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Technical steps towards one-to-one electrode–neuron interfacing with neural circuits reconstructed in vitro

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Abstract

The electrical interfacing with cultured networks of neurons by means of multielectrode arrays (MEAs) is an area of intensive research given the potential of this technique to aid in the extraction of the algorithms that support neurocomputation in brain tissue. To this end, we have previously described the polymer-on-multielectrode technology (PoM [E. Claverol-Tinturé, M. Ghirardi, F. Fiumara, X. Rosell, J. Cabestany. Multielectrode arrays with elastomeric microstructures for neuronal patterning towards interfacing with uni-dimensional neuronal networks. J. Neural Eng. 2(2) (2005) L1–7] and demonstrated single-site recordings from patterned invertebrate cells. The realisation of the full potential of PoM is dependent on its successful application to vertebrate cells and to the recording of activity in ensembles of neurons. Here, we describe progress in these directions, specifically towards the development of vertebrate neuronal cultures devoid of glial layers compatible with PoM devices, the connection of pairs of invertebrate neurons threading microchannels and the recordings of synaptic and spike-like activity.

Keywords: Neural interfaces; Multichannel electrophysiology; Neuronal culture; Cell patterning; Microfluidics; Snail neurons; Helix aspersa; PDMS

1. Introduction

Neurocomputation as implemented by aggregates of living neurons depends on the electrical interplay of large numbers of synaptically connected cells. To achieve a better understanding of network level information processing phenomena, it is advantageous to build realistic computer models. These rely heavily on detailed physiological data describing neuron–neuron interactions [9] and, therefore, are in need of experimental techniques suitable for monitoring neuronal activity in networks of neurons.

Conventional multielectrode arrays (MEAs) are capable of multi-neuron electrical recordings [7,11,13] typically by means of multiple microelectrodes embedded in a flat substrate on which neurons can be grown to form networks. In the most common experimental configuration, neurons are cultured in large quantities (of the order of 5×10^4) and are monitored by up to 100 electrodes. The obvious difference in number between cells and recording sites makes one-to-one neuron–electrode interfacing problematic and, as a result, MEAs have fallen short of the expectations created by the first developments in the area.

To realise one-to-one interfacing, every neuron must be grown in the proximity of a single electrode with a pre-specified geometry because the position of the recording site with respect to the cell body and processes (dendrites and axons) influences greatly the magnitude and shape of the recorded signal [5].

Significant progress towards neuron patterning on MEAs has recently been achieved employing either chemical or physical guidance of cell growth. Chemical strategies rely on the selective coating of cell culture substrates with promoters or inhibitors of cell adhesion and growth. A popular realisation of this approach is the microcontact printing of adhesion molecules such as poly-L-lysine [3] by physical contact of a pre-soaked rubber stamp on a substrate. While this technique is relatively straightforward, issues of long-term cell compliance and

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clustering remain. On occasions, typically after days or weeks of culture, neurons can cross over areas coated with adhesion inhibitors, loosing compliance with the pre-defined growth pattern. Moreover, while chemical strategies can force neuron growth over specific regions, within these areas the continuous movement of cell bodies remains difficult to restrain, often resulting in cell aggregation and precluding one-to-one neuron–electrode interfacing.

A second family of cell patterning techniques addresses the issues of compliance and clustering by means of physical confinement of neuronal growth. To this end, 3D structures of dimensions in the order of tens of micrometres are produced using well established microfabrication techniques so that cell growth is confined by solid features such as walls and pillars. Along these lines several proof-of-principle results have been achieved over the last decade using microwells in silicon [10], Parylene cylindrical cages [15] and SU-8 pillars to form fences [17]. Yet, this success has not resulted in widespread adoption of microfabrication-based cell patterning techniques on MEAs partly due to the high complexity and cost of the device production procedures involved.

