10.1109/ULTSYM.2015.0072

Improving targeting of ultrasound-mediated blood-brain barrier opening using chirp and random-based modulations

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***Abstract*—Beam distortions of focused ultrasound (FUS) caused by skull may compromise accurate targeting of transcranial therapeutic ultrasound applications. Although, the efficacy of the random and chirp modulations in improving targeting have been proven in simulations, *ex-vivo* and in phantom studies, many other parameters that can be observed i*n vivo* are disregarded such as the heterogeneity of the soft tissue, the variability of the drug uptake and clearance among subjects. In this study, the capability of the chirp- and random-based coded ultrasonic excitation in improving the targeting is investigated using a FUS-mediated blood-brain barrier (BBB) opening protocol in mice. The coded ultrasonic excitation signals were generated with frequency bandwidth: 1.5-1.9 MHz, pressure: 0.52 MPa, and duration: 30 s. Fifteen mice were divided in three groups (n=5 each). One group was sonicated in the right caudate putamen with chirp-based signal (frequency varying linearly), the other group was sonicated with random-based coded signal (randomly varying frequency) and they were compared with a third group sonicated with standard mono-frequency ultrasound (1.5 MHz, 0.52 MPa, burst duration: 20 ms, total duration: 5 min). The mean BBB opening volumes assessed by contrast enhanced magnetic resonance imaging were 9.38 ± 5.71 mm^3, 8.91 ± 3.91 mm^3 and 35.47 ± 5.10 mm^3 for the chirp, random and standard sonication, respectively. The mean cavitation levels assessed by passive cavitation detection were 55.40 ± 28.43 V.s, 63.87 ± 29.97 V.s and 356.52 ± 257.15 V.s for the same groups. The coded excitation methods improved the targeting precision, generating lower cavitation levels and more confined opening volumes than the conventional sonication. The coded excitation methods may thus enable more precise drug delivery and it may benefit other FUS applications that use higher-pressure levels and require precision to ablate or stimulate the targeted region.**

Keywords—attenuation; chirp; coded ultrasonic excitation; therapeutic ultrasound; skull

I. Introduction

The combination of focused ultrasound (FUS) with microbubbles has been demonstrated being capable of noninvasively, transiently, and locally open the blood-brain barrier (BBB) [1, 2]. The controlled BBB disruption with FUS allows the passage of a variety of pharmacological agents, which is presented as a potential therapy for the treatment of e.g. Alzheimer's disease [3] and Parkinson´s disease [4]. Furthermore, other transcranial ultrasound-based techniques have demonstrated the capability of ultrasound to dissolve blood clots [5], ablate brain tumors [6], reduce hyper-excitable activity of neurons [7], and modulate neuronal activity [8, 9].

However, the use of ultrasound for the treatment of neurological disorders remains challenging due to ultrasound beam distortion caused by the skull. As a result, standing wave formation, attenuation, and aberration of focus may prevent the correct targeting in the brain. Large phased arrays have been used to correct targeting, which allow computed tomographic-based corrections of the phase and amplitude [10]. Short ultrasound pulses have also been reported *in vivo* to suppress standing wave formation in the brain [11, 12]. However, the efficacy of chirp- and random-based modulations for therapeutic applications have only been assessed in simulations or phantoms [13, 14, 15] where many other parameters are disregarded such as the heterogeneity of the soft tissue, the variability of the drug uptake and clearance among subjects.

In this study, the use of chirp- and random-based coded ultrasonic excitation is evaluated to improve targeting of ultrasound-mediated BBB opening.

II. Materials and Methods

A. Coded ultrasonic excitation methods

The chirp and the periodic selection of random frequency (PSRF) coded ultrasonic excitation were assessed in this study. The signals are described by

$$s(t)=K(f(t)).\sin(\emptyset(t)) \quad (1)$$

where $\emptyset(t)$ is the time domain function of the phase and $K(f(t))$ is the experimental calibration factor that varies the amplitude of the signals according to the frequency $f(t)$ taking

This study was supported in part by NIH (R01EB009041 and R01AG038961) and FAPESP (2011/10809-6 and 2013/08116-8). The opinions, assumptions, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of FAPESP.

into account the transducer frequency response and the mouse skull attenuation. The signals were customized to allow N full cycles of pulses for each f (t) making Ø(t) constant for a period of time N/f(t).

