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Microfabrication technologies for a coupled three-dimensional microelectrode, microfluidic array

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Abstract

Planar or two-dimensional (2D) microelectrode arrays (MEAs), which are used for *in vitro* culturing of neurons and tissue slices, have been in existence for over 30 years. However, in order to study complex network morphologies and tissue slices which contain substantial 3D neuronal structures, 3D MEAs with microfluidic ports are required. Integrated fabrication of 3D MEAs with embedded microfluidic ports for nutrient perfusion through these relatively thick tissues typically requires non-planar lithography, which is not easily accomplished. This paper reports a laser-scribing technique coupled with electroplating to fabricate 3D MEAs coupled with microfluidic ports. An excimer laser has been used to define patterns in a polymer mold layer that is conformally vapor-deposited on a 3D microfluidic SU-8 substrate. Metal is electroplated through this mold to fabricate electrodes at multiple heights. To demonstrate 3D MEAs, a standard design was chosen consisting of an array of three-dimensional protrusions ('towers') optionally with microfluidic functionality on which electrodes can be formed extending to the top of each tower. Additional electrodes are formed on the substrate resulting in a multi-level electrode structure. Since microfluidics can exist both in the substrate as well as along the towers, a coupled three-dimensional electrical and microfluidic functionality is achieved. The resulting 3D MEAs have been analyzed electrically using impedance spectroscopy and baseline noise measurements. They have further been evaluated fluidically using micro-particle image velocimetry measurements.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Microelectrode arrays (MEAs) provide important information about single neurons and functional organization of neural networks and tissues. Due to the size of the cells involved which are in the micron-scale, an attractive technique for manufacturing MEAs is microfabrication, which has been used to make planar (2D) devices. Several microfabricated 2D arrays have been reported in the last 35 years for extracellular stimulation and recording from cultured neuronal cells and brain slices [1–3]. However as these cultures mature to a 3D form (and since brain slices contain substantial 3D

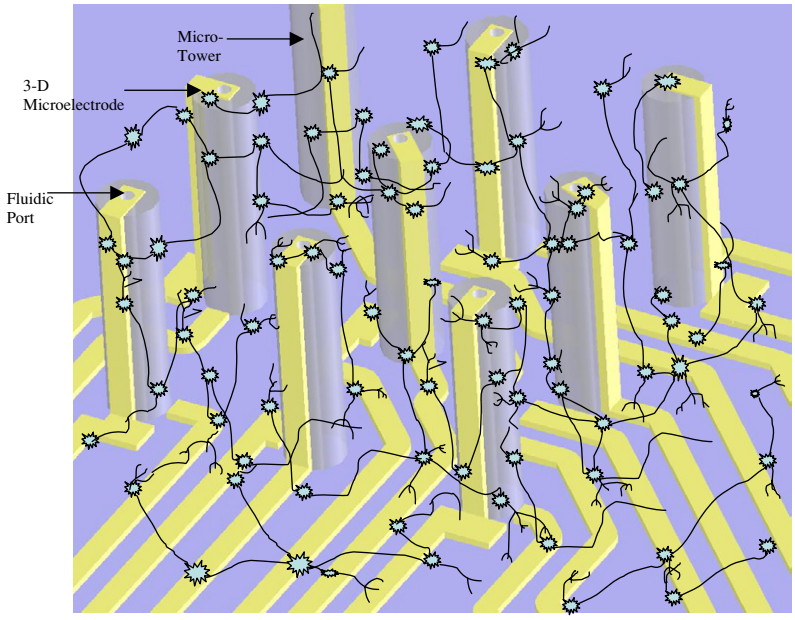

Figure 1. Conceptual view of the 3D coupled microelectrode, microfluidic array with neuronal culturing.

neuronal structure), they become difficult to study using planar MEAs. Signals from cells that occur at a certain height above the planar structure can be lost since the electrodes are not in proximity to these cells. Heuschkel *et al* [4] have simulated 2D and 3D microelectrodes and have found in *in vitro* experiments that the signals from a 3D microelectrode are 2–3 times superior in magnitude than those from a 2D counterpart. Also the three-dimensional nature of neuronal networks *in vitro* mimics the actual nature of networks inside the human body, and in order to attain more information about them 3D MEAs are required. Since the objective of these devices is to enable culturing biological material outside the body for longer periods of time, nutrients need to be supplied to the biological material in order that they can be kept alive for longer periods of time. Microfluidic ports can serve this purpose. Bai *et al* [5] report a microassembly technique of a series of microfabricated 2D MEAs to achieve 3D implantable MEAs. Thiebaud *et al* [6] and Metz *et al* [7] report fabrication of 3D MEAs in silicon and glass, respectively: but the reported heights of the electrodes are less than 100 μm. In this paper, MEAs with microfluidic ports with electrode heights of up to 500 μm are reported. These devices are targeted specifically to house 3D dissociated cultures of neurons and perform electrophysiological measurements from these cultures.

