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Micropatterning of poly(dimethylsiloxane) using a photoresist lift-off technique for selective electrical insulation of microelectrode arrays

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Abstract

A poly(dimethylsiloxane) (PDMS) patterning method based on a photoresist lift-off technique to make an electrical insulation layer with selective openings is presented. The method enables creating PDMS patterns with small features and various thicknesses without any limitation in the designs and without the need for complicated processes or expensive equipments. Patterned PDMS layers were created by spin-coating liquid phase PDMS on top of a substrate having sacrificial photoresist patterns, followed by a photoresist lift-off process. The thickness of the patterned PDMS layers could be accurately controlled (6.5–24 μm) by adjusting processing parameters such as PDMS spin-coating speeds, PDMS dilution ratios, and sacrificial photoresist thicknesses. PDMS features as small as 15 μm were successfully patterned and the effects of each processing parameter on the final patterns were investigated. Electrical resistance tests between adjacent electrodes with and without the insulation layer showed that the patterned PDMS layer functions properly as an electrical insulation layer. Biocompatibility of the patterned PDMS layer was confirmed by culturing primary neuron cells on top of the layer for up to two weeks. An extensive neuronal network was successfully formed, showing that this PDMS patterning method can be applied to various biosensing microdevices. The utility of this fabrication method was further demonstrated by successfully creating a patterned electrical insulation layer on flexible substrates containing multi-electrode arrays.

Keywords

Microfabrication; PDMS patterning; electrical insulation

1. Introduction

Microfluidic systems requiring integrated microelectrodes are widely used in various bio/medical applications such as in miniaturized electrochemical sensors, electrical impedance measurement microdevices, and microchip electroporators [1–4]. Integrated microelectrodes are also widely used for electrophysiological recording on neuronal cells in the form of multi-electrode arrays (MEAs) [5–7]. In such applications, arrays of embedded electrodes for sensing or stimulation have to be electrically insulated from the solution and from each other except for the sensing/stimulation sites of the electrodes to maximize the signal to noise ratio (SNR) and to prevent cross-talking of signals between electrodes. Insulation of these electrodes is typically done by depositing one or multiple layers of SiO_2 or Si_3N_4 on top of the electrodes using plasma-enhanced chemical vapor deposition (PECVD) processes [8–11]. The most

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widely used method to create openings in this insulation layer is photolithography followed by reactive ion etching (RIE). However, this method requires expensive equipments such as a PECVD and a RIE, and involves complicated multi-step fabrication processes. In addition, this method may not be suitable for flexible devices that require electrode insulation due to the cracking of hard insulating materials. Flexible neural probes fabricated by sandwiching sensing/stimulation electrodes between polyimide layers have been introduced but the method also involves complicated processes and requires specialized equipments for patterning the polyimide insulation layer [12–14]. A simple method to selectively insulate microelectrodes leaving wire bonding pads and sensing/stimulation part of the electrodes open would have significant advantages over conventionally available processes.

Poly(dimethylsiloxane) (PDMS) is a widely used polymer material in bio/medical microchips due to its ease of fabrication, biocompatibility, low cost, and optical transparency [15–17]. In addition, PDMS is electrically non-conductive, hence can be used as an electrical insulation layer. One limitation of this material is that there is no easy way to directly pattern PDMS. PDMS is not a photosensitive material and although few methods have been proposed to make PDMS photo-definable, the material preparation steps are complicated and not easy to follow, limiting its use [18–20]. Other PDMS patterning methods include plasma etching, blade scratching, and a parylene lift-off process [21–24]. Patterning PDMS using blade scratching is a method that allows a relatively thick layer of PDMS to be patterned [21]. However, it is a manual process and cannot be used to create small patterns. Eon et al. demonstrated that PDMS can be directly patterned using oxygen plasma etching, but the slow etch rate (7 nm/min) due to silicon oxide-like layer formation on top and the rough surface after plasma etching is limiting its use [22]. Tong et al. introduced a precision PDMS patterning method using a parylene C layer lift-off process that provided a relatively smooth surface [23]. In this method, PDMS was spin-coated and cured on a parylene C layer that was deposited and patterned by a low-pressure chemical vapor deposition and a RIE. Parylene C was then peeled off by hand, leaving PDMS only at the area where the parylene C layer had openings. Although this method could provide thin PDMS layers (4 μm) having small features (5 μm), the type of patterns that can be generated is very limited. Since PDMS was removed by lifting off the parylene C layer, areas where PDMS has to be lifted off had to be all connected so that they could be removed along with the parylene C layer, making this method useful for only a limited sets of designs. This method also requires equipments such as a parylene coater and a RIE, limiting its wide spread use as well as increased cost.

