Three-dimensional Tip Electrode Array Technology for High Resolution Neuro-Electronic Systems used in Electrophysiological Experiments *in-vitro*

Neil Joye*, Maurizio Lavagnino, Alexandre Schmid, *Member, IEEE*, and Yusuf Leblebici, *Senior Member, IEEE*

***Abstract*—A three-dimensional tip electrode array technology for *in-vitro* electrophysiological experiments is presented. Based on simulation results obtained with a finite element model of the neuron-electrode interface, it has been shown that the electrical coupling between the neural cells and the three-dimensional tip electrode array is improved compared to standard planar electrodes. Consequently, three-dimensional microelectrode arrays (MEAs) exhibiting a higher spatial resolution than classical integrated MEA systems have been manufactured using the proposed fabrication process. Three-dimensional tip electrode arrays with an electrode diameter of 3-4μm, a height of 1.75μm, and a pitch dimension of 5-6μm have been manufactured on silicon substrate. Future *in-vitro* electrophysiological experiments are expected to confirm the superiority of the three-dimensional electrodes over the planar electrodes.**

***Index Terms*—Finite element model of the neuron-electrode interface, high-density microelectrode array, *in-vitro* electrophysiological experiments, three-dimensional tip electrode.**

I. Introduction

The study of neural cellular functionality and electrical behavior is considered as a fundament and prerequisite to better understand the operation of the brain. As technology has improved, neuroscientists have shown increased interest in the *in-vitro* experimentation methodology to support advanced studies of the brain and the operation of its constituting neurons. Using microelectrode arrays (MEAs), it is now possible for populations of neural cells to be examined simultaneously [1]–[7], thereby providing better insight into the functionality and interconnectivity of cellular networks [1], [2].

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However, important technological challenges related to recording and stimulating the electrical activity of neuron cultures need to be solved. One of the main limitations of MEAs is their low spatial resolution. Current MEAs have inter-electrode spacings of 18-200μm [2]–[7]. These dimensions are much larger than the 10μm typical size of neuron cells used in *in-vitro* electrophysiological experiments. Therefore, when recording the electrical activity of a neuron culture, a large number of cells are not sensed by the MEA, causing a spatial under-sampling of the neural network activity.

A new approach based on three-dimensional tip electrodes is presented in this paper, as a way to increase the electrical coupling between the neural cells and the individual sensors in the electrode array. Using this technique, MEAs with higher spatial resolution are fabricated, thus eluding the under-sampling limitation of classical MEAs.

This paper is organized as follows. In section II, the three-dimensional tip electrode concept is described. The electrical model of the neuron-electronic interface and early simulation results are discussed in section III. The manufacturing process of the three-dimensional tip electrodes is presented in section IV. Finally, conclusions are taken, and future perspectives are projected in section V.

II. Three-dimensional tip electrode array

Arrays of three-dimensional tip electrodes with a base diameter limited to 2-5μm, a height of 1-2μm, and a pitch dimension of 4-6μm have been considered in order to guarantee that every neuron in the cell culture are lying on the top of several electrodes, as depicted in Fig. 1. Neuron cells obtained by dissociation of pre-natal hippocampal rat cells are cultured on the surface of the silicon microelectrode array. Each cell will grow and form connections with its neighbors during an initial incubation period

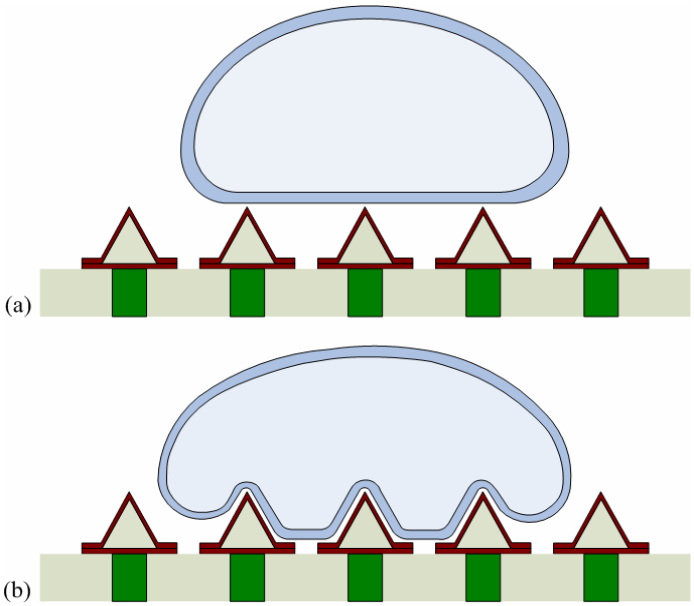

Fig. 1. Conceptual cross-section of a neural cell lying on the tips of a three-dimensional electrode array with (a) the cell lying on the top of the tips and with (b) the attached membrane having the same topology as the surface of the MEA.

of two to three weeks. During this period, every cell is expected to move on the surface of the silicon MEA, and locally adapt to the surface topology in one of the two possible schemes depicted in Fig. 1. Cells may lie on the tips of the electrodes where the membrane rigidity prevents any adaptation, as described in Fig. 1(a). In this case, the distance between the electrode tips and the neuron membrane is expected to be reduced compared to planar electrodes, thus enabling an improved electrical coupling as proved in the cell-electrode interface simulations described in section III. Alternatively, the cell membrane may attach to the surface and follow its topology, as depicted in Fig. 1(b). In this case, the electrical coupling between an electrode and a neuron is improved due to an increased cell-electrode contact area.

This innovative technology must not be confused with three-dimensional MEAs that have already been designed [8]–[10]. In these cases, the three-dimensional electrodes are used to penetrate the dead cell layer at the tissue slice border of acute brain slices. Consequently, their heights are much more significant, and range from 47μm to 100μm.

III. Neuron-Electrode Interface Modeling

The current flow distribution of a neural cell with a typical size of 10μm, lying in a cell culture environment and adequately lying on top of an electrode has been simulated using COMSOL Multiphysics™, a finite element method simulation software. In these simulations, an intracellular potential equal to 100mV is applied in order to study the behavior of the dc current in the neuron-electrode environment.

The current flow simulation of a 10μm diameter neural cell with an intracellular potential equal to 100mV lying on top of a 4μm diameter planar electrode is depicted in Fig. 2(a) for a cell-electrode distance equal to 100nm, in a qualitative three-dimensional graphical representation. The distance of 100nm corresponds to an average neuron-electrode distance [11]. In Fig. 2(b), the same simulation has been performed using a 1.5μm high and 4μm diameter three-dimensional tip electrode instead of the planar electrode. The distance between the cell and the top of the three-dimensional tip electrode is equal to 20nm. This small distance is an estimation of how close will be the tip electrode from the cell membrane. As shown in Fig. 2(a), a significant amount of the current flowing out of the cell is collected by the planar electrode. However, for the three-dimensional tip electrode, practically all the current is collected by the electrode. Therefore, sensing the membrane current from a neuron cell is expected to be improved using this type of three-dimensional tip electrode.

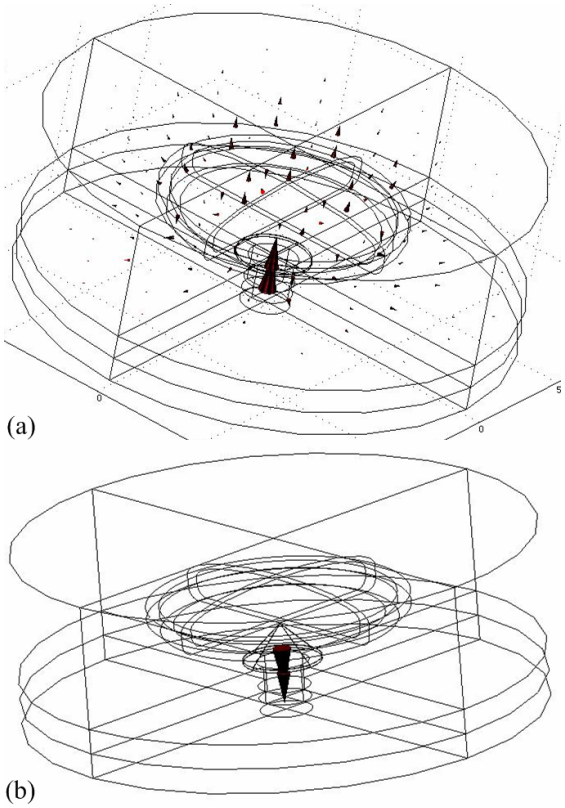

Fig. 2. Current flow simulation of a 10μm diameter neural cell with an intracellular potential equal to 100mV lying on top of (a) a 4μm diameter planar electrode at a cell-electrode distance equal to 100nm and (b) a 1.5μm high and 4μm diameter three-dimensional tip electrode at a cell-distance equal to 20nm.

Future work will consist in simulating the current flow and electric potential in the cell-electrode environment using dynamic intracellular potentials in order to model action potential waveforms, as well as modeling the simulation of an action potential in a neural cell. During electrical simulation of neural cells, Faradaic reactions can occur if the applied electrical stimulation stimulus is too large. This mechanism implies that electrons are transferred between the electrode and the electrolyte, resulting in reduction or oxidation of chemical species in the electrolyte [12]. Therefore, simulating the electrical field and the electric potential at the tip of three-dimensional electrodes, where the electrical field is expected to be maximum, could be very useful for fixing a limit value on the applied electrical stimulation stimulus in order to guarantee that the electrode interface reactions remain in the non-Faradaic domain.

IV. MEA MANUFACTURING

An original fabrication process for the three-dimensional tip electrode array has been developed. The main process steps are described in the following.

First, a 10nm Ta layer used as an adhesion layer is evaporated, prior to the evaporation of a 100nm thick layer of Pt, on a Si_3N_4 substrate. Then, as depicted in Fig. 3, these two layers of Ta and Pt are etched in a Cl_2/Ar plasma in order to form planar electrodes and their output wiring. The planar electrodes are used as a base for the three-dimensional tip electrodes. In order to create the three-dimensional structure, a 1.75µm thick layer of SiO_2 is first sputtered on the surface of the MEA, as shown in Fig. 4(a). The thickness of this layer will set the height of the three-dimensional electrodes. Then, as depicted in Fig. 4(b), an isotropic etching in BHF is performed in order to create the three-dimensional tips. The Si_3N_4 layer is used as an etching stop. Metallization of the tip electrodes is conducted in a further step by evaporating a 10nm thick Ti adhesion layer followed by a 100nm thick Pt layer. Finally, a 300nm thick SiO_2 passivation layer is sputtered on the surface of the chip. The final cross-section of the three-dimensional electrode array is depicted in Fig. 4(c).

The shape of the three-dimensional tips depends on several process parameters: the pitch dimension, the thickness of the SiO_2 layer, the size d_{resist} of the photoresist array (Fig. 4(b)), and the isotropic etching time. After having manufactured various configurations, three-dimensional MEAs with a minimum tip diameter of 0.5µm have been obtained, as depicted in Fig. 5. The pitch dimensions ranges from 5-6µm, and the electrode size from $3x3\mu m^2$ to $4x4\mu m^2$.

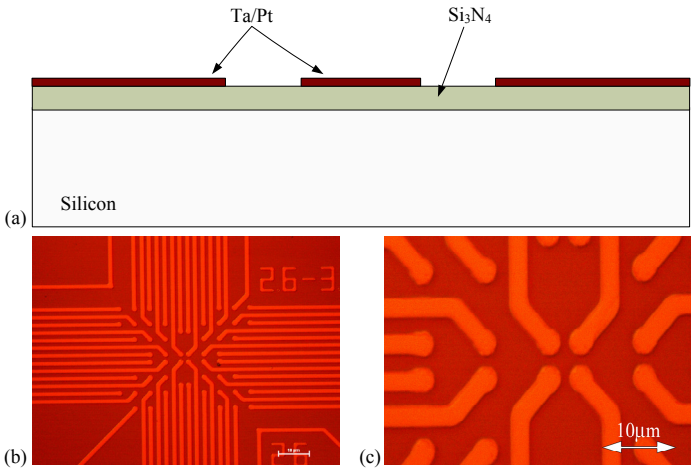

Fig. 3. (a) Schematic view of the cross-section of Pt planar electrodes deposited on a Si_3N_4 layer. (b) Optical microscope micrograph of the Pt planar electrodes and their output wiring. (c) Zoom of (b).

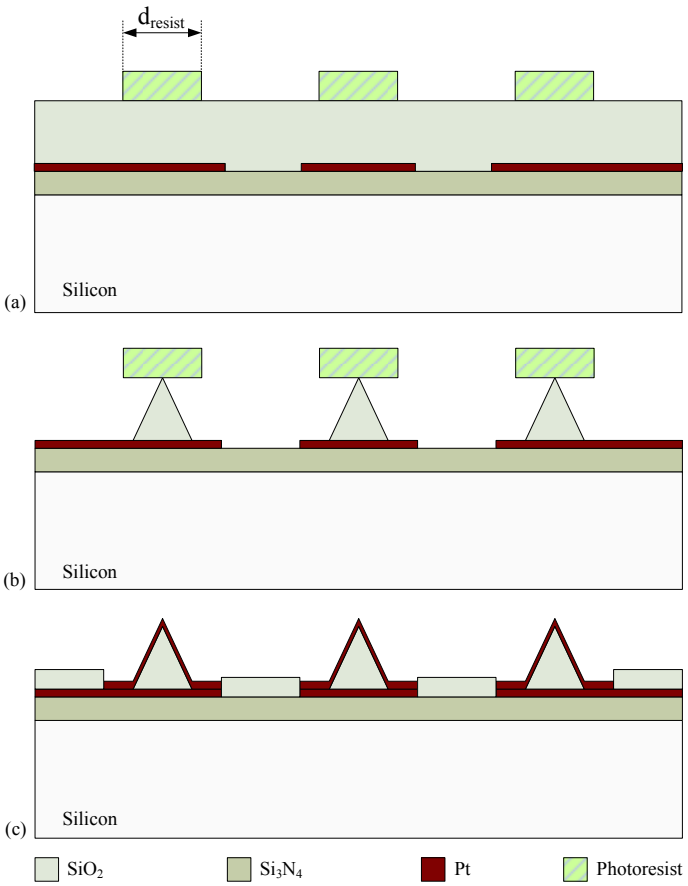

Fig 4. Schematic view of the cross-section of selected steps of the three-dimensional tip electrodes fabrication process.

