring on-line MRI.

To effectively apply PCD transcranially in primates, however, the main challenge lies in the skull effects such as attenuation and scattering. These factors affect the reliability of the transcranial PCD, and hence its sensitivity needs to be assessed. Studies reported on primate skulls dealt with the acoustical properties of the skull [6-7] and the transcranial focusing quality [8], but none have systemically investigated the transcranial PCD sensitivity. In this study, the PCD response through the in vitro non-human primate (NHP) skull [9] and the human skull was investigated, the metrics for separately evaluating the stable cavitation dose using harmonics (SCD_h), ultraharmonics (SCD_u), and the inertial cavitation dose (ICD) were used in both the in vitro skull experiment and the in vivo BBB opening experiment in NHP.

The objective of this study was thus to determine the PCD threshold through a monkey and a human skull in vitro, and the feasibility of guidance of the BBB opening using PCD in NHP in vivo for generating a uniform disruption spot with reproducibility at the pressures of safety. In order to measure the sensitivity of the PCD through the primate skulls, the phantom experiments with the use of monodisperse microbubbles (median diameter: 4-5 μm) were performed at 500 kHz, with a wide-band hydrophone serving as PCD, and the B-mode imaging for guidance and comparison. The SCD_h, SCD_u, and the ICD were also quantified and compared in between the cases of the presence and the absence of the skull. The SCD_h, SCD_u, and the ICD were then used in the in vivo

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experiments to realtime assess the extent of cavitation during the treatment of BBB opening.

II. Methods

A. Experimental System

A single-element focused transducer (H-107, Sonic Concepts, WA, USA) was used for sonication, and a spherically focused, flatband hydrophone (Y-107, Sonic Concepts, WA, USA) coaxially and confocally aligned with the transducer served as the passive cavitation detector. A work station (T7600, Dell) with a customized program in MATLAB® (Mathworks, MA, USA) was developed to automatically control the sonication through the function generator (33220A, Agilent Technologies, CA, USA) whose signal was amplified 50 dB (ENI, NY, USA) and then drive the FUS transducer through a 50 Ohm matching box, the acquisition of the PCD signals through a digitizer (Gage Applied Technologies, QC, Canada) after a 10-20 dB amplification (5800, Olympus NDT, MA, USA), and achieve real-time monitoring of the PCD spectra and the cavitation dose.

B. In Vitro Experiment

A monkey (rhesus macaque) skull and a human skull were cleaned and degreased by a commercial company (Skull Unlimited, OK, USA). In order to fit the phantom setup, both the skulls were cut so that facial bones of the monkey skull were removed and for the human skull a segment connecting frontal and parietal lobes was excised. The thickness of the monkey skull was 2.2 mm and 5-8 mm for the human skull. The monkey skull was degassed for 24 h and 48 h for the human skull prior to the experiment. The pressures in the focus of the FUS transducer with and without the skulls were calibrated using a bullet hydrophone (ONDA, CA, USA).

Using the setup shown in Fig. 1, the degassed water and the in-house lipid-shelled, monodisperse MB [10] (median diameter: 4-5 μm) were diluted to 2×10^5 bubbles/mL and injected respectively to the 4-mm-in-diameter channel in the phantom made of acrylamide with and without the skull placing on top to mimic a set of brain capillaries within the focal spot. During the sonication (FUS frequency: 0.5 MHz, PNP: 50-450 kPa, pulse length: 100 cycles=0.2 ms and 5000 cycles=10 ms, PRF: 10 Hz, duration: 2 s), a linear array transducer of a diagnostic ultrasound scanner (Terason Ultrasound, MA, USA) was placed transverse to the ultrasound beam to acquire the B-mode images of bubble disruption and to ensure the alignment of the focus to the channel, and the then PCD signals through the hydrophone. The PCD signals were acquired without the B-mode monitoring to avoid interference.

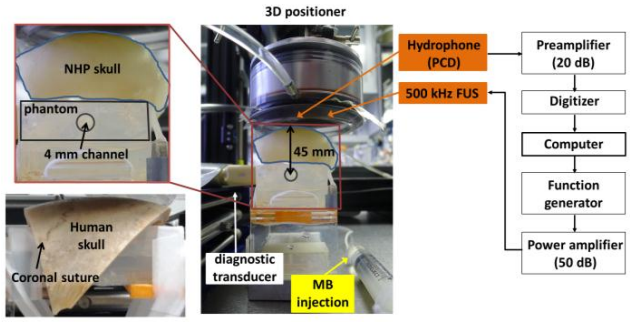

Fig. 1. In vitro experimental setup. In order for the skull to fit on top of the phantom, the NHP skull was excised to remain only the cranial bone with a thickness of 2.2 mm, and the human skull was cut to remain the piece connecting the frontal lobe and the the parietal lobe with a thickness of 5-8 mm.

