Progress Report: FUS-mediated reversible modulation of region-specific brain function

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We examined whether or not local cortical function can be safely and reversibly regulated by the application of pulsed FUS (FUP) in an animal (rabbit) model. *We originally proposed to conduct FUS experiment in-vitro first to examine the range of safe FUS parameters for the animal testing; however, we opted to pursue the animal experiment based on the review of literatures on biological effects of FUS*. New Zealand White rabbits (n=5; all male) underwent craniotomy 15 days prior to the FUP application. Functional MRI (fMRI) was used to locate the rabbit's visual areas that were activated by the 3 Hz white light stimulation (via LED strobe). Real-time fMRI (rtfMRI) was employed to monitor the blood oxygenation level dependent (BOLD) signal response from the region-of-interest in the visual areas while the FUP intensity and duration (to the same area) was adjusted from 25 W/cm^2 to 350 W/cm^2 (temporal peak intensity). A 690 KHz transducer (known to allow the transmission of acoustic energy transcranially) with 8 cm focal depth was used. Injection of microbubbles, which often used to amplify the effects of acoustic blood-brain-barrier (BBB) opening, was not adopted.

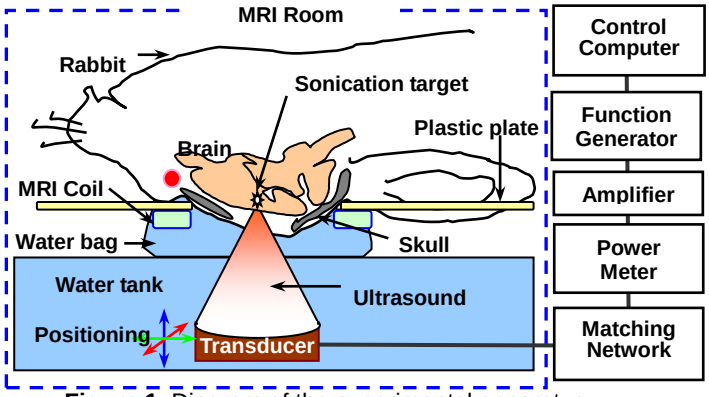

Figure 1. Diagram of the experimental apparatus.

We determined that FUP (500 μs duration and 10 msec inter-pulse-interval) administered at 50W/cm^2 intensity for the duration of 9 sec robustly activated the cortical region (size of 4 x 4 x 8 cubic mm; example shown in Fig.2A), followed by the temporary deactivation (hypothesized to reflect the 'relative' refractory period) for more than 10 min. Original degree of cortical activity, as measured by the BOLD, was recovered after ~15 min. MR thermometry was used to measure the temperature at the FUP site and virtually no temperature change was detected during the 27 s of FUP application (Fig. 2B). Intravenous injection of Trypan Blue revealed no apparent BBB disruption. H&E staining of the brain tissue showed no presence of local hemorrhaging or tissue damage. Two animals were allowed to survive for more than 2 weeks after the FUP application. Normal behavior and histology suggested that the given FUP parameter can be safely used for reversible modulation of cortical function. We also have observed selective excitation, as measured by the increase in BOLD activity, from motor areas above the sonication window when higher acoustic energy (350 W/cm^2) was applied; however, this phenomenon was not reproduced at this stage since we would like to confirm the inhibitory nature of the FUP.

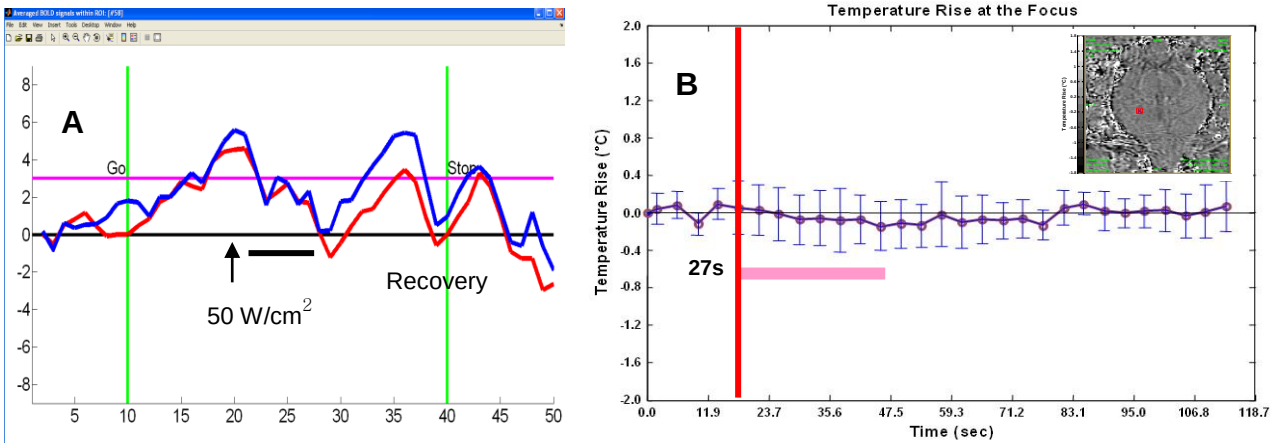

Figure2. (A) rtfMRI results showing the suppression of BOLD signal upon application of FUP. Red and blue lines indicate the BOLD signal averaged across 5 pixels from two adjacent ROIs (3 mm apart) from the targeted acoustic foci in visual cortex. Green vertical lines indicate the starting and end of the visual stimulation. (B) MR Thermometry results showing there is no temperature elevation from the FUS locus during the 27s-long application of the FUP.