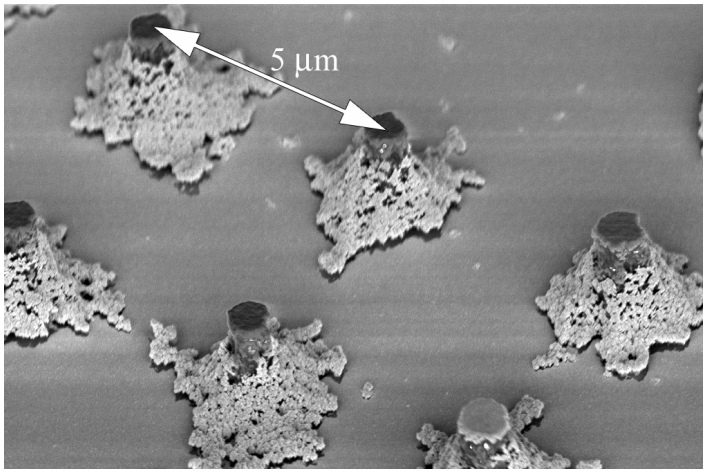

Fig. 5. SEM microphotograph of a three-dimensional tip electrode array with a tip height of 1.75µm, a pitch of 5µm, and a tip size of around 0.5µm.

V. Conclusion and Perspectives

In this paper, an innovative three-dimensional tip electrode array technology for *in-vitro* electrophysiological experiments is presented. Based on current flow simulation results obtained with a finite element model of the neuron-electrode interface, it has been shown that the electrical coupling between the neural cells and the three-dimensional tip electrode array is improved compared to standard planar electrodes. Therefore, a manufacturing process for three-dimensional tip MEAs has been developed.

Three-dimensional tip electrode arrays with an electrode diameter of 3-4µm, a height of 1.75µm, and a pitch dimension of 5-6µm have been manufactured on silicon substrate. The minimum tip diameter that has been obtained is around 0.5µm. Future electrophysiological experiments will determine if this dimension is satisfactory. However, work is already being performed in order to sharpen these tips.

Packaging of the samples silicon-substrate MEAs is currently undertaken, as described in Fig. 6 and Fig. 7. *In-vitro* electrophysiological experiments using these MEAs will then be carried out in a further step, where planar and three dimensional silicon-substrate MEAs will be characterized. The experiments results are expected to confirm the superiority of the three-dimensional electrodes in terms of electrical coupling.

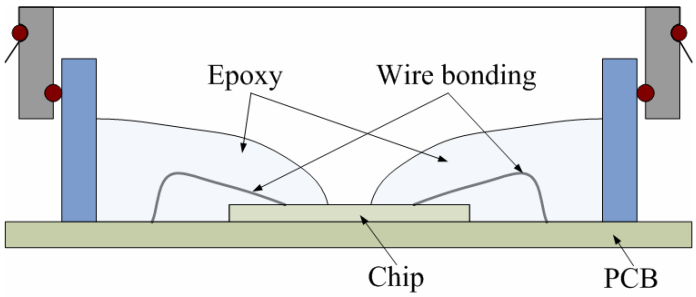

Fig. 6. Cross-section of the final MEA system.

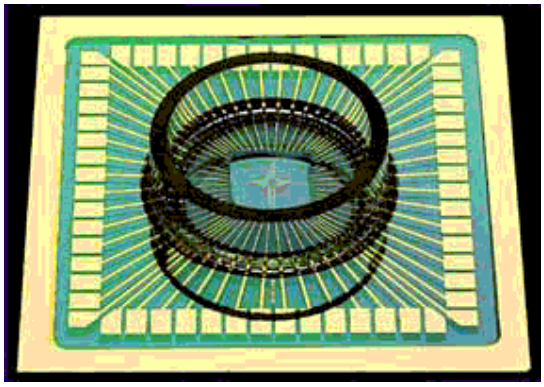

Fig. 7. Packaged MEA showing the cell culture chamber, after Multi Channel Systems MCS GmbH (from [13]).

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