Development of a planar microelectrode array offering long-term, high-resolution neuronal recordings

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Abstract — **All nervous system functions, ranging from simple reflexes to learning and memory, rely on networks of interconnected brain cells called neurons, which initiate and convey electrical information. Monitoring neuronal activities of a large neuronal ensemble, non-invasively and over an extended time period, is pivotal for understanding all brain functions. A variety of neuro-electronic interfaces now allow monitoring of neuronal and ion channel activities. These neuronal monitoring devices are however limited vis-à-vis their efficacy, fidelity and longer-term recording capabilities. Here we report on a novel microelectrode array technology that allows for the detection and characterization of neural activity from individual cells and networks, over long periods of time and with a higher signal-to-noise ratio than commercially available devices.**

Our in-house development of planar microelectrode arrays has focused on modifying design parameters and fabrication techniques to improve their performance. One such device, the Soma-Soma Chip (SS-Chip), allows us to record activity from single and paired cells (pre- and post-synaptic neurons) continuously over extended periods of time with a signal-to-noise ratio higher than similar standard devices. This allows for the analysis of neural activity, which can help to characterize firing patterns of neurons at various developmental-stages. Of particular importance is the precise "signatures" of neuronal firing pattern that offers a unique opportunity to decipher how neuronal activity influences brain network connectivity. Our data also underscore the importance of further development of novel microelectrode array technologies. These developments will provide novel tools and open new research opportunities critical for understanding the fundamental cellular and network properties underlying network activity under both normal and disease conditions.

Keywords — **Microelectrode array, long-term recording, single cell activity, signal amplitude**

I. Introduction

The sophistication and complexity of micro- and nano-fabrication processes have considerably impacted the development of biomedical devices, such as neuro-electronic hybrids and microelectrode arrays (MEAs). The neuroscience and computational fields have used these techniques to explore fundamental biological and electrophysiological principles of cellular excitability. Several trans-disciplinary research groups have since established themselves by proposing new technological advances offering the ability to study neural connectivity, network activity, sub-threshold potentials, or brain plasticity. There have been a wide range of innovative designs to improve MEAs, from non-penetrating nanopillar electrodes, mushroom-shaped protruding microelectrodes, to planar patch-clamp MEAs [1]. These efforts have focused on improving the electrical signal that can be recorded. The strength of this signal is typically defined as the electrical coupling coefficient, which is the ratio of the signal amplitude recorded with extracellular techniques compared to intracellular recording with sharp electrodes. Ideally, these extracellular recording MEAs will allow the detection of action potentials (APs) as well as sub-threshold currents with a resolution equivalent (coupling coefficient of ~1) to standard intracellular methods over long-term periods up to several months.

The main advantage of MEAs is their ability to record and stimulate neural activity *in-vivo* or *in-vitro* over extended periods of time without damaging the cell membranes [2]. However, three-dimensional microelectrode arrays that penetrate or are engulfed by the cell's membrane have all but eliminated this essential benefit. By partially protruding into the cell membrane, thereby limiting the slight movements of the cell in its environment, three-dimensional electrodes tend to damage the cell, which significantly affects their viability and thus the potential for long-term recordings. While these new configurations are validated in their ability to record activity with a high resolution, they do so while neglecting the long-term recording capabilities of MEAs – the original purpose of extracellular recordings. This in turn severely limits their usefulness. For example, long-term recording on fully biocompatible MEAs is critical to study the long-term effects of drugs on neural network formation [3] and synaptic plasticity [4].

The need to record neuronal activity over an extended time period with high fidelity while also monitoring synaptic activity led us to develop an in-house novel planar MEA. The goal was not only to investigate activity changes from

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pre- and post-synaptic single cells simultaneously and over the long-term, but also at a resolution higher than commercially available devices. These improvements will support the neuroscience and computational fields as well, providing an "easy-to-use" and low-cost tool, thus allowing researchers to monitor neuronal and synaptic activities.

II. Materials and Methods

A. Animals

In order to overcome the challenges posed by the complexity of the mammalian neural network, we opted to use the freshwater snail *Lymnaea stagnalis* as a model system for the study of fundamental neuronal properties, synaptogenesis (formation of synapses), and network formation. This invertebrate model provides us with larger neurons (50 – 80 µm diameter, compared to 6 – 8 µm for mammalian neurons) that are both structurally and functionally well characterized and can be manipulated on MEAs with great ease at the level of individual neurons. Our approach was to use specific neurons with well-defined activity to study neurons' electrophysiological activity over time and better characterize our in-house developed chips.