Recently, a method combining both PDMS plasma etching and lift-off process to fabricate glass-PDMS-glass microfluidic channels was introduced [24]. The method provided relatively faster PDMS etch rate using O_2 and CF_4 gas mixture, but the process produces particles on the PDMS surface after plasma etching and required a reactive ion etcher. Ostuni et al. and Jackman et al. proposed a method to obtain PDMS layers with openings by spin-coating PDMS on top of a SU-8 patterned substrate and peeling it off after polymerization [25,26]. This PDMS layer could be then used as a stencil/shadow mask or as an electrical insulation layer by placing the PDMS layer on top of a sample substrates or electrodes. The main challenges of this method were difficulties in aligning the PDMS layer to the substrate and handling of the thin PDMS layers.

In this paper, we present a simple, low-cost, and easy to follow PDMS layer patterning method without any limitation to the type of designs. This photoresist lift-off based method could be used to pattern thin PDMS layers regardless of whether all patterns are connected or not, hence can be easily used for selective passivation of microelectrode arrays, allowing an all PDMS microchip. How processing parameters such as PDMS dilution ratios, spin-coating speeds, and sacrificial photoresist layer heights affect the thickness of the final PDMS layer was

characterized. The electrical insulation capability of patterned PDMS layers and the biocompatibility of this fabrication processes were also characterized.

2. Materials and Methods

2.1 Design and Fabrication

Most electrical insulation layers used in microfluidic devices for biosensing, including multi-electrode array (MEA) for neuronal signal recording, cover the whole substrate except for the sensing part and bonding pads where electrodes have to be exposed bare. PDMS layers having arrays of up to 1225 circular openings in three different diameters (15 μm , 30 μm , 50 μm) with four different center-to-center distances (100 μm , 200 μm , 300 μm , 500 μm) have been fabricated over a 147 mm² area on glass and poly(methyl methacrylate) (PMMA) substrates. The different sizes were selected to characterize how pattern sizes and distances between patterns affect the openings in the PDMS layer.

The PDMS patterning steps are illustrated in Figure 1. To characterize how different thicknesses of sacrificial photoresist layers (NR2-20000P and NR4-8000P, Futurrex, Inc., Franklin, NJ) influence the PDMS patterns, photoresist patterns having thicknesses of 6.5 μm , 13 μm and 24 μm were fabricated. PDMS pre-polymer (10:1 mixture, Sylgard[®] 184, Dow Corning, Inc., Midland, MI) was then spin-coated at different spin-coating speeds (2000, 4000 and 6000 rpm with acceleration of 200 rpm/sec) on the photoresist patterned substrate (glass or PMMA film) and baked inside an 85°C oven for 15 minutes for polymerization. For the PDMS layer to function as an electrical insulation layer without affecting the overall device, a relatively thin PDMS layer has to be obtained. PDMS is a material with relatively high viscosity (3900 mPa·s) thus to obtain a thin insulating layer, PDMS was diluted in toluene (Sigma Aldrich, St. Louis, MO) at 1:1 and 1:2 ratios by weight. PDMS was diluted right before the spin-coating process in order to prevent the evaporation of toluene which could change the viscosity of diluted PDMS. Substrates coated with polymerized PDMS was then immersed in photoresist remover (RR4, Futurrex, Inc., Franklin, NJ) and sonicated for 5 minutes to lift-off the sacrificial photoresist layer, followed by rinsing in isopropyl alcohol (IPA) and drying inside an 85 °C oven. When patterning PDMS layers on top of electrode arrays, Cr/Au (10/250 nm thick) was deposited on the substrate and patterned using a conventional photolithography process followed by metal film etching. Also, to demonstrate that this fabrication method is not limited to discrete circular openings, straight, zigzag and serpentine shaped lines having width of 100 μm and length of 13–17 mm were patterned as well.

