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Novel disposable microelectrode array for cultured neuronal network recording exhibiting equivalent performance to commercially available arrays

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ABSTRACT

Microelectrode arrays (MEAs) enable non-invasive recording of supra-threshold signals, i.e. action potentials or spikes, from a variety of cultured electrically active cells. While this label-free technology has been shown to be useful for pharmacological and toxicological studies, a major limitation has been the reliance on expensive recording substrates that have been manufactured with the intent of re-use. Prior work by our group has demonstrated an approach for fabricating MEAs using conventional liquid crystal display manufacturing techniques. Here, we describe and characterize the UT Dallas planar MEA which is fabricated with low cost materials and processes. We compare the performance of the UT Dallas MEAs, which consist of exposed gold microelectrodes with patterned parylene insulation over traces, with well-established commercially available MEAs using cultured murine cortical networks. Detailed electrophysiological comparisons show virtually identical performance between MEA types with respect to network metrics including recording yield across the array, network spike rate and burst rate, and virtually identical pharmacological responses to a diverse set of neuropharmacological agents.

1. Introduction

Neuronal networks cultured on planar microelectrode arrays (MEAs) have utility in wide range of in vitro applications including neuropharmacology [1–4], assessing biocompatibility of novel materials [5], studying neural network plasticity [6–8], connectivity [9], and response to electrical stimulation [10,11], as well as the emergence of dynamic states [12,13]. Murine primary cultures derived from embryonic neural tissue mature to form spontaneously active networks on the surface of MEAs. In fact, the use of cultured cortical tissue on MEAs for pharmacological assays has been cross-validated across multiple laboratories [14]. Compared to other electrophysiological assay or imaging approaches, MEAs offer label-free, non-invasive, and long-term recording capabilities.

To create MEAs with features on the order of tens of microns, fabrication processes and materials common in the silicon microelectronics industry are used. Common conductive materials comprising MEAs include indium tin oxide (ITO), platinum, gold, or titanium nitride, whereas the insulating materials include SU-8, silicon nitride, polymimide, parylene, or polydimethylsiloxane. Advances in MEA technology have mainly focused on increasing spatial resolution and channel density through the incorporation of on-chip CMOS electronics [15–18]. Unfortunately, MEAs are typically expensive, fragile, and consist of materials that are distinctly different from conventional polystyrene culture dishes used and discarded in typical cell biology laboratories. Manufacturers expect re-use and specialized handling of these devices.

We previously reported on the application of liquid crystal display fabrication technologies to create a low-cost functional MEA [19] that made use of gold microelectrode contacts and traces insulated with parylene-C, a biocompatible polymer often used in implantable device applications [20–22].

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In the present paper, we demonstrate reproducible fabrication of low-cost MEAs leveraging our fabrication process that exhibit stable electrochemical impedance profiles of gold contacts and conducting polymer modified sites under culture conditions. Developed at the University of Texas at Dallas (UT Dallas), we show that the performance of these MEAs with respect to cultured neuronal network recording is entirely consistent with that from well-established commercially available MEAs from Multi Channel Systems (Reutlingen, Germany). Our data show that the UT Dallas MEAs offer a platform for low-cost, disposable high content assays from neuronal networks.

2. Methods

2.1. Fabrication and characterization

An array of 60 gold square microelectrodes 30 μm in length was patterned on the substrate with insulation over the leads with parylene-C. The outer dimensions of the array substrate were 3.8 cm × 3.8 cm with 2 mm × 2 mm bond pads separated by 400 μm. As shown in Fig. 1, the substrate was a 500 μm thick layer of polycarbonate, a stiffer material than polyethylene naphthalate used previously [19], which improves ease of handling. The gold microelectrodes were deposited by e-beam evaporation and patterned using standard photolithography and wet etch. Parylene-C was deposited by chemical vapor deposition and then exposed to oxygen reactive ion etching for patterning.

Electrochemical characterization of the MEA was performed by electrochemical impedance spectroscopy (EIS) on individual microelectrodes from a typical array. The measurements were performed using a two-electrode setup using a potentiostat/galvanostat (CH 600D, CH Instruments, Texas, US) equipped with an electrochemical analyzer module (CHI Version 9.03, CH Instruments). Measurements were made in the presence of phosphate-buffered saline (PBS) at pH of 7.4 at room temperature by applying a sinusoidal signal with 20 mV amplitude over a range of frequencies from 10 Hz to 100 kHz to characterize the complex impedance of the working electrode and the electrolyte solution.