Figure 1 shows a schematic of the proposed 3D MEA. Towers are provided at a certain height above a substrate with metal lines defined on them. Fluidic ports are also provided in the center of the tower. Neuronal cultures are shown schematically as a network all around the towers. It is proposed that we can record from and stimulate these cells using the electrodes and also flow nutrients through the fluidic ports to keep the cells alive for longer periods of time.

Fabrication of this type of device is challenging due to the patterning of distinct features in 3D. Conventional proximity lithography with large mask-substrate gaps to accommodate the 3D substrate topography becomes very challenging due to non-uniform resist coatings and diffraction of light during exposure. Thus novel non-lithographic approaches in combination with technologies to easily fabricate high aspect ratio structures are needed for 3D MEAs. In this paper two such technologies are reported: (1) fabrication of high aspect ratio towers using double-side exposure of the negative-tone epoxy SU-8; (2) definition of metal lines on a polymer mold coated on SU-8 towers using an excimer laser followed by electroplating. These two key technologies along with packaging and electrical/fluidic validation of the device are described below.

2. Fabrication of tower arrays with double-side exposure technology

Complex 3D fabrication technologies for MEMS devices have been developed by many groups [8–14] using the negative-tone epoxy SU-8 (Microchem Inc., Newton, MA). The properties of SU-8 that make it attractive for MEMS fabrication are: chemical resistance, thermal stability and photo definition. SU-8 has been utilized as the structural material in this work.

Structures for both MEAs (solid towers) and MEAs with fluidic ports (hollow towers) have been fabricated. The solid tower arrays with electrodes can be used to study the morphological organization and electrophysiological activity in short-term cultures. The fabrication process for solid and hollow (microfluidic) tower arrays is detailed in figure 2. The substrate consists of a 4 inch chrome mask that serves a dual function: it not only acts as a temporary substrate for tower fabrication, but is also used in the double-side exposure which will be described later in this section. The process begins with definition of the tower structure locations onto the substrate (step 1). A 100 μm thick SU-8 layer (SU-8 2025) is then coated onto the substrate and soft-baked for 30 min at 95 °C. A second mask is then used to expose circular SU-8 regions (which will ultimately become the device

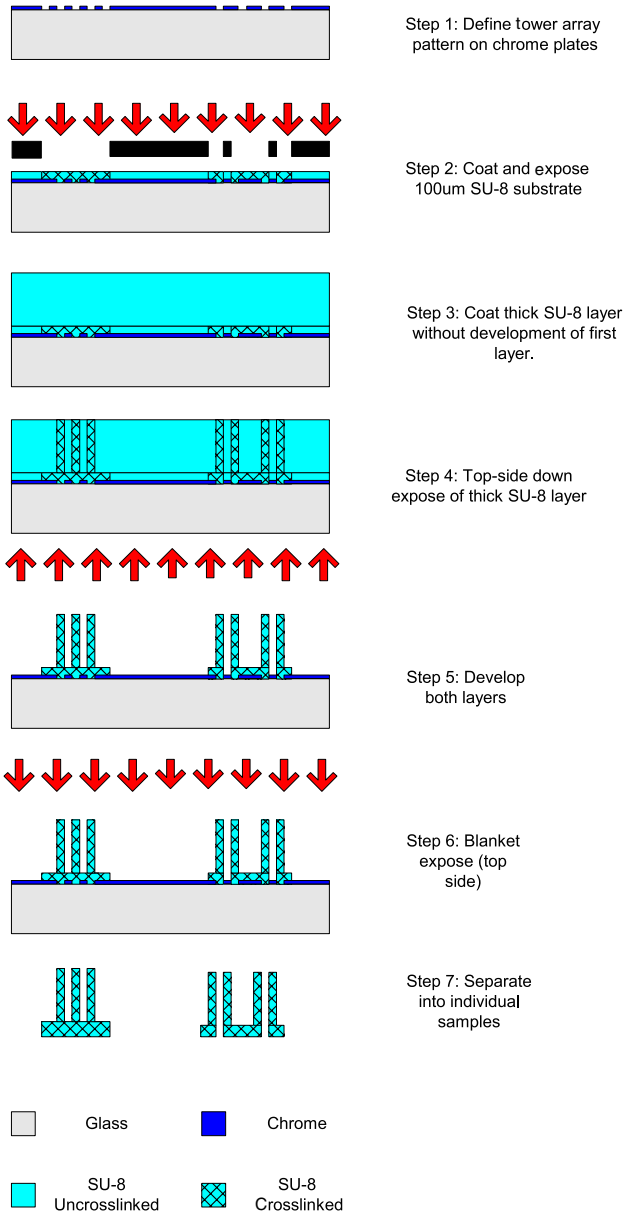

Figure 2. Fabrication process flow for solid (left side) and hollow (right side) tower arrays.

substrate) from the top using standard photolithography (step 2), followed by post-exposure baking. A second layer of SU-8 is coated without developing the first layer and soft-baked for 6 h at 95 °C (step 3). The sample is then exposed from the backside (step 4) using a larger energy dose to accommodate the thicker SU-8. The mask for this step is already defined on the chrome plate (double-side exposure technique). The 4 inch chrome plate is then developed in PGMEA (Microchem Inc., Newton, MA) with stirring for 1.5 h (step 5) after post-exposure baking (1 h at 95 °C). After the development process, the 4 inch chrome plate is blanket exposed from the topside (step 6) to fully cross-link the structures and baked (1.5 h at 95 °C) to aid the separation of the tower arrays from the plate. The tower arrays are then mechanically released from the substrate (step 7). Two designs (12 tower hollow array with dimensions: average inner diameter ∼140 μm; average height ∼460 μm; and 13 tower solid array with dimensions: average diameter ∼130 μm, average height ∼450 μm) were selected for fabrication of 3D microelectrodes. Figure 3(*a*)–(*d*) shows optical micrographs and SEM pictures of the separated tower arrays.