2.2 PDMS Pattern Evaluation

Patterned PDMS layers both with and without electrodes were first evaluated using an upright optical microscope (Eclipse LV 100D, Nikon America Inc., Melville, NY) equipped with a digital camera (DS-2Mv, Nikon America Inc., Melville, NY). Next, surface profiles of the patterned PDMS layers were measured using a surface profilometer (Dektak3, Veeco Instruments Inc., Plainview, NY). Since PDMS is an elastic material and the thickness measured with a stylus based profiler may not be accurate, thermally curable polyurethane (PU) (Smooth-Cast[®] 310, Smooth-On Inc., Easton, PA) was used to create an inverse replica of the patterned PDMS layer and their surface profiles measured as an alternative. Thermally curable PU pre-polymer was mixed with curing agent and poured on top of the patterned PDMS substrate followed by 10 minutes of degassing inside a vacuum connected desiccator to ensure that no bubbles were trapped between the PDMS and the PU layer. It was then left overnight on a leveled table for polymerization. The fully cured PU replicas were then peeled off from the PDMS substrates and their surface profiles were measured. A scanning electron microscope (SEM) (JEOL 6400, JEOL Ltd., Tokyo, Japan) was used to image the patterned PDMS layers.

2.3 Electrical Insulation Assessment

The electrical insulation characteristics of the patterned PDMS layers were tested by measuring the electrical resistance between adjacent electrodes. Glass and PMMA substrates having an array of 64 Cr/Au electrodes with 100 μm diameter sensing sites and separated by 500 μm were used. Electrical resistances between two adjacent electrodes without any PDMS coating were measured using a digital multi-meter (34410A, Agilent Technologies Inc., Santa Clara, CA) before and after applying 200 μl of phosphate buffered saline (PBS) solution over the electrodes, and were used as a reference. Then, resistances between electrodes that were fully coated with PDMS without any patterned openings were measured. Finally, electrodes coated with PDMS but with sensing sites exposed by the newly developed PDMS patterning technique were used and the resistances between two adjacent electrodes were measured. The measured resistance values were compared with the reference resistance value to evaluate how the patterned PDMS layers affect the electrical conductivity between the open sensing sites while insulating the rest of the electrodes.

2.4 Biocompatibility Assessment through Cell Culture

To evaluate the biocompatibility of the patterned PDMS layer, primary rat central nervous system (CNS) neurons were cultured for up to two weeks on top of the patterned PDMS layers and the cell viability as well as axonal network formation was evaluated. An inverted microscope (IX71, Olympus America Inc., Center Valley, PA) equipped with a digital camera (DP 70, Olympus America Inc., Center Valley, PA) was used to image cells. Primary CNS neurons were prepared from forebrains of embryonic day 16–18 Sprague-Dawley rats. Briefly, forebrains free of meninges were dissected in ice-cold dissection buffer ($\text{Ca}^{2+}/\text{Mg}^{2+}$ -free Hank's Balanced Salt Solution containing 10 mM HEPES), dissociated with L-cysteine activated papain (10 units/ml) in dissection buffer for 5 minutes at 37 $^{\circ}\text{C}$, and resuspended in dissection media containing trypsin inhibitor (10 mg/ml) for 2–3 minutes. Following two more washes with the trypsin inhibitor solution, the tissue was resuspended in a plating media (Neurobasal media containing 2 % B27, 1 mM Glutamine, 25 μM glutamic acid and 100 units/ml of penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin) and triturated with a fire-polished glass Pasteur pipette until all clumps disappeared. The cells were then passed through a 70 μm cell sieves and live cells were counted using a hemocytometer and trypan blue exclusion assay. The viability of isolated cells using this method is constantly greater than 90 – 95 %. $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free Hank's balanced salt solution, Neurobasal media, B27, penicillin, streptomycin and goat serum were from Invitrogen (Carlsbad, CA). Poly-d-lysine, papain, trypsin inhibitor, glutamine, glutamic acid, paraformaldehyde and Triton X-100 were from Sigma (St. Louis, MO).

MEA insulated with patterned PDMS layers were treated with plasma for 90 seconds (PDC-001 Plasma cleaner, Harrick Plasma, Ithaca, NY) and immersed in 70 % ethanol for 30 minutes for sterilization followed by poly-d-lysine (PDL) overnight coating prior to cell seeding. Cells were plated at an area seeding density of 1000 cells/ mm^2 and cultured at 37 $^{\circ}\text{C}$ in a humidified 5 % CO_2 incubator. Cells were cultured for up to two weeks and their viabilities tested through Calcein AM staining (Molecular Probes, Inc., Eugene, OR).

