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Short communication

Neural recording and stimulation of dissociated hippocampal cultures using microfabricated three-dimensional tip electrode array

Yoonkey Nam [a,*], Bruce C. Wheeler [a], Marc O. Heuschkel [b]

[a] *Department of Bioengineering, University of Illinois at Urbana-Champaign, 405 N. Mathews Ave., Urbana, IL 61801, USA*

[b] *Ayanda Biosystems SA, PSE Parc Scientifique, EPFL, CH-1015 Lausanne, Switzerland*

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Abstract

There is increasing interest in interfacing dissociated neuronal cultures with planar multielectrode arrays (MEAs) for the study of the dynamics of neuronal networks. Here we report on the successful use of three-dimensional tip electrode arrays (3D MEAs), originally developed for use with brain slices, for recording and stimulation of cultured neurons. We observed that many neurons grew directly on protruding electrode surface, appearing to make excellent contact. A larger than usual portion of extracellular spikes had large positive peaks, while most of the spikes from conventional two-dimensional electrode arrays had large negative spikes. This may be due to the direct capacitive coupling situation provided by relatively large electrode surface area.

Keywords: Multielectrode array; Three-dimensional tip electrode; Cultured neurons; Extracellular recording; Electrical stimulation; Spike shapes

1. Introduction

A planar multielectrode array (MEA) is a culture dish with embedded microelectrodes for the purpose of recording and stimulating interfaced tissues, e.g., acute slices (Wheeler and Novak, 1986; Boppart et al., 1992; Hiroaki et al., 1999) or dissociated cultures (Pine, 1980; Gross et al., 1982; Jimbo et al., 1993). This has been extensively used to investigate the learning and memory paradigm in neuroscience (Jimbo et al., 1999; Shahaf and Marom, 2001) or to build cell-based biosensors for drug screening (Gross et al., 1995; Pancrazio et al., 2003).

Recently, a three-dimensional tip electrode array (3D tip MEA) has been developed to enhance the quality of the recording signal for acute slice experiments (Heuschkel et al., 2002). Protruding 3D tip electrodes allow access through surface dead cell layers to the intact cell layer in the brain slice allowing the detection of larger signals than can be obtained with conventional 2D type electrodes. From acute slice preparations, single unit extracellular action potentials as well as evoked excitatory postsynaptic potentials were recorded. The low electrode impedance due to the relatively large surface area resulted in a low noise level and higher safe-charge injection limit for electrical stimulation.

To date this new type of MEA has been used principally for acute slice experiments, and to our knowledge, no reports have been made of its use with dissociated neuronal cultures. Hence, we were interested in demonstrating basic biocompatibility as well as recording and stimulation capability. It was expected that one could grow cells on these MEAs. However, in as much as an electrode integrates the potential present at its surface, it was expected that these larger than average surface area electrodes would report smaller than average signals from neurons that happened to grow nearby. Previously, we have reported empirical data indicating that somata needed to be within 20 μm of an electrode to be recordable in sparse dissociated culture (Nam et al., 2004). As shown in the report below, we were surprised by the tendency of neurons to adhere tightly to these electrodes and for the recorded signals to be easily large enough to warrant use in dissociated culture.

* Corresponding author. Tel.: +1 217 244 2693; fax: +1 217 244 5180.
E-mail address: ynam1@uiuc.edu (Y. Nam).

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2. Methods

2.1. Multielectrode arrays and culture

3D tip MEAs were received from Ayanda Biosystems (Lausanne, Switzerland). Electrodes were made of platinum (60 electrodes per MEA, geometric area: 60 μm × 60 μm, height 50–70 μm) and they were insulated with SU-8. Two different types of conventional MEAs with two-dimensional electrodes served as control groups. One had a thin silicon nitride insulator (0.5 μm) which provided a flat type electrode (TiN, 10 or 30 μm in diameter, Multi Channel Systems, Reutlingen, Germany). The other had a relatively thick polymer insulator (SU-8, 5 μm) which formed a recessed electrode (indium–tin oxide, 40 μm × 40 μm, Ayanda Biosystems).

Poly-D-lysine (PDL, 0.1 mg/ml in deionized water, MW 70,000–150,000, Sigma–Aldrich) was coated for 2 h. After the coating, the MEA surface was rinsed with deionized water once and sterilized with 70% ethanol for 30 s and stored in sterile-petri dish.

