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Neural Sensing of Electrical Activity with Stretchable Microelectrode Arrays

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***Abstract*—Sensing neural activity within mechanically active tissues poses particular hurdles because most electrodes are much stiffer than biological tissues. As the tissue deforms, the rigid electrodes may damage the surrounding tissue. The problem is exacerbated when sensing neural activity in experimental models of traumatic brain injury (TBI) which is caused by the rapid and large deformation of brain tissue. We have developed a stretchable microelectrode array (SMEA) that can withstand large elastic deformations (> 5% biaxial strain) while continuing to function. The SMEA were fabricated from thin metal conductors patterned on polydimethylsiloxane (PDMS) and encapsulated with a photo-patternable silicone. SMEA were used to record spontaneous activity from brain slice cultures, as well as evoked activity after stimulating through SMEA electrodes. Slices of brain tissue were grown on SMEA in long-term culture and then mechanically injured with our well-characterized in vitro injury model by stretching the SMEA and the adherent culture, which was confirmed by image analysis. Because brain tissue was grown on the substrate-integrated SMEA, post-injury changes in electrophysiological function were normalized to pre-injury function since the SMEA deformed with the tissue and remained in place during mechanical stimulation. The combination of our injury model and SMEA could help elucidate mechanisms responsible for post-traumatic neuronal dysfunction in the quest for TBI therapies. The SMEA may have additional sensing applications in other mechanically active tissues such as peripheral nerve and heart.**

I. Introduction

TBI is a significant public health concern with approximately 1.4 million occurring annually in the U.S., resulting in 50,000 deaths and permanent disability for 80,000, according to the Centers for Disease Control. To study TBI in a highly controlled environment, we have developed an *in vitro* or tissue culture model of mechanically-induced injury which allows for the precise control of brain tissue deformation[1-3]. Previous studies have determined that the primary mechanisms of cell and tissue damage in TBI is tissue stretch[4]. Our model incorporates a complex tissue culture substitute of the *in vivo* brain called an organotypic brain slice culture[5;6]. We have used this model previously to define the tolerance of living brain to mechanical stimuli[1;2;7].

In addition to cell death, TBI induces additional effects such as disruption of neuronal electrophysiological function. Some of the devastating consequences of TBI, such as loss of consciousness, coma, epilepsy, motor deficits, and cognitive impairment, may be due to neuronal dysfunction, even in the absence of cell death[8;9].

Microelectrode arrays (MEAs) allow for the simultaneous recording of neuronal activity from multiple electrodes and have enabled studies of ensemble neuronal function and long-range connectivity. Higher order behaviors may depend on the coordinated activity of neuronal networks which cannot be studied with single-electrode approaches[10;11]. However, current MEA designs are not compatible with *in vitro* model of TBI which induce injury by deformation of the culture substrate[12]. MEAs are typically patterned on glass or other rigid substrates or flexible polyimide foils. But these substrates cannot withstand deformations necessary to induce mechanical injury, i.e. > 5%. An alternative strategy would be to use traditional arrays and remove them for the injury event, but recording from the same location after injury would be unlikely. In addition, culture sterility would be compromised, limiting studies to the acute phase.

To meet the need for a substrate embedded, stretchable electrode array, we have constructed SMEAs using elastically stretchable conductors[13].

II. Methods

A. SMEA Fabrication

A metal stack (3nm Cr, 75nm Au, 3nm Cr) was sequentially deposited by electron beam evaporation on PDMS and patterned by conventional photolithography and etching. The metal conductors were insulated with a photo-patternable silicone, and vias were opened at their ends to form 11 electrodes (100um x 200um) and a twelfth reference electrode[14;15]. SMEA were packaged between two printed circuit boards to interface with a multichannel

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amplifier (MultiChannel Systems), and polycarbonate cylinder formed a culture well. Electrodes were electroplated with platinum black.