Table 1. Parameters for laser scribing of 1 μm parylene.

Parameter	Value	Comments
Energy	200 mJ	Constant
Pulses	30	Start with 20 and increase by 2 in the *X* direction (20–38). Process window of ±5
Power attenuation	10%	Start with 6% and increase by 2 in the *Y* direction (6–24). Process window of ±2
Velocity	120 μm s^{-1}	Constant
Frequency	90 Hz	Constant

3. Fabrication of microelectrodes using excimer laser scribing and electroplating

3D microfabrication to define metal patterns in deep trenches or high aspect ratio structures is a difficult problem in MEMS. Several techniques have been developed to address this issue including electrodeposited resists [15], optimized photoresist coatings [16] and 3D metal transfer micromolding [17]. In this research, laser micromachining [18] has been used to address this complex problem. Lasers are attractive tools for 3D microfabrication because it is possible to directly machine material with substantial topography in a non-contact manner. An excimer laser (Resonetics Inc., Nashua, NH) operating at 248 nm has been used to ablate polymeric substrates and polymer layers. Unlike standard cleaning of parylene from printed wiring board (PWB) substrates, in this work the use of the excimer laser to *scribe* lines (pattern) of varying width (lowest size of 20 μm) into parylene, followed by using the scribed parylene as an electroplating mold, is reported.

The laser ablation process is characterized on glass substrates before the actual tower array is ablated as shown in figure 4(*a*). The glass substrates are cleaned using a 2:1 solution of sulfuric acid and hydrogen peroxide (J T Baker Inc., Phillipsburg, NJ) for 10 min at 120 °C. A seed layer of Ti/Cu (300 Å/9000 Å) is then sputtered (CVC Products Inc., Rochester, NY) onto these substrates. A parylene layer (1 μm thick) is then conformally coated onto the substrates using a parylene deposition system (Specialty Coating Systems, Indianapolis, IN). The substrate is then placed in the excimer laser and a 10 × 10 matrix of shots are fired onto it using a 20 μm laser spot size, with each shot increasing in power in the *Y* direction and number of bursts in the *X* direction (table 1). The same process is repeated for an effective spot size of 65 μm. The electrode lines on the substrate and the towers are designed to be 20 μm in width. The bond pads at the edge of the substrate are designed to be 65 μm in width. Table 1 summarizes the optimized laser ablation parameters. The substrate is then observed under the microscope to determine the correct parameters for parylene ablation. Optical micrographs of the matrix of laser shots are shown in figure 4(*b*). The ablation process is then confirmed

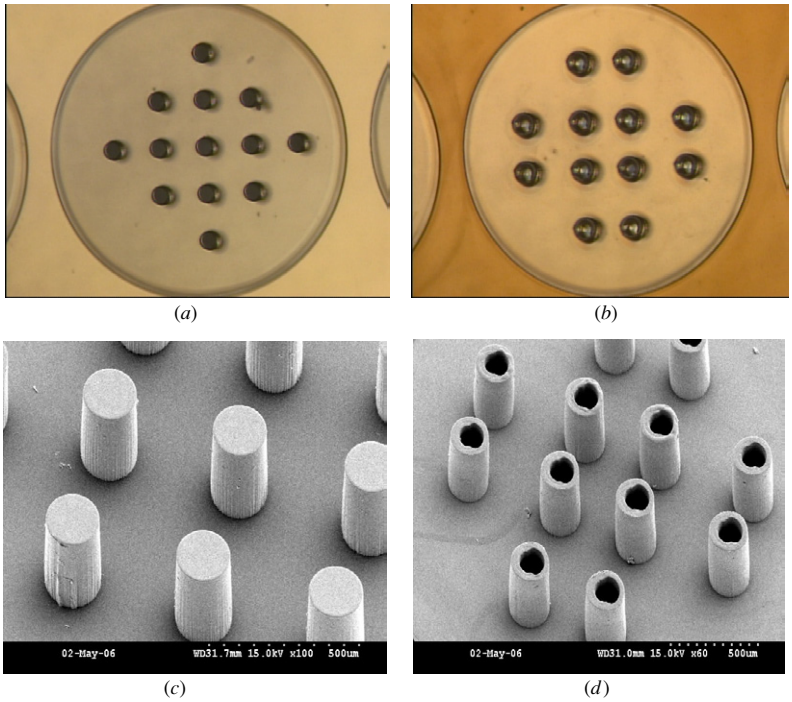

Figure 3. Images of tower arrays: (*a*) and (*b*) optical images of solid and hollow towers; (*c*) and (*d*) SEM of solid and hollow tower arrays.

