AN IMAGE-GUIDED FOCUSED ULTRASOUND SYSTEM FOR GENERATING ACOUSTIC SHOCK WAVES THAT INDUCE TRAUMATIC BRAIN INJURY IN WILD-TYPE ZEBRAFISH

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1 Introduction

Unintentional injuries such as traumatic brain injury (TBI) are the leading killer and disabler of Canadians under the age of 44 [1]. TBI is induced by biomechanical forces caused by a direct blow to the head, face, or elsewhere on the body with an impulsive force transmitted to the head, yielding a rapid but short-lived impairment of neurologic function [2].

In order to investigate TBI mechanisms, we have developed an image-guided focused ultrasound system that generates acoustic shock waves to induce TBI in wild-type zebrafish. This system permits the development of a TBI model for zebrafish that adequately recapitulates mild (closed-head) traumatic brain injury and subsequent secondary injury mechanisms. Acoustic shock waves of short pulses (pulse length = 50 ms) of intense focused ultrasound (pressure = 11 MPa) within a 7.5 mm × 1.2 mm focal zone cause brain injury by inducing mechanical stress and transient cavitation. Since zebrafish have a high degree of genetic homology and cell signaling pathways relative to mammalian species, this research may provide insight into shockwave-induced dysfunction leading to TBI and disruption of the blood-brain barrier in humans.

2 Method

This shock wave generation system incorporates a 1 MHz focused ultrasound transducer (focal length = 10 cm in water), which is excited by pulsed 1 MHz signals that are amplified and transmitted via an impedance-matching transformer. A calibrated radiation force balance is used to correlate input electrical power with output acoustic power, which is subsequently correlated with focal-zone pressure using measurements from a calibrated hydrophone. In our system, the output acoustic power is approximately 64% of the input electrical power [3].

Focal-point acoustic intensities and pressures are simulated using a Linear Acoustic and Temperature Simulator (LATS) program developed by our group [4]. LATS simulates the axial and cross-sectional acoustic intensity profiles (Fig. 1) for a given transducer geometry and input power. Corresponding calculated acoustic pressures reveal good agreement between the simulated and measured acoustic pressures (Fig. 2). The profiles also provide insight into the intensity distributions and beam-widths at various distances from the transducer.

An imaging probe embedded within the focused transducer generates confocal B-mode images (Fig. 3) to enable visualization of the zebrafish location, and to locate the zebrafish brain consistently within the focal zone.

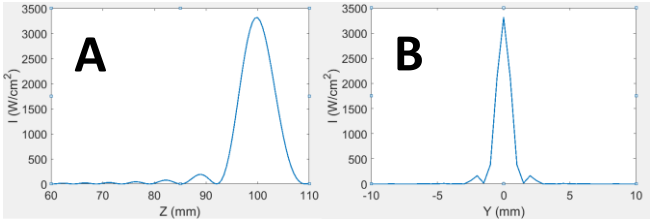

Figure 1: A) Axial and B) cross-sectional intensity profiles.

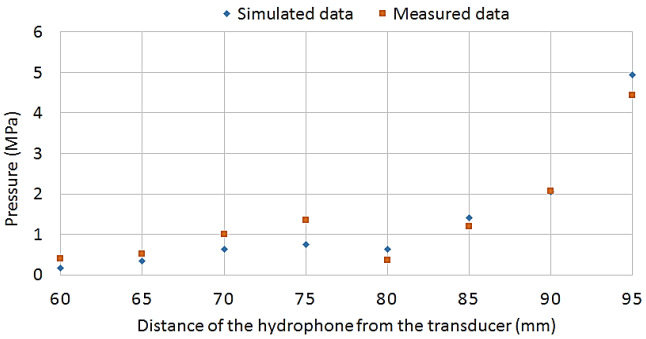

Figure 2: Simulated and measured acoustic pressures vs. distance from the transducer.

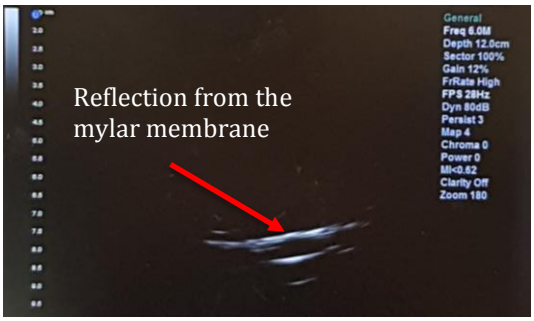

Figure 3: Confocal B-mode images enable visualization of the zebrafish location. When the zebrafish head is aligned with the imaging transducer, gill movement is visible in the B-mode image.