B. Organotypic Hippocampal Brain Slice Cultures

Animal procedures were approved by the Columbia University IACUC. The hippocampi of a post-natal day 9 rat pup were removed and sectioned with a McIlwain tissue chopper (Brinkmann Instruments)[3]. Slices were transferred to Millicell (Millipore) membrane inserts or SMEA pre-coated with laminin and poly-L-lysine[3]. Cultures were maintained under standard conditions (37ºC, 5% CO_2).

C. Controlled Mechanical Deformation

The SMEA and hippocampal cultures were mechanically stretched by pulling the SMEA down over a rigid, tubular indenter[2]. This model has been extensively characterized previously and reproduces the complex 3-D strain field experienced by brain tissue during TBI[1-3]. The induced strain was verified from images taken during stretch. Lagrangian strain was calculated according to Eq. 1 using the area of the slice or an electrode before stretch (A_0) and at the maximum stretch (A_{max}).

$$E = \frac{1}{2}\left(\frac{A_{\max}}{A_0} - 1\right) \quad (1)$$

D. Electrophysiological Recordings

At indicated time points, neural activity was recorded from hippocampal slice cultures perfused with artificial cerebrospinal fluid at 37 °C. Electrical neural activity was sampled at 20 kHz and low pass filtered at 5 kHz. In some cases responses were evoked with constant current stimuli generated with a programmable stimulator (MultiChannel Systems) to generate stimulus response (S/R) curves. Stimuli of varying magnitudes (0μA-200μA in 10μA steps) were applied through SMEA electrodes to the mossy fibers while field potentials were recorded from the other electrodes. The response was plotted as a function of stimulus intensity and fit to a sigmoid function:

$$R(S) = \frac{R_{\max}}{1 + e^{m \bullet (I_{50} - S)}}. \quad (2)$$

R_{max} was the maximum response, I_{50} the current which induced a half maximal response, m the slope, and S the stimulus intensity.

Fig. 1. A) Recording of spontaneous activity from a hippocampal slice culture placed on an SMEA after the latter was repeatedly stretched (one channel only). B) The biological origin of the signal was confirmed pharmacologically by eliminating spontaneous activity with 1uM tetrodotoxin.

III. Results

An SMEA was stretched multiple times up to 20% biaxial strain before a brain slice culture was transferred to it. Spontaneous neural activity was detected above a low background noise of less than 2μV (Fig. 1). The biological origin of the signal was verified pharmacologically as tetrodotoxin, a voltage sensitive sodium channel antagonist, eliminated it. Electrical stimuli were applied to brain slice cultures to generate S/R curves (Fig. 2). These results show that after large, biaxial deformations the SMEA can record spontaneous activity and can be used to stimulate tissue to record evoked responses.

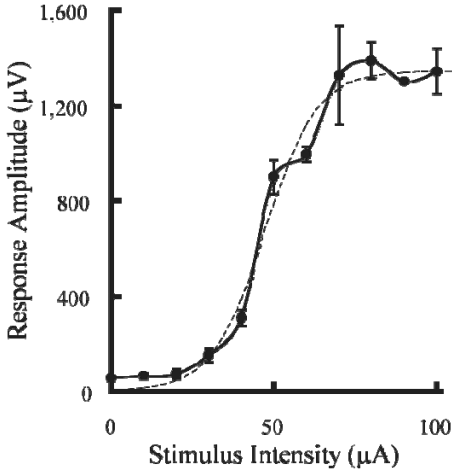

Fig. 2. Recording of evoked activity from a hippocampal slice culture placed on an SMEA to generate an S/R curve. Activity was evoked by stimulating through two SMEA electrodes and recording from the other electrodes.

Hippocampal slice cultures were grown for 5 days on SMEA before being injured by stretching the underlying SMEA together with the adherent culture. Image analysis confirmed deformation of both the SMEA and the adherent tissue (8%, Fig. 3). Furthermore, it was evident that the relative positions of the electrodes in the tissue were not altered by the deformation event, such that neural activity was recorded from the same location before and after injury. Spontaneous electrical activity immediately before injury was relatively quiescent with sparse single unit activity.

