EX-VIVO VALIDATION OF A PASSIVE CAVITATION DETECTION SYSTEM FOR HOLOGRAM-ASSISTED BLOOD-BRAIN BARRIER OPENING IN NON-HUMAN PRIMATE

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ABSTRACT.

A safe blood-brain barrier (BBB) opening is characterised by a reversible and temporary disruption of the tight junctions. Within a range of ultrasound excitation levels, injected microbubbles cavitate in a stable regime leading to a safe BBB opening. However, above a maximum threshold, the cavitation becomes inertial and microbubble collapse can cause irreversible damages. Passive Cavitation Detection (PCD) allows a real time control of the cavitation state of the microbubbles by analysing the scattered signals. In this study, a system for PCD measurement is developed for hologram-assisted BBB opening and tested in a non-human primate *ex-vivo* skull. An *ex-vivo* skull is combined with a 3D-printed head to obtain a closed cavity. Brain is replaced by water and microbubbles are continuously injected into the head phantom. Measurements are performed with a dual transducer setup, a 0.5 MHz focused ultrasound transducer aligned with a 3.5 MHz passive cavitation detector. The presence of harmonics and ultra-harmonics in the spectrum of the received signals indicates that microbubbles are cavitating, and the absence of broadband noise indicates that no inertial cavitation is occurring. This system enables emission adjustments, allowing to monitor the treatment and ensuring the safety of the procedure.

RESUMEN.

Una apertura segura de la barrera hematoencefálica se caracteriza por una ruptura reversible y temporal de la conexión estrecha entre células. Dentro de un rango de niveles de excitación ultrasónica, las microburbujas inyectadas cavitan de manera estable implicando una apertura segura de la barrera. Sin embargo, pasado un cierto umbral, la cavitación empieza a ser inercial y el colapso de las microburbujas puede causar daños irreversibles. La detección pasiva de cavitación permite un control en tiempo real del estado de cavitación de las microburbujas analizando las señales recibidas. En este trabajo se desarrolla un sistema de detección pasiva de cavitación para la apertura de la barrera hematoencefálica asistida por holograma y se realizan unas pruebas con un cráneo *ex-vivo* de un primate. El cráneo *ex-vivo* está unido a la cabeza impresa en 3D del mismo primate para obtener una cavidad cerrada. El cerebro está sustituido por agua desgasificada y las microburbujas están inyectadas en continuo en el phantom de la cabeza. Las medidas se realizan usando un sistema de dos transductores, un transductor focalizado de frecuencia central 0.5 MHz alineado con un transductor de cavitación de 3.5 MHz. La presencia de los armónicos y ultra-armónicos en el espectro de las señales recibidas indican que las microburbujas están cavitando y la ausencia de ruido de banda ancha

indica que no hay cavitación inercial. Este sistema permite ajustar los parámetros de emisión, y por lo tanto permite la monitorización del tratamiento y asegura un procedimiento seguro.

1. INTRODUCTION

The blood-brain-barrier (BBB) is a physical barrier in the Central Nervous System (CNS) blood vessels and capillaries composed of endothelial cells connected by tight junctions that protects the brain by limiting entry of potentially neurotoxic plasma components, blood cells, and pathogens [1]. However, in cases of CNS disease, the extremely low permeability of the BBB blocks the delivery of many potentially effective diagnostic or therapeutic agents [2]. Focused ultrasound (FUS) in combination with microbubbles can lead to a transient and non-invasive opening of the BBB, thus facilitating targeted drug delivery to the brain [3]. Recent research also has shown that holographic acoustic lenses allow skull aberrations correction and a control of the acoustic beam direction and the focal zone shape in order to target specific affected areas of the brain [4].

Even when ultrasound focusing can be efficiently achieved using holograms, the problem of monitoring the treatment is still an open issue. Since one of the most robust indicators of the opening of the BBB is the acoustic emission produced by the cavitation of microbubbles [5], spectral algorithms have been developed for the detection of sub-harmonic, harmonics and ultra-harmonics generated by cavitation activity [6]. Quantitative indexes can be calculated to evaluate the cavitation dose [7], and be used to implement acoustic feedback systems to dynamically control the power delivery system and limit in real time the dose delivered [8][9].

