This manuscript describes a convenient method for the fabrication of freestanding, microperforated membranes in photocurable polymers using only one step of photolithography. We used photosensitive prepolymers to make the membranes and photolithography to define the micropatterns. We demonstrated the fabrication of single- and multilayer microperforated membranes in SU-8 photoresist and Norland Optical Adhesive prepolymer. These membranes can be used to pattern surfaces in various materials and to fabricate complex three-dimensional microfluidic channel structures. © 2010 American Institute of Physics. [doi:10.1063/1.3491474]

I. INTRODUCTION

This paper describes a convenient method for the generation of thin, perforated polymer membranes that contain single- or multilayer structures and their applications in microfluidics. Freestanding, perforated membranes (or stencils) have three main functions in microfluidics. First, the membrane can be used as a filter to separate particles with different sizes. Second, the membranes can act as a shadow mask for surface patterning1–12 (e.g., using chemical vapor deposition). This application simplifies the patterning of materials that are incompatible with conventional photolithography because it does not require the use of photoresists or organic solvents. Third, the membranes can be used as components in the fabrication of three-dimensional (3D) microfluidic devices: The perforations in the membrane can be used as a filter or can interconnect channels that are positioned above and below the membrane to form networks of 3D channels.13–17

Several methods are available that fabricate freestanding, perforated membranes containing ordered patterns of microstructures. Direct photolithography can pattern well-defined perforated membranes in photoresists, but it is limited to photoresists and most photoresists cannot generate freestanding membranes. Similarly, reactive ion etching (RIE) can fabricate freestanding, perforated membranes in more materials, e.g., parylene2,6,8,11,12 and parylene-SU-8 bilayer membranes.9 Such membranes are reversibly sealable and can pattern cells, lipid bilayers, and proteins; however, three steps are required to produce the membranes (vapor deposition, photolithography, and RIE). In contrast, phase separation micromolding uses a substrate that contains microposts and a polymer solution that contracts during phase separation to fabricate perforated membranes.18,19 The contraction of the polymer along the vertical axis creates perforations in the polymer film; however, contraction along the horizontal axis deforms the perforations.18 Alternatively, soft lithography provides a simple way that involves spin-coating a thin layer of liquid prepolymer on a substrate that contains microposts; the prepolymer, when cured, is peeled off from the substrate to produce a membrane that contains holes defined by the microposts. This method is used predominantly to fabricate perforated polydimethylsiloxane (PDMS) membranes;3–5,10 however, the meniscus of the liquid prepolymer at the microposts produces irregular features at the surface of the...
membrane. In addition, thin PDMS membranes tear easily and are difficult to manipulate (they are very easy to stick to other materials and to itself). To produce smooth surfaces, a perforated membrane can be obtained by squeezing a solution of polymer between a flat substrate and a master patterned with microposts; however, because it is difficult to completely remove the polymer liquid thin layer between the substrate and microposts, nonthrough holes are often observed. The pressure required by the method can also easily damage the microposts. Overall, all the soft lithographic methods require a master that contains microposts that is generated with conventional photolithography.

Here, we introduce a photolithographic method that fabricates high-quality perforated membranes that are characterized by flat surfaces, well-defined thicknesses, and that can be detached easily from substrates. The membranes can be used to pattern surfaces and to fabricate 3D microstructures. The principle of this method is simple (Fig. 1): We fill the space between two planar substrates using photosensitive prepolymer; we pattern the photosensitive prepolymer using a mask that selectively blocks light; we remove the two planar substrates from the photopatterned polymer to produce a perforated membrane. The method requires a single step of photolithography only. This method is different from conventional photolithography in several ways: (1) The patterned material is not limited to photoresists but can be fabricated using all photocurable liquids (e.g., optical adhesives); (2) because the liquid is confined in two substrates, it is not necessary to treat the substrates to promote adhesion of each photoresist; (3) the thickness of the membrane is defined using spacers, with which more accurate and more uniform membranes are easier to fabricate compared with methods that use spin-coating; (4) multilayer structures can be formed in
the perforated membranes using similar procedures. Finally, perforated membranes with moderate quality can be generated with a handheld UV lamp in a common laboratory, eliminating the need for a mask aligner and cleanroom.

