Development of a dual-compartment microelectrode array for investigation of neuronal network media exchange

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This work is focused on designing, testing and optimizing dual-compartment microelectrode arrays (MEAs) to facilitate media exchange and communication between two *in vitro* hippocampal networks. The proof of principle will be to assess the impact of conditioned cell culture media exchange between two initially isolated neuronal networks, specifically how nicotine-modulated neuronal networks interact with non-nicotine modulated neuronal networks via media exchange.[1] Nicotinic acetylcholine receptors are known to play a role in cognitive functions of the hippocampus, such as memory consolidation.[2] The hippocampus is a region of the central nervous system that has a major role in memory and learning. We will test feasibility of the dual-compartment microelectrode arrays by investigating the differences in neuronal firing activity in networks before and after a network of naive hippocampal neurons is exposed to cell media previously conditioned by nicotine-treated neurons.
Multiple dual-compartment microelectrode arrays are fabricated using the photolithography process (Fig. 1). Each MEA consists of two chambers with a total of sixty 100 nm thick titanium electrodes, 38 μm in diameter, that records electrical activity; two of the electrodes are used for internal electrical reference (Fig. 1a). The electrodes are fabricated on top of a quartz substrate that provides transparency in both visible and ultraviolet illumination. This illumination is required for monitoring the cell growth, network connectivity and performing fluorescence measurements. A 200 nm SiO_2 isolation layer is patterned on the surface, allowing 30 μm wide openings only at the neural recording and measurement sites (Fig. 2). An autoclavable biocompatible resin ring with a removable silicon plug is 3D printed and adhered to the device using a biocompatible autoclavable epoxy (Fig. 3). Since both inorganic and organic materials are used for the fabrication, sterilization of the dual-chamber array is performed via an autoclave machine and UV radiation. The design of the ring was optimized to ensure fast media exchange, avoid leaks and support via an easy removal of the partition. The materials were carefully chosen to support the sterilization process needed. Surface functionalization with poly-D-lysine and laminin provides biocompatibility for cell growth. This dual-chamber system allows two neuronal cell cultures to develop separately; media exchange will occur once the silicon plug is removed. The developed MEA enables the electrical monitoring of developmental changes in two *in vitro* neural networks and can be used for a variety of studies in neurophysiology.

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[1] A. Luchicchi, B. Bloem, J. N. M. Viaña, H. D. Mansvelder, and L. W. Role, Front Synaptic Neurosci. 2014, vol. 6, p. 24
[2] S. Djemil *et al.*, J Neurochem. 2020 May, vol. 153, p. 468–484

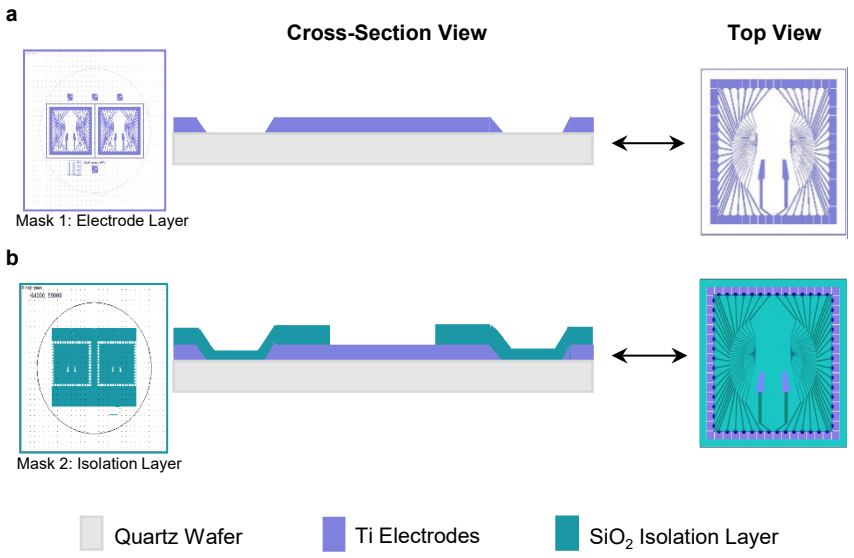

Figure 1: Fabrication Flow. (a) Sketch showing the photolithography mask and the fabrication of the electrode layer on 4" wafer. (b) Sketch showing the photolithography mask for the fabrication of the isolation layer.

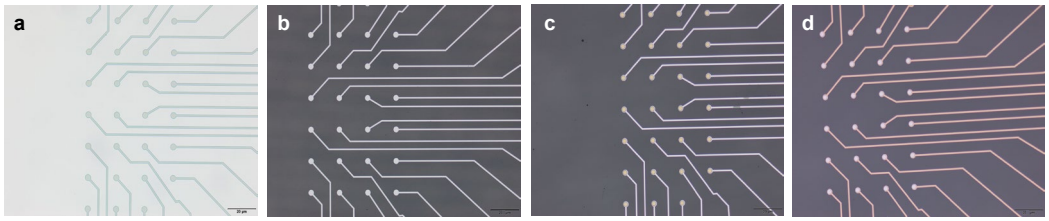

Figure 2: Patterning results. (a) Electrode layer after develop and (b) after lift off; (c) Isolation layer after develop and (d) after lift off

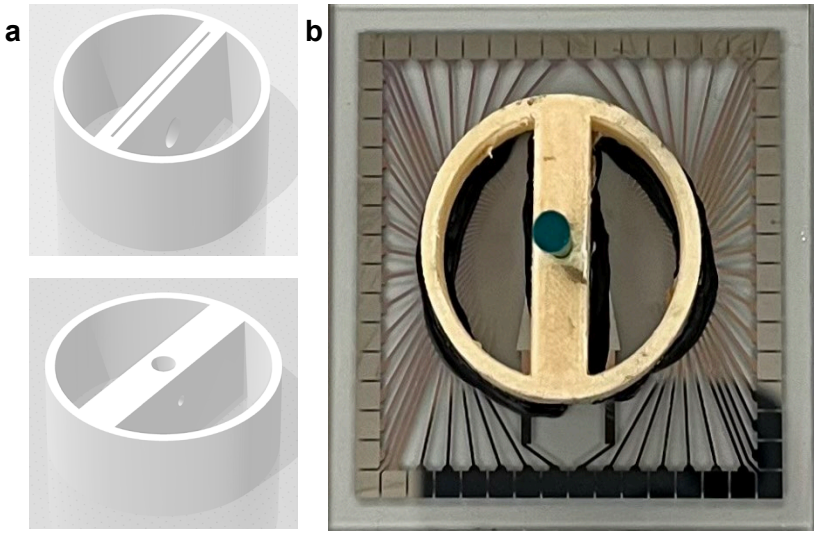

Figure 3: Dual-chamber microelectrode arrays. (a) Various ring designs investigated, e.g. with slide partition (top) and with vertical plug (bottom) and (b) completed structure with biocompatible and autoclavable materials.