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Review Article

Brain cells and neuronal networks: Encounters with controlled microenvironments

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ABSTRACT

This review is an attempt to shed light on the wealth of the close association between micro/nanotechnologies and neurobiology. With this aim, we have identified four specific areas in the field of neuroscience that fruitfully exploit the conceptual approaches of physics together with the engineering tools originally developed for microelectronics. Each of these areas are developed in this review within a dedicated section. The first section "Isolating environmental parameters" illustrates how fabricating specific microenvironments allows one to isolate the cell response to specific physiological stimuli, either chemical or topographical. In a complementary way, the section "Revealing the properties of brain cells" shows how the design of rather artificial *in vitro* culture conditions can give precious insights into the field of neurobiology. The acquired knowledge concerning the response of isolated neurons to external physical cues may inspire methodologies for mastering the architecture, polarity and connectivity of *in vitro* neuronal assemblies. These aspects are developed in the section "Controlling neuronal architectures". Finally, a section entitled "Instrumentation of neuronal networks" reports some salient examples of recent innovative achievements in the field of neuron–electronic interfacing, illustrating how the issue of neuronal contact with micro-structured environments supports the development of advanced multiplexed electrophysiology methods. We will finally conclude this review by introducing some medical applications originating from the contribution of micro/nanotechnologies to the field of neuroscience. Our take-home message is that configuring the cellular microenvironment using microengineering tools has a strong positive impact in neuroscience at different levels, from the most fundamentals of cellular neurobiology to medical applications.

1. Introduction

The field of neuroscience constitutes a natural playground for physicists and engineers. Historically, this is illustrated by the emergence of electrophysiology as an independent discipline and by the continuous implementation of up-to-date technologies for neuronal interfacing. The original glass micropipettes used to measure neuronal intracellular potentials have been replaced by integrated extracellular sensors, either passive like microelectrode arrays, or active thanks to the use of miniaturized silicon devices.

Instrumentation is however not the only area where micro/nanotechnology and neuroscience can come together to great advantage. Recording neuronal activity requires understanding and control of the properties of the neuron/electronic interface. This concerns either the insertion of stimulation electrodes within the brain or the *in vitro* growth of neuronal networks upon electronic devices. A new highly multidisciplinary field has thus emerged from the basic requirements to create neuronal *in vitro* architectures and to optimize the neuron–electrode interface. Its aim is to explore the neuron, and more globally the brain cell responses to the chemical and topographic properties of the microenvironment. The issue of neuronal adhesion on various substrates has thus been extensively studied during the past years. As a result, the electrical picture of neurons has been enriched by many other biophysical aspects, related for example to biomechanics and more generally to the structure and dynamics of the cytoskeleton in controlled environments.

In this context, this review is an attempt to draw a picture of the contribution of microengineering tools to the field of neurobiology. The wealth of the close association between these two scientific domains will be developed in four parts following a short introduction to brain cells. We will mainly focus on primary cells as a first step towards a better understanding of brain tissue.

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In the preamble, we introduce some fundamental notions of neurobiology necessary to grasp the issues presented in this review after a brief overview of the different types of brain cells. Then, a first section entitled "Isolating environmental parameters" illustrates how fabricating specific microenvironments allows one to isolate the cell response to specific stimuli, either chemical or topographical. More generally, this highly informative *in vitro* approach gives precious insights into the field of neuron biology: this is the subject of the next section entitled "Revealing the properties of neurons". All the knowledge accumulated on isolated cells has inspired various methodologies for mastering the architecture, polarity and connectivity of *in vitro* neuronal assemblies. These aspects are developed in the section: "Controlling neuronal architectures". We then return to the initial concern of electrophysiology, i.e. the achievement of the highest recording sensitivity of neuronal activity, by giving some examples of the most innovative achievements in the field of neuron–electronic interfacing in a penultimate section entitled "Instrumentation of neuronal networks". Finally we conclude this review by presenting a few perspectives opened by the contribution of micro/nanotechnologies to the field of neurosciences, focusing in particular on medical applications.

2. Preamble: biological background

We gives in this section the basics of neurobiology necessary to understand the issues associated to the design of novel cellular microenvironments. This section will also introduce the specific vocabulary associated to the biology of brain cells (highlighted in bold italic). For a generalist introduction to neurobiology, the reader can refer to the book "From Neuron to Brain" [1].

