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Application of a PDMS microstencil as a replaceable insulator toward a single-use planar microelectrode array

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Abstract Here we present a novel idea for a replaceable insulator, and thus advance toward the goal of a single-use planar microelectrode array (MEA) for the study of electrogenic tissues. The concept of a replaceable insulator is motivated by insulator degradation after repeated usage of an MEA. Instead of fabricating a more durable insulator for repeated MEA usage, we propose replacing the insulator and effectively producing a fresh MEA for each experiment. We chose a polydimethylsiloxane (PDMS) microstencil as a candidate for the replaceable insulator as it is biocompatible, shows reversible adhesion to surfaces, and can be easily and controllably fabricated. As a proof-of-concept, we demonstrate two applications using microstencils: the rejuvenation of an old MEA and the fabrication of a single-use MEA. These MEAs were tested with dissociated neural cell cultures and neural recordings were performed at 14 days *in vitro*. Inexpensive and quick supply of insulators with micrometer-sized holes provides a way of constructing an MEA that can be treated as a disposable component in high throughput cell-based biosensor applications.

Keywords Microelectrode array · Microstencil · Hippocampal cell culture · Neural recording

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1. Introduction

Planar microelectrode arrays (MEAs) were developed to study electrogenic tissues such as dissociated neuronal cultures and brain tissue slices. They have been widely used with dissociated cultures for a variety of neuroscience investigation including learning and memory (Jimbo, 1999; Marom, 2002; Segev, 2004) and cell-based biosensors for the detection of neurotoxins (Gross, 1995; Pancrazio, 2003; Selinger, 2004).

There are a number of requirements for MEAs to function properly. First, fabrication materials should be biocompatible or non-toxic for cell cultures. Second, the insulation layer should maintain high impedance for the entire length of the experiment as poor insulators attenuate neural signals (Gross, 1979). Third, the electrode impedance should be low enough to record extracellular signals which can be as small as a few tens of microvolts (Pine, 1980).

MEAs are fabricated by microfabrication processes including photolithography, metal deposition, coating of inorganic or organic insulators, and etching. One of the main advantages of the use of microfabrication processes is the potential for mass production that leads to devices in many application areas. In practice, neither commercially-available nor fabricated MEAs are sufficiently inexpensive to be considered disposable. Therefore, MEAs are routinely recycled after experiments in most laboratories. However, recycling generally cause insulator degradation, which can be

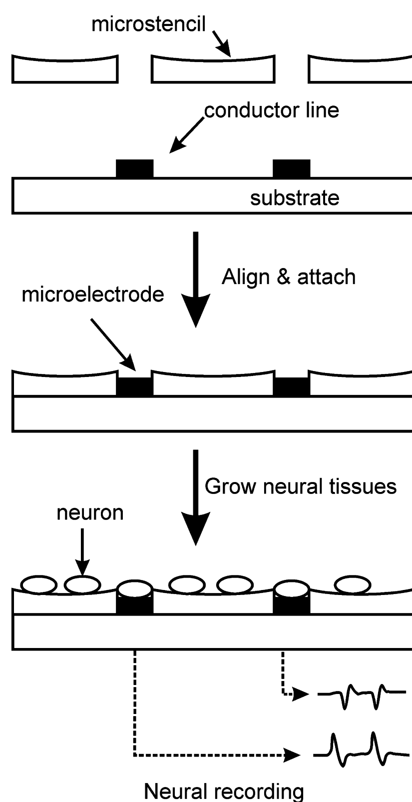


Fig. 1 Schematic illustration of a single-use MEA

problematic for MEA function (Wagenaar, 2004). From our long experience of fabricating custom MEAs and recycling commercial MEAs (Novak, 1986; Novak, 1988; Boppart, 1992; Chang, 2000; Nam, 2004), we have noticed that most of the MEAs suffered from the delamination or leakage in the insulation layer while electrodes and metal lines were remain intact after long repeated recycling. Recycling also makes it difficult to obtain reproducible surfaces for special cell culture techniques such as cell patterning.

Here we present a novel replaceable insulator approach to overcome the insulator degradation problem. It stands in contrast to the alternative approach of developing a more durable insulation layer that would withstand recycling and surface cleaning processes. Figure 1 illustrates this approach in detail. A replaceable insulator (thin membrane with micrometer-scale holes) is placed on top of a metal-patterned substrate to form an MEA structure. Cells are cultured and extracellular recordings are performed. After the experiment, the insulator is removed leaving the original metal-patterned substrate. After proper cleaning, a new insulator is attached to create an MEA with a fresh surface. This concept obviates recycling the same surface for multiple cell cultures. As long as the electrodes are intact, replacing the insulator will produce an MEA with a fresh insulation layer. Used insulators can be disposed after each experiment.

In this paper, we designed and tested an inexpensive replaceable insulator using a PDMS microstencil. A PDMS microstencil has been developed by other researchers for cell patterning and protein patterning assays (Folch, 2000; Ostuni, 2000). The following properties of the PDMS membrane made it suitable for this application: 1) PDMS has been routinely used with cell cultures; 2) It can be easily attached and detached from planar surfaces; 3) It can be fabricated to have holes tens of micrometers in diameter; and 4) It is fabricated by soft-lithography that is manageable in most biology laboratories. Here we demonstrate the use of the replaceable insulator in two applications. First, a microstencil was used to rejuvenate recycled commercial MEAs. Second, it was used to fabricate an MEA originally with no insulator. Electrical impedances were measured under culture condition and multichannel recordings were performed at two weeks with dissociated hippocampal neurons.

Some of these results have been presented in abstract form (Nam, 2004; Musick, 2005).

2. Method

2.1. Fabrication of microstencil

The microstencil was fabricated by a spin-coating method adopted from elsewhere (Ostuni, 2000). Briefly, to fabricate the mold, SU-8-2050 (negative photoresist, MicroChem, Newton, MA) was spin-coated on a silicon wafer (3-inch) for 30 s at 2000 rpm with a targeted final thickness of 75 μm. The coated wafer was baked on a hotplate for 9 min at 95°C. The photoresist was selectively exposed to UV under a transparency mask (feature size $50 \times 50\ \mu\text{m}^2 - 100 \times 100\ \mu\text{m}^2$, high resolution inkjet printer, 5080 dpi, Printing department at University of Illinois at Urbana-Champaign) for 3 min and baked for 7 min at 95°C on a hotplate. After slowly cooling the wafer, the photoresist was developed in SU-8 developer (MicroChem Corp., Newton, MA), rinsed with isopropanol (IPA), and dried with a nitrogen stream.

To fabricate a PDMS microstencil, the mold was first coated with a thin photoresist (AZ5214) for 30 s at 3000 rpm and baked for 60 s at 110°C on a hotplate. The thin photoresist layer served as a sacrificial layer to release a cured PDMS membrane. The mixture of PDMS prepolymer and curing agent (10:1 (w/w), Sylgard 184 silicone elastomer kit, Dow Corning Corp.) was spin-coated on top of the photoresist layer for 40 s at 4000 rpm. The coated mold was cured for 15 min at 110°C in a convection oven. A thick PDMS annulus (thickness: 3–5 mm) was attached on the partially cured PDMS layer and cured for another 15 min in the same oven. The PDMS annulus made it possible to handle the thin PDMS microstencil during the following assembly process. The fully-cured PDMS-coated mold was soaked in an

acetone ultra-sonication bath until the PDMS layer released from the mold. The detached microstencil was rinsed with IPA and DI water.

2.2. Preparation of substrates

For the purpose of the rejuvenating a degraded insulator, recycled commercial MEAs originally purchased from Multi Channel Systems (Reutlingen, Germany) were used. These MEAs had 60 TiN electrodes (10 μm in diameter, 200 μm spacing) and silicon nitride insulator (500 nm in thickness).

To fabricate an MEA structure using a microstencil, glass slides with indium-tin oxide (ITO) or gold lines were used. Sixty metal lines were patterned to form a multielectrode array with no insulator. To make an ITO-patterned slide, an ITO-coated glass slide was photo-lithographically defined by a positive photoresist (AZ 5214, Clariant Corp., Sommerville, NJ) and chemically etched in HCl for 40 s. To create a gold-patterned slide, Piranha-etched glass slides were placed in a dual source metal evaporator and chromium (2 nm) followed by gold (50 nm, 99.99% pure) was evaporated onto the slides. A metal pattern was defined by micro-contact printing process and the slide was chemically etched in a gold etchant solution (Kumar, 1993).

2.3. Assembly of microstencil with a substrate

The assembly process was performed under a low-magnification objective (5x or 10x, reflected or transmitted light microscope). A drop of ethanol was placed on top of an MEA and a microstencil was aligned to the electrodes manually. The MEAs were then placed on a hotplate set to 60°C for 1 h to dry ethanol. For relatively large holes (100 μm × 100 μm), it was also possible to align and attach the insulator without an ethanol drop.

2.4. Cell culture and substrate preparation

To enhance the cell adhesion, microstencils and substrates were coated with poly-D-lysine (PDL, MW 70 000–150 000, Sigma-Aldrich) solution (0.5 ml, 0.1 mg/ml in DI water) for 60 min. Then the PDL was removed, and the microstencil was rinsed with DI water to remove any unbound PDL.

Before cell plating, the assembled substrates were incubated in 0.5 ml of culture media under house vacuum for at least 15 min to remove any trapped bubbles. Without the degassing step, cells seldom landed inside the stencil holes.

Dissected hippocampal tissues (18-day gestation Sprague/Dawley or Fisher 344 rat hippocampus) were purchased from Brain Bits™ (www.BrainBitsLLC.com), arriving in a 2 ml tube containing embryonic day 18 hippocampus in 2 mL B27/Hibernate. This was immediately stored at 4–8°C until cells were plated (usually within 7 days). For the cell plating, 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 75–200 cells/mm^2. Cultures were stored in an incubator at 37°C, 5% CO_2, 9% O_2. Media changes were done with glutamate-free media. The first media change was done at 4 days in vitro (DIV) and half of the media was changed weekly or after recording if necessary. All animal procedures were done in accordance with approved animal use protocols at the University of Illinois.

2.5. Impedance measurement

AC (alternating current) impedances were measured with an HP 4284A Precision LCR meter at 1 kHz (input voltage 10–50 mV_{rms}) with an Ag/AgCl electrode as a ground electrode. Test solution was 1 × PBS (phosphate buffer solution, pH 7.4).

For a shunt impedance measurement of a PDMS film, a PDMS film with no holes was placed on top of a fully coated gold slide and electrical impedance was measured in a PBS solution. The area covered by the PBS solution ranged from 33 mm^2 to 79 mm^2.

2.6. Neural recording

An MEA was connected to an MEA1060 amplifier (Gain 1200, 10 Hz–3 kHz, Multi Channel Systems, Reutlingen, Germany), and amplified signals were fed to the MC card (sampling frequency 40 kHz, Multi Channel Systems, Reutlingen, Germany). Software (MC Rack) provided by manufacturer (Multi Channel Systems) was used to display and store the digitized data.

3. Results

3.1. Fabrication and assembly of microstencil attached MEAs

The microstencil insulator had 64 holes in 8 × 8 matrix configuration (hole center-to-center: 200 μm, hole size: 50 μm × 50 μm–100 μm × 100 μm) to match with a commercial MEA configuration. Figure 2 shows a completed microstencil with holes of 88 μm × 88 μm. The spin-coating speed while applying the PDMS prepolymer on the mold was an important parameter in obtaining a microstencil with complete through-holes. When the speed was too slow (2000–3000 rpm), the holes often were covered by thin PDMS films. For the molds we used (thickness: 86 μm ± 10 μm, mean ± SD, $n = 3$), reproducible PDMS thicknesses were achieved at spin speeds between 4000 and 4500 rpm (Fig. 2), resulting

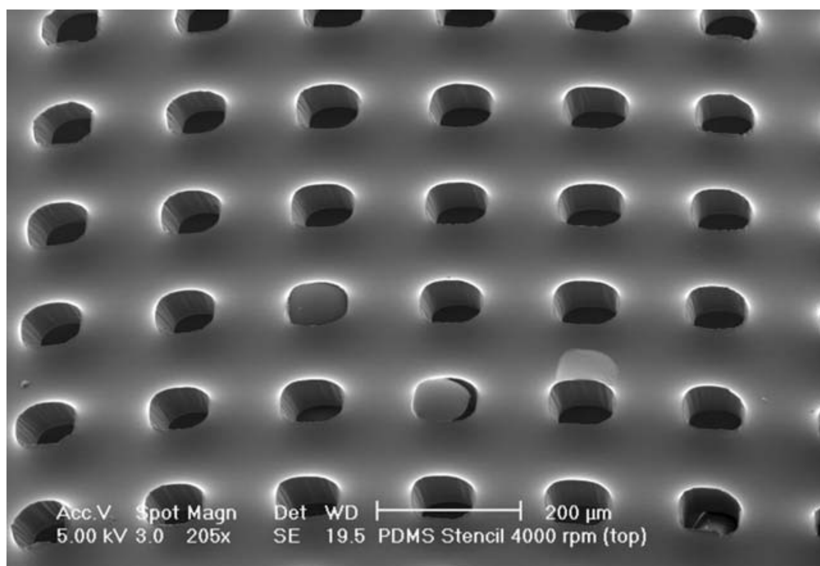


Fig. 2 PDMS microstencil fabricated with a spin speed of 4000 RPM. Scale bar: 200 μm

in a thickness of 41 μm $\pm$ 5 μm (mean $\pm$ SD, $n = 3$). The resulting PDMS microstencils were mechanically durable for repeated attachments and detachments.

The microstencils were easily aligned manually under 5x or 10x magnification. The formation of a reversible seal could be confirmed visually when the PDMS membrane contacted the substrates. To determine the accuracy of hand-aligning, microstencils with 70 μm $\times$ 70 μm holes were repeatedly aligned to an MEA. Of 12 trials, one trial resulted in a completely unaligned insulation, and one trial resulted in a torn insulation film. In the remaining 10 trials, an image of the aligned insulation and underlying pattern was taken at 5x magnification. After all trials had been completed, three electrodes in the pattern were randomly selected. The alignment in a diagonal direction from the center of the insulation hole to the center of the electrode was measured for these same three electrodes on the 10 successful trials. The distance measured between ideal alignment and actual alignment had an average value of 5.5 μm with a standard deviation of 3.6 μm. This suggests that with practice an experimenter could routinely achieve adequate hand alignment.

3.2. Rejuvenation of old insulation MEAs

A conventional MEA was rejuvenated by placing and aligning the microstencil on top. An old MEA that had been used nine times over seven months was chosen as a model for an MEA with a degraded insulator. When the electrode tip impedance in parallel with the insulator shunt impedance was compared before and after the assembly, there was a noticeable difference. By attaching the insulator, the measured impedance including both electrode and insulation in shunt increased from 307 kΩ $\pm$ 47 kΩ $\angle -81^\circ \pm 3^\circ$ (mean $\pm$ SD, $n = 60$) to 1.10 MΩ $\pm$ 0.28 MΩ $\angle -69^\circ \pm 4^\circ$ (mean $\pm$ SD, $n = 60$), implying that the attached insulator effectively reduced the parasitic (shunt) leakage through the insulator. We note that reducing the leakage through the insulator should also increase the efficiency of local current delivery for the electrical stimulation (Wagenaar, Pine, 2004).

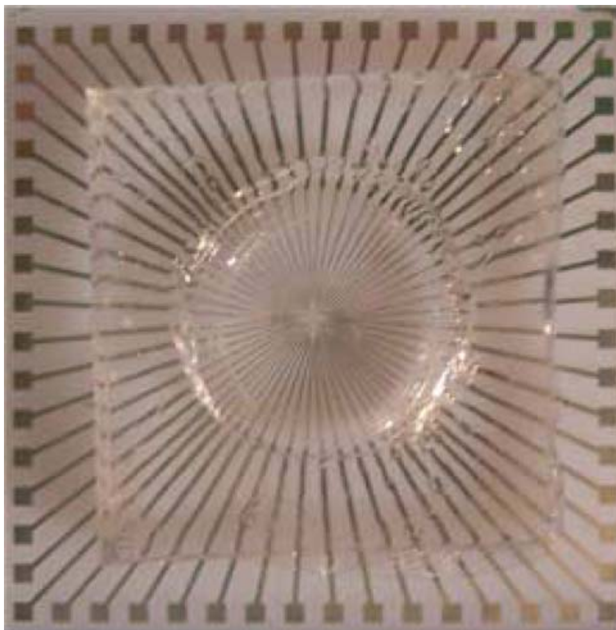
3.3. Construction of a single-use MEA

A single-use MEA was constructed by placing the microstencil on top of bare metal (ITO or gold) pattern substrates. Microelectrodes were defined by the dimension of the metal line and the size of the microstencil hole. In this work, we used 30 μm (ITO) or 50 μm (gold) wide metal lines which were fabricated from inexpensive transparency masks. Figure 3 shows an MEA assembled by placing a microstencil on top of a gold-patterned glass slide. Microelectrodes formed by ITO lines with the stencil hole size 96 $\times$ 96 μm^2 had an area of 3058 μm^2 $\pm$ 1060 μm^2 ($N = 12$ electrodes, SD) depending on the alignment relative to the metal pattern. For the ITO electrode that had an area of 2240 μm^2, the electrode impedance was 535 kΩ . For the arrays fabricated with gold as a conductor, electrode impedances were 384 kΩ $\pm$ 178 kΩ (SD, $n = 53$) with an electrode area of 2400 μm^2 $\pm$ 530 μm^2 (SD, $n = 53$, hole size: 70 μm $\times$ 70 μm). The size and impedance of the electrodes were comparable to MEAs used for recording extracellular action potentials in dissociated neuronal cultures or brain slice experiments.

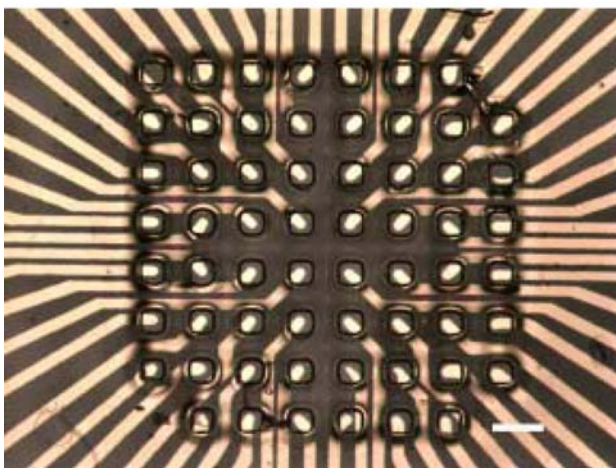
The electrical impedance through a PDMS film without holes was measured in an electrolyte solution to test the possibility of water permeation through the film. Measured shunt impedances are listed in Table 1. The impedance on three of the five thicknesses measured decreased over the first two days, suggesting the possibility of water permeating the film. However, after that point, the impedance leveled off

Table 1 Shunt impedances for PDMS films and their change over 4 days

Thickness (μm)	Area of PDMS film (mm^2)	|Z| (MΩ) (1 kHz)	Change over 4 days (MΩ) Day 1 (20–24 h)	Day 2 (33–48 h)	Day 4 (108 h)
13	50	2.0	1.9	2.0	2.0
18	33	3.7	3.8	3.1	3.1
36	50	4.5	4.3	3.9	3.9
69	50	8.2	7.5	7.2	7.2
126	79	11.0	11.0	11.0	11.0



(a)



(b)

Fig. 3 An MEA consisting of gold lines on a glass slide with a microstencil insulator. (a) A fully assembled device (22 mm × 22 mm). (b) Magnified view of the center of the MEA shown in (a). Scale bar: 200 μm

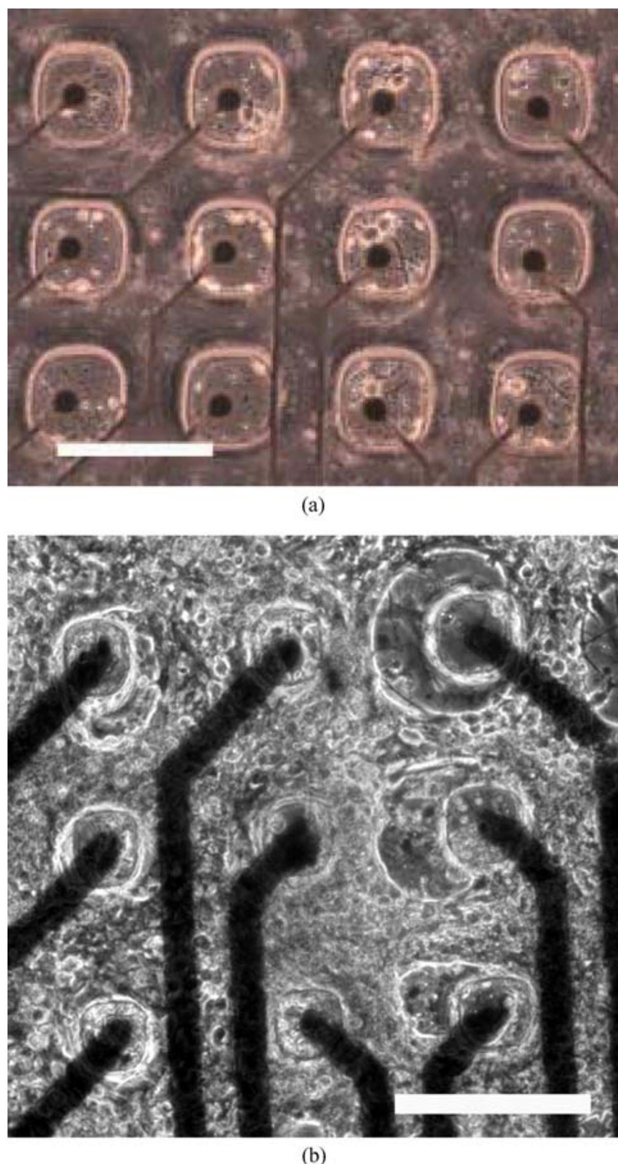
and was stable for all thicknesses assessed. To monitor the stability of the shunt impedance of an actual microstencil, a microstencil (thickness 40 μm, hole size 100 μm × 100 μm) was misaligned on purpose with metal lines so that a few metal lines were completely covered by the PDMS insulator. In this way shunt impedances of multiple electrodes were monitored simultaneously. Four of six sites tested maintained high impedance value for 12 days with slight decreases (impedance values from 49, 50, 52, 80 MΩ to 39, 48, 50, 77 MΩ at 1 kHz). Judging from these results (short and long-term tests with and without holes), PDMS microstencils can be applied so as not to leak and hence should be adequate as insulation layers for the arrays.

3.4. Cell culture and neural recordings

Air bubbles were trapped in most of the holes when cell culture media was loaded for cell plating. The degassing step (exposure to vacuum) was essential to load cells inside the holes, which increases the chance of cell-electrode coupling. In the degassing step, we visually inspected for trapped bubbles with a microscope and adjusted the degassing time (from 15 min to a few hours) accordingly. Once there were no bubbles, cell plating usually resulted in successful plating of neurons in the holes. Figure 4 shows dissociated hippocampal neurons growing and forming a dense network on either a rejuvenated MEA (Fig. 4(a)) or a single-use MEA (Fig. 4(b)). Hippocampal cultures were maintained for more than two weeks and spontaneous activity was successfully measured (Fig. 5). Spike amplitudes were comparable to those from conventional MEA recordings.

