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Novel Broadband Acoustic Neuronal Cell Stimulator for High Throughput Cell Investigation

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***Abstract*—Ultrasound brain stimulation recently takes a lot of attention for the capability of modulating neuronal activities with mouse or human models. However, fundamental studies in the cellular level have not been performed with *in-vitro* studies. With the use of conventional focused ultrasound beam, semi-uniform acoustic energy only can be delivered to couple of cells, and unexpected artifacts such as shear waves and reverberations would cause additional neuronal responses which need to be avoided. In this paper, a novel high throughput coverslip based transducer was developed, and astrocytes were directly cultured on the surface of transducer to deliver the uniform acoustic energy to the group of cultured cells. Fluorescence calcium responses from the group of astrocytes could be measured. Astrocyte showed suppressive responses to the acoustic stimulations, and absolute peak amplitude of the calcium response was generally increased as the acoustic stimulation frequencies decreased.**

Keywords— Neuromodulation, Coverslip transducer, Astrocyte, Broadband, High throughput.

I. Introduction

Recently, the feasibility of the acoustic neuro modulation has been demonstrated in many forms [1],[2]. Although, the outcomes of the stimulation seem to be quite promising, the study is usually performed with *in-vivo* experiments without investigating fundamental effects on the neuronal cells with *in-vitro* studies. With the conventional focused ultrasonic beams applied externally to the cultured brain cells on a glass substrate, it is hard to study the pure cell response to the ultrasound stimulation because only a few neuronal cells can be stimulated with semi-uniform acoustic intensity [3]. Also, nonlinear distortions in the stimulation waveforms caused by shear wave along the substrate and reverberations can cause unexpected additional responses and make the cell investigations difficult [4]. To acquire statistically meaningful results, the importance of delivering uniform acoustic stimulations over a group of neuronal cells is paramount. In this paper, a novel high throughput coverslip based transducer was developed, and astrocytes were cultured on the surface of the transducer. The fluorescence calcium ion channel response to the acoustic stimulation was demonstrated, and the dependencies on frequencies were investigated.

II. Method

A. *Transducer fabrication*

A polyvinylidene fluoride-trifluoroethylene (PVDF-T(rFE)) transducer was developed with the substrate of a glass coverslip of 18mm in diameter. A square centimeter bottom electrode was fabricated by sputtering Au/Cr layer on the coverslip, and the spin coating of PVDF-T(rFE) was followed for 60 seconds at a speed of 500 rpm. After curing the PVDF-T(rFE) layer in an oven with 135 degree for 2 hours, top electrode of 8mm x 8mm was loaded. And then, poling process was performed by applying high voltage of 80MV/m. Finally, a parylene layer of 12 μm was loaded for an acoustic matching, water passivation and electric field passivation.

B. *Transducer output measurement*

Output pressure level of the transducer was measured by needle hydrophone (NH0200, Precision Acoustics, Dorchester, UK) in the frequency range from 1 to 10 MHz for acoustic output calibrations. To mimic the actual measurement system, an acrylic lens sample was machined. To prevent the reverberations from the bottom, water surface and lens, ultrasound absorber (Aptflex F28, Precision Acoustics, Dorchester, UK) were laid on those boundaries as shown in fig. 1(a). Needle hydrophone was inserted through the hole in the custom made 10x lens phantom. For investigating the neuronal cell responses, astrocytes from a mouse brain were cultured on the fabricated transducer. The cellular output was recorded for the different excitation frequencies by using confocal microscopy.

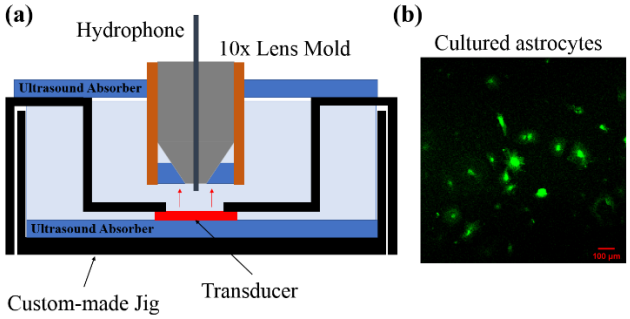

Fig 1.(a) Output measurement setup. (b) Calcium image of cultured astrocytes

C. *Experimental procedures*

Astrocytes were cultured on the transducers placed in a 12 well plate for 10 days. Due to the parylene layer, the transducers became biocompatible and protective to the alcoholic agent for cleaning the transducers. Once the cells were cultured, the calcium ion indicator fluo-4 was loaded into the astrocytes.
After loading the transducer in a custom-made chamber properly, astrocytes were imaged by confocal microscopy (LSM880, Carl Zeiss, Oberkochen, Germany) while being stimulated by transducer at 120 seconds interval. Real-time imaging was performed to monitor the fluorescence calcium signal level changes of the cultured cell. The cellular responses to the different frequencies from 4 to 10 MHz were recorded with the pressure, PRF, duty cycle and total stimulation period of 0.1MPa, 1.5 kHz, 50% and 500ms, respectively.

