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# Quantification of Stable Cavitation Dose during FUS-induced Blood-Brain Barrier Opening in Mice and in Non-Human Primates

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*Abstract*— **A passive cavitation detector (PCD) has previously been developed and used to transcranially acquire the acoustic emissions stemming from the interaction between the microbubble and the brain tissue during FUS-induced blood-brain barrier (BBB) opening, thereby determining the pressure threshold of inertial cavitation (IC) based on the quantification of the broadband response, i.e. inertial cavitation dose (ICD). Given that at certain pressures the BBB opens as a result of stable cavitation only, the stable cavitation dose (SCD) is introduced and quantified during BBB opening in mice and in non-human primates using monodisperse bubbles at varying pressures. In mice, the SCD was quantified with respect to the microbubble diameter and shell properties. Three different diameters (1-2, 4-5, and 6-8 μm) with C18 acyl-chain length of the lipid shell, and three shell acyl-chain lengths (C16, C18, and C24) with a diameter of 4-5-μm were used to induce BBB opening in the right hippocampus (1.5-MHz frequency; 100-cycles (67 μs) pulse length; 10-Hz pulse repetition frequency; 1 minute duration, 0.15 or 0.30 MPa peak-rarefactional pressure). In monkeys, 4-5-μm monodispersed bubbles were used to target different brain regions (500 kHz frequency; 5000 cycles (10 ms) pulse length; 2 Hz pulse repetition frequency; 2 minute sonication duration; 0.20 or 0.25 MPa peak-rarefactional pressure). A 10-MHz Pulse/Echo transducer and a broadband hydrophone were used as a passive cavitation detector (PCD) in mice and monkeys, respectively. The RMS PCD signal amplitude corresponding to the ultra-harmonics ($SCD_u$) and at harmonics ($SCD_h$) in the range of 4-16 MHz (mice) or 1-5 MHz (monkeys) was estimated. Due to the skull effect, there was no difference in $SCD_u$ between before and after microbubble administration in mice or monkeys, but the $SCD_h$ was found to be significantly higher in the 4-5-μm and 6-8-μm bubble cases compared to the 1-2-μm case at 0.30 MPa in mice. In addition, the BBB opening threshold and SCD were not affected by the acyl-chain length of the shell, although the $SCD_h$ in the case of all bubble shells studied was significantly higher than the sham at 0.30 MPa. In monkeys, the $SCD_h$ was found to be significantly higher than the sham. As a result, the $SCD_h$ can serve as an indicator for BBB opening occurrence in mice and monkeys.**

***Keywords- blood-brain barrier, BBB, cavitation, microbubble, inertial, SCD***

## I. INTRODUCTION

Microbubbles, initially used merely as contrast agents for ultrasonic imaging, have been shown critical in focused ultrasound (FUS) induced blood-brain barrier (BBB) opening recently [1, 2]. Typically, most reported studies use commercially available and poly-dispersed microbubbles, such as Definity®, Optison®, or Sonovue®. Thus, it has been difficult to determine the role of the microbubble properties in those applications. Mono-dispersed microbubbles have been used in the field of FUS-induced BBB opening to elucidate the effect of the bubble size on the BBB properties [3, 4]. The BBB can be opened only through nonlinear bubble oscillation at 4-5 and 6-8-μm, but not with the 1-2-μm microbubbles for a given set of acoustic parameters [4]. Since microbubbles are required to induce safe BBB opening, their role needs to be thoroughly investigated. Besides the diameter, the shell has been shown to dictate bubble behavior [5, 6]. The main shell constituent is lipid for the Definity® and Optison®, and albumin for the Sonovue bubbles. The pressure threshold of inertial cavitation has been shown to differ between the aforementioned contrast agents [6]. Because the shell and the diameter range distribution of commercial microbubbles are different, both factors need to be investigated. It has been shown that the acyl-chain length (i.e., lipid hydrophobic chain) dictates the dissolution behavior of the lipid monolayer-coated microbubbles [7] and the ultrasound-induced microbubble fragmentation [8]. Therefore, shell effects are expected to affect the acoustic response from microbubbles.

