Microfluidic channels for the study of axonal properties on CMOS micro-electrode array

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We have constructed a platform for studying the connections between neurons based on our high density CMOS microelectrode array (MEA) [1] and a novel microfluidic culture device featuring axonal isolation channels. We are able to perform extracellular electrophysiology on cell soma as well as on confined axons with very high spatial and temporal resolution, which can be exploited to study the growth, development, and maturation of neuronal networks.

The custom microfluidic cell culture device was designed and then realized in polydimethylsiloxane (PDMS) using a three-layer SU-8 soft lithography process. The layout of the device is shown in Figure 1a-c: two culture chambers that house the cell bodies are connected by axonal isolation channels whose dimensions are 12 µm in width and 4-7 µm in height, such that soma cannot breach them and only axons can grow through. This simple implement is bonded to the high density CMOS MEA previously developed in our lab that has been demonstrated on brain slices and acute retinal preparations [2]. The MEA comprises 11,011 platinum electrodes in a 1.7 x 2.0 mm^2 area and 126 reconfigureable read-out channels. Precise alignment of the device on the MEA is not necessary since channel spacing does not correspond to electrode pitch; still bonding is complicated by surface topology, most significantly the 1.6 µm recesses in which the electrodes sit.

In the present work, dissociated rat cortical neurons were plated in each of the two PDMS culture chambers and their subsequent activity, both spontaneous and stimulated, was observed over the course of weeks or months. After only seven days *in vitro*, axons had grown through the 950 µm long channels and their action potentials or spikes could be observed propagating through the channels and evoking electrical activity in the opposite chamber.

Alongside their utility in guiding axons, the channels also offer a strong enhancement of the electrical signal since axons are able to grow directly over the electrodes and the channels limit ion diffusion. In open cultures, tens of traces had to be averaged in order for axonal signals to be detected [3], whereas in the channels this is not necessary, as Figure 1d clearly shows. This amplification effect can be employed to investigate properties of the axons such as the propagation velocity of spikes. Measurements in many different channels and in a number of different cultures have yielded a velocity of 0.5 ± 0.1 m/s, which is in agreement with previous results assuming the axons are not myelinated.

By using the features of the high-density electrodes underneath, activity in the cultures can also be triggered by targeted stimulation either at the soma or along the axons with voltage or current pulses. Elicited signals move throughout the culture, tracing the neural processes and demonstrating the interconnectedness of the culture.

The work done so far provides a foundation for numerous studies. The unique fluidic and electrical seclusion provided by the channels offers unprecedented access to an important neuronal element, the axon, which has thus far been elusive [4], but whose attributes are proving more interesting and complex than previously thought. While the system is necessarily artificial, its advantages lie precisely in its constructed nature. Variations in the microfluidic pathways allow many interesting phenomena to be studied such as coupling between axons, conduction failures, and axonal branching. The two compartment design permits confinement of medium or chemicals to just half of the cell culture, *i.e.* one chamber. The design is suitable for co-cultures of complementary cells or tissue.

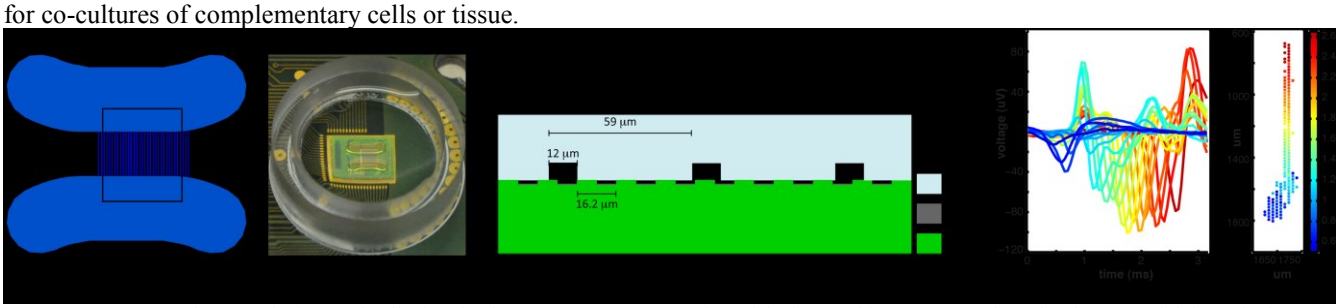

Figure 1: **a**. Layout of PDMS device; **b**. detail of packaging on PCB; **c**. cross section of device on chip; **d**. propagation of action potential through channel.

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