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Characterization of growth and electrical activity of nerve cells cultured on microelectronic substrates: towards hybrid neuro-electronic devices

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The main purpose of this paper is to describe several techniques for characterizing the functional coupling of a population of *in vitro* cultured neurons to a planar array of microtransducers. Both experimental aspects and computer simulations are considered. Experimental data include a comparison of neuron growth on various substrata and multichannel extracellular monitoring of neuron activity. Simulations dealing with impedance measurements and extracellular stimulation are also described.

1. Introduction

The growing interest in experimental and theoretical characterizations of nerve cells coupled to microtransducers is opening up a new research area on the borderline between neuroscience and microelectronic technology. Hybrid neuro-electronic devices, consisting of neurons functionally coupled to microelectronic devices, can be used as preliminary models towards the development of neuro-prostheses. In the literature, a variety of neural electrodes have been proposed, which can be used to stimulate and record electrical activity in nerve cells of the peripheral nervous system. [1, 2]. The neuritic regeneration process is strongly influenced by the electrical and micromechanical properties of microelectronic devices, and the biocompatibility of electrode materials is essential to ensuring neuritic survival and accurate signal transduction, especially for long-term use.

The main purpose of this paper is to characterize the functional coupling of a population of cultured neurons to a planar array of microtransducers. At present, this technique is being developed in several laboratories [3–6], including ours [7], and shows great promise as a new powerful tool for *in vitro* testing of experimental conditions basic to *in vivo* experimentation.

2. Materials and methods

2.1. Neuron culture

Experiments were performed on primary culture neurons from Dorsal Root Ganglia (DRG) of 10–12 day old chick embryos. The culture was established by the method described by Greene [8]. About 50–60 ganglia were dissected from two–three embryos (thoracic-lumbar region). During dissection, the ganglia were collected in culture medium RPMI-1640 (Sigma), without serum, and then placed in Ca^{++}- and Mg^{++}-free phosphate-buffered saline containing 0.125% trypsin. After 20 min incubation at 37 °C the ganglia were washed twice with a medium containing heat-inactivated horse serum 1%, and dissociated by tituration in a siliconized Pasteur pipette. The culture medium was composed of RPMI-1640, with 1% FCS, 1% horse serum, 1% antibiotics, and 10 ng/ml of NGF. The culture was maintained at 37 °C in a water-saturated atmosphere with 5% CO_2.

2.2. Substrata

Two different kinds of arrays of planar microelectrodes were utilized. The microelectrodes were designed and fabricated according to thin-film technology; they differed in the substrata utilized for the fabrication. One kind of device was based on an array produced at the Center for Integrated Systems, University of Stanford (USA), within the framework of a cooperation with our Bioelectronics Laboratory. It consisted of 32 gold electrodes, 10 μm × 10 μm and 50 μm apart; the substrate was Si_3N_4 on SiO_2. The other kind of device consisted of 64 ITO (Indium–Tin–Oxide) electrodes on quartz. These arrays were fabricated in the Basic Research Laboratories, NTT, in Tokyo (Japan) and were a generous gift of Dr Kawana.

DRG neurons were transferred and plated with a density of 10 000 cells/ml in drops of 100–150 μl onto an array of microelectrodes, previously coated with adhesive natural protein such as Laminin, or with synthetic proteins such as Poly-Lysine and with specific neuritic promoting factors such as the Nerve Growth Factor (NGF).

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2.3. Automated workstation for signal recording

Signals were extracellularly recorded by using a PC-based automated workstation, which consisted of a personal computer 486 equipped with an A/D conversion board (National Instruments AT-MIO16 F5; maximum sampling rate: 200 kHz), with two DMA channels and 16 input channels. In order to avoid electrically induced artifacts and to increase the amplitudes of extracellular signals (see Section 3), an amplifier stage with a gain of 190 and a filter stage with a bandwidth ranging from 400 Hz to 8 kHz were designed. Every experiment was performed in a Faraday cage to avoid electromagnetic noise. During each experiment, a stereomicroscope with a TV camera was used to check the positions of the cells on the microelectrode array and to correlate the recorded signals with images of the cells on the array.