4. Discussion and conclusions

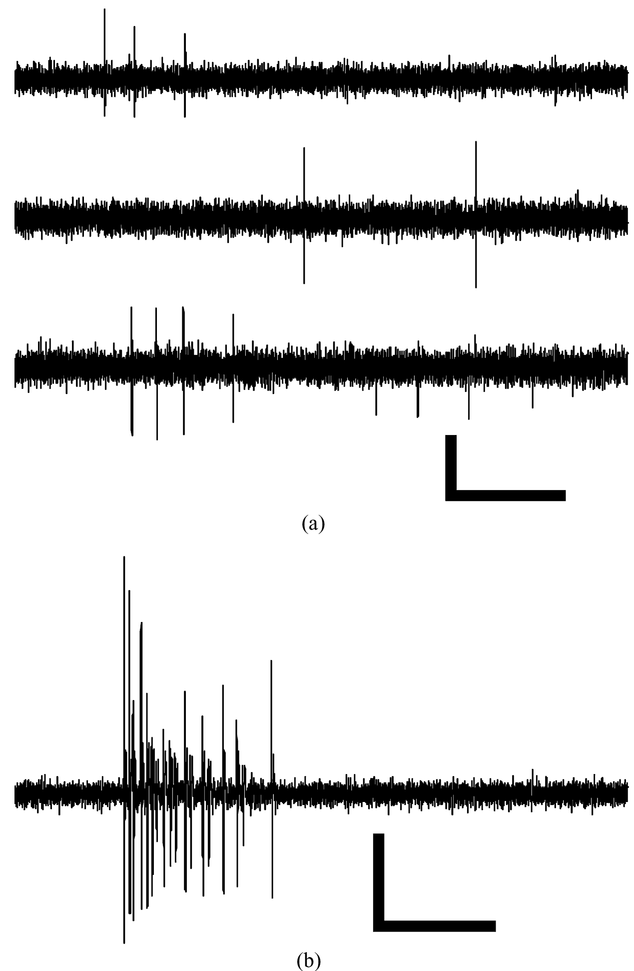
MEAs have been used to study the dynamics of neuronal networks (Jimbo, 1999), drug discovery (Stett, 2003), verify cardiac tissue formation from stem cells (Kehat, 2004), and detect neurotoxins as biosensors (Gross, 1995). All of these applications would benefit from the availability of single-use MEAs. As the insulator is the weakest component in determining the lifespan of an MEA, we propose the



(a)

(b)

Fig. 4 Hippocampal neuronal cultures on assembled devices. (a) A microstencil (hole size of 100 μm) on top of an old commercial MEA (14 DIV, cell plating density: 200 cells/mm^2). (b) A microstencil (hole size of 50 μm) on a gold pattern substrate on a glass slide (28 DIV, cell plating density: 1000 cells/mm^2). Scale bar = 200 μm



(a)

(b)

Fig. 5 Extracellular recordings from a rejuvenated old MEA (14 DIV) (a) and a microstencil attached gold substrate MEA (28 DIV) (b). Scale bar: 50 μV, 200 ms

concept of a replaceable insulator using a PDMS microstencil. PDMS microstencils with micro-holes were fabricated using soft lithography and attached to metal-patterned substrates (commercial MEAs with degraded insulators and metal patterns without insulators). Functional tests showed that the attached insulator could effectively rejuvenate a leaky insulator and perform normal electrical recordings from dissociated neuronal cultures after two weeks. In addition, we were able to construct a single-use MEA when the PDMS microstencil was attached to a bare metal-patterned substrate. The attached insulator maintained high shunt impedance over 12 days and microelectrodes that were suitable for neural recordings could be readily fabricated by isolating the tip of the metal line by PDMS holes. However, some electrodes showed a gradual decrease over 12 days. This could be due to the imperfect PDMS-substrate seal which can lead to a gradual leak underneath the PDMS membrane. The exact mechanism causing gradual decrease in impedance needs to be investigated further as, based on our measurements, it is most likely not insulation failure. The relatively simple procedure to fabricate a single-use MEA was the key result of the demonstration.

Our effort here was to find a quick and easy way to construct a functional MEA for biological researchers with limited access to specialized fabrication equipment. There are many issues to be optimized to make this idea practical. The PDMS microstencil thickness should be reduced to a few micrometers to be comparable with commercial MEAs and to avoid deeply recessed electrode structures. As the film thickness reduction could impact electrical insulation quality and handling, careful design compromises are needed. Other

synthetic materials may provide comparable quality of reversible sealing and biocompatibility. For truly disposable insulators, all procedures should be done outside of a cleanroom facility. If the MEAs were made with off-the-shelf components, potentially even with printed circuit board technology (Giovangrandi, 2006; www.multichannelsystems.com), the entire MEA might be disposable. We hope this preliminary study toward a single-use MEA will boost efforts to provide cell-based biosensor research with more affordable units.

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References

S.A. Boppart, B.C. Wheeler, and C.S. Wallace, IEEE Transactions on Biomedical Engineering **39**, 37 (1992).

J.C. Chang, G.J. Brewer, and B.C. Wheeler, Biomedical Microdevices **2**, 245 (2000).

A. Folch, B.H. Jo, O. Hurtado, D.J. Beebe, and M. Toner, Journal of Biomedical Materials Research **52**, 346 (2000).

L. Giovangrandi, K.H. Gilchrist, R.H. Whittington, and G. T.A. Kovacs, Sensors and Actuators B **113**, 545 (2006).

G.W. Gross, IEEE Transactions on Biomedical Engineering **26**, 273 (1979).

G.W. Gross, B.K. Rhoades, H.M. Azzazy, and M.C. Wu, Biosens Bioelectron **10**, 553 (1995).

Y. Jimbo, T. Tateno, and H. Robinson, Biophysics Journal **76**, 670 (1999).

I. Kehat, L. Khimovich, O. Caspi, A. Gepstein, R. Shofti, G. Arbel, I. Huber, J. Satin, J. Itskovitz-Eldor, and L. Gepstein, Nature Biotechnology **22**, 1282 (2004).

A. Kumar, and G.M. Whitesides, Applied Physics Letters **63**, 2002 (1993).

S. Marom, and G. Shahaf, Quarterly Reviews of Biophysics 35, 63 (2002).

K. Musick, Y. Nam, and B.C. Wheeler, Biomedical Engineering Society 2005 Annual Fall Meeting, Baltimore (2005).

Y. Nam, J.C. Chang, B.C. Wheeler, and G.J. Brewer, IEEE Transactions on Biomedical Engineering **51**, 158 (2004).

Y. Nam, and B.C. Wheeler, Biomedical Engineering Society 2004 Annual Fall Meeting, Philadelphia (2004).

J.L. Novak, and B.C. Wheeler, IEEE Transactions on Biomedical Engineering **33**, 196 (1986).

J.L. Novak, and B.C. Wheeler, Journal of Neuroscience Methods **23**, 149 (1988).

E. Ostuni, R. Kane, C.S. Chen, D.E. Ingber, and G.M. Whitesides, Langmuir **16**, 7811 (2000).

J.J. Pancrazio, S.A. Gray, Y.S. Shubin, N. Kulagina, D.S. Cuttino, K.M. Shaffer, K. Eisemann, A. Curran, B. Zim, G.W. Gross, and T.J. O'Shaughnessy, Biosens Bioelectron **18**, 1339 (2003).

J. Pine, Journal of Neuroscience Methods **2**, 19 (1980).

J.V. Selinger, J.J. Pancrazio, and G.W. Gross, Biosens Bioelectron **19**, 675 (2004).

R. Segev, I. Baruchi, E. Hulata, and E. Ben-Jacob, Physical Review Letters 92, 118102 (2004).

A. Stett, U. Egert, E. Guenther, F. Hofmann, T. Meyer, W. Nisch, and H. Haemmerle, Analytical and Bioanalytical Chemistry **377**, 486 (2003).

D.A. Wagenaar, J. Pine, and S.M. Potter, Journal of Neuroscience Methods **138**, 27 (2004).