Apart from the animal testing, we developed a robotic platform to pattern rat neurons and astrocytes in 3D collagen scaffold. This technique, so called on-demand 3D freeform fabrication (FF), is expected to provide in-vitro neural tissue composites for further testing of safe FUP parameters. *The platform was used in order to build a in-vitro neural tissues to conduct the AIM#1 of the original proposal (which aimed to test different FUS parameters applied to neural tissues created in vitro).* The printing hardware is shown in Fig. 3. The printer consists of modules of 4-channel array microvalves (SMLD Fritz Gyger AG, Thun-Gwatt, Switzerland) and a 3-axis Cartesian robotic stage that controls the timing and location of dispensing of cells in suspension and collagen precursor. The dispensing array, with a pneumatically-driven control mechanism (shown in the later section), was mounted to the robotic stage (Newmarksystems, CA; with bidirectional reproducibility of 5 µm). The target substrate was mounted to another robotic stage that moves along the vertical direction. The cell containing culture media and hydrogel precursors in aqueous form were placed in disposable plastic syringes (equivalent to the ink cartridges in commercial printer) and continuously fed to the dispensing array under pneumatic pressure. The entire device was housed in a laminar flow hood (StreamLine, FL) with two cameras (Pixelink, PL-A741, Ottawa, Canada and , UBV-49, Logitech, CA) used (1) to measure the droplet size and for (2) visual inspection of tissue constructs. The one of the dispensers and target substrates were temperature controlled (at 20ºC, operating temperature between 5ºC to 40ºC) by solid-state thermoelectric device (TED, TE Technology, Traverse City, MI). All cell/solution compartments and tubing used in this experiment were disposable and replaceable. All the machine parts were designed in detachable modules for easy assembly and modification.

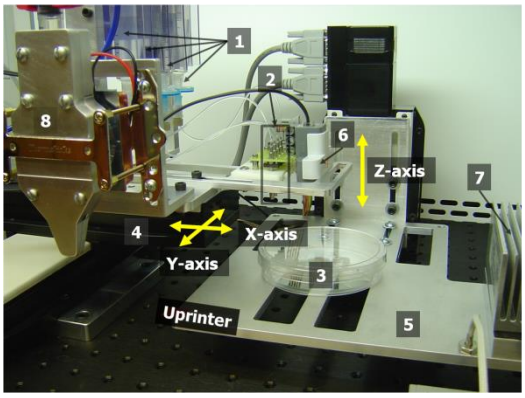

Figure 3 Picture of the modular cell printing platform shown with [1] fluid cartridges for cells and hydrogel precursors; [2] a dispenser array; [3] target substrate; [4] X-Y stage; [5] vertical stage; [6] range finder; [7] vertical stage heater/cooler; [8] optional independent heating/cooling unit for the dispenser.

Figure 4 illustrates the volume rendered Immunostaining image of 3-D neuron cylinder created by the platform. The 3-D artificial neuron cylinder was constructed by layer-by-layer printing of neurons in ring-shaped patterns on to the collagen scaffold. With 10x objective lens, the 3 different layers of neuron rings were distinguished. Neurite outgrowth of printed neurons in collagen was also observed. This shows that the printing platform can be used to create the neural tissues in hydrogel scaffold for in-vitro testing of FUS parameters.

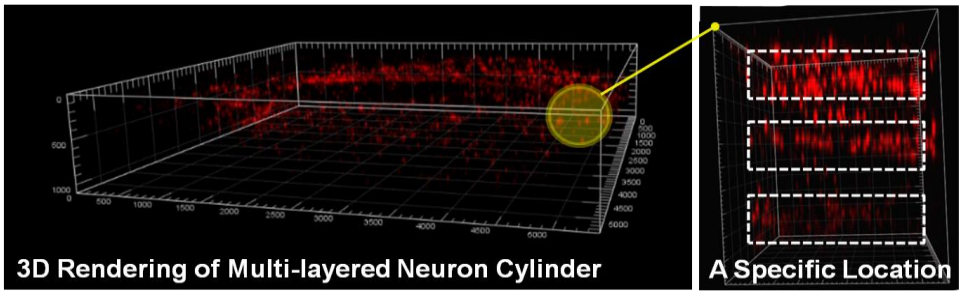

Figure 4 3D-rendering of collagen hydrogel block containing rings of rat neural cells from confocal microscopic imaging (MAP-immuno-stained).

Expectation for the immediate future:

Based on the robust and encouraging results so far, we will mainly focus on the continuation of the animal experiment to confirm our findings (inhibitory effects on neural activity) while validating the safety of the method via histological analysis. This focus on animal experiment, in addition to the proposed testing on in-vitro brain/neural tissue, will provide more practical evidence for (later) clinical application. We will also devote significant effort to systemically examine the FUP parameters, which lead to FUS-mediated *excitation* of regional cortical activity.