2.4. Computer simulations

An *ad hoc* modified version of the electric circuit analysis program SPICE was used to simulate both the electrical behaviour of a neuron stimulated by means of an extracellular microelectrode coupled to it and the mechanical behaviour (adhesion) of a neuron growing on an array of microelectrodes.

3. Results

This section deals with experimental data and computer simulations concerning the morphology, adhesion and electrical activity of chick embryo DRG neurons.

3.1. Experimental results

3.1.1. Cell growth conditions

The *in vitro* reconstruction of a network of neurons is modulated by several biochemical factors including adhesion-promoting molecules and differentiating agents such as the NGF. Fig. 1 gives a summary of typical morphological growths as a function of chemical agents, obtained in our laboratory. The data refer to ITO arrays; similar results were obtained by the silicon-nitrate array.

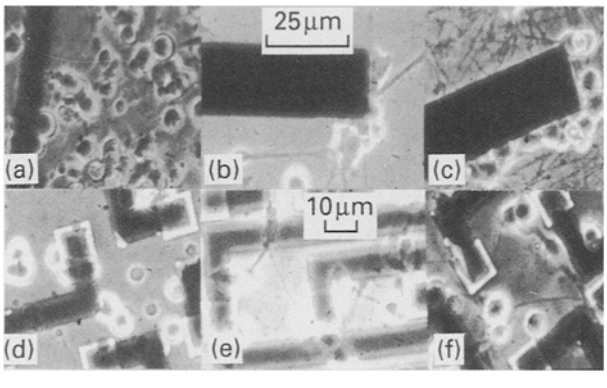

Figure 1 Typical morphological growths on ITO arrays: (a) Laminin without NGF after 18 h; (b) Laminin with NGF after 6 h; (c) as in (b), after 18 h; (d) Poly-D-Lysine without NGF after 18 h; (e) Poly-D-Lysine with NGF after 6 h; (f) as in (e), after 18 h.

3.1.2. Recording of spontaneous electrical activity

Neural electrical activity was simultaneously recorded from multiple sites (typically, eight channels) on the electrode array. Examples of recorded signals are shown in Fig. 2. As described in the previous section, DRG neurons were seeded on the ITO array precoated with Laminin. The maximum amplitude of the signals was around 250 µV and the noise was around 25 µV. Signal shape and amplitude can be related, in a complex way, to the functional coupling of different neuronal units (cell bodies and/or arborizations) to (part of) microelectrodes [9]. Spontaneous electrical activity was typically recorded from several units for a few hours.

3.2. Simulation results

The use of microelectronic techniques to record/stimulate the electrical activity of neurons cultured *in vitro* by using planar microelectrodes introduces a new kind of device that cannot be classified according to the traditional distinction between extracellular and intracellular recording devices. Hence modelling and computer simulations of the critical parameters (e.g. cell/microtransducer coupling) of this new recording technique are fundamental tools to interpret and predict experimental data. An *ad hoc* modified version of the electrical circuit analysis program SPICE was developed for this purpose [10].

The implementation (in the SPICE code) of new subroutines describing the Hodgkin–Huxley equations resulted in a general-purpose simulation program for detailed modelling of neurons. Moreover, the efficiency of SPICE in simulating electronic devices makes our modified version a very appropriate tool for the characterization of the electrical coupling between microtransducers and neurons.

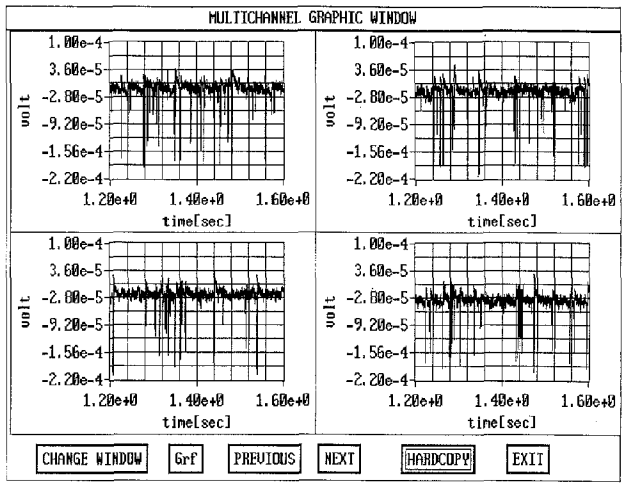

Figure 2 Multichannel recording of electrical activity by using a planar microelectrode array. Four simultaneously recorded extracellular signals of DRG neurons are shown.