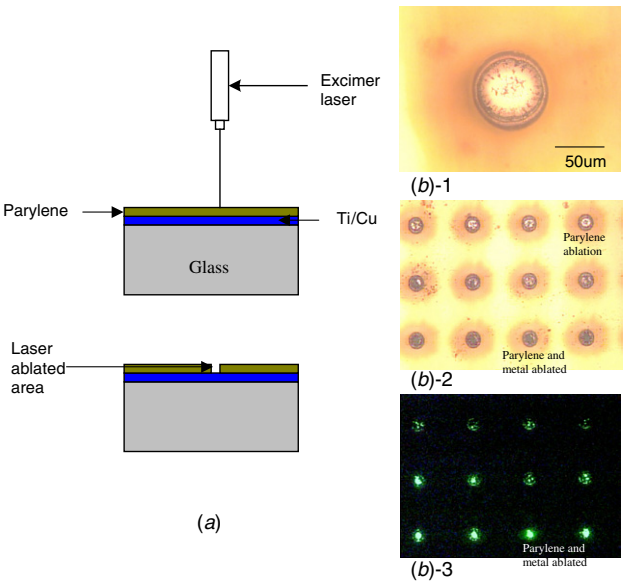

Figure 4. (*a*) Schematic of the laser ablation process; (*b*)-1: optical micrograph of a spot with parylene ablation and seed layers still remain; (*b*)-2: array of 65 μm laser spots with varying degrees of ablation of parylene and seed layers (optical micrograph top illumination); (*b*)-3: array of 65 μm laser spots with varying degrees of ablation of parylene and seed layers (optical micrograph bottom illumination).

by Ni electroplating, which will be described later in this section.

Figure 5 details the process flow of laser scribing of microelectrodes on SU-8 tower arrays. Prior to laser scribing, the tower sample is mounted on a glass slide of dimensions 10 mm × 20 mm using Shipley S1813 photoresist (Rohm and Haas, Marlborough, MA) and cured at 100 °C for 10 min. The tower array is then coated with a seed layer of Ti/Cu (300 Å/9000 Å) using a dc sputterer (step 1). A parylene layer (1 μm thick) is then conformally coated onto the substrates (step 2). Parylene is then patterned using the excimer laser with the parameters that are determined from the test samples as described above (step 3). The sample is then electroplated using a current density of 3 mA cm^{-2} (step 4). Prior to electroplating the sample is dipped in a solution of 15% hydrochloric acid (J T Baker Inc., Phillipsburg, NJ) in DI water, followed by a solution of 25% nitric acid (J T Baker Inc., Phillipsburg, NJ) in DI water. This is done in order to remove the native layer of copper oxide. The parylene is subsequently blanket etched using O_2 plasma followed by seed layer (Ti/Cu) removal (step 5). Optical and SEM images of the device after parylene etching are shown in figures 6(*a*)–(*d*). The tower array is then dipped in acetone for 5 min to release it from the glass substrate.

4. Tower array packaging

The fabricated tower arrays need to be packaged for a complete device. For packaging fused-silica substrates are used. Other conventional packaging substrates such as alumina ceramic, FR-4 epoxy-glass composite PWB, liquid crystal polymer (LCP) can also be used for this purpose but fused-silica was preferred due to the fact that it is completely biocompatible and also laser micromachinable [19] for fluidic ports. Fused silica wafers (500 μm thick, 4 inch diameter; Mark Optics Inc., Santa Ana, CA) were diced into 49 mm × 49 mm chips (size of commercial 2D MEA). The fabrication process for the packaging chips is a standard lift-off process that has been described elsewhere [20]. The external fluid access ports were drilled using a CO_2 laser (LS500 Laser Engraving System,