The time-domain function of the phase for the chirp signal is given by

$$Ø(t) = 2.\pi.f_0.\exp(t.\Delta f/N).t \quad (2)$$

In the random signal, the frequencies were randomly sorted with derivative of the phase angle expressed by [15]

$$dØ_{rand}(t)= 2.\pi.f_{rand}\,(t).dt \quad (3)$$

B. Calibration factor K(f (t))

The calibration factor K(f(t)) was obtained by measuring the ultrasonic signal generated by the therapeutic transducer (center frequency: 1.94 MHz; −6 dB frequency bandwidth: 1.28–2.31 MHz; diameter: 70 mm; Imasonic SAS, Voray-sur-l'Ognon, France) using a hydrophone (model HGL-0200, ONDA Corp., Sunnyvale, CA, USA). The calibration was conducted with the hydrophone placed on the caudate putamen (anterior/posterior: 6 mm, medial/lateral: ±2.2 mm, dorsal/ventral: 3 mm referenced from Lambda) of 2 freshly excised mouse skulls in a degassed water tank.

C. Animals preparation

All procedures involving mice were approved and conducted in accordance with the Columbia University Institutional Animal Care and Use Committee. A total of 15 wild-type adult male mice (strain: C57BL/6, masses: 20-28 g; Harlan, Indianapolis, IN, USA) were assigned into three groups Chirp, Random, and Regular with 5 mice each group. First, the animals were placed inside an acrylic box for 5 min with a mixture of isoflurane at 2% and oxygen at 0.8 L/min. After the animals were anesthetized, they were immobilized within a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The fur on head and tail was removed. The animal's body temperature was maintained at approximately 40ºC using a heating pad and a mixture of isoflurane at 1% and oxygen at 0.8 L/min was continuously delivery during the whole procedure (SurgiVet, Smiths Medical PM, Inc., Waukesha, Wisconsin, USA).

D. Sonication procedure

A therapeutic single-element FUS transducer (center frequency: 1.94 MHz, focal depth: 60 mm, diameter: 60 mm; Imasonic, Besancon, France) was driven by an arbitrary waveform generator (33220; Agilent, Palo Alto, CA, USA) through a nominal 50 dB gain power amplifier (325LA; E&I, Rochester, NY, USA). A pulse-echo transducer (center frequency: 10 MHz, focal depth: 60 mm, diameter: 22.4 mm; model U8517133, Olympus NDT, Waltham, MA, USA) and the therapeutic transducer with their foci overlapping were mounted together in a 3D positioning system. The transducers were acoustically coupled to the animal's head using an acrylic cone attached to the transducers filled with deionized degassed water, a water container placed over the animal´s head, and ultrasonic coupling gel between the animal´s head and the water container. A PC remotely controlled the arbitrary waveform generator via USB. The chirp and the PSRF signals were generated in Matlab and uploaded to the arbitrary waveform generator.

Lipid-shelled, polydisperse microbubbles (microbubbles size: 1.38 μm, concentration: $6.5.10^9$/mL) were injected through the tail in all mice immediately prior to sonication. All mice were sonicated in the right caudate putamen, leaving the left hemisphere intact as control. The animals from the Regular group were sonicated with pulsed ultrasound with f = 1.5 MHz, 20 ms pulse duration, 5 Hz of burst rate, 0.52 MPa (PNP, peak negative pressure) for 5 min, providing a total ON-time of 30 s. The Chirp group was sonicated using a signal with frequencies varying linearly from 1.5 MHz to 1.9 MHz with frequency steps of 10 kHz, PNP = 0.52 MPa, and 3 cycles, providing a 70.7 us pulse length repeated continuously for 30 s. The PSRF group was sonicated with the same parameters, but with the frequencies randomly assorted in the same frequency interval of the Chirp group (1.5-1.9 MHz).