II. MATERIALS AND METHODS

A. Materials

We purchased PDMS prepolymer kit (RTV 615) and SU-8 photoresist (and SU-8 developer) from GE Silicones, Wilton, CT, USA and MicroChem, Newton, MA, USA, respectively. Norland Optical Adhesive 73 (NOA 73) was obtained from Norland Products Inc., NJ, USA. The sources of other chemicals and equipment will be mentioned in the text.

B. Fabrication of SU-8 membranes

We thoroughly mixed the two components of PDMS prepolymer (A:B=10:1) and degassed the mixture under vacuum in a desiccator. This PDMS mixture was spin-coated (4000 rpm, 30 s) on to silicon wafers or other substrates and cured to form a thin PDMS layer on the substrates. We poured ~2 ml SU-8 2050 onto a PDMS-coated silicon wafer and heated the photoresist at 120 °C in an oven for 1.5 h to remove solvent in the photoresist. After being cooled down to room temperature, the prebaked SU-8 became solid and was stored for use.

We spin-coated PDMS prepolymer on a glass slide (4000 rpm, 30 s) and cured the PDMS at 70 °C oven for 1 h. The excess PDMS at the edges was cut away with a razor to ensure a flat substrate. To make an SU-8 membrane, we took proper amount of prebaked SU-8 and placed it between two PDMS-coated glass slides, which were separated by two spacers at the ends of the slides. We used three kinds of spacers: Teflon membrane (~15-μm-thick), cover slide (~150-μm-thick), and silicon wafer (~500-μm-thick). Other thicknesses were achieved using combinations of these spacers. After being heated in a 120 °C oven for 5 min, the SU-8 melted and reflowed to fill the gap between the two PDMS layers on the slides. Then a pair of office clips was used to fix the assembly together until it cooled to room temperature. After the top PDMS-coated slide was carefully removed from the SU-8, we gently placed a photomask with the desired pattern onto the SU-8 layer. Subsequently, we exposed the SU-8 layer by illuminating UV light through the photomask (the exposure time depends on the thickness of SU-8). After a postbake at 95 °C in an oven for 10 min, we disassembled the glass slides and spacers, and immersed the SU-8 membrane in its developer until the desired pattern was obtained. The perforated membrane was dried carefully using a nitrogen air gun and stored in a Petri dish for examination under an optical microscope and for future use.

C. Fabrication of NOA membranes

We spin-coated PDMS prepolymer on a glass slide (4000 rpm, 30 s) and cured the PDMS at 70 °C oven for 1 h. The excess PDMS at the edges was cut away using a razor; a blank transparency film (PP2900, 3M Visual System Products, Europe) was placed onto the PDMS-covered glass slide. After spacers (e.g., two layers of Scotch™ tape for ~80 μm) were placed at the edges of the transparency film, we added a few drops of NOA 73 on the transparency film. The NOA on the film was degassed under vacuum in a desiccator for 10 min. We placed another transparency film that contained printed patterns on one side onto a PDMS-coated glass slide with the blank side of the film contacting the PDMS. This film was directly placed onto the NOA with the patterned side of the film in contact with NOA. We fixed the device using two clamps, which were positioned at the sides of the glass slides; the NOA was sandwiched into a thin membrane between the substrates. We used UV light (365 nm, 18 mJ/cm², 20 s) from a mask aligner to polymerize the NOA membrane. We also used UV light from a thin-layer chromatography chamber (3 min) to polymerize another NOA membrane. We disassembled the device to separate the NOA membrane from both substrates. The NOA membrane was subsequently developed in acetone to remove the unexposed polymer. After development, the membrane was exposed
to UV light to complete polymerization (365 nm, 18 mJ/cm², 120 s or handheld UV lamp for 15 min). The fabricated NOA membrane was examined under an optical microscope and stored in a Petri dish for future use.

