BIOMIMETIC DENDRITIC STRUCTURES FOR CELL-CHIP COUPLING ENHANCEMENT

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The nervous system plays a fundamental role. The development of a functional nervous system requires neurons to interact with and promptly respond to a wealth of biochemical, mechanical and topographical cues found in the neural extracellular matrix (ECM). Among these, ECM topographical cues have been found to strongly influence neuronal function and behaviour[1]. Here, I will discuss how the design of the architectural organization of the brain ECM can be useful as a source of inspiration to design biomimetic substrates to enhance neural interfaces and dictate neuronal behaviour at the cell-material interface. The nervous system is composed by two parts: the central nervous system and the peripherical nervous system. The first one controls most functions of the body and mind. Each neuron is composed by a cell body, which houses the nucleus. The dendrites and axons form extensions from the cell body. Neurons connect with another to send and receive messages in the brain and spinal cord[2,3]. Messages are passed from neuron to neuron through synapses, small gaps between the cells, with the help of chemical compound called neurotransmitters. The messages are electrical impulses that are called action potential. To transmit an action potential across synapse, the neurotransmitters are released from the pre-synaptic neuron, across the gap to a post-synaptic neuron[4]. In recent years, the recording of the neural network communication has been investigated, and in this scenario the interaction between cell and electrode plays a fundamental role. Importantly, the cell- chip coupling between cells and micro- and nano structures depends on cells adhesion and on the cell's ability to rearrange its cytoskeletal architecture to wrap around vertically aligned structures[5,6]. The aim of this work is to design a biomimetic micro-structured inspired by the shape and designs of dendritic spines, a biological structure essential in neuronal communication. We have identified three structures' geometry: thin that can initiate contacts with presynaptic terminals and are therefore essential in the initial stages of spinogenesis and synaptogenesis; the mushroom shape as a result of the plastic and dynamic reshaping of the neuronal circuits during development or learning-dependent synaptic strengthening and the stubby[7]. These topographical architectures dictated engulfment event, as well as reducing the cleft and improving the cell-chip coupling. We investigated the membrane to material interface with high resolution methods. Therefore, experimental strategy involves the focused ion beam assisted milling and scanning electron microscopy (FIB-SEM) imaging. This procedure is able to: (i) identify a region of interest, (ii) bypass the substrate removal since the cut can be applied to any type of material, (iii) preserve subcellular structures due to plastic layer embedding, (iv) establish the cleft between electrogenic cells and electrodes, (v) perform the 3D intracellular space reconstruction. The method for sample preparation is the ultra-thin plasticization (UTP) that leads the preservation of the subcellular structures[8,9]. The UTP involves: the cell fixation by using glutaraldehyde to freeze cells structures; RO-T-O procedure in which the staining with osmium and uranium enhances the contrast sensitivity for the imaging; the resin infiltration provides a solid support for the subsequent FIB milling; and in the end resin excess removal and polymerization. The biomimetic substrate is fabricated by using the

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[1] Chklovskii, «Synaptic Connectivity and Neuronal MorphologyTwo Sides of the Same Coin».
[2] Bourne e Harris, «Balancing Structure and Function at Hippocampal Dendritic Spines».
[3] Kandel et al., *Principles of neural science*.
[4] Kandel et al.
[5] Santoro et al., «Interfacing Electrogenic Cells with 3D Nanoelectrodes».
[6] Mariano et al., «Advances in Cell-Conductive Polymer Biointerfaces and Role of the Plasma Membrane».
[7] Maiti et al., «Molecular Regulation of Dendritic Spine Dynamics and Their Potential Impact on Synaptic Plasticity and Neurological Diseases».
[8] Zhao et al., «Nanoscale Manipulation of Membrane Curvature for Probing Endocytosis in Live Cells».
[9] Santoro et al., «Revealing the Cell–Material Interface with Nanometer Resolution by Focused Ion Beam/Scanning Electron Microscopy».

two-photon polymerization lithography. The figure 1 shows the interface and the membrane deformation on biomimetic micropillar-based surfaces. Interesting is appreciating the membrane adhere on the electrode shape and the clear cell-substrate distance. Moreover, our preliminary findings suggest that the shape of the micro-structured electrode may indeed influence the neuron-chip coupling.

In conclusion, for in-cell recordings it is necessary to investigate which shape and in particular, which dimensions are suitable for promoting the tight contact between neuronal cells and 3D nanostructures. An optimal contact at the interface, improves the complete signal transfer from the neuronal cell to the recording device.

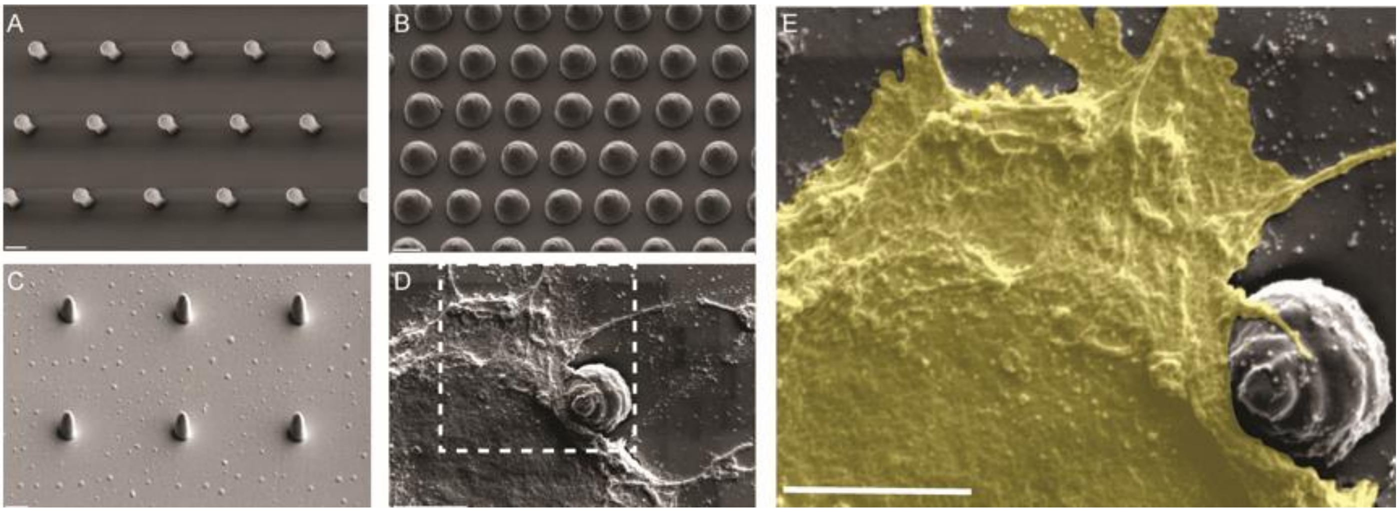

Figure 1: Scanning Electron micrographs of primary neurons on biomimetic substrates, scale bar 2μm. A) Mushroom array pitch 10μm, head diameter 2μm and high 1μm. B) Stubby array, pitch 4μm and head diameter 2μm. C) Thin array, pitch 10μm and high 1μm. D) Primary neurons on stubby array fixed at DIV4. E) Enlargement and false colouring of the neuron wrapping a stubby shaped pillar.

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