C. In Vivo Experiment

Four male rhesus macaques (Macaca mulatta) weighing between 6-11 kg were used in this study. The experimental procedure was described in the previous studies [1-2]. In order to induce the BBB opening, the same in-house made MB were injected intravenously with a dosage of 2.5×10^8 bubbles/kg of the animal and the sonication (PNP in situ: 250–600 kPa, pulse length: 10 ms, PRF: 2 Hz, duration: 2 min) started at the same time of injection. In order to calibrate the PCD in vivo, a boost of MB (1.25×10^8 bubbles/kg) were injected after the 2 min sonication and sonicating 10 s (pulse length: 0.2 ms and 10 ms) in 50-700 kPa without inducing opening. 40 sonications to open the BBB in the caudate and the putamen and 20 sonications for the PCD calibration were performed.

MRI scanning (3T, Philips Medical Systems, MA, USA) using Spoiled Gradient-Echo T1-weighted sequence (TR/TE=20/1.4 ms; flip angle=30º; NEX=2; spatial resolution: 500×500 μm^2, slice thickness: 1 mm with no interslice gap) with and without the injection of gadodiamide (0.2 mL/kg, Omniscan®, GE Healthcare, NJ, USA), T2-weighted sequence (TR/TE=3000/80 ms; flip angle=90º; NEX=3; spatial resolution: 400×400 μm^2, slice thickness: 2 mm with no interslice gap), and Susceptibility-Weighted Image sequence (SWI, TR/TE=19/27 ms; flip angle=15º; NEX=1; spatial resolution: 400×400 μm^2, slice thickness: 1 mm with no interslice gap) was performed 0.5 h after the sonication to detect the BBB opening, the occurrence of edema and hemorrhage, respectively.

The study was approved by the Institutional Animal Care and Use Committee at Columbia University and the New York State Psychiatric Institute.

D. Quantification of Cavitation Dose

The PCD signals, frequency spectra, spectrograms (8-cycle Chebyshev window, 98% overlap, 4096-point Fast Fourier Transform) were used to monitor the cavitation online and offline in MATLAB®. In order to quantify the cavitation dose, the spectra were used with the harmonic, ultraharmonic, and broadband signal amplitudes within 1-5 MHz separated for stable cavitation dose (SCD_h for harmonics and SCD_u for ultraharmonics) and the inertial cavitation dose (ICD), respectively. The harmonics (1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 MHz) and the ultraharmonics (1.25, 1.75, 2.25, 2.75, 3.25, 3.75, 4.25, 4.75 MHz) were the maxima in the harmonic and ultraharmonic passband (width: 20 kHz). The broadband signals were acquired by comb filtering the harmonics (stopband width: 360 kHz) and ultraharmonics (stopband width: 100 kHz). The SCD_h, SCD_u, and ICD for each sonication are the integration of every pulse over the entire sonication duration.

$$SCD_h = \sum_N \sqrt{\overline{S_h{}^2}} \quad (1)$$

$$SCD_u = \sum_N \sqrt{\overline{S_u{}^2}} \quad (2)$$

$$ICD = \sum_N \sqrt{\overline{S_b{}^2}} \quad (3)$$

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where N is the number of pulses, $\overline{S_h^2}$ the mean squared amplitude of the harmonic signals in a single pulse, $\overline{S_u^2}$ the mean squared amplitude of the ultraharmonic signals in a single pulse, and $\overline{S_b^2}$ the mean squared amplitude of the broadband signals in a single pulse.

III. Results

A. In Vitro Cavitation Monitoring

In monitoring the B-mode images of MB cavitation, the bubbles were found to collapse at 200 kPa since the contrast enhanced bubble channel lost echogenicity in the focal region as shown in Fig 2. On the other hand, the SCD_h, SCD_u, and ICD from the PCD signals showed significantly increase ($p<0.05$) in Student's t-test (two-tailed, unpaired, unequal variance) at 50 kPa and above for the MB cavitation as compared to the control.