D. Fabrication of NOA membranes that contain a 3D microstructure

We fabricated PDMS slabs that contained features using soft lithography as described previously. We used a glass slide to support one PDMS slab. We aligned the other PDMS slab to a photomask that was attached to a glass slide. We used two 150-μm-thick glass slides as spacer. We applied NOA between the two patterned PDMS slabs and we exposed the device to UV light (365 nm, 18 mJ/cm², 1 min). We removed the exposed NOA membrane, developed it in acetone (0.5 h), and dried it in air. We used this NOA membrane and two flat PDMS slabs (one of which has holes as inlets and outlets) to form a sealed 3D microfluidic system.

E. Micropatterning of protein on PDMS

We exposed an ~100-μm-thick SU-8 membrane to oxygen plasma (1 min) and we sealed a layer of PDMS to the SU-8 membrane. We placed fluorescein isothiocyanate labeled bovine serum albumin ([FITC-BSA], 0.1 ml of a 0.05 mg/ml solution in phosphate buffered saline (PBS), pH 7.4; Sigma) on the membrane. We placed the PDMS in vacuum (5 min) so that the air bubbles that were trapped when adding the solution were removed. We incubated the device (6 h, 4 °C), we removed the FITC-BSA solution, and we rinsed the device in PBS (pH 7.4). We removed the SU-8 membrane and imaged its surface using fluorescence microscopy (Nikon Eclipse TE2000-U, Japan).

F. Micropatterning of metallic thin films on glass

We fixed SU-8 membranes on a glass slide using Scotch tape or directly attached NOA membranes to a glass slide. We sputtered metallic thin films (5 nm Cr as adhesive layer and then 100 nm Au or Pt) with the desired thickness onto the slides using a dc magnetic sputtering system (ARC-12M, Plasma Science Inc., USA). The perforated membranes were removed and the patterned slides were examined under an optical microscope.

G. Micropatterning of cells on a Petri dish

HeLa cells were handled in sterile tissue culture hoods and we maintained the cells in Eagle’s minimum essential medium (MEM)/10% fetal bovine serum supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (all chemicals and reagents were purchased from Gibco, Invitrogen, NY, USA). We incubated the cells in an incubator at 37 °C with 5% CO₂. We passed the cells using 0.25% trypsin (Gibco, Invitrogen, NY) and we neutralized the trypsin using MEM medium. We centrifuged the cell suspension (1250 rpm, 3 min) and we resuspended the cells in MEM medium. We directly attached an ~80-μm-thick NOA membrane to a sterile Petri dish and sterilized it using UV light (30 min). We seeded the cells to the NOA covered dish (2 × 10⁵ cells/ml). After 2 days of incubation, we peeled off the NOA membrane and we examined the patterns of cells using optical microscopy.

III. RESULTS AND DISCUSSION

We demonstrated our method with two photosensitive polymers: SU-8, a negative photoresist that seals reversibly to PDMS, and NOA, a photocurable adhesive that, when cured, seals reversibly to transparency film and PDMS.