E. Blood-brain barrier opening quantification

The volume of the BBB opening was determined by contrast-enhanced magnetic resonance images (MRI; 320 by 320 matrix size, spatial resolution of 80 by 80 μm^2, slice thickness of 400 μm). Gadodiamide (287 mg/mL, OmniscanTM Novaplus, Novation LLC, TX, USA) was injected intraperitoneally immediately after sonication and 30 minutes before the MRI acquisition for all mice with a 2-D FLASH T1-weighted sequence using a 9.4-T microimaging MRI system (DRX400, Bruker BioSpin, Boston, MA, USA). The BBB volume was determined counting the number of voxels in the MRI with intensity value equal to or above 2.5 standard deviations of the reference value obtained from the control hemisphere.

F. Microbubbles activity quantification

The microbubble activity was measured in real-time using passive cavitation detection (PCD) [16]. The pulse-echo transducer was connected to a pulser-receiver (NDT-5800, Panametrics, MA, USA) in receiving mode with a 20 dB amplification, and the PCD signals were digitized at 100 MHz (Gage Applied Technologies Inc., Lachine, QC, Canada) and the Fast Fourier Transform of the PCD signal was then calculated on Matlab providing the real-time frequency spectra. The stable cavitation dose (SCDh) was determined by measuring the root mean square of the harmonics (from 2^{nd} to 6^{th}) voltage amplitude of the pulse-echo signals.

G. Safety evaluation

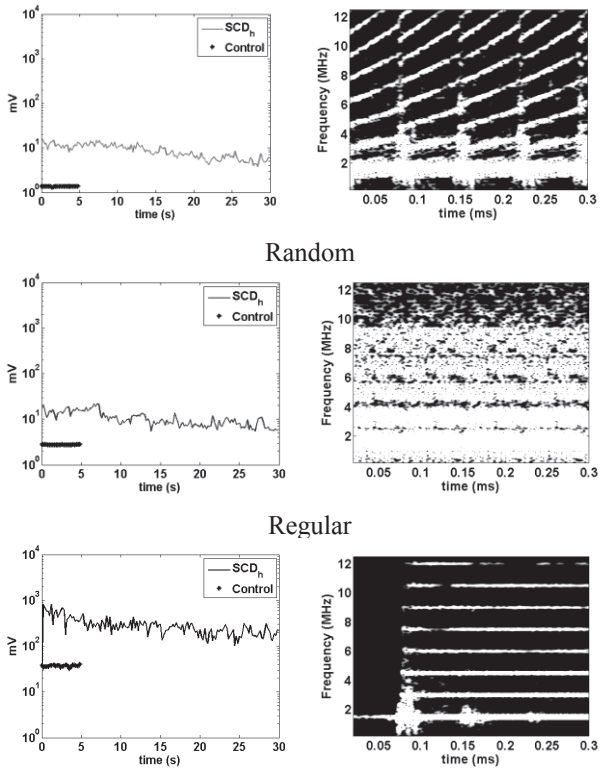

Fig. 1 – (left column) Cavitation levels (SCDh) obtained for each method and (right column) real-time spectrograms of the PCD signals for each method.

Seven days after sonication, all mice were analyzed with histology of the brain sections using Hematoxylin and Eosin staining. The animals were transcardially perfused and fixed in 4% paraformaldehyde. After that, the brains were paraffin embedded and the caudate putamen was sectioned horizontally at 6-µm thickness in 8 levels with 180-µm distance between sections. The sections were stained and bright-field images were acquired using light microscope. A trained observer evaluated if there was cell/tissue loss or red blood cell extravasation into the brain parenchyma.

III. Results

The cavitation levels SCDh were calculated for up the 6th harmonics of the real-time PCD signals during the sonication of the groups chirp, random, and regular. Fig. 1 shows representative cavitation levels (SCDh) and real-time spectrograms of the PCD signals for each sonication method.

Fig. 2 shows representative T1-FLASH weighted MR images obtained from animals sonicated with chirp, random, and regular methods. The brighter regions in the MR images show the diffusion of the gadolinium into the brain parenchyma revealing the BBB disruption.