B. Transcranial PCD In Vitro

The detectability of PCD changed after placing the skull. In the case of placing the monkey skull, the SCD_h was detectable ($p<0.05$) at the same pressure of 50 kPa and above, whereas both the SCD_u and ICD were detectable at higher pressures (150 kPa and above). In the case of placing the human skull, the detectability decreased for all cavitation doses, which became 100 kPa for the SCD_h, 250 kPa for the SCD_u, and 350 kPa for the ICD.

In order to investigate the PCD signal reduction through the skulls, the cavitation contrast was calculated by taking the ratio of cavitation dose of sonicating bubbles to that of sonicating water (Fig. 3). The cavitation contrast without the skull in place was found to be 2-39 dB for SCD_u and ICD, and 29-49 dB for the SCD_h. After placing the monkey skull, it decreased to ±0-15 dB for SCD_u and ICD and 10-29 dB for the SCD_h. With the human skull in place, it decreased even more; ±0-3 dB for SCD_u and ICD and ±0-6 dB for the SCD_h.

By comparing the cavitation contrast in the case of no skull in place to the cases of skull in place, the transcranial PCD thresholds were defined by separating the cavitation contrast significantly higher than the control ($p<0.05$) or not. It was found that when the cavitation contrast was above 15.4 dB, all of the cavitation doses were significant through the monkey skull, and 34.1 dB for the human skull. Since the cavitation dose significantly higher than the control means that it is detectable transcranially, 15.4 dB and 34.1 dB are found to be the PCD thresholds for monkey and human in this study, respectively. The skull attenuation was thus 7 dB/mm.

C. Transcranial PCD In Vivo

The detectability of transcranial PCD in the NHP was studied within 50-700 kPa using 100 and 5000 cycles in vivo (Fig. 4). In the case of 5000 cycles, the SCD_h was detectable ($p<0.05$, 0.06) at 100 kPa and above, and for the ICD was 250 kPa and above. However, the SCD_u had large variation and was not reliable until the pressure went up to 700 kPa. In the case of 100 cycles, the detectability decreased. The SCD_h was detectable ($p<0.05$, 0.06) at 200 kPa and above with an exceptions (250 kPa) due to large variation of the control, for the ICD was 600 kPa and above, and for the SCD_u was 700 kPa.

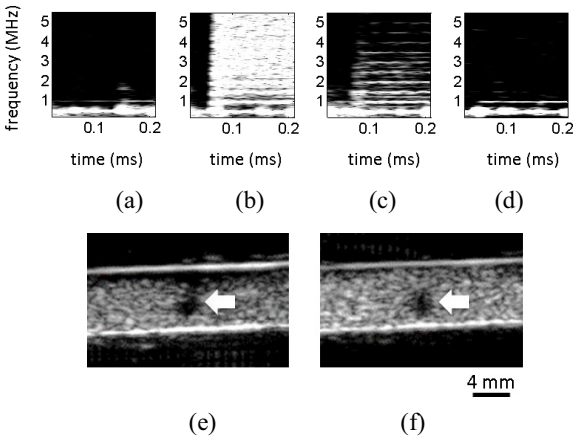

Fig. 2. In vitro cavitation monitoring. (a) Spectrogram for sonicating water. (b) Spectrogram for sonicating MB without the skull in place. (c) Spectrogram for sonicating MB with the monkey skull in place. (d) Spectrogram for sonicating MB with the human skull in place. (e) B-mode images of bubbles collapse at 200 kPa without the skull in place. (f) B-mode images of bubbles collapse at 200 kPa with the monkey skull in place.

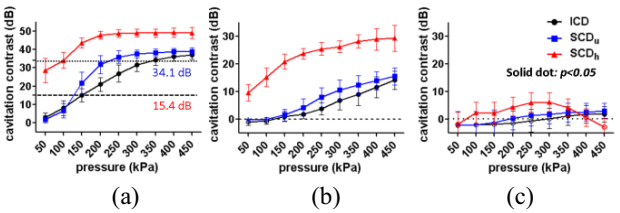

Fig. 3. In vitro cavitation contrast (a) without the skull in place, (b) with the monkey skull in place, and (c) with the human skull in place. The SCD_h, SCD_u, and ICD were significantly increased ($p<0.05$) when sonicating bubbles in the case of no skull. In the case of NHP skull in place, the SCD_u, and ICD showed no significance for pressures at 50 and 100 kPa, corresponding to the cavitation contrast below the dash line in (b). In the case of human skull in place, the SCD_h, SCD_u, and ICD showed no significance for pressures at 50/400/450 kPa, 50-200 kPa and 50-300 kPa, respectively, corresponding to the cavitation contrast below the dash line in (c).

