Bilateral blood-brain barrier opening in mice using acoustic holograms

Background (character limit 1000): Microbubble-enhanced low-intensity focused ultrasound is the only non-invasive technique to disrupt the blood-brain barrier (BBB) temporally and locally, which allows targeted drug delivery into the central nervous system. However, it is not possible to simultaneously target two or more brain structures, using either a single-element or multi-element transducer. In this work, we present a novel technique using acoustic holograms to perform BBB opening in two mirrored regions in mice brain. The system consists of a single-element transducer and a 3D-printed holographic lens designed to simultaneously create two symmetric foci in anesthetized mice in vivo. This approach has three main advantages: (1) simple and low-cost; (2) aberrations due to skull and water container are corrected; and (3) a single sonication enables multiple BBB opening locations, becoming a time- and cost-effective therapeutic system for neurological diseases.

Materials and Methods (character limit 1500): The process of hologram generation is divided in five steps. First, we extract the geometry and acoustic properties of a mouse skull from x-ray micro-CT images and we identify the target structure based on MRI scans. Second, simulated back-propagation and phase-conjugation are used to calculate the acoustic wavefront at the holographic surface. Third, a phase-only lens is generated from both the phase and magnitude of the recorded wavefront. Fourth, the lens is manufactured using Formlabs ClearResin (sound speed: 2580 m/s; density: 1171 kg/m^3) by stereolithographic 3D-printing. Finally, the target acoustic image is generated by exciting the lens with the single-element transducer. For the in-vivo experiment, 1µl/g Definity microbubbles were injected intravenously and 1.68-MHz FUS (400 kPa peak-negative pressure, PRF: 5 Hz, pulse length: 1 ms) was applied for 2 minutes. Then, 0.2 ml of gadolinium tracer was injected intraperitoneally, and post-treatment BBB opening was assessed using T1-weighted MRI. Figure 1 shows the experimental and simulated configuration consisting of the single-element FUS transducer (diameter: 64mm, focal depth: 62.6mm; H-204, Sonic Concepts, WA, USA), the acoustic lens to compensate the skull aberrations and split the focus in two, the high-frequency 10-MHz PCD was used for targeting purposes, the water cone for acoustic coupling between the lens and the head of the animal, and the shaved head of the mouse where the bifocal target was reconstructed.

Results (character limit 1000): T1-MRI shows gadolinium extravasation at two symmetric focal spots. The two experimental BBB openings are separated 3.0 ± 0.7 mm (n=5 mice) compared to 5.3 mm in full-wave simulations. The discrepancy was likely due to the differences between the skull scanned with micro-CT for manufacturing the lens and the skulls of the treated mice. Furthermore, air pockets trapped within crevices of the lens may have contributed to the field distortion. Using the micro-CT scan of the treated subjects would lead to a better ultrasound location prediction. Nevertheless, we show here the capability of bifocal ultrasound generation in different animals using a separate unique micro-CT scan.

Conclusions (character limit 750): A bilateral BBB opening is possible employing one single sonication with a holographic lens in mice, thus improving the treatment efficiency for several neurodegenerative diseases targeting symmetric brain structures, e.g. hippocampus, putamen and caudate. This work demonstrates the feasibility of hologram-assisted BBB opening for targeted drug delivery in the central nervous system in symmetric regions in separate hemispheres.

Take Home Message (character limit 280): We demonstrate how a single-element transducer with a 3D-printed holographic lens allows to (1) simultaneously produce bilateral BBB opening

in anesthetized mice in vivo, and (2) compensate the aberrations due to both the skull and the water cone.

Biography of the presenting author (character limit 1000): Sergio Jiménez-Gambín received his BSc in Telecommunication engineering at University of Alicante in 2015. Under a research internship, he has worked on the detection of accelerated corrosion in concrete using non-linear ultrasound-based techniques. In 2016, he received a MSc in Acoustic engineering at Polytechnic University of Valencia. Since 2017, he is a PhD student led by Prof. Paco Camarena at i3M working on focused ultrasound modelling and hologram generation for therapeutic transcranial ultrasound, funded by an FPI grant of the Generalitat Valenciana. In 2020, he was a visiting researcher at Ultrasound Elasticity Imaging Laboratory (UEIL) at Columbia University, led by Prof. Elisa E. Konofagou, working on BBB opening using acoustic holograms.