3.2.1. Computer simulations of impedance measurements

Nerve-cell adhesion to planar electrodes can be characterized by monitoring the electrode impedance [11]. An equivalent circuit representing the impedance measurement system was modelled. The circuit consisted of a sinusoidal signal generator connected via a 1 Mohm resistor to the tissue culture medium electrode. The generator frequency ranged form 1 kHz to 4 kHz and the voltage amplitude was 1 mV. The tightness of the cell/electrode junction was quantified as a function of the output voltage of the measurement circuit. Fig. 3a shows the equivalent circuit for impedance measurements in the case of a junction between a neuron membrane patch and a planar microelectrode (10 μm × 10 μm). Fig. 3b shows a simulation of an experiment based on impedance measurements using the circuit shown in Fig. 3a. The value of the output voltage changes as a function of the sealing resistance, R_{seal}, between the nerve cell and the microelectrode. In this case the presence of a peak in the graph is due to the fact that the cell, after initial stabilization (e.g. attachment to electrode), begins to exhibit motility causing a fluctuation in the output signal as described in [11].

3.2.2. Computer simulations of electrical activity stimulation

The neuron regeneration process can be induced by chemical neuritic-promoting factors (e.g. NGF), by applied electrical fields, or by direct DC stimulation [12]. The arrays described could be exploited for these kinds of experiments. Toward this end, modelling and computer simulations of the stimulation of electrical activity in nerve cells by means of planar microelectrodes were performed.

Fig. 4a presents the equivalent circuit of a nerve-cell–microelectrode junction for extracellular stimulations. A simulation of the response of a nerve cell to a train of stimulating current pulses is shown in Fig. 4b. A train of pulses of 0.7 ms duration and a current

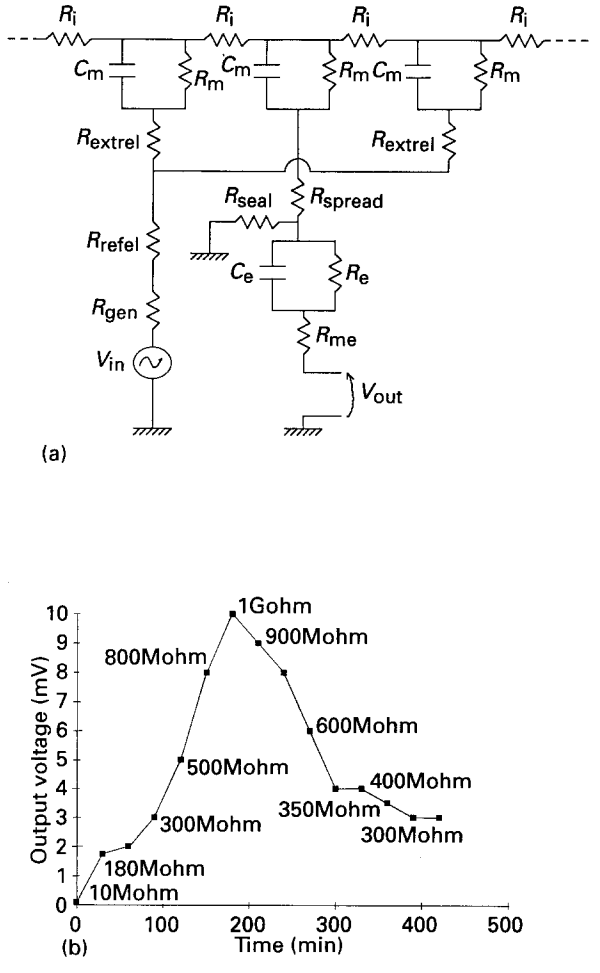

Figure 3 (a) Equivalent circuit of the junction between a membrane patch (described by a compartmental model) and a passive (i.e. noble metal) microtransducer modelled for impedance measurements (Cm: membrane capacitance; Rm: membrane resistance; Ri: cytoplasmatic resistance; Rspread: spreading resistance; Rseal: sealing resistance; Re,Ce: equivalent elements of the electrode-electrolyte interface; Rme: metallic resistance; Rgen: voltage source resistance; Rrefel: reference electrode resistance; Rextrel: extracellular resistance). (b) Response of the impedance measurement circuit to different values of the sealing resistance R_{seal}.