Tab. 1 presents the mean cavitation levels (SCDh) and the BBB opening volume obtained from each sonication method. ANOVA statistical analysis for both SCDh and BBB opening volume show significant difference between the coded sonication methods and the regular sonication method. No

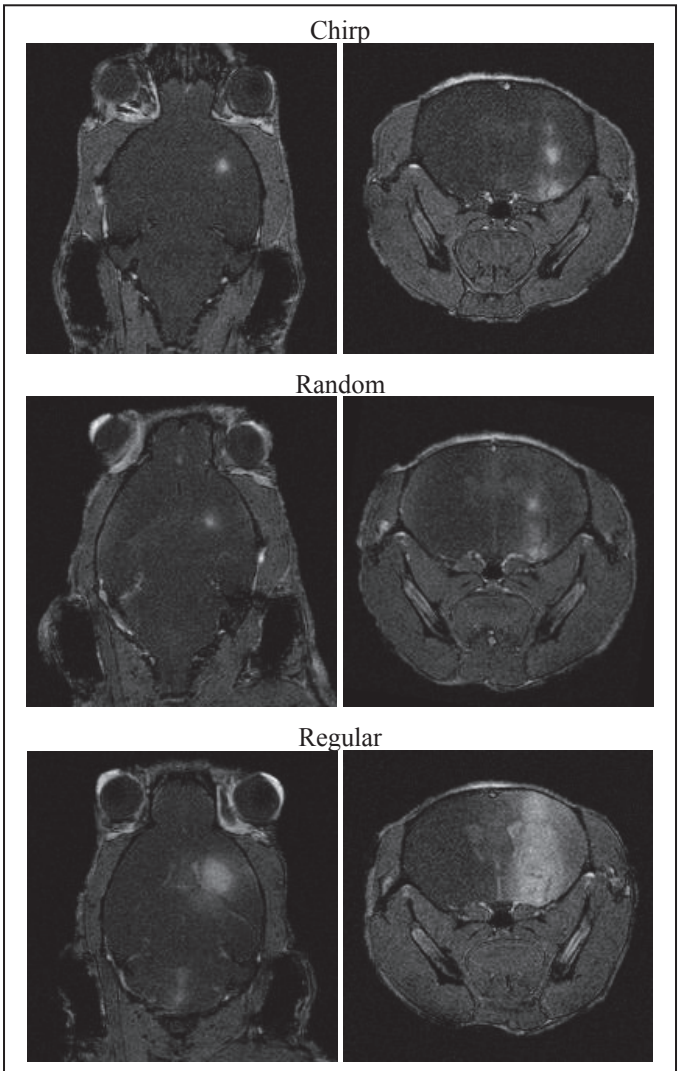

Fig. 2 – T1-FLASH weigthed MR images with contrast agent (gadolinium).

significant difference were found between the chirp and random sonication.

The histological examination revealed no brain damages in the mice sonicated with chirp coded ultrasonic excitation. Four out of five animals from the Random group presented dark neurons as minor tissue damage. Three animals from the Regular group expired on the second day during MRI acquisition (potentially due to prolonged anesthesia) and two presented similar minor damages to the found in the Random group.

TABLE I. Cavitation levels (SCDh) and BBB opening volumes for chirp, random, and regular sonications.

	Sonication methods		
	Chirp	***Random***	***Regular***
SCDh	55.40±28.43	63.87±29.97	356.52±257.15
BBB opening volume (mm^3)	9.38±5.71	8.91±3.91	35.47±5.10

IV. Discussion

The coded excitation methods improved the targeting precision, generating lower cavitation levels and more confined BBB opening volumes than the standard mono-frequency sonication. The coded methods did not present standing wave formation, even though they were driven in continuous wave mode to facilitate standing wave formation. However, the coded methods were not capable of completely avoiding BBB openings close to the brain base at distances 2 mm and 2.48 mm from the focal spot, which were also observed in the standard sonication. The coded excitation methods may thus enable more precise drug delivery and it may benefit other FUS applications. Further studies are necessary to explore different microbubbles size distribution, shell properties and their optimum frequency bandwidths.

V. Conclusion

This study presented an *in vivo* quantification of improvements in transcranial ultrasound targeting using chirp- and random-based ultrasonic excitation methods. The results presented here have shown the capability of these techniques to induce more confined BBB opening volumes. The improvement in transcranial ultrasound targeting can also benefit other therapeutic ultrasound techniques that use higher-pressure levels and require precision to ablate or stimulate the targeted region without causing damage to the normal tissue.

Acknowledgment

We thank Hong Chen, Ph.D. and Tenysson W. Lemos, Ph.D. for their important input and technical support.

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