B. Chip fabrication

MEAs were fabricated at the Advanced Micro/Nanosystems Integration Facility (AMIF), University of Calgary. Following a custom design, they were fabricated using a two-mask photolithography process on 49x49mm glass, 1mm thick. The electrodes are ~200 nm gold deposited on a 10 nm chrome adhesion layer. An epoxy based photoresist layer of thickness 5µm (SU8) was then deposited to provide an insulation layer over the electrode traces. Openings in the SU8 layer left the main electrode arrays bare for stimulation/recording. Sizes and intervals between electrodes can be adjusted according to our experimental needs by modifying the photomask designs. Using a standard photolithography technique, we maintained a relatively easy fabrication process, adaptable and functional for our needs. We named this new MEA design the Soma-Soma-Chip (SS-Chip), as it offered the opportunity to study pre- and post-synaptic neurons simultaneously when cultured in a soma-soma configuration (both cell bodies are contacting each other) (Figure 1).

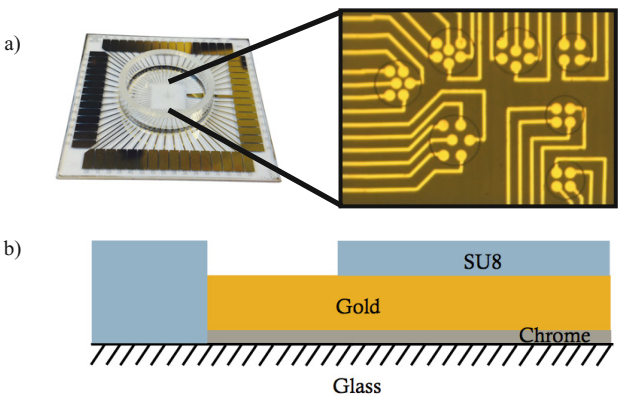

Fig. 1: a) Picture of the SS-Chip (left) and design of 30µm diameter electrodes at the center of the SS-Chip; b) Schematic of the glass, metals and photoresist layers.

C. Cell culture

Syed, et al [42], have already published a detailed *Lymnaea* cell culture protocol. Briefly, we removed the central ring ganglia from 1 to 2 month old *Lymnaea stagnalis* animals and treated it with trypsin (2 mg/mL; T- 4665; Sigma-Aldrich, St Louis, MO, USA). After 20 minutes we used a trypsin inhibitor (2 mg/mL; T-9003; Sigma-Aldrich) for 15 minutes to stop the enzymatic reaction. For the purpose of our experiments, we isolated the specifically identified pre- and post-synaptic neurons, VD4 and LPeD1, respectively, by gentle suction applied through a fire-polished, Sigmacote®-treated glass pipette (SL2; Sigma-Aldrich). The cells were then plated on the multi-electrode units of a poly-L-lysine coated SS-Chip in conditioned media (CM) containing trophic factors necessary for growth and synapse formation. Trophic factors present in the conditioned medium were obtained by incubating isolated *Lymnaea stagnalis* central ring ganglia from 2 to 6 month old animals for 3 to 7 days in defined medium (DM; L-15 Special Order; Life Technologies, Gaithersburg, MD, USA – no added trophic factors). The neurons were allowed to settle overnight and used for experiments the next day (12-18 hours post culture).

D. Electrophysiology

Neuron action potentials (electric potential changes) were recorded using our SS-Chip microelectrode array connected to an MEA amplifier and PCI acquisition card (MEA1060; Multichannel Systems, Reutlingen, Germany). In addition, sharp glass microelectrodes were used (1µm tip with a resistance of 30-40 mega Ohms ID, World Precision Instruments, FL, USA) to perform intracellular recordings. Electrophysiological recordings were analyzed using Axoscope 9.0 (Axon Instruments Inc.) for intracellular recordings, and MC_Rack (Multichannel Systems, Reutlingen, Germany) for extracellular recordings. Individual action potentials with their associated timestamps were extracted using a spike detector function and processed by Excel (Microsoft; Redmond, WA, USA).