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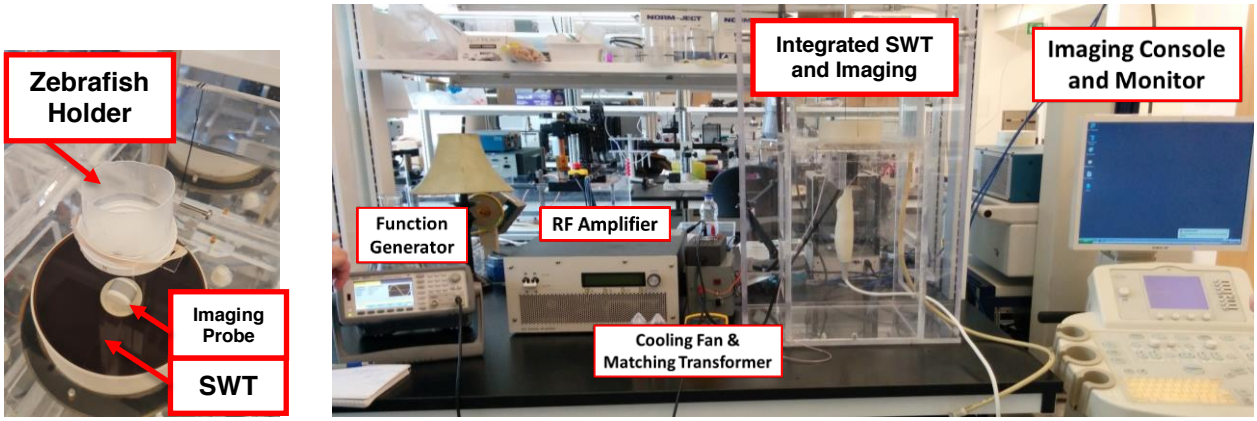

Figure 4: Left, zebrafish holder positioned at the focal length of the shock wave transducer (SWT). Right, full image-guided focused ultrasound system.

Zebrafish are anaesthetized using 100 ppm clove oil, and then individually positioned in a holder within a water tank (Fig. 4). The holder contains a 3 mm hole covered by a thin layer of ultrasound-transparent mylar membrane, which is located under the zebrafish head to ensure that acoustic shock waves are delivered only to the head.

3 Impact on zebrafish

In our previous post-TBI studies, zebrafish shocked with 50 ms pulses of 11 MPa typically experienced longer recovery times, decreased swim distances and velocities, heightened anxiety, and altered group social dynamics [5]. However, after injection of neuroprotective compounds such as CB3 and SD1 into shocked zebrafish, preliminary results indicate that these compounds can yield a reversal of the symptoms experienced by shocked zebrafish.

CB3 and SD1 have an additive effect on increasing swim distance, lowering anxiety, and increasing the mobility of shocked zebrafish. For instance, control fish explore the tank floor for ~60 s before swimming near the surface (Fig. 5a), and shocked fish explore the tank floor for significantly longer (> 200 s) before swimming near the surface (Fig. 5b). Adding CB3 (Fig. 5c) and then SD1 (Fig. 5d) are found to induce the shocked fish to swim at shallow depths after times comparable to the control group (~60 s).

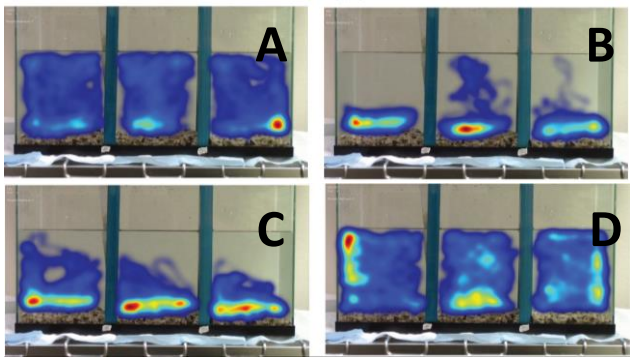

Figure 5: Heat maps representing the swim coverage of zebrafish in water tanks under various conditions: A) Control; B) Shocked; C) Shocked and injected with CB3; D) Shocked and injected with CB3 and SD1.

4 Conclusion

An image-guided ultrasound system has been developed to deliver acoustic shock waves effectively to induce TBI in zebrafish. Proper alignment of the zebrafish with the shock wave transducer can be checked using confocal B-mode imaging. Simulated and measured acoustic pressures are found to be in good agreement. Zebrafish shocked with a 50 ms shock wave at 11 MPa exhibit longer recovery times and exhibit erratic swim patterns when compared to those of control fish. Further investigations on the impact of neuroprotective compounds will be conducted with this system to gain insight into the mechanisms of TBI.

Acknowledgments

The authors acknowledge research funding support from the Ontario Research Fund - Research Excellence (ORF – RE) and the Ryerson Dean's Research Fund.

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