In this work, we implement a passive cavitation detection system and validate it experimentally through a non-human primate *ex-vivo* skull. Experimental setup consists of a dual transducer system, a passive cavitation detector and a FUS transducer coupled with the holographic lens, and a primate head-mimicking in which microbubbles are injected. A parametrical study is conducted by varying the amplitude of the emitted signal and the impact on microbubbles behaviour is analysed, based on cavitation index values.

2. MATERIAL AND METHOD

2.1. Assembly of the primate head

The head of a non-human primate is mimicked by combining the upper part of an *ex-vivo* skull with the rest of the head printed in 3D. Madrid hospital provided us an *ex-vivo* skull of a macaque with the corresponding CT scan and the MRI of the entire head. A 3D model of the head removing the part corresponding to the *ex-vivo* skull and the brain is created and printed. This way, we can stick together both parts, the *ex-vivo* skull and the 3D-printed head, and obtain a closed cavity. The cavity is then filled with degassed water.



Figure 1 - Head mimicking of a non-human primate by assembling an ex-vivo skull and a 3D-printed head.

2.2. Sonication

A 0.5 MHz Focused Ultrasound (FUS) transducer is used for transcranial sonication. A 3D-printed holographic lens is placed at the exit surface of the FUS transducer. Two different lenses are designed in order to have, in one case, a 5.6 cm deep focal point in axis with the geometrical centre of the transducer and, in the other case, a focal point located at the same depth but off-axis. During this initial study we only take the centred focus lens into account. The coupling between the system and the skull is made by using a thin membrane which is directly in contact with the ex-vivo skull. The space between the lens and the membrane is fulfilled with degassed water. The cavitation detector is inserted in the centre of the FUS transducer in such a way that origins of both transducers are aligned. A total sonication of 150 s is applied at the location of the focal point. 10 ms bursts are generated at a pulse repetition frequency of 1 Hz. A parametric study is carried out by varying the amplitude of the generated signal. A calibration of the system allows us to determine the amplitude at the focal point corresponding to a fixed input voltage. This way, we estimated that for a pressure level of 0.5 MHz at the focal point, an input voltage of 200 mV is needed.

2.3. Neuronavigation system

The exact positioning of the transducer is guided by the Brainsight neuronavigation system. Previous to the experiment, a calibration of the system is needed by using a 3D camera and trackers that determine the position and orientation of the transducer relative to the head of the primate. For this, the printed head is fixed to a table and the dual transducer setup is mounted on a mechanical arm. The tracking is visualized on the Brainsight software where the CT scans have been imported.

2.4. Microbubble preparation

For the experiments, microbubbles (Luminity, Lantheus Medical Imaging) were injected by using an infusion pump (Perfusor fm, B Braun) at an infusion rate of 0.02 ml/s. The initial concentration is fixed at 20 µL/kg and the solution is diluted in 5 mL of PBS. A parametric study is carried out by varying this concentration value.

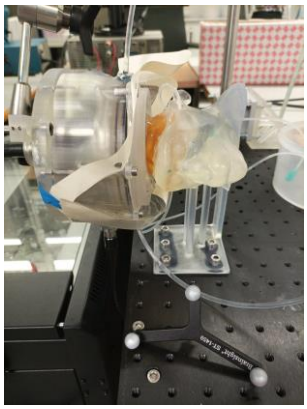


Figure 2 - Experimental setup for passive cavitation detection through an *ex-vivo* skull.

3. RESULTS

For each series of measurements, a first one is performed without microbubbles as a reference. The values of the cavitation indexes corresponding to harmonics, ultra-harmonics and broadband noise are calculated for different sonication levels. For a low input level (100 mV), no significant change in the cavitation index value is detected when microbubbles are injected (Figure 3). When increasing the input level to 200 mV, corresponding to a pressure amplitude of 0.5 MHz at the

focal point, the level of the harmonics increases whereas no changes for the ultra-harmonics and broadband noise curves are observed. Thus, at this applied pressure, stable cavitation is detected, and the absence of ultra-harmonics and broadband noise indicates that no unstable regime is reached.