III. Results

A. Biocompatibility

The SS-Chip MEA allowed cultured neurons to grow at a rate of up to 1mm per 24 hours, indicating a high degree

of biocompatibility with the substrate materials used in our fabrication process. When pre- and post-synaptic neurons (VD4 and LPeD1, respectively) were cultured together in a soma-soma configuration, action potentials could be triggered in VD4 using intracellular sharp electrodes which elicited 1:1 EPSPs of constant amplitude and latency, as seen when cultured on our SS-Chip (95% of paired cells formed a synapse within 24 hours on the SS-Chip, n = 20) (Figure 2).

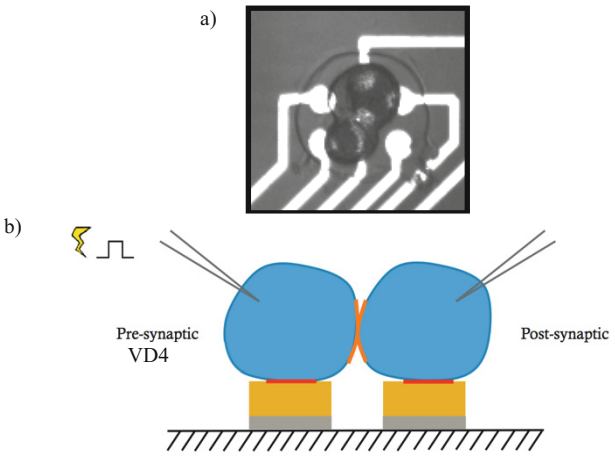

Fig. 2: a) VD4 and LPeD1 cells cultured in a soma-soma configuration on a multi-electrode unit; b) Protocol for recording activity from both the pre- and post-synaptic cells as well as post-synaptic potentials

This first study demonstrates that synaptogenesis can occur concurrently with growth on our in-house MEA. Therefore, our SS-Chip MEAs are fully biocompatible and all materials used during the fabrication process can be safely used for further development.

B. Long-term single cell recording

To test the ability of our chip to continuously monitor action potentials from healthy neurons for more than 14 days, neurons were cultured on the chip and their activity was monitored for several days. We discovered that activity could be reliably recorded from single cells over a period longer than 7 days, mainly due to the custom arrangement of electrodes on the substrate. Indeed, cultured single or paired cells generally had the tendency to move away from their original recording sites, which considerably affected our ability to record neural activity. This natural phenomenon occurs when neurons grow and extend neural processes, which generate a mechanical stress along the axon and the cell membrane - thus pulling the soma away from the electrodes upon which they were initially plated. Moreover, when the pre- and post-synaptic cells VD4 and LPeD1 were cultured in a soma-soma configuration, they had a greater tendency to pull each other and hence move off the recording electrodes. However, the design of the multi-electrode unit, with electrodes close to each other, allows the cells to move away from their original location while remaining in contact with another adjacent electrode. This approach simplified the protocols needed to continuously record the activity from single neurons over much longer time periods as compared to commercial MEAs (Figure 3).

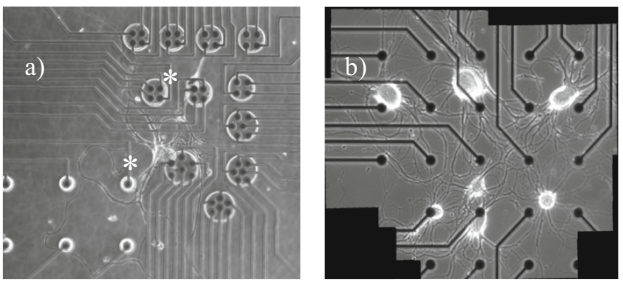

Fig. 3: a) Two LPeD1 cells (indicated by *) cultured on our SS-Chip after 14 days still remained on their respective multi-electrode units; b) Seven LPeD1 cells cultured on a standard commercially available 8x8 MEA after 7 days. Six of them moved off from their original recording sites.

C. Better signal-to-noise ratio than traditional planar electrodes

The signal-to-noise ratio and the amplitude of potentials recorded depend on a variety of factors. Several reported factors [1] have been identified as crucial for determining the signal-to-noise ratio and therefore the electrical coupling coefficient: 1. the sealing resistance, which is a function of the adhesion between the cells and the electrodes; 2. the input impedance of the dielectric microelectrodes; 3. the neuronal current magnitude, which is a function of the cell size, ion channel concentration, and their location on top of the microelectrodes. However, increasing the sealing resistance (R_{seal}) appears to be the most efficient and reliable way to increase the signal-to-noise ratio.