3. Results and Discussions

3.1 PDMS Lift-off Patterning

Table 1 shows how the different processing conditions affect the resulting PDMS patterns. 'O' in the table denotes that the PDMS was successfully patterned over the whole substrate area and 'Δ' indicates that only parts of the overall substrate area was successfully patterned with boundaries of the circular PDMS openings having residues. 'X' in the table denotes that the PDMS layer was not properly patterned and that most of the PDMS layer coated on top of the

sacrificial photoresist patterns remained even after the photoresist lift-off process. Scanning electron microscopy (SEM) images representing each category ('O', 'Δ', 'X') are shown in Figure 2. When PDMS layers were patterned using appropriate processing conditions ('O' in Table 1), size of the pattern openings as well as distances between the patterns did not affect the resulting PDMS patterns. On the other hand, PDMS layers were not patterned perfectly ('Δ' and 'X' in Table 1) by the developed lift-off process when the spin-coating speed or PDMS dilution ratio was too low compared to the height of the sacrificial photoresist layer. Under these improper processing conditions, the size of the circular openings and the distance between the patterns influenced the final patterning results. Typically, patterns with smaller opening diameters and longer separation distances resulted in better patterning whereas circular patterns too large or too close to each other resulted in poor lift-off of the PDMS layer on top of the sacrificial photoresist layer. Figure 3(a) shows arrays of successfully patterned circular openings in a PDMS layer with different diameters (15 μm, 30 μm, and 50 μm) but same 100 μm center-to-center distance. Figure 3(b) shows arrays of 30 μm diameter openings with 100 μm, 200 μm, and 300 μm center-to-center distances all showing good results. This capability of creating holes in PDMS could not be achieved by the previously reported parylene lift-off based PDMS patterning technique since it would have been impossible to remove the parylene patterns from each of the holes by peeling them off [23].

For some processing conditions, PDMS layers resulted in uneven surface thickness around the photoresist patterns even when the PDMS openings were clearly defined. This is due to the surface tension of the PDMS pre-polymer around the sacrificial photoresist patterns and occurred when the spin-coated PDMS thickness was too thin compared to the height of the photoresist pattern. Figure 4 shows undiluted PDMS spin-coated at 6000 rpm on 24 μm thick photoresist patterns followed by photoresist lift-off. It can be seen that the PDMS around the sacrificial photoresist patterns was thicker than the rest of the area. However, such uneven surface is typically not of any concern for most biosensing microdevice applications. Even for applications requiring even surface, this problem was solved by selecting proper photoresist pattern thicknesses and PDMS spin-coating speeds, as can be seen in the smooth pattern surfaces in Figure 2 and Figure 3.

From the processing parameters resulting in good PDMS patterns, we further investigated how each processing parameter affects the final thickness of the patterned PDMS layer by measuring the thickness of the PDMS layer around the pattern boundaries. Figure 5 shows the effect of each parameter on the final thickness of the patterned PDMS layer. It can be seen that the height of the sacrificial photoresist pattern had the most influence in determining the final thickness of the patterned PDMS layer, where increased photoresist thickness resulted in thicker PDMS pattern. Thickness of the patterned PDMS layer decreased as the spin-speed and the dilution ratio of PDMS increased. These results show that PDMS layers can be successfully patterned and their thickness can be controlled as needed by adjusting the processing parameters.

The PDMS patterning method developed here works not only for circular patterns but also for continuous line patterns. One hundred micrometer wide and 13–17 mm long straight, zigzag, and serpentine openings were successfully patterned in PDMS (Figure 6). NR4-8000P photoresist patterns with a thickness of 24 μm were used as a sacrificial photoresist layer and diluted PDMS (1:2 by weight in toluene) was spin-coated at 6000 rpm.

3.2 Electrical Insulation

The measured electrical resistance after applying PBS between adjacent bare sensing electrodes was 5–7 MΩ. When the electrodes were fully covered with PDMS without any openings, the measured resistance showed infinite after applying PBS solution, indicating that the PDMS layer properly functioned as an electrical insulation layer. Finally, electrodes were insulated with a PDMS layer with circular openings made at the sensing sites of the electrodes (Figure

7(a)). The measured electrical resistance between adjacent electrodes after applying PBS was 5–7 M Ω , which is identical to the previously measured reference resistance value. The substrate containing PDMS patterned electrodes were left in PBS for up to 3 days and electrical resistances were re-measured, but no change in resistivities were observed. These results show that the patterned PDMS layer can function properly as an electrical insulation layer.