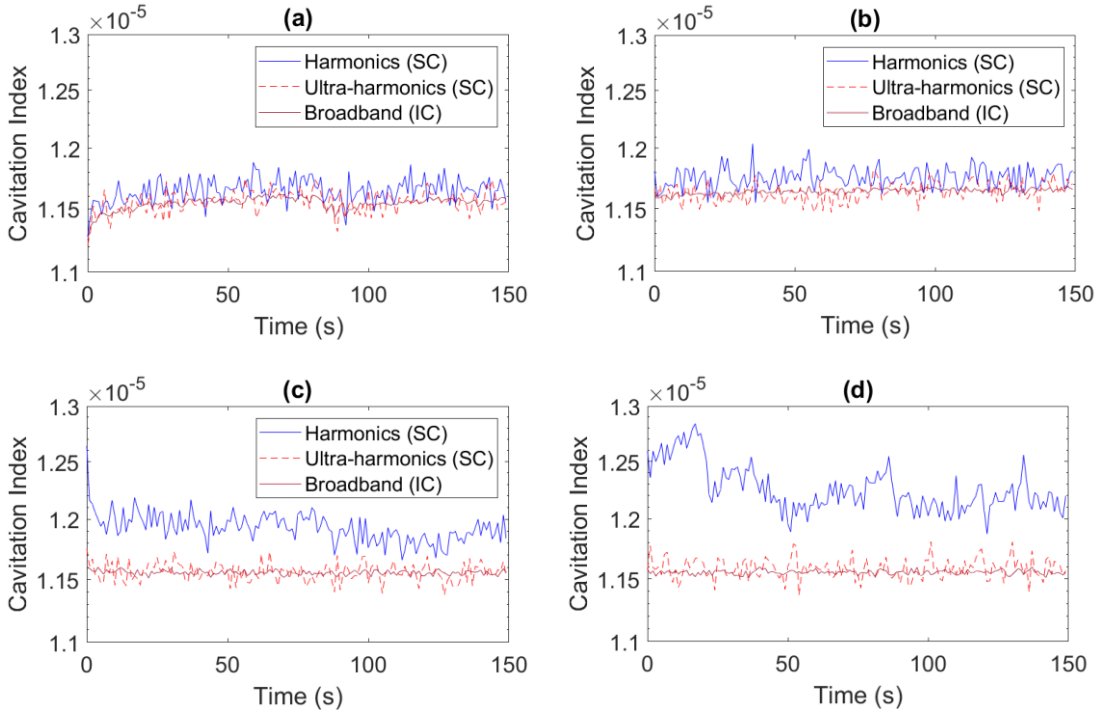


Figure 3 - Cavitation index in the case of (a) an input voltage of 100 mV without microbubbles and (b) with 20 µL/kg of microbubble concentration, and (c) an input voltage of 200 mV without microbubbles and (d) with 20 µL/kg of microbubble concentration.

4. CONCLUSION

In this study, the objective was to develop a PCD system coupled to a holographic lens system for transcranial sonication ex-vivo. We were able to mimic a real macaque head by assembling an ex-vivo skull with a 3D-printed part of the head in such a way that the FUS transducer could be coupled to the ex-vivo skull. By knowing the correction provided by the lens to the geometrical focal point of the FUS transducer and by using the 3D camera of the neuronavigation system, we could find the exact positioning of the dual transducer setup. A 0.5-MHz FUS transducer was used and a reference pressure level at the focal point was fixed at 0.5 kPa, corresponding to an input voltage of 200 mV. For a microbubbles concentration of 20 µL/kg injected to the brain phantom, we were able to detect stable cavitation. We also saw that below this threshold no microbubble activity is detected. Also, the absence of broadband noise indicates that no inertial cavitation is generated. This passive cavitation detection system allows a real time monitoring of the sonication and applying the parameters employed for this study, an in-vivo application could be possible assuring the safety of the treatment.

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