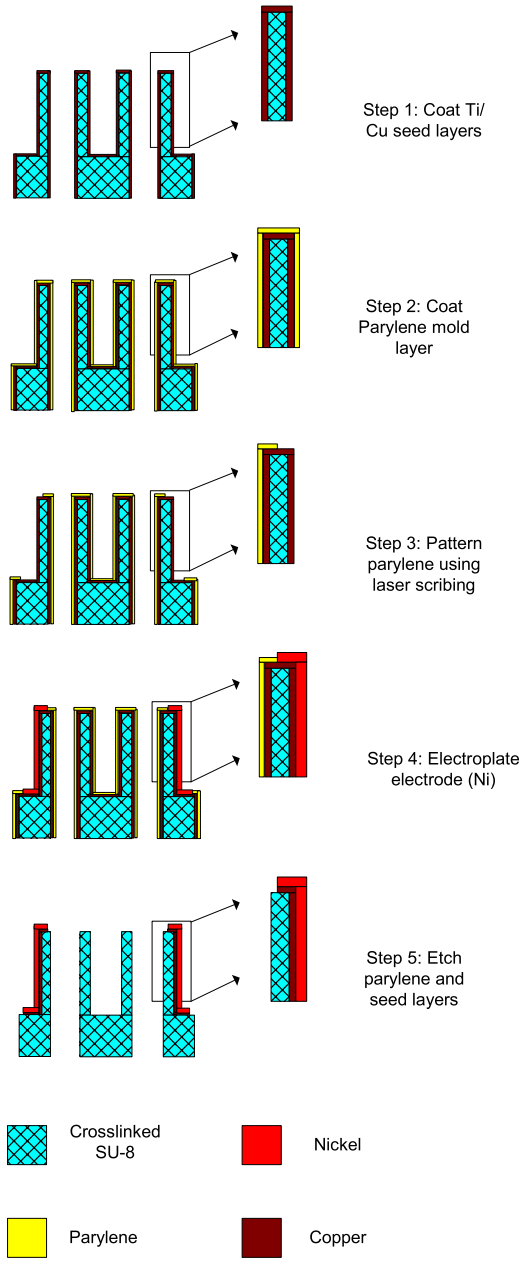

Figure 5. Process flow for laser scribing of microelectrodes on hollow tower arrays.

New Hermes-Gravograph Inc., Duluth, GA). After the drilling of the laser ports, the chips are treated with O2 plasma to remove surface contamination. Once the chips are fabricated, the tower arrays (from section 3) are mounted onto the chips with a thin layer of polydimethylsiloxane (PDMS). The tower array is then wire-bonded to complete the packaging.

5. Recording sites electroplating

Commercial 2D MEAs typically have a 5 μm layer of biocompatible insulation deposited [21] and recording sites opened for electrical stimulation and recording by planar lithography. An additional requirement for the insulation layer of 3D MEAs is that it should be laser micromachinable so that the definition of recording sites can be carried out in a similar way as the electrodes. Parylene has been selected as the insulation material in this work. It is highly biocompatible [22] and laser micromachinable as we have shown in section 3. There is conflicting evidence about the toxicity of SU-8. Copper and nickel also have biocompatibility issues [23, 24]. However, all these materials are commonly used for MEMS processing. Thus a thick insulation layer of parylene (25 μm) was used according to the guidelines established by Vernekar *et al* [25]. Excimer laser ablation of this thicker parylene layer was evaluated as described in section 3 on test substrates. The spot size used for this ablation was 50 μm. The parameters for this process are described in table 2. Recording sites were ablated at both 2D and 3D electrodes sites. A 5 mm tall PDMS culture ring (ID: 15 mm; OD: 25 mm) was attached to the chip using a thin layer of PDMS. PDMS is known to be completely biocompatible [26], thereby having no negative effect on the MEA assembly. It serves two purposes: acts as containment ring for both the static cell culture and platinum-black electroplating solution. Electroplating platinum-black increases the surface area of the electrodes and also serves to reduce the impedance of the electrodes [27]. If some measure of control can be achieved over this plating process, uniform impedances can be achieved across all the electrodes in the MEA chip. Ross *et al* [28] describe the design and implementation of a platinum electroplating setup in more detail. Platinum-black is electroplated onto the recording sites of the 3D MEA using a current density of 4.9 μA cm^{-2} at room temperature using a solution of 1% chloroplatinic acid with 0.0025% HCl and 0.01% lead acetate (all diluted in DI water). Optical micrographs of electroplated recording sites are shown in figure 7. Figure 8 shows a photograph of the completed chip.

Table 2. Parameters for laser scribing of 25 μm parylene.

Parameter	Value	Comments
Energy	250 mJ	Constant
Pulses	200	Start with 100 and increase by 20 in the *X* direction (100–300). Process window of $\pm$ 25
Power attenuation	30%	Start with 10% and increase by 2 in the *Y* direction (10–30). Process window of $\pm$ 2
Frequency	60Hz	Constant

6. Electrical evaluation

6.1. Electrical impedance analysis

Electrical impedance spectroscopy of the fabricated 3D MEA device may be used to evaluate the electrical properties of individual electrodes. In some cases, platinum-black does not adhere well to the recording site due to re-deposition of parylene during laser micromachining. The occurrence of this phenomenon (in-spite of the parameters for the process being optimized) can be traced to the narrow process window of ablation, visualization and focusing errors on 3D recording sites. Therefore, it is critical to establish the viability of each electrode before biological testing. This is accomplished with a Stanford Research SR785