Until now, the inertial cavitation dose (ICD) has been quantified during BBB opening as an indicator of inertial cavitation occurrence. Since the stable cavitation is capable of inducing BBB opening, the quantification of stable cavitation dose (SCD) may provide useful information for ultrasound-induced BBB opening. Therefore, in this study, we propose a method to quantify SCD and study its dependence on three parameters/effects, including the effect of microbubble diameter in mice, the effect of lipid shell composition in mice, and BBB opening in monkeys. In order to exclude the impact of bubble size, the diameter of the microbubbles was kept constant at 4-5-μm in all experiments unless otherwise stated. The acoustic emission from microbubbles was detected transcranially and noninvasively. In addition, since the applied pressure (0.30 MPa) would open the BBB in the absence of inertial cavitation, the ICD is not investigated.

## II. METHODS

### A. Ultrasound and Magnetic resonance imaging

All mouse and monkey experiments were carried out in accordance with the Columbia University Institutional Animal Care and Use Committee. The acoustic parameters

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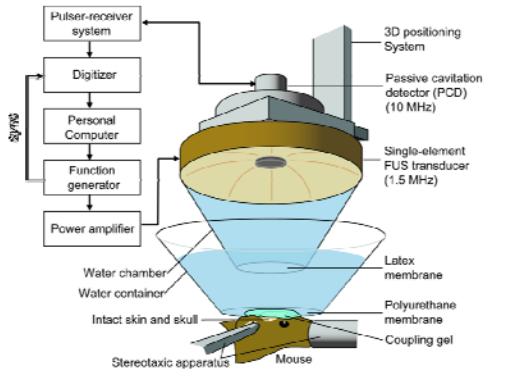
were shown in Table 1. Fig.1 showed the experimental setup for mice and monkeys. A 1.5-MHz FUS transducer (focal depth: 60 mm; outer radius: 30 mm; inner radius 11.2 mm, Imasonic, Besançon, France), and a 500-kHz transducer (focal depth: 64 mm; outer radius: 32 mm; inner radius 11.3 mm) with a flat-band hydrophone at the center (Sonic Concepts Inc., WA, USA) were used for mice and monkeys, respectively. The transducer was driven by a function generator (Agilent Technologies, Palo Alto, CA, USA) through a 50-dB power amplifier (ENI Inc., Rochester, NY, USA). A cone filled with degassed and distilled water was attached to the transducer system. The acoustic emissions from the microbubbles were captured with the PCD and collected using a digitizer (model 8349, Gage Applied Technologies, Inc., Lachine, QC, Canada) through a 20-dB amplifier (model 5800, Olympus NDT, Waltham, MA, USA). The MRI parameters used in mouse and monkey experiments were shown in Table 2.

Table 1. Acoustic parameters for mice and monkey experiments

| Parameter | Mice | Monkeys |
|---|---|---|
| Frequency: | 1.5 MHz | 0.5 MHz |
| Pressure (P-N): | 0.15, 0.30 MPa | 0.20, 0.25 MPa |
| Pulse Length: | 100 cycles (67 µs) | 10 ms |
| Pulse Rate: | 10 Hz | 2 Hz |
| Total Duration: | 1 min (600 pulses) | 2 min (240 pulses) |
| Microbubble: | Mono-dispersed | 4-5 µm diameter |
| Number : | 76 | 8 sonications |
| PCD: | 10 MHz | Hydrophone |

Table 2. MRI parameters for mice and monkey experiments

| Parameter | Mice | Monkeys |
|---|---|---|
| System: | Bruker 9.4 T | Philips 3 T |
| Contrast agent (CA): | Omniscan™ | Omniscan™ |
| Volume of CA: | IP, 0.30 mL | IV, 1 mL |
| Resolution ($T_1$-w, µm$^3$): | 86 x 86 x 500 | 500 x 500 x 1000 |
| Scan time ($T_1$-w): | 10 min | 18 min |



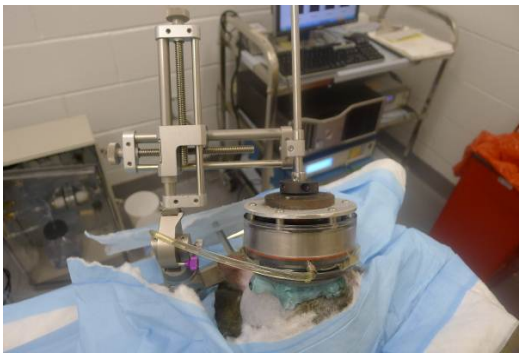



Fig 1. Experimental setup for mice (top) and monkeys (bottom).