Increasing the signal resolution will have an impact on our ability to characterize neural activity over long time periods, since our capacity to analyze neural signals is dependent on how accurately action potential spikes can be detected over the noise. As a result, a higher resolution will significantly facilitate data analysis. By modifying the fabrication process, we developed novel electrodes which dramatically increased signal amplitude (from less than 1mV [1] to 4.2 mV peak-to-peak, *Figure 4*) and thus the coupling coefficient (from 0.001-0.01 [1] to 0.07). These improvements rest in our fabrication process whereby the structure of the electrodes likely offers an increased sealing resistance (R_{seal}). The new planar SS-Chips allowed us to record activity at a resolution higher than certain devices with three-dimensional electrodes (*Figure 5*), while still maintaining our capability to record long-term activity (weeks).

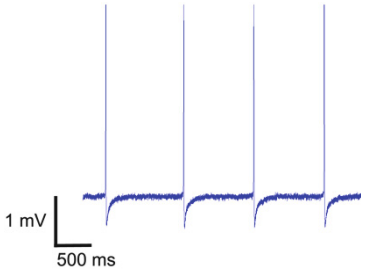

Fig. 4: Action potentials recorded with our SS-Chips showing a peak-to-peak amplitude of 4,2mV.

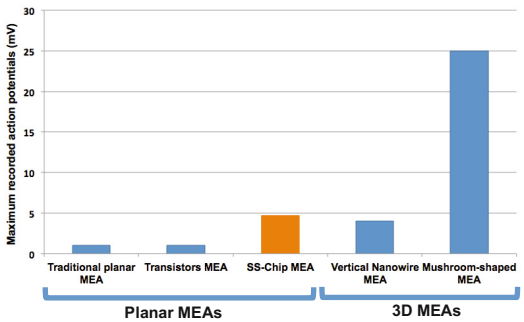

Fig. 5: Maximum peak-to-peak recorded action potentials (mV) of various MEA designs as reported in the literature [1]. Our SS-Chips (orange) with planar electrodes allowed us to record activity at a resolution higher than certain devices with three-dimensional electrodes and at a resolution greater than any other planar MEA reported.

IV. Conclusions

Our in-house designed and fabricated SS-Chip enables us to record pre- and post-synaptic activity over extended periods of time at a higher resolution than commercial devices. Exploiting parameters such as the size and spatial pattern of the electrodes, or the types and thickness of materials, we have optimized the signal-to-noise resolution of our custom designed MEAs while maintaining a relatively simple fabrication process. The most recent version of the SS-Chip not only enables activity to be recorded at a resolution never attained before using planar electrodes, but also allows us to specifically characterize the evolution of single cells and synaptic activity during synaptogenesis. This innovative tool will enable us to better understand how neurons communicate over time and offers relevant opportunities to study long-term drug effects on neural activity.

This study demonstrates that there is still significant room for improvement in the development of planar MEAs, since they can achieve high signal-to-noise ratios while allowing long-term recordings. In addition, they are a relatively low-cost and highly customizable solution. Innovating on the simple design of other planar MEAs, we have been able to apply our MEA to fundamental neuroscience research, such as neural circuitry formation and synaptogenesis. The main purpose of developing *in vitro* extracellular recording technologies is to provide neuroscientists with a tool to better understand cell activity (single cell, paired cells, networks, and brain slices), synaptic plasticity and network phenomena over long time periods (up to several days). The planar electrodes we developed for our SS-Chip allows us to record activity for several days without damaging the cell membrane, while allowing processes to freely grow. While three-dimensional MEAs (for example, “mushroom” or spike electrodes) do not permit long-term recording, our SS-Chip fills a technological gap between traditional planar MEAs and three-dimensional MEAs, since it records higher resolution activity long-term.

Our study suggests that there is still work to be done to improve the signal-to-noise ratio of planar MEAs. The ability to conduct long-term recordings should not be considered optional when designing new MEAs, as this is critically important for fundamental and clinical neuroscience research.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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