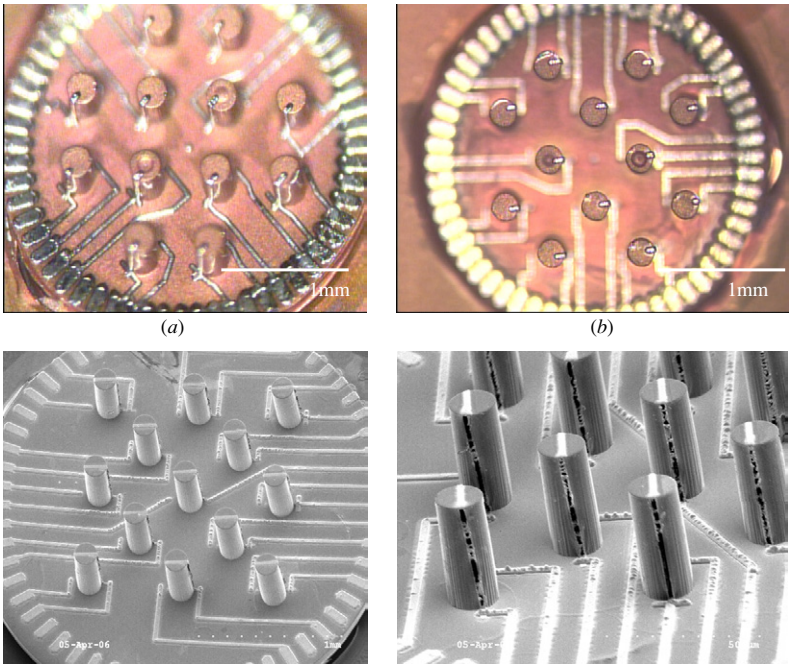

Figure 6. Optical micrographs of hollow tower arrays with electrodes after parylene etching (holes are filled with photoresist, which will be removed after etching seed layer) (*a*) and (*b*); SEM images of solid tower arrays with electrodes after parylene etching (*c*) and (*d*).

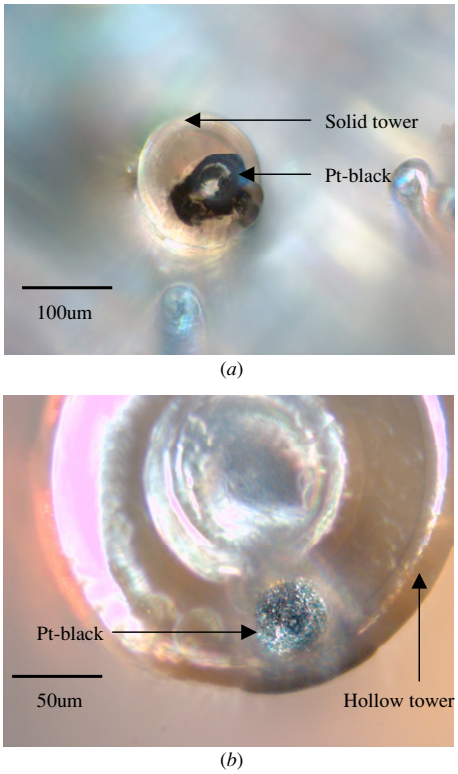

Figure 7. Optical micrograph of the recording sites: (*a*) solid tower array and (*b*) hollow tower array.

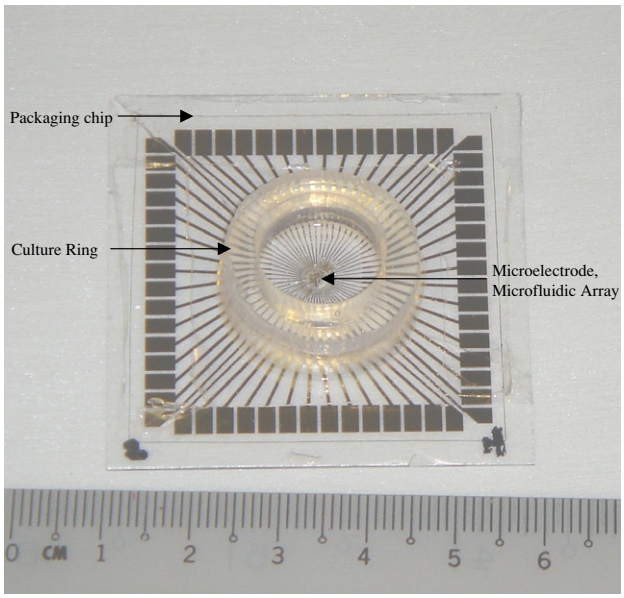

Figure 8. Photograph of the completed 3D MEA chip.

(Stanford Research Systems, Sunnyvale, CA) two-channel dynamic signal analyzer augmented with a custom-built, controlled switching board that allows for rapid, automated measurements of the magnitude and phase of microelectrode impedances across a large range of frequencies (1 mHz to 100 kHz). Impedance measurements were performed between the microelectrode, ground and the cellular conducting media (Hank's Balanced Salt Solution, Invitrogen Corporation, Carlsbad, CA). These testing procedures ensure that the microelectrodes perform as expected, and allow biologists to calibrate stimulation protocols. These tests also identify any problems with the fabrication process. The fabricated and packaged 3D MEAs were interfaced with the system