## B. *Mircobubbles*

Perfluorobutane-filled and lipid-shelled microbubbles with three different diameters (1-2, 4-5, and 6-8 µm) with C18 acyl-chain length of the lipid shell and PEG-40S, as well as three different acyl-chain lengths (C16, C18, and C24, purity > 99%, Avanti Polar Lipids, Alabama, USA) and PEG-5000 with a 4-5-µm diameter were manufactured and size-isolated in-house using differential centrifugation as described in Feshitan et al. [9].

## C. *Acoustic emission signal acquisition and analysis*

The frequency response of the first pulse of each sonication was obtained using a FFT in MATLAB® (2011a, Mathworks, Natick, MA). In order to quantify the stable cavitation, peak amplitude around each harmonic and ultra-harmonic frequency each pulse was measured. The SCD was quantified based on the RMS of the amplitude at ultra-harmonics ($SCD_u$) and at harmonics ($SCD_h$) in the range of 4-16 MHz (mice) or 1-5 MHz (monkeys). The SCD was defined as the area under the time-amplitude curve over the entire pulse duration. The SCD without microbubble administration was also quantified as the sham. A Student's t-test was used to determine whether the SCD was statistically different across different microbubble properties or the sham sonication. A P-value of $P < 0.05$ was considered to denote statistically significant difference in all comparisons.

# III. Results

The SCD at 0.15 and 0.30 MPa at three bubble diameters is shown in Fig. 2. Unlike the $SCD_u$ which shows no difference between all diameters and sham, BBB opening is indicated by $SCD_h$. At 0.30 MPa, the $SCD_h$ at 4-5-µm and 6-8-µm diameter bubbles is significantly higher than at 1-2-µm and sham. This confirms our previous findings which indicate that the BBB opening threshold is 0.30 MPa, with the absence of the inertial cavitation, at 4-5-µm and 6-8-µm, but not at 1-2-µm diameter. In addition, the $SCD_h$ at 4-5-µm and 6-8-µm diameter bubbles at 0.15 MPa is similar to 1-2-µm diameter bubbles at 0.30 MPa. Therefore, no BBB opening is induced at 0.15 MPa.

The T1-weighted MR images and corresponding spectra of the first pulse of each acyl-chain length case at 0.15 and 0.30 MPa are depicted in Fig. 3 and three main observations can be made: 1) the BBB opening pressure threshold is around 0.30 MPa at all acyl-chain lengths, which means that the BBB opening pressure threshold is not affected by the acyl-chain length; 2) the spectrum shows that the BBB was opened without the occurrence of inertial cavitation at 0.30 MPa for all acyl-chain lengths with a diameter of 4-5 µm, which is the same as what was shown in our previous study on the effect of microbubble diameter [4]; 3) Compared to 0.15 MPa, the BBB is opened at 0.30 MPa with higher amplitude of harmonics.

The corresponding SCD is shown in Fig. 4. Unlike the $SCD_u$ which showed no difference between all acyl-chain lengths and sham, BBB opening was indicated by $SCD_h$ related to the nonlinear oscillation at 0.30 MPa for all acyl-chain lengths. In addition, at 0.30 MPa, the $SCD_h$ with PEG-40S (Fig. 2) is higher than with PEG-5000 (Fig. 4) microbubbles with C18 acyl-chain length and a diameter of 4-5 µm.

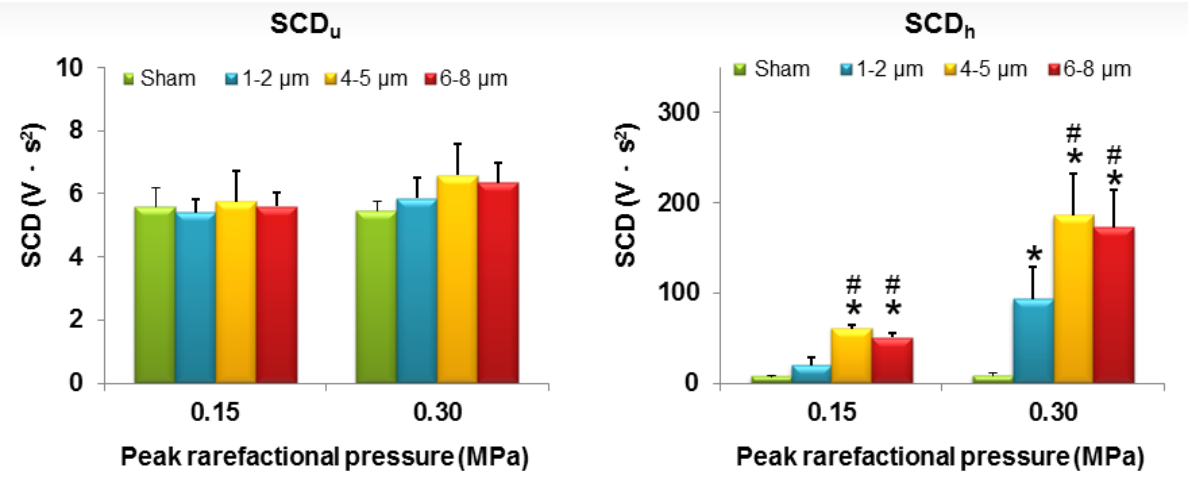



Fig. 2. The $SCD_u$ and $SCD_h$ at three bubble diameters and two pressure amplitudes as indicated. Unlike the $SCD_u$ which showed no difference between all diameters and sham, BBB opening was indicated by $SCD_h$ related to the nonlinear oscillation at 0.30 MPa for 4-5-μm and 6-8-μm diameter bubbles (but not at 1-2-μm). *: $P < 0.05$, compared to sham, #: $P < 0.05$, compared to 1-2 μm.

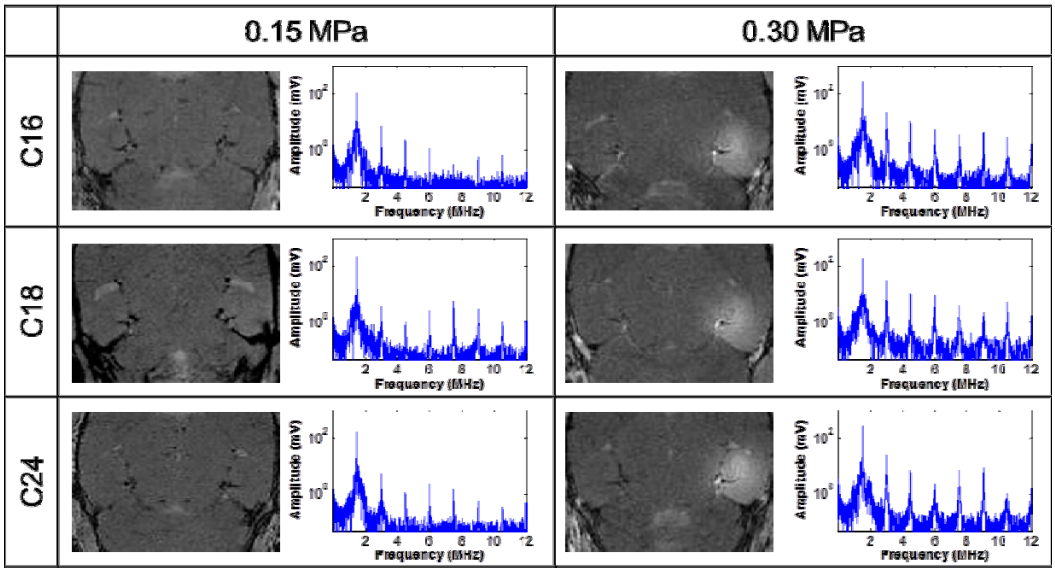



Fig. 3. MR images and corresponding spectrums at three acyl-chain lengths and two pressure amplitudes as indicated. Compared with 0.15 MPa without BBB opening, the BBB was opened at 0.30 MPa for all acyl-chain length at 4-5-μm diameter microbubbles with higher amplitude of harmonics.