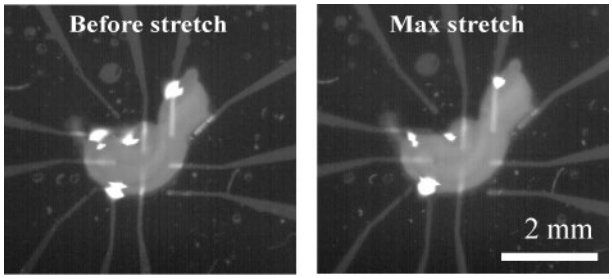

Fig. 3. Image analysis of SMEA and tissue deformation. As the SMEA substrate was stretched, so was the adherent hippocampal slice culture which was deformed 8%. The relative location of the electrodes within the tissue did not change after injury.

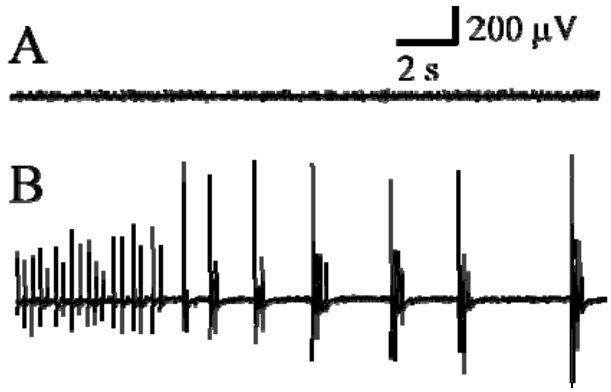

Fig. 4. Neuro-electrical activity recorded immediately before (A) and after (B) injury. Prior to injury, neurons were generally quiet, firing only an occasional spontaneous action potential. After injury, bursting was evident on multiple electrodes simultaneously (only one electrode shown). The adhesion of the culture to the SMEA enabled recording this activity within seconds of the injury event.

Immediately after injury, spontaneous and continuous neuronal discharges were readily evident, lasting less than a minute (Fig. 4). This bursting decreased over about 5 minutes and eventually subsided, which would have been missed if the slices were not cultured directly on the SMEA. This bursting activity may be due to a hypothesized mechanism called mechanoporation in which transient defects in the plasma membrane are generated by the applied deformation. The defects may allow the influx of sodium and calcium ions, resulting in depolarization followed by glutamate release in a positive feedback look causing excitotoxicity[16]

S/R curves were generated before injury, immediately after injury, and at 4d and 9d post-injury in the same cultures so that sigmoidal fit parameters could be normalized to pre-injury values (Fig. 5). Over time, R_{max} decreased by 35% while I_{50} decreased by 9%. In contrast, S/R curve parameters in control cultures changed little during the 14d culture period indicating good biocompatibility of the SMEAs (not shown).

IV. Discussion

We have developed a stretchable microelectrode array which can withstand large, rapid, and repeated biaxial deformation up to 20%. Our SMEA can perform traditional electrophysiological measures of neuronal function such as recording spontaneous activity, electrically stimulating tissue, and recording evoked potentials. While adhering to the SMEA, cultures were stretched to induce injury. Because the electrodes were integrated into the culture substrate, recordings were made from the same locations over time, allowing for changes in electrical activity to be normalized to pre-injury values.

One advantage of MEA over single electrodes is the simultaneous recording from multiple locations within a neuronal network, which can provide insight into long-range connectivity and mechanisms of information processing [17;18]. Distributed processing and storage of information