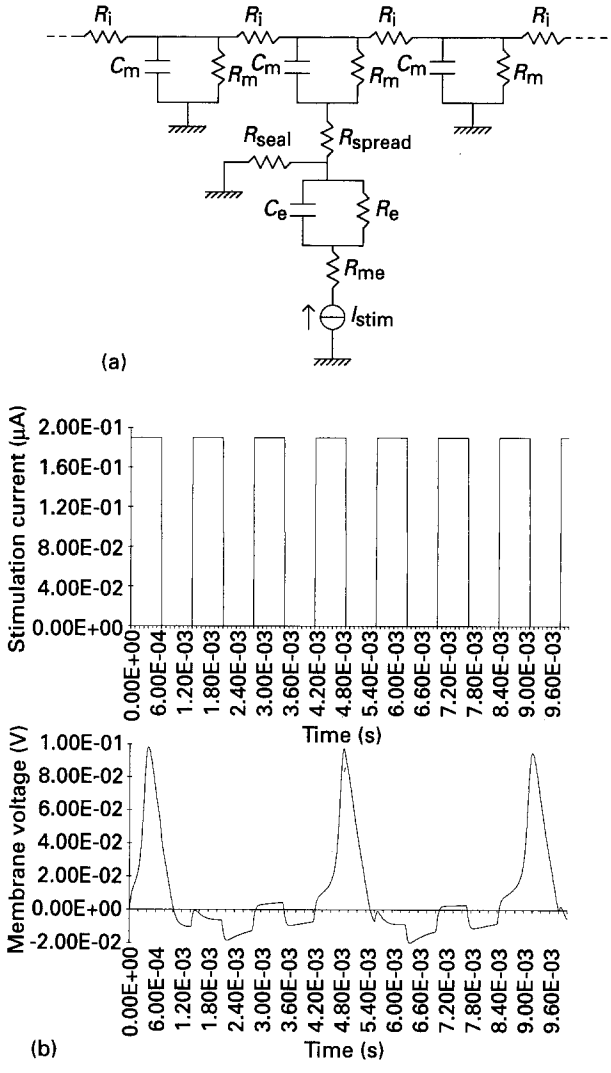

Figure 4 (a) Equivalent circuit of the nerve-cell–microelectrode junction for extracellular stimulation (Cm: membrance capacitance; Rm: membrane resistance; Ri: cytoplasmatic resistance; Rspread: spreading resistance; Rseal: sealing resistance; Re,Ce: equivalent elements of the electrode-electrolyte interface; Rme: metallic resistance). (b) SPICE output representing the response of a nerve cell to a train of pulses of stimulating current.

amplitude of 190 nA was applied. Action potentials were generated every third stimulating pulse. These simulation results are very similar to experimental data reported in the literature [13], and could be used to design new experimental protocols.

4. Prospects

To obtain a stable functional coupling between neuronal units and biocompatible microelectronic devices is a basic step towards the design of neuro-electronic interfaces aimed at transducing and stimulating nerve electrophysiological activity.

In vitro experiments with networks of active neurons coupled to microelectronic elements, are a fundamental tool for attaining the final goal of *in vivo* implantation.

As described in this paper, the effectiveness of this tool is critically based on the parallel development of several techniques, ranging from the preparation of short-term cultures of neuron populations to *ad hoc* designed bioelectronic measurements and simulations.

At present, the coherent integration of these techniques is opening up a new research area on the borderline between neuroscience and microelectronics and holds great promise for both basic research and clinical applications.

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