A. Fabrication of SU-8 membranes

Figure 1(a) schematically illustrates the method that we use to fabricate perforated membranes in SU-8 photoresist. We take an appropriate amount of stock SU-8 photoresist (prebaked) and place it between two PDMS-coated glass slides that are separated by two spacers at the sides. We
heat this prebaked SU-8 photoresist at 120 °C in an oven for 5 min. After the SU-8 photoresist is melted, we quickly fix the two PDMS surfaces in position with clamps and allow the assembly to cool. After cooling down to room temperature, a dried SU-8 prepolymer membrane is formed between the two flat PDMS surfaces. Because the strength of the clamps is weak and the PDMS layers are relatively thin (~100 μm), the thickness of the formed SU-8 membrane is equal to the thickness of the spacers. The top PDMS is removed and replaced by a photomask for photolithography. After UV exposure and postbake, SU-8 polymer that has not been exposed to UV light is washed away in SU-8 developer. We fabricate three SU-8 membranes with feature sizes down to 10 μm and thickness from 15 to 500 μm using this method [Figs. 2(a)–2(c)]. Our method has two technical improvements that facilitate the process: (1) The thickness of formed membranes is accurately controlled. The thickness of the membrane is determined only by the thickness of the spacers. It is also possible to make membranes with thicknesses up to 1 mm using thicker spacers. (2) Pretreatment of SU-8 prepolymer in large amounts at one time saves time: A stock supply of soft-baked SU-8 can be stored in lieu of an experiment. For other methods that require the fabrication of SU-8 structures with thicknesses greater than 200 μm, it takes a long time (a few hours) to dry the thick photoresist during the soft-baking step. In our method, the prebaked SU-8 prepolymer is stored and divided into several small portions; it is only necessary to reheat an appropriate amount of the prebaked prepolymer at 120 °C for only ~5 min to make it ready for subsequent UV exposure.

B. Fabrication of NOA membranes

Figure 1(b) illustrates the method for fabricating a perforated membrane using NOA and either 3M transparency film or PDMS as the substrate. We describe the method using 3M transparency film in the following [Fig. 1b(i)]. The polymer solution is placed onto the transparency film, which is supported by a PDMS-coated glass slide. We use a layer of PDMS so that the film that contains printed micropatterns will seal conformally to the glass slide; we cut the higher edge of a spin-coated layer of PDMS to ensure a flat substrate. The degassing step is used to avoid bubbles while sandwiching the NOA between two substrates. Figure 2(d) shows a pattern of square holes that is produced using photolithography with collimated light. In the example described, 3M film—the photomask—is placed in direct contact with the NOA polymer. Because NOA prepolymer is liquid before it is photocured, it can easily stick to the photomask transparency and contaminate it. To protect the pattern on the film, a thin layer of PDMS is spin-coated and cured on the patterned side of the top transparency film [Fig. 1(b)(ii)]. With the layer of PDMS in the middle, the transparency does not directly contact the NOA liquid. This PDMS layer is required to be thin enough (less than 1/10 of the smallest features on the pattern) to maintain pattern fidelity during pattern transfer with UV exposure. Using PDMS also gives this method, the potential to fabricate multilayer structures in NOA membranes (see below).

Figure 2(e) shows a pattern of circular holes that is produced by exposing the NOA liquid to a noncollimated light source (handheld UV lamp commonly used in thin-layer chromatography). Although the fidelity of pattern transferred is not perfect, the elimination of mask aligner and cleanroom makes this method convenient and accessible to almost all researchers in related fields.

C. Smallest feature size of membranes

The smallest feature size of our membranes depends on the thickness of the membranes and the largest aspect ratio that can be achieved. Thin membranes always suffer from low mechanical strength, which makes the membranes delicate and hard to remove from the substrate. As SU-8 and NOA have different mechanical strengths and different bonding strengths to the substrates, membranes fabricated from these materials are easily handled when the thickness of the SU-8 membrane is greater than ~15 μm or the thickness of the NOA membrane is greater than ~50 μm. The aspect ratio is largely determined by the light source; collimated light generates
FIG. 2. Microphotographs of SU-8/NOA membranes that contain various patterns. (a) An 80-μm thick SU-8 membrane. (b) A 500-μm-thick SU-8 membrane. (c) An array of 10 μm diameter circles in a 15-μm-thick SU-8 membrane. (d) An array of 100 μm side squares in an 80-μm-thick NOA membrane. (e) An array of circles that is fabricated using a membrane that contained an array of 300 μm diameter circles and a noncollimated light source. The scale bars in (c), (d), and (e) represent 100, 200, and 500 μm, respectively.
features with higher aspect ratios than noncollimated light (e.g., handheld UV lamp) does. Currently, the smallