Stretchable Microelectrode Arrays
A Tool for Discovering Mechanisms of Functional Deficits Underlying Traumatic Brain Injury and Interfacing Neurons with Neuroprosthetics

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Abstract—**Traumatic brain injury (TBI) can be caused by motor vehicle accidents, falls and firearms. TBI can result in major neurological dysfunction such as chronic seizures and memory disturbances. To discover mechanisms of functional deficits underlying TBI, we developed a stretchable microelectrode array (SMEA), which can be used for continuous recording of neuronal function, pre-, during, and post-stretch injury. The SMEA was fabricated on a polydimethylsiloxane (PDMS) substrate with stretchable, 100µm wide, 25nm thick gold electrodes patterned there on [1]. The electrodes were encapsulated with a 10-20µm thick, photo-patternable PDMS insulation layer. Previous biocompatibility tests showed no overt necrosis or cell death caused by the SMEAs after 2 weeks in culture [2]. The electrical performance of the SMEAs was tested in electrophysiological saline solution before, during and after biaxial stretching. The results showed that the electrode impedance increased with the strain to reach 800kΩ at 8.5% strain and then recovered to 10kΩ after relaxation. The working noise level remained below 20µVpp during the whole process. New methodologies for improving the patterning of the encapsulation layer were tested on gold electrode arrays supported on glass. With these prototype arrays, robust population spikes were recorded from organotypic hippocampal slice cultures of brain tissue. Additionally, seizure-like activity induced with 1 mM bicuculline was also recorded. Our results demonstrate that the prototype arrays have good electrical performance compatible with existing multielectrode array systems. They also indicate the ability to record neuronal activity from hippocampal slices. This novel technology will enable new studies to understand injury mechanisms leading to post-traumatic neuronal dysfunction.**

I. INTRODUCTION

Approximately 2% of the US population lives with disabilities cause by TBI which disrupt the function of the brain. The epileptic-like activity in the central nervous system (CNS) after head injury is believed to be the result of post-traumatic hyperexcitability; the mechanism underlying this is still debated. Understanding how mechanical input induces electrophysiological pathology can help to identify therapeutic targets for the development of strategies to mitigate and prevent post-traumatic damage. Various models have been developed to study post-traumatic damage. According to prior work, the initial pathophysiologic process is likely to be caused by deformation of brain tissue during head injury. In our laboratory, a TBI model for the precise and reproducible injury of hippocampal cultures was developed [3]. Hippocampal cultures are grown on silicone membranes and injured by bi-axially stretching the membranes, mimicking induced brain deformation during TBI.

Previous studies have attempted to determine the mechanisms underlying TBI-induced functional deficits including regulation of intracellular signaling cascades, changes in cell viability, tissue activity, and organism behavior in different TBI models. The development of electrophysiologic tools for interfacing the brain with electronic devices to monitor and treat neurological diseases may be of particular use to the neurophysiologist and the clinician. By recording the extracellular electrical activity of neuronal populations in a neural network, specific post-traumatic alterations can be identified and analyzed. As a tool for extracellular recording from neural circuits, microelectrode arrays (MEAs) enabled long-term and simultaneous multi-site recording of functional activity of neural circuits [4-6]. However, traditional rigid MEAs, are incompatible with TBI models which induce injury via

tissue deformation because they can not deform with the tissue. Thus a stretchable MEA is required for monitoring brain activity before, during, and after traumatic injury, with no change of recording sites. We designed stretchable microelectrode arrays (SMEAs) with the novel ability to deform with the tissue during injury, allowing for continuous recording of neuronal function, pre-, during, and post-stretch. The SMEAs are fabricated on elastomeric substrates which are compatible with our TBI model. Stretchable gold electrodes were patterned on a polydimethylsiloxane (PDMS) substrate and encapsulated with a photo-patternable silicone (WL-5150, Dow Corning) insulation layer. After packaging and integration with a multichannel electrophysiology amplifier, the SMEAs will allow for long-term and simultaneous multi-site recording of extracellular activity from hippocampal cultures, before and after injury. Fig.1 shows how SMEAs work for *in-vitro*-TBI model, recording from the same hippocampal slice culture before and during stretch.

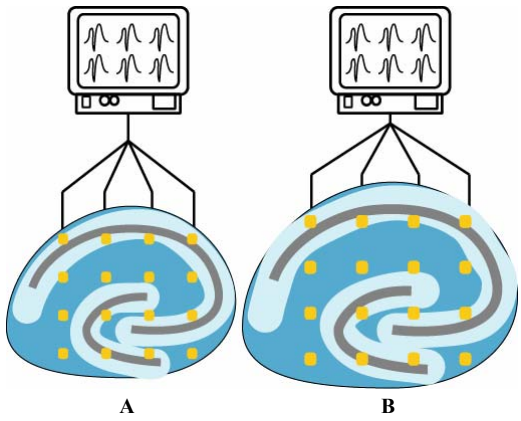

Fig.1 Continuous multichannel recording during deformation: A) pre-stretch; B) 20% 2-D stretch.

In our present work, the electrical performance of SMEAs was tested in electrophysiological saline solution before, during and after biaxial stretching. The recorded electrode impedance and working noise level were found to be well suited for the purpose of extracellular recording when compared to commercial MEAs. Additionally, to test new fabrication schemes, gold electrodes were patterned on a glass substrate and then insulated with the patternable PDMS. Using these test arrays, we were able to monitor robust spontaneous activity from hippocampal slice cultures and detect epileptiform activity induced with 1 mM bicuculline. This novel technology will enable studies to increase our understanding of post-injury changes in neuronal electrophysiological function.

II. METHODS & RESULTS

A. Traumatic Brain Injury Model

Organotypic hippocampal slice cultures were cultured on and adhered to a silicone culture substrate. A stretch injury device was designed to stretch the cultured tissue by the rapid deformation of the culture substrate [7]. Strain of the tissue during application of the stretch injury was verified with high speed video.

B. SMEA Design, Fabrication & Packaging

Fig.2 shows a top view of a 2×2 stretchable microelectrode array that we have designed [2]. The recording sites were 100μm×100μm square with an inter-electrode spacing of 300μm. The fabrication process has been described in detail in previous work [1, 8].

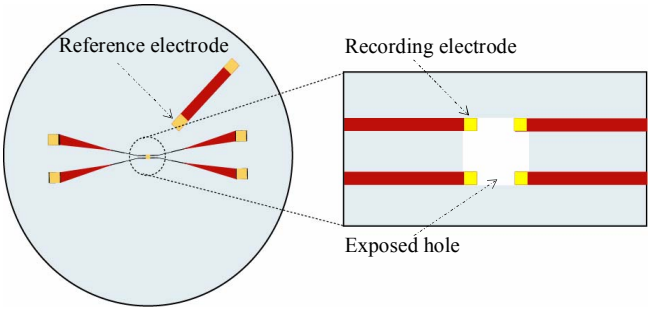

Fig.2 SMEA design (2×2 arrays)

For maintenance of the culture environment and interfacing the SMEAs with a multi-channel biosignal amplifier, the SMEAs were packaged as shown in Fig.3. Two PCB boards were used to fix the SMEA and form an electrical connection from the gold contact pads on the top PCB#1 to the gold electrodes on the SMEA. Conducting silver paste was used to achieve a robust connection. The gold pads on the top side of PCB#1 interfaced with the multi-channel amplifier through a series of spring-loaded contacts. A plastic culture well was mounted on the top of PCB#1 for maintenance of culture medium or recording solution.

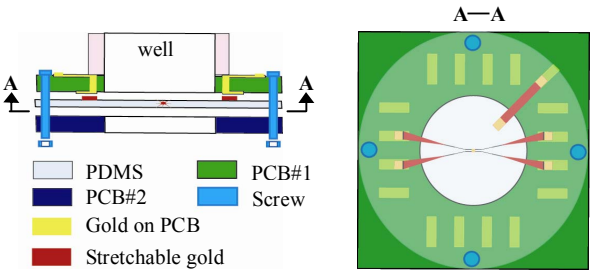

Fig.3 SMEA packaging

After chip packaging, the SMEAs were mounted on a MultiChannel System (MCS) amplifier (MEA1060-Inv-BC, MultiChannel System).

C. SMEA Mechanical Performance

The induced strain within the SMEA, as well as that of cultured hippocampal slices, during deformation was

analyzed from high speed video of the stretch event (as shown in Fig.4). During dynamic electrode stretch, no obvious alteration of resistance was found before and after stretch (data not shown).

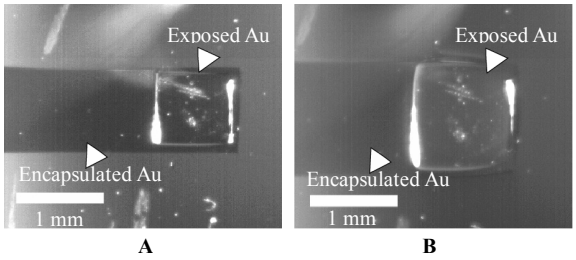

Fig.4 Photos (reference electrode) taken by high speed camera: A) pre-stretch; B) 29% stretch at a strain rate of 2,000% per second;

D. *SMEA Electrical Performance*

The amplifier together with the SMEA was mounted on an injury device (shown in Fig.5); the PCB holder could be fixed at any displacement relative to the indenter so that the SMEA could be held at different biaxial strains for impedance test.

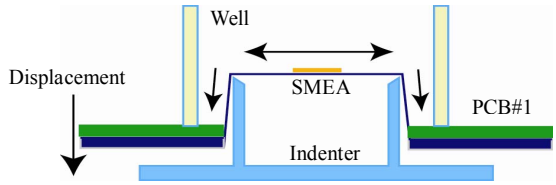

Fig.5 Local part of the injury device

Culture medium (conductance ~1.6S/m) was added to the well to simulate experimental recording conditions. A 0.1 uA peak to peak 1 kHz sine wave was applied to the microelectrodes and the potential difference across the SMEA was recorded at different strain levels. Since the impedance of the medium plus the reference electrode (Pt wire) was negligible compared to the impedance of the microelectrodes, their impedance (at 1kHz) was calculated by Ohm's Law.

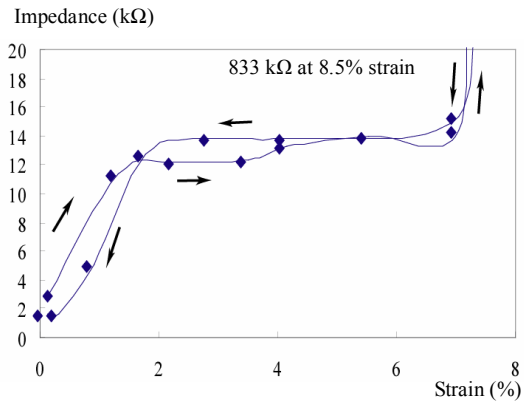

Fig.6 SMEA impedance (at 1kHz) vs. strain

As shown in Fig. 6, the impedance of SMEA increased with strain and recovered after relaxation. The impedance reached 800kΩ at 8.5% strain. Below 7.1% strain, the SMEA impedance was low (~15 kΩ) and is well suited for the purpose of extracellular recording as compared to commercial MCS MEAs (30 kΩ-400 kΩ).

E. *Recording from Hippocampal Slice Cultures*

To test new fabrication schemes for the critcal encapsulation layer, gold micro-electrode arrays were patterned on a glass substrate and then insulated with the patternable PDMS. Using these test arrays, spontaneous activity from hippocampal slice cultures was monitored. Hippocampal slice cultures (12 days in vitro) were used for recording. Artificial cerebrospinal fluid (aCSF) was used as the recording solution and was constantly aerated with with 95% O_2 and 5% CO_2. Two of the four electrodes were positioned underneath the granule cell layer (see Fig.7), and spontaneous activity was recorded with the MCS readout systems. As shown in Fig.8, robust spontaneous activity was detected with the prototype array.

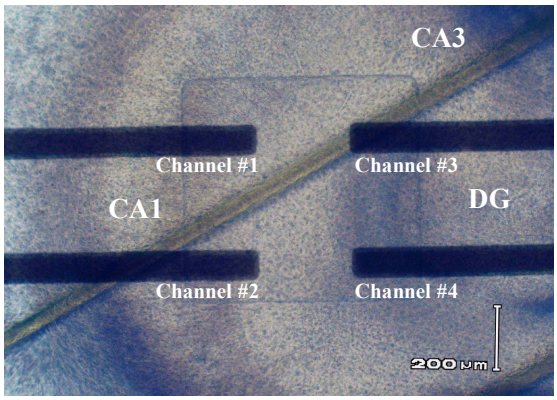

Fig.7 A hippocampal slice culture on SMEA

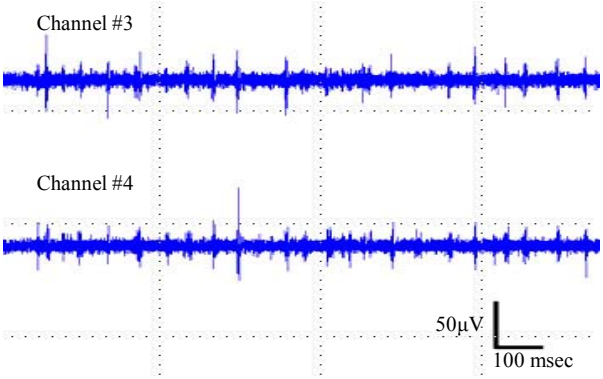

Fig.8 Spontaneous activity of a hippocampal slice culture (recorded in the granule cell layer)

To examine the ability of prototype arrays to detect alteration of neural activity after treatment, the effect of bicuculline on hippocampal slice cultures was tested. Bicuculline is a competitive antagonist of $GABA_A$ receptors, which mediate the main inhibitory circuits in hippocampus. Loss of $GABA_A$-mediated inhibitory networks, caused by high concentration of bicuculline, will induce hyperexcitability of the hippocampus. Fig.9 shows that spontaneous epileptiform activity induced by 1 mM bicuculline was clearly simultaneously detected by the two recording electrodes in the granule cell layer. Obvious alteration of spontaneous activity was found several seconds after bicuculline was added, due to lag of perfusion and delayed pharmacokinetic action. This result demonstrates that the prototype array can detect the effect of bicuculline on hippocampal slice cultures.

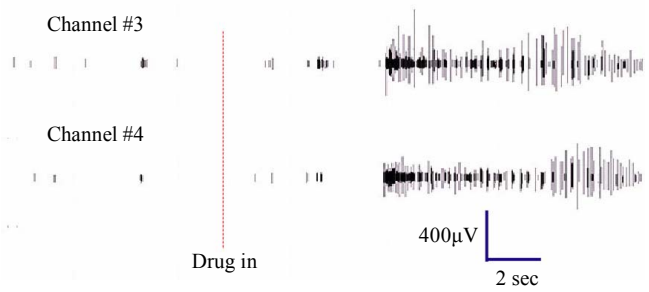

Fig.9 Spontaneous epileptiform activity induced with 1 mM bicuculline (recorded in the granule cell layer)

III. CONCLUSION

Our results show good electrical performance of the SMEAs, compared to the commercial MCS arrays, and the ability of the prototype array to record neuronal activity from hippocampal slices. This novel technology will enable studies to increase our understanding of post-injury changes in neuronal electrophysiological function. Future work will improve the manufacture and performance of the SMEAs.

IV. REFERENCES

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