D. Monitoring the BBB opening

Realtime monitoring of the PCD signals has been used during the treatment to ensure the bubbles perfused to the brain and remained persistent toward the end of the treatment. Fig. showed four cases of monitoring with safe opening (Fig. 5). The time for the perfusion was 10-30 s according to the time-resolved SCD_h. The SCD_u increased spontaneously and the ICD varied less than 6 dB during the entire treatment. No edema and hemorrhage were detected in T2-weighted and SWI images. No physiological values including heart rate, breathing rate, blood pressure and weight were changed before and after the treatment.

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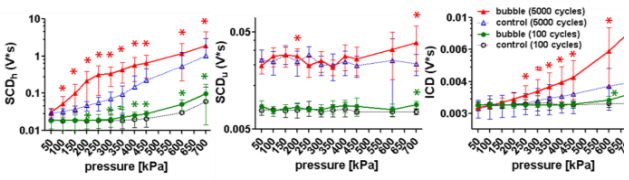
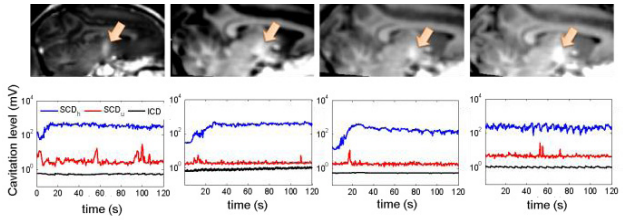


(a) (b) (c)

Fig. 4. In vivo cavitation dose using 100 and 5000 cycles. (a) SCD_h. (b) SCD_u. (c) ICD. *: p<0.05. #: p<0.06. Red: 5000 cycles. Green: 100 cycles.



(a) (b) (c) (d)

Fig. 5. In vivo PCD monitoring of BBB opening at (a) 275 kPa, (b) 350 kPa, (c) 450 kPa, and (d) 600 kPa. The upper row shows the contrast-enhanced T1-w images (sagittal view), and the lower row shows the realtime PCD monitoring.

IV. Discussion and Conclusion

The study was aimed at investigating the transcranial PCD sensitivity in primates and the in vivo cavitation detectability during the treatment of BBB opening using FUS and MB. Bothe the in vitro experiment using the NHP skull and the human skull and the in vivo experiment in NHP have been performed and the detection thresholds have been found in both human and monkey in vitro and were compared with the in vivo case.

In the in vitro experiments, the SCD_h, SCD_u, and ICD were detectable at 50 kPa and above, and the B-mode imaging showed bubble collapse at 200 kPa and above. The sensitivity of PCD decreased after placing the skulls, with the signal reduction of 15.4 dB for the monkey skull and 34.1 dB for the human skull. The attenuation of the monkey skull decreased the detectability of the SCD_u, and ICD to 150 kPa, and the SCD_h to 100 kPa, SCD_u to 250 kPa, and ICD to 350 kPa for the human skull. In the in vivo experiments, the SCD_h and ICD was detectable at and above 100 kPa and 250 kPa, respectively, and the SCD_u was less reliable due to spontaneous occurrence. The BBB was found to be disrupted in 250-600 kPa without edema, hemorrhage, and physiological changes were found. In conclusion, the SCD_h was more detectable and reliable than the SCD_u in assessing stable cavitation in vivo, and the inertial cavitation was detectable at 250 kPa and may occur at lower pressures.

The PCD (SCD_h, SCD_u, and ICD) in vivo were found to be less sensitive as compared to the in vitro case. This may due to the fact that the tissue attenuation was neglected in vitro and the number of bubbles in the focal region in vivo could be less depending on the density of the capillaries. The SCD_h is a good indicator for accessing the BBB opening since the cavitation contrast is the highest and is the most detectable cavitation dose, while the SCD_u is less detectable due to its spontaneous occurrence characteristic. The ICD is often used as an indicator of brain damage. However, the treatment could still be safe with the occurrence of inertial cavitation in NHP as shown in this study. On the other hand, the lack of significant inertial cavitation in PCD may due to the attenuation of 7 dB in 1-5 MHz for the primate skull.

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