2.2. Cell culture

Dissected hippocampal tissues (18-day gestation Sprague/Dawley or Fisher 344 rat hippocampus) were purchased from Brain Bits™ (http://www.brainbitsllc.com). Tissues were mechanically dissociated and plated in serum free B27/Neurobasal medium (Invitrogen, Gaithersburg, MD) with 0.5 mM glutamine and 25 μM glutamate at the density of 100 cells/mm^2. Cultures were stored in an incubator at 37 °C, 5% CO_2 and 9% O_2. After 4 days in vitro (DIV), the media was changed to serum free B27/Neurobasal medium with 0.5 mM glutamine. Thereafter, half of the media was changed weekly or after recording if necessary. Cultures were inspected under transmitted light inverted microscope (phase contrast) and scanning electron microscope (SEM).

2.3. Neural recording and stimulation

MEA 1060 (gain 1 200, bandwidth 10–3000 Hz, Multi Channel Systems) and MC Rack software (Multi Channel Systems) were used for data acquisition. Raw data were digitized and stored at 40 kHz for off-line analysis. Each recording session lasted for 2 min and seven cultures were used to collect data from 11 recording sessions (5 recordings from 14 DIV, 6 recordings from 21 DIV).

For electrical stimulation of the culture, voltage pulses were delivered by STG-1008 (Multi Channel Systems). We chose positive first biphasic stimulus (voltage controlled, ±0.8 to ±1.4 V, pulse width 200 μs), as recommended for effectiveness in stimulating cultured neurons (Wagenaar et al., 2004). The intensity of the stimulus was selected such that stimulation artifact permitted monitoring of evoked responses as early as 5 ms after stimulus delivery.

Raw data were filtered using the second order Butterworth digital filter and spikes were detected by setting the threshold level at five to seven times the standard deviation of background noise level. Channels that exceeded spike rate of 0.1 Hz were regarded as active.

3. Results

3.1. Biocompatibility of 3D tip MEAs

The insulator and electrode tip material was completely compatible for short (hours to days) and long (weeks) term. Neurons showed normal cell adhesion and neurite extension after the plating and formed a dense neural network after a few weeks in vitro. Most of the cultures in this study remained healthy for at least a month (one survived for 7 weeks). Data reported below are from cultures in either the third or fourth week since plating.

In order to observe cell growth on top of the electrode, we used scanning electron microscopy. Images showed that somata as well as neurites adhered to the surface of the electrode and grew without any noticeable difference when compared to cell growth on flat surfaces. Some electrodes were completely covered by the mixture of somata and entangled neurites (Fig. 1(a)). Others had a single soma or multiple somata with extending neurites

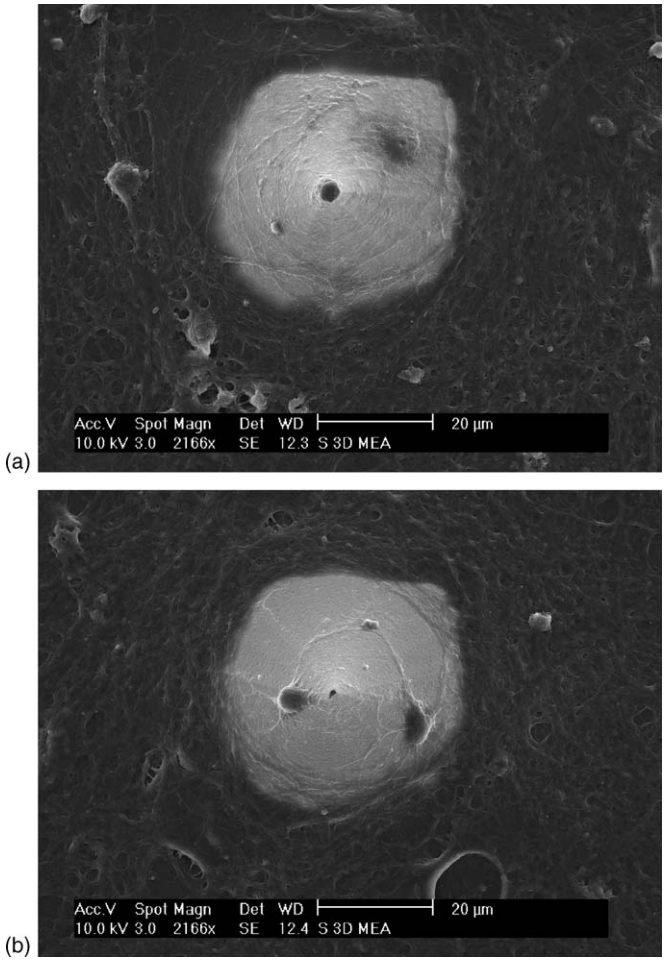

Fig. 1. Scanning electron micrograph of neuronal growth (hippocampal neurons) on the surface of the 3D tip electrode at 24 DIV. Positive (a) or negative going spikes (b) were recorded from these electrodes (see text). Top view. Scale bar = 20 μm.