III. Results

A. *Transducer output measurement*

Acoustic reverberations shown in the hydrophone output signal were successfully reduced by the use of the absorbing material. The output negative pressure of 0.1MPa shown in fig. 2(i) was achieved until the frequency reduced to 4MHz. Under 4MHz, 0.1MPa was barely acquired. The acoustic output of 0.2MPa could be only acquire from the frequency range higher than 8MHz.

B. *Astrocyte calcium response*

Fig. 2 shows the fluorescence calcium signal changes for the different stimulation frequencies from 4 to 10MHz in fig. 2(a)-(g), respectively. In each image, the stimulation was applied at 5s and takes resting time for 115s. With 0.1MPa, suppressive neuronal activities were recorded, and the absolute peak signal level was increased as the stimulation frequencies were decreased as shown in fig. 2(h).

IV. Discussion

Previously, investigations on acoustically stimulating group of neuronal cells *in-vitro* were barely reported because the conventional focused ultrasound beam stimulates only couple of cells and may trigger unintended neuronal activities. In the present study, a novel broadband acoustic cell stimulator was developed to overcome those limitations. Astrocytes were cultured directly on the surface of the developed transducer, and the frequency dependencies of the neuronal activities were successfully demonstrated.

As shown in fig. 2, all of the cell was presenting suppressive responses which may be the amplitude of the stimulation. The higher stimulation amplitude may induce excitatory responses which need to be investigated in the future.

The acoustic output of the transducer in the lower frequencies were limited as seen in fig. 2(i). The use of other piezoelectric material would solve the concerns of presenting lower acoustic output in the lower frequencies.

References

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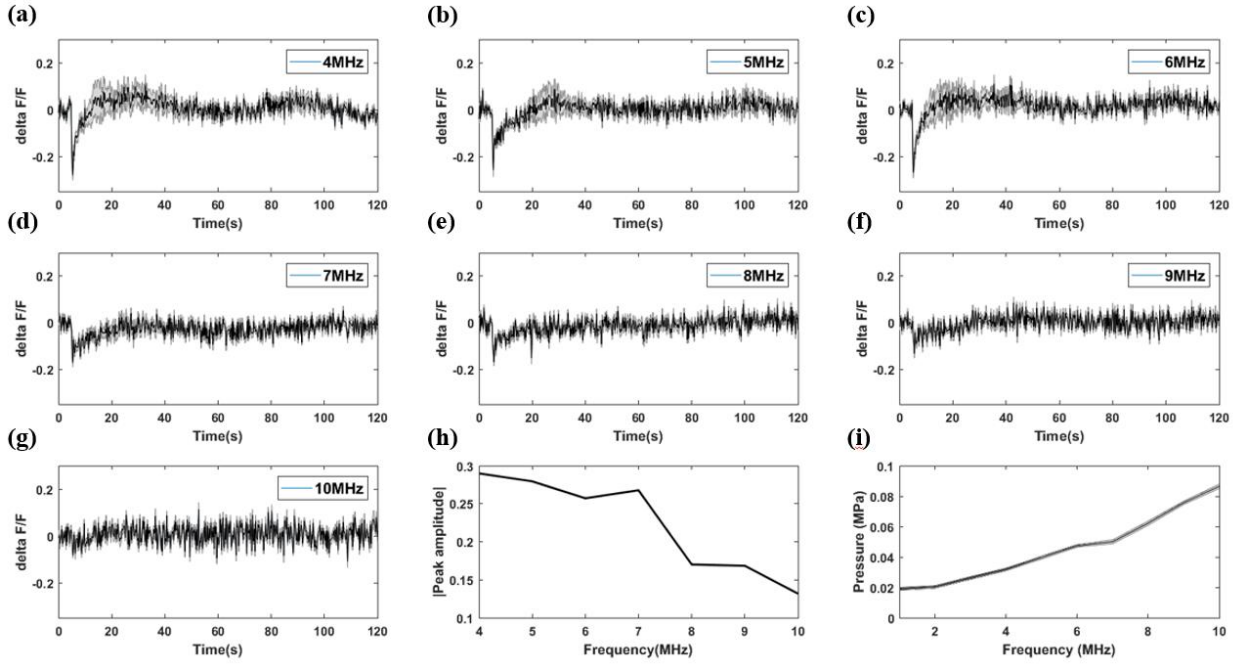

Fig 2.(a)~(g) Fluorescence intensity change frequency range from 10MHz to 4MHz. (F=Fluorescence intensity). (h) Absolute value of peak amplitude after stimulation. (i) Transducer output negative pressure. (Input electrical amplitude = 90V_{PP})