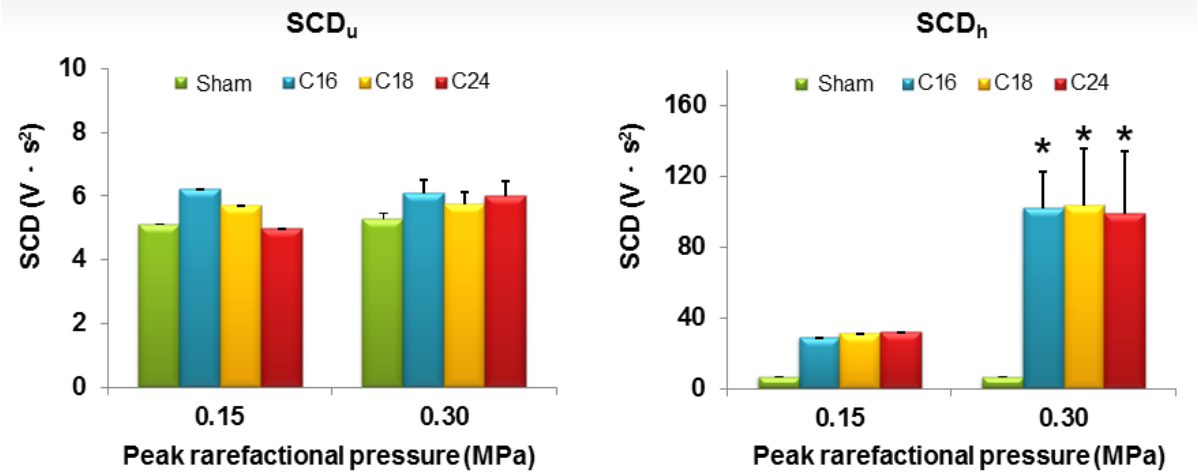



Fig. 4. The $SCD_u$ and $SCD_h$ at three acyl-chain lengths and two pressure amplitudes as indicated. Unlike the $SCD_u$ which showed no difference between all acyl-chain lengths and sham, BBB opening was indicated by $SCD_h$ related to the nonlinear oscillation at 0.30 MPa for all acyl-chain lengths. *: $P < 0.05$, compared to sham.

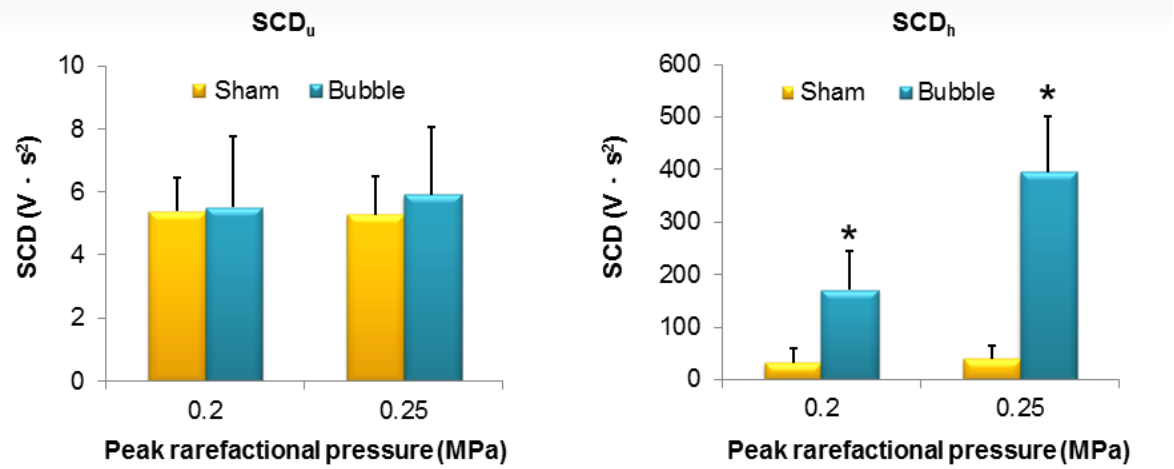



Fig. 5. The $SCD_u$ and $SCD_h$ during BBB opening in monkeys at two pressure amplitudes as indicated. Unlike the $SCD_u$ which showed no difference between microbubbles administration and sham, BBB opening was indicated by $SCD_h$ related to the nonlinear oscillation at 0.2 and 0.25 MPa at 4-5-μm microbubbles. *: $P < 0.05$, compared to sham.

The SCDs during BBB opening in monkeys at 0.20 and 0.25 MPa are shown in Fig. 5. Similar with Figs. 2 and 4, the $SCD_u$ showed no difference between all acyl-chain lengths and sham, but BBB opening was indicated by $SCD_h$. In the monkey experiments, harmonics can serve as a good indicator for BBB opening. For example, at 0.25 MPa, the $SCD_h$ is almost 70 times higher than $SCD_u$.

## IV. Conclusion

In this study, our findings indicated that 1) the pressure threshold of BBB opening was not affected by the acyl-chain lengths. 2) Due to the skull effect, ultra-harmonics may not serve an indicator for BBB opening. However, the detection for harmonics were not affected by the skull and the $SCD_h$ showed significant difference when the BBB opened. Further studies need to be performed to investigate the threshold of $SCD_h$ for BBB opening in mice and in monkeys.

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