2.2. MEA preparation and cell culture

Commercially available MEAs from Multi Channel Systems (Reutlingen, Germany), model MCSMEA-S2-GR which consist of 60 ITO microelectrodes, were used for comparison with the novel MEAs. Microelectrodes had a diameter of 10–30 μm and inter-electrode spacing of 200 μm. As described in [5], MCS MEAs were first disinfected by 70% ethanol for 20 min under laminar flow in a biohood and then rinsed with sterile de-ionized water. To obtain better cell adhesion, the center regions of the MCS MEAs were coated with 50 μg/ml of poly-D-lysine (PDL) (Sigma–Aldrich, St. Louis, MO) overnight. After the incubation with PDL, the arrays were then washed with sterile deionized water three times to remove any excess PDL. The arrays were then coated at the center with 20 μg/mL of laminin (Sigma–Aldrich, St. Louis, MO) for at least an hour. Prior to the cell seeding the laminin was removed from the MCS MEAs.

Small, yet important modifications to the above preparation steps enabled immediate and reliable use of the UT Dallas MEAs for cell culture. First, UT Dallas MEAs were exposed to oxygen plasma treatment with the oxygen pressure of 15 psi for 1 min at 75 W (PE-50, Plasma Etch Inc, Carson City, NV). UT Dallas MEA wells were filled with PBS and allowed to soak for 24 h at room temperature. Under a biological containment hood, UT Dallas MEA wells were treated with 70% ethanol for 20 min, washed with sterile water, filled with cell culture medium consisting of DMEM (Life Technologies), 2% B27 (Life Technologies), 5% horse serum (Atlanta Biologicals, Lawrenceville, GA), 5% fetal bovine serum (Life Technologies), and 0.2% 4 mg/ml ascorbic acid (Sigma–Aldrich) and incubated overnight at 37 °C in the cell culture incubator. After removal of the culture medium, ~50 μl of 50 μg/ml of PDL was applied to the center of each UT Dallas MEA recording well and allowed to incubate at 37 °C in a CO_2 incubator overnight, with care taken to avoid evaporation. Approximately 2–3 h prior to cell plating, PDL was washed from the recording wells three times with sterile water and the surface was allowed to dry. Finally, ~50 μl of 20 μg/ml of laminin was applied for at least 1 h with the excess removed immediately prior to plating. Pilot experiments revealed that strict adherence to the above preparatory steps enabled reliable adhesion of dissociated primary embryonic cortical tissue and subsequent maturation into active networks.

The primary neuronal culture method was similar to that described in [23]. The procedure was approved by the Institutional Animal Care and Use Committee of George Mason University (Fairfax, VA). Timed pregnant, embryonic day 17, CD-1 mice (Charles River, Wilmington, MA) were euthanized with carbon dioxide followed by decapitation. Embryos were extracted in ice cold L15 (Life Technologies, Grand Island, NY). Upon isolation of the frontal cortex, the tissue was stored up to 24 h in a hibernate media (BrainBits, Springfield, IL) supplemented with 2% B27 (Life Technologies) and 0.5 mM Glutamax (Life Technologies), Later, the hibernate

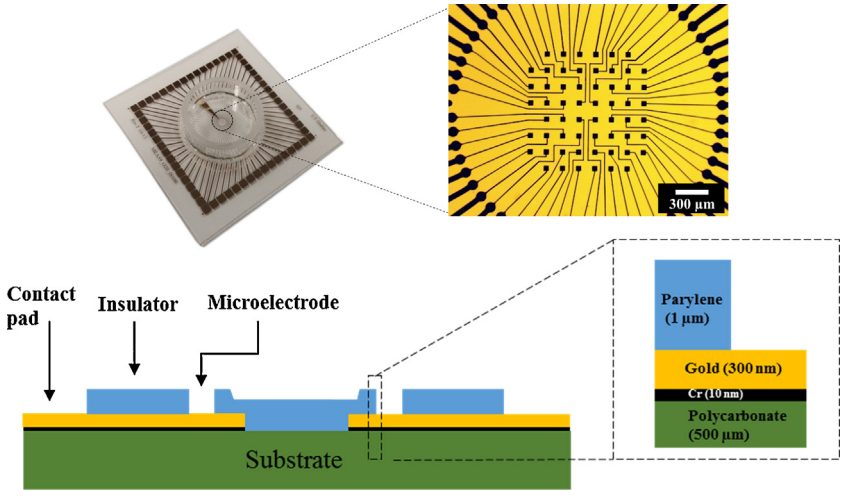

Fig. 1. An optical image of the MEA with magnification showing the microelectrodes (Top). A cross-sectional drawing of the UT Dallas MEA showing the different layers, thicknesses, and the material used in each layer (Bottom).