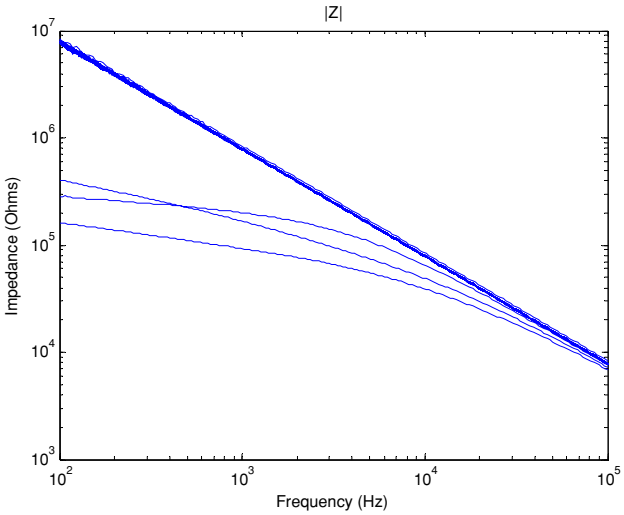

Figure 9. Impedance spectroscopy (magnitude of impedance of the electrodes as a function of frequency) results: impedance of the viable electrodes is clearly separated from the non-viable ones in the 10^5–10^6 Ω range.

and each electrode was scanned. Figure 9 shows the clear distinction between viable and non-viable electrodes among the 24 possible ones. The viable electrodes are in the range 10^5–10^6 Ω measured at 1 kHz. Even though 100% viability has not been achieved, these devices are very useful for biological experiments (that are currently underway).

6.2. Baseline noise measurement

Measurement of ambient noise of the electrodes is very important for biological testing. The baseline noise of an electrode needs to be low in order to record electrophysiological activity from a neuronal culture. This measurement was performed using MEA-1060 amplifier and MC Rack Software (Multichannel Systems (MCS), Reutlingen, Germany). The packaged 3D MEA was interfaced with the MCS set-up and the measurements were made under minimum surrounding noise (no ambient lighting or blowers in hoods) between the microelectrodes, ground and the cellular conducting media (Hank's balanced salt solution). It was observed that several channels had baseline noise levels of 10–15 μV. A snap shot of the data recorded from all the

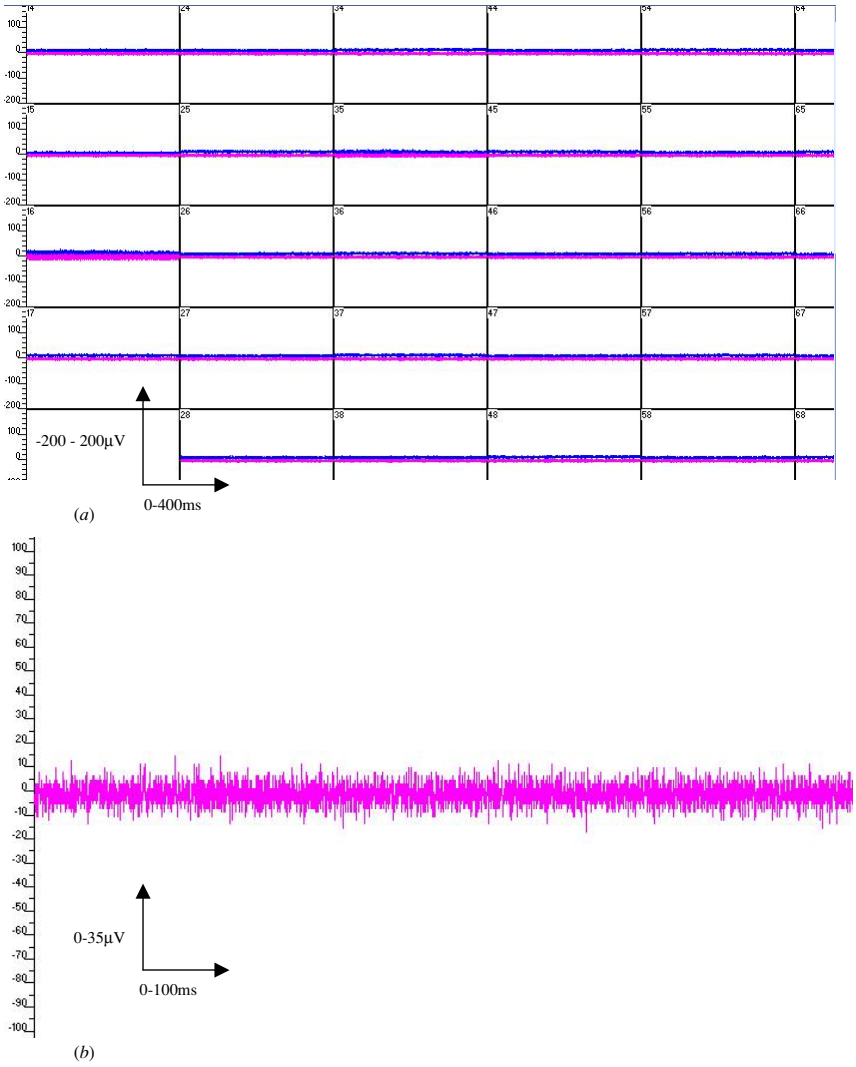

Figure 10. Baseline noise measurement. Note: the data is snap shots of a certain time during the measurement and the scales are as indicated : (*a*) all channels; (*b*) one representative channel (channel 16) with low noise.

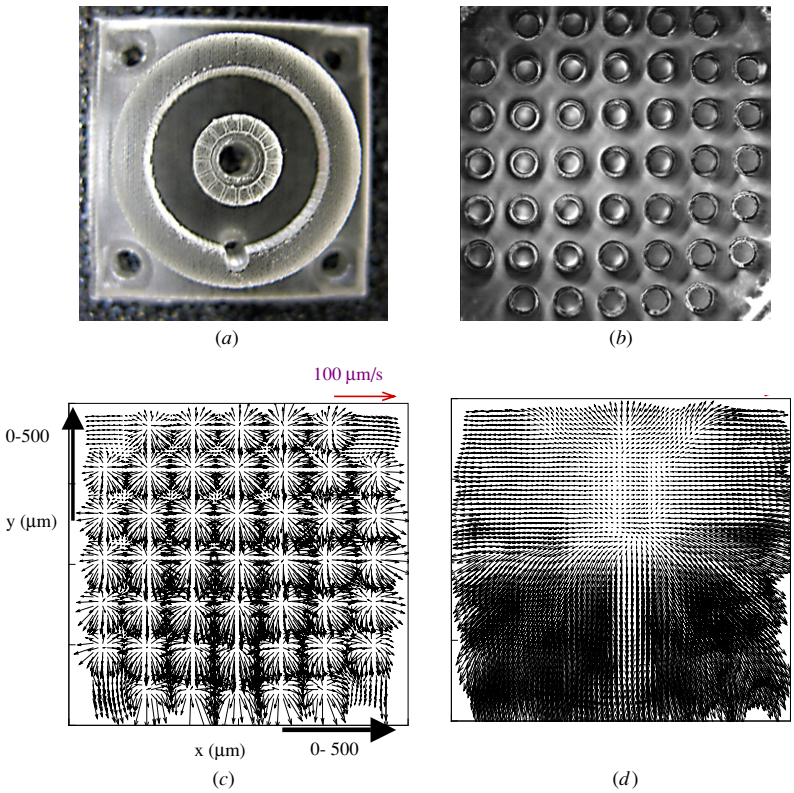

Figure 11. Micro-PIV measurement—(*a*) optical micrograph of perfusion chamber; (*b*) hollow tower array with 45 towers shown here with backside illumination; (*c*) velocity jets from mPIV at the top of the tower arrays; (*d*) velocity jets from mPIV at a height of 700 μm on top of the substrate.

channels and one of the channels with low noise is shown in figures 10(*a*) and (*b*). This shows that these devices have the capability of measuring the action potentials from viable neuronal cultures.

7. Microfluidic evaluation: micro-particle image velocimetry (mPIV)

3D cultures are essential for long-term neurophysiological studies. Their survival, however, is often limited by poor interstitial diffusion of media and gases necessary for cellular metabolism, and poor waste removal. In order to establish the fluidic validity of the 3D tower arrays a perfusion chamber [29] was fabricated and a hollow tower array with 45 towers ($\sim$130 μm inner diameter and $\sim$500 μm in height) was attached to the chamber using PDMS and cured. Figures 11(*a*) and (*b*) depict the perfusion chamber and the tower array (pictured using back-side illumination) respectively. The induced flow within the chamber is studied experimentally using micro particle image velocimetry (microPIV) [30]. In this non-intrusive technique, the working fluid is seeded with 500 nm fluorescent tracer particles. Particle images are recorded with a CCD camera attached to an upright microscope in an episcopic darkfield fluorescence arrangement. Velocity distributions, normal to the axe of microjets issuing from the towers, are shown in figures 11(*c*) and (*d*) for two elevations: near the top of the towers (*c*) and about 100 μm above the top of the towers (*d*). Nominal volume flow rate though the substrate is 3 μl min^{-1}. This proves with certainty that the towers are in fact open all the way to the top and fluid can be perfused through the tower arrays without clogging or any other disruptions.

8. Conclusions

In this work the fabrication and testing of three-dimensional coupled microelectrode, microfluidic arrays for neuronal and tissue slice interfacing is reported. Fabricated micro-tower arrays measuring up to 500 μm in height and 130–140 μm in diameter using the negative-tone epoxy SU-8 are reported. Two new fabrication technologies have been developed in this work: (*a*) double-side exposure technology to create high aspect ratio structures in SU-8; (*b*) microelectrodes on these tower arrays were patterned on a parylene mold using the excimer laser. Nickel was electroplated on this patterned mold to form the microelectrodes. Both 2D and 3D electrodes were fabricated on the same chip to compare their relative performance. The MEA chip was then packaged on a fused-silica substrate with metal lines and bond pads for electrical interfacing. Thick parylene was used as the insulation material and coated on the entire packaged chip. Recording sites were then laser-ablated and platinum-black was electroplated. The chip was then electrically evaluated using impedance spectroscopy (100 Hz–100 kHz). Viable electrodes were in the impedance range of 10^5–10^6 Ω at 1 kHz. Baseline noise measurements on the device yielded a noise level of 10–15 μV which demonstrate the ability to pick up action potentials from a neuronal culture. Microfluidic evaluation

was performed using mPIV measurements and these clearly showed that the ports were open and can be perfused at a flow rate of 3 μl min^{-1}.

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