

Identification of synaptic activities in microelectrode array-based neural networks

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The Microelectrode Arrays (MEAs) have been used for several decades to investigate neuronal networks *in vitro*. In most of the studies, the neuronal networks have been studied statistically due to complexity of cultured neuronal networks. However, in order to understand the behaviours of neuronal networks dynamically, the identification of synaptic activities of individual neurons is crucial. In this study, we observed individual synaptic activities by utilizing low density neuronal networks arranged orthogonally on MEA's.

1 Aligned microcontact printing

Rat hippocampal neurons were finely patterned by soft lithography technique on microelectrode array fabricated as previously reported. The polydimethylsiloxane (PDMS) stamp was moulded from the stamp master designed to match the patterns of MEAs as depicted in Fig. 1. The fabricated PDMS stamp has the pattern of 2 μ m-wide line and 20 μ m circles at the crossing points. The circles were intended to induce the attachment of neuronal cell bodies for recording neural activity through the underlying electrodes. Fluorescein isothiocyanate (FITC) labeled poly-L-lysine (PLL) was inked to the PDMS stamp for 1 hour after dissolved at 1mg/1ml in borate buffer solution. After blowing off PLL, the PDMS stamp was mounted at the custom-made alignment tool and made to contact with the surface of the MEA for more than 1 hour.

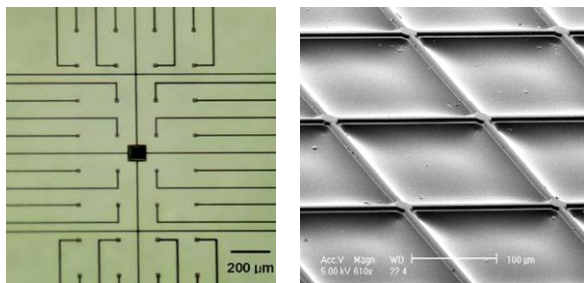


Fig. 1 Enlarged picture of MEA sites area (left), and SEM image of moulded PDMS stamp(right)

2 Electrophysiology

From rat hippocampal neural networks cultured on PLL-printed MEAs, signal propagations were identified as a proof of the synaptic connectivity, for the first time to our knowledge, from MEA-based neuronal networks. When cells were plated at 200cells/cm², spontaneous activity could be recorded as early as 7 days in culture. Recordings were made for as long as 71 days. Electrical stimulation (200 μ A current step, 50 μ s pulse width) was also used to evoke activity (Fig. 2). It was also shown that activities were evoked by chemical stimulations of high-K⁺ solution.

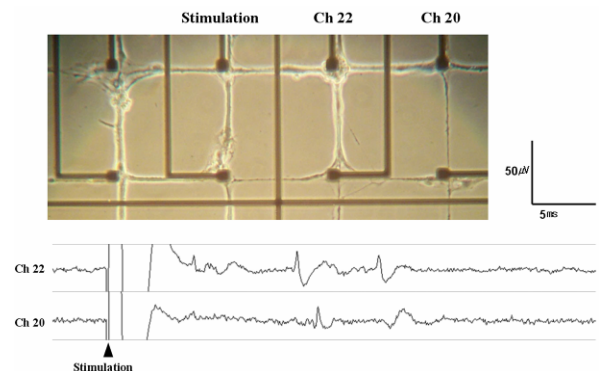


Fig. 2 Patterned neural network (top) and electrically evoked neural responses (bottom)

3 Immunocytochemistry and Scanning Electron Microscopy

After a series of electrophysiological experiments, the neurons were exposed to a mixture of rabbit polyclonal anti-MAP2 and mouse monoclonal anti-synaptophysin. In order to identify these primary anti-

bodies, Texas Red goat anti-rabbit and Alexa 488 goat anti-mouse were used as secondary antibodies. MAP-2 is a maker for the identification of the neuronal cell bodies and dendrites, and synaptophysin is one of the presynaptic proteins which show the locations of the synapses. After the immunostaining, cells were applied with DAPI for staining nuclei. Presynaptic proteins (Fig.3b) were located along neurites and near cell bodies (Fig.3c). It shows that synapses have been developed between neurons successfully in this patterned neuronal network. After critical point drying process, scanning electron microscopy was followed to identify the cells directly.

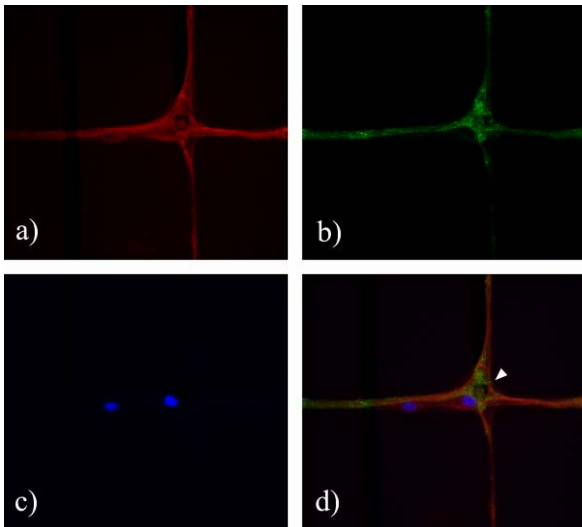


Fig. 3 Immunohistochemistry images for (a) dendrites, (b) presynaptic proteins, (c) nuclei, and (d) merged image

4 Summary

Neurons were successfully cultured in low density for long term survival. From the neuronal networks cultured in low density, propagation of signal along the network was recorded for the first time. The evoked potentials were also observed following elec-

trical current stimulations. The formation of synapses and the location of cell bodies were identified by immunocytochemistry.

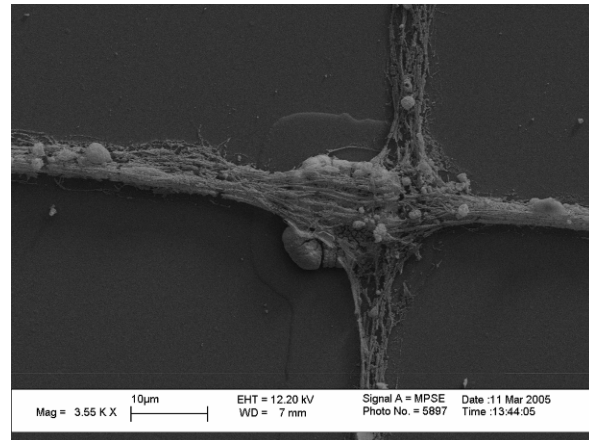


Fig. 4 SEM image of a patterned neural network

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