To address this issue, we are pursuing an experimental programme towards novel devices capable of physical cell guidance and extracellular recordings while remaining suitable for production at moderate costs. We have previously described the polymer-on-MEA (PoM) technology [4] which combines polymeric films containing microwells and microchannels fabricated with soft-lithography techniques [8,16] and standard planar MEAs and achieved physical confinement and guidance of individual mollusc neurons in the proximity of recording sites and demonstrated multisite extracellular recordings.

However, to realise the full potential of PoM, its optimisation for the monitoring of ensembles of interconnected neurons, rather than isolated cells, would be advantageous. Moreover, culture of vertebrate neurons would be preferable to invertebrate ones as the former could support the study of high-level cognitive functions.

Here, we describe progress towards these two goals: (1) demonstrating the culture of vertebrate cells devoid of glial carpet (non-electrogenic companion cells that facilitate neuron survival but can insulate the electrodes and deteriorate the electrical signals), (2) patterning both vertebrate and invertebrate neurons by polymeric films and (3) recording electric potentials of synaptic origin produced by pairs of interconnected neurons.

2. Methods

2.1. Vertebrate neuron culture

The protocol used for culturing cortical neurons was similar to those described previously [1,2,12] with some modifications. The frontal cortex of Sprague–Dawley pups at stages P1–P7 was dissected and pieces of approximately 2×2 mm cut with a scalpel. The dissection was performed in L15 at 4 °C. Digestion with papain (12 units/ml in L15) was performed at 37 °C for 30 min in a 15 ml tube on a rotating platform. The tissue was then rinsed thoroughly with dissection medium and mechanically dissociated by 40 suction/expel cycles with a plastic pipette tip cut to an opening of 2 mm in diameter. The cells were plated on poly-L-lysine treated Petri dishes or glass coverslips (0.1 mg/ml for 24 h) in neurobasal supplemented with B27 and glutamine and kept at 37 °C in an atmosphere of 5% CO_2. Serum was not used in order to control growth of glial cells. This protocol sufficed to achieve healthy cultures for up to 2 weeks.

Recently, a coculture step [1] has been introduced in order to facilitate long-term survival. Glass coverslips with neurons cultured for 2 days as described above were turned upside-down (cell bodies facing down) and dropped on a glial feeder layer which had been cultured for 10 days to reach confluency. The protocol to culture this layer on a glass coverslip is identical to that described for neurons with the exception that 10% horse serum (Sigma) was added to the culture medium. As a result, the bottom layer was rich in glial cells and poor in neurons and was close enough to the top layer, where glia was absent, to deliver the factors that support long-term survival of the neurons.

2.2. Invertebrate neuron culture

Culture of Helix aspersa neurons followed procedures described previously [4,6,14]. Briefly, snails were collected locally or provided by a breeder (Cal Jep, Spain) and kept in hibernation until wetted prior to the dissection. An active snail was selected and anaesthetised by injection of 1–2 ml of 0.08 mM $MgCl_2$. The circum-oesophageal ring was removed, pinned on cured silicone, trimmed free of surrounding tissue and cut into eight pieces with fine scissors. Using a plastic pipette, these were transferred to a 35 mm Petri dish with culture medium (40% L15 Leibovitz medium supplemented to the following concentrations: 62 mM NaCl, 5 mM KCl, 7 mM $CaCl_2$, 5 mM $MgCl_2$, 20 mM HEPES, 10 mM D-glucose, peniciline 100 units/ml, streptomycine 100 mg/l, fungizone 2.5 mg/l, adjusted to pH 7.6 with NaOH). The ganglia were then torn with hypodermic needles, resulting in the release of multiple neurons from the neuropiles. After a 20 min settling period, healthy cells were identified by their round shape (intact membrane) and transparent/bright cell body as seen under the dissection microscope. Cells with a diameter of 20–50 μm that had survived the procedure were sucked with blunt-opening capillaries (0.58 mm ID, WPI, USA) and expelled into a Petri dish with filtered medium. This cleaning step was repeated twice with two additional dishes filled with clean medium in order to limit the amount of debris and microorganisms carried over from the snail to the PoM device. Finally, the neurons were dropped inside PoM wells.