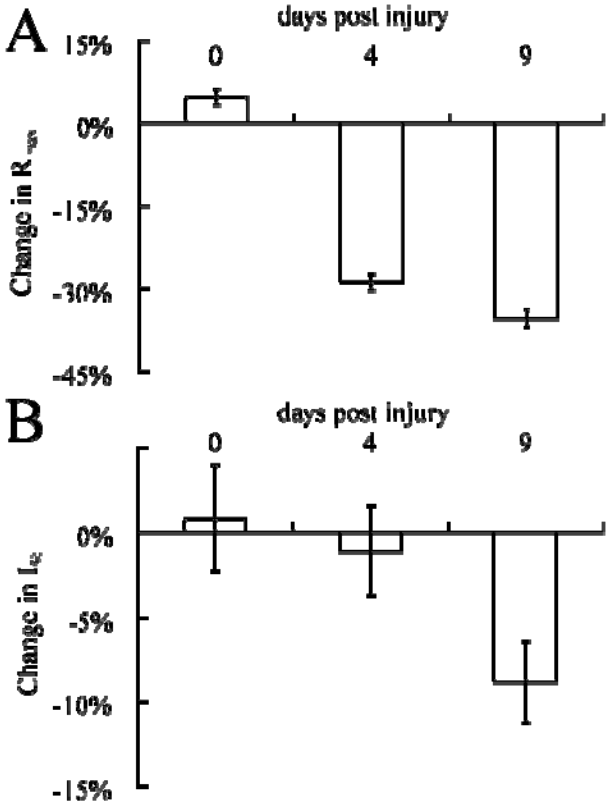

Fig. 5. Quantification of evoked activity post-injury normalized to pre-injury values. Injury induced decreases in both R_{max} and I_{50} which developed over time in culture. Taking multiple measurements in a single culture was enabled by the substrate embedded SMEA because sterility was not broken to make recordings.

may be relevant for higher order brain processes which are difficult to study with single electrode techniques[19;20].

Most MEA are built on rigid substrates using standard integrated circuit manufacturing processes. These materials fail under low strain[21] and are not compatible with most of TBI models. In contrast, PDMS is a hyperelastic material which can undergo large deformations and is being introduced more widely to the manufacture of circuitry and MEAs. For example, it has been used as a substrate for foldable and elastic semiconductor circuits[22;23] and surface stimulation of the spinal cord[24]. Here we have used our SMEA to not only stimulate, but to also record both evoked and spontaneous neuro-electric activity after SMEA stretch. Although we did not measure the number of stretch cycles an SMEA can withstand in the current study, stretchable gold conductors on PDMS can withstand more than hundreds of cycles before failure[13]. Others have reported MEAs fabricated on polyimide substrates which are flexible to an extent, however, they cannot undergo deformations large enough (>5%) to induce neural injury[25-27].

A substantial advantage of our approach is that it employs materials which can be photolithographically patterned. The benefit is that features can be patterned with sub-millimeter accuracy as demonstrated by our 100µm x 200µm

electrodes. We continue to refine the photolithographic process with soft materials (PDMS, silicone) to further reduce the feature size. However, in its current form, the electrode dimensions are larger than commercially available rigid arrays which have feature sizes in the 10-50μm range. As such, our relatively large electrodes limit our ability to interpret the anatomical origin of electrical activity. We are also striving to increase the number of electrodes, from the current 11 closer to 60 of the commercially available rigid MEA.

As we have demonstrated, SMEA can be used to mechanically stimulate and record from injured brain tissue, which enables a number of possibilities for TBI research. The combination of an SMEA, a complex brain slice culture preparation, and precise injury model could form the basis of a rapid screening platform for the discovery of novel TBI therapies. These studies would be facilitated by normalizing post-injury functional outcomes to pre-injury function. This could reduce data variability associated with recoding from separate injured and control cultures, which in turn would speed up discovery by reducing group size. Functional measures of electrical activity serve as *in vitro* correlates of behavior, cognition, and consciousness at the whole organism level. They may be sensitive measures of neuronal health and capture subtle changes induced by mechanical stimulation. Because sterility is maintained before and after injury, SMEA enable long-term studies which could quantify the effect of therapeutic strategies directed at repairing damaged neuronal circuits.

V. Conclusion

A greater understanding of the pathobiological changes initiated by mechanical stimuli has the potential to reduce the healthcare costs, mortality, and morbidity associated with TBI. Our SMEA technology could enable new studies to uncover injury mechanisms, particularly in the acute and sub-acute temporal window after injury. Therapeutic targets could be identified, and new therapies, directed against them, tested in our *in vitro* TBI model. In addition to TBI, our technology has other applications as flexible or conformable interfaces with mechanically active tissues and organs such as the heart, spinal cord, or peripheral nerves.

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