To show that this patterning method can be also applied for electrically insulating multi-electrode arrays on flexible devices, a PDMS layer having arrays of 50 μm openings at sensing sites were patterned over a flexible PMMA film (thickness, 500 μm) (Figure 7(b)). The measured electrical resistances of 5–7 M Ω indicate that the patterned PDMS layer fully insulated the electrodes. Electrical resistances were measured again after bending the PMMA substrate multiple times using hand, but no noticeable changes in resistances were observed.

3.3 Biocompatibility

To investigate whether this PDMS patterning technology results in any biocompatibility issues, primary forebrain neuron cells from embryonic day 16–18 rats were cultured on top of a glass substrate having electrode arrays insulated with a patterned PDMS layer. Patterned PDMS layer did not show any adhesion problem with the glass substrate with electrodes during the culture period. Neuron cells successfully attached to the substrate and started to form extensive neuronal network on top of the PDMS insulation layer by 4 days *in vitro* (DIV 4) as shown in Figure 8. Cells were successfully cultured on the device for up to two weeks and no noticeable negative effects from the patterned PDMS layer on cultured neuron cells were observed and confirmed through a cell viability assay.

Conclusion

We have developed a PDMS patterning method that utilizes photoresist as a sacrificial layer for a PDMS lift-off process. The processing steps are simple and do not require expensive equipments other than a standard photolithography tool, and PDMS layers with different sizes and thicknesses could be successfully patterned on both glass and flexible PMMA film substrates without any limitations. This method could be used to create any type of PDMS pattern designs regardless of whether they were all connected or not, making this a universal one-stop method for PDMS patterning. The patterned PDMS layer successfully functioned as an electrical insulation layer. Neuron cells were successfully cultured on top of the patterned PDMS insulation layers for up to two weeks as demonstrated by extensive neurites outgrowth. We expect that this simple PDMS micropatterning method can be widely used to create electrical insulation layers for various bio/medical microdevices.

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Figure 1. Schematics of the PDMS micropatterning steps using a photoresist lift-off process to selectively insulate electrodes.

Figure 2. SEM images of patterned PDMS layers representing good ('O', left), partial ('Δ', center), and poor ('X', right) patterns. Pattern diameters were 50 μm. Scale bars, 10 μm.

Figure 3. SEM images of successfully patterned PDMS layers having (a) circular openings of 15, 30, and 50 μm diameters (from left to right) with 100 μm center-to-center distances and (b) arrays of 30 μm circular openings with 100, 200, and 300 μm center-to-center distances (from left to right). Scale bars, 100 μm .

Figure 4. SEM images of PDMS layers having 50 μm diameter circular openings but with uneven thickness. Scale bars, 100 μm (left) and 20 μm (right).

1:2 dilution / 6000 rpm	
PR Thickness	Thickness (μm)
6.5 μm	6.50 ± 0.36
13 μm	9.42 ± 0.12
24 μm	15.83 ± 0.21

1:2 dilution / 13 μm thick pattern	
Spin-speed	Thickness (μm)
2000 rpm	12.50 ± 0.22
4000 rpm	10.13 ± 0.35
6000 rpm	9.42 ± 0.12

4000 rpm / 24 μm thick pattern	
PDMS Dilution	Thickness (μm)
Undiluted	24.02 ± 0.22
1 to 1 (w/w)	19.86 ± 0.72
1 to 2 (w/w)	16.80 ± 0.28

Figure 5. Effect of processing parameters (sacrificial photoresist layer thickness, PDMS spin-coat speed, PDMS dilution ratio) on the final thickness of the PDMS patterns. Thickness of the patterned PDMS layers was measured from 38 sites of 16 different samples.

Figure 6. SEM images of 100 μm wide and 13–17 mm long (a–c) straight, serpentine, and zigzag line shaped PDMS patterns. Scale bars, 500 μm . (d) Close-up view of a serpentine line pattern. Scale bar, 50 μm .

Figure 7.

(a) A microscopic image of a PDMS layer with circular openings patterned on top of an electrode array embedded glass substrate. Scale bar, 100 μm . (b) Photographic image of a flexible PMMA substrate with multi-electrode array insulated by a patterned PDMS layer.

Figure 8. Fluorescence images of neuron cells at DIV 4 showing successful culture on top of Au electrode array insulated with patterned PDMS layer. Cell bodies and neurites were stained with Calcein AM (green). Scale bars, 50 μm .

Table 1

PDMS patterning results at different processing conditions. 'O' in the table denotes that the PDMS was successfully patterned over the whole substrate area and 'Δ' indicates that only parts of the overall substrate area was successfully patterned with pattern boundaries having torn residues. 'X' denotes that the

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