2.1. Introduction to brain cells

The central nervous system (CNS) is mainly composed of *neurons* and of far more numerous ***glial cells*** (Fig. 1a) grouped in three principal types: astrocytes, microglia and oligodendrocytes. This third kind of glial cells has a similar role than Schwann cells in the peripheral nervous system (PNS), i.e. to wrap a myelin sheath around neuronal extensions. The name of "glial cells" comes from the Greek word "glue", thanks to the first role of support and protection of the nervous tissue attributed to these cells. Glial cells, unlike neurons, can divide and this capacity is one reason of the glial origin of most brain cancers. There is growing interest for these cells and for their active contributions to various brain functions, including the regulation of the strength of neuronal connections [2,3].

Neurons are excitable cells characterized by a resting intracellular potential (around −70 mV) that can locally switch to positive values in neuronal processes conveying electrical signals. The neuron morphology reflects its function to collect, process and transmit electrical signals through chemical junction named ***synapses***. From a mature neuron cell body, or ***soma***, emerge one ***axon*** (sometimes two in specific neuronal types) that conveys the output signal toward post-synaptic neurons, and multiple ***dendrites*** organized into a tree structure that collects the electrical activity produced by pre-synaptic neurons. In mammals, the typical size of a soma is on the order of 10 μm, the axon and dendrites diameter of about 1 μm and less in their distal part [4].

Embryonic cortical and hippocampal neurons development *in vitro* has been divided into five stages identified from the morphological changes that occur during maturation [5–7] (Fig. 1b). Soon after plating, dynamic sub-cellular structures named ***lamellipodia*** and ***filopodia*** are produced at the edges of the soma. After

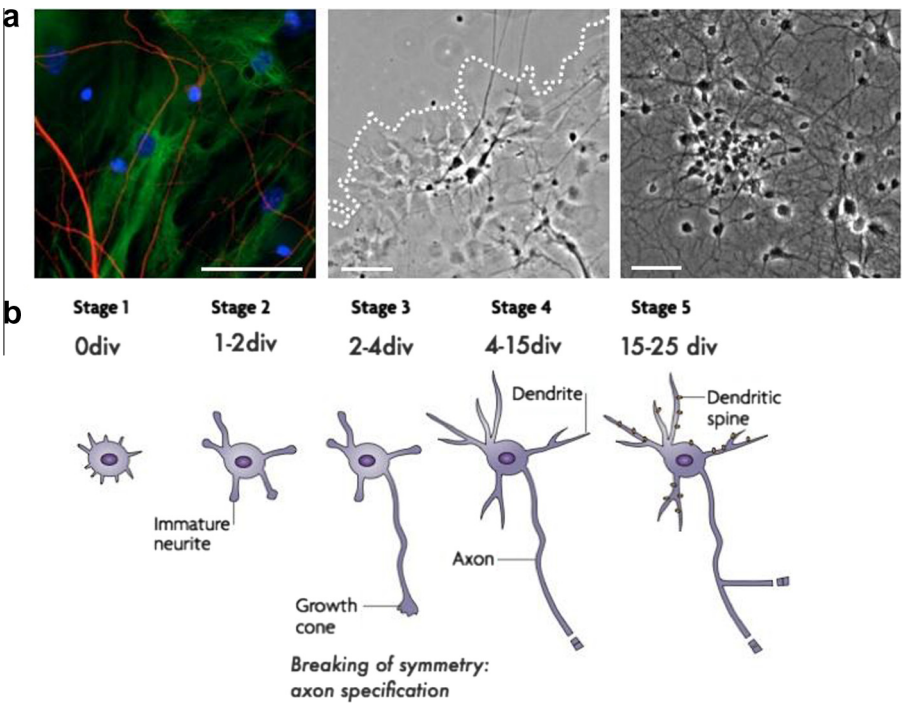

Fig. 1. Preamble: biological background. (a) Morphologies of co-cultured neurons and glial cells on hard gels (rigidity of 100 kPa). The left and middle images show a low density culture by fluorescence and phase contrast, respectively, as compared to a high density culture (right). Glial cells are evidenced by their flattened shapes (see the white dashed line contour in the central image) and neurons from the bright halo around their cell bodies and from their dark thin branches. Red (MAP2 staining, revealing neuronal branches), green (GFAP staining specific of glial cells), blue (Hoechst staining, nucleus). Scale bars: 50 μm. (b) The generic stages of development of cortical/hippocampal neurons (times refer to mice hippocampal neurons, DIV: Days In Vitro) [7]. Adapted with permission from Nature Neuroscience. Copyright © 2007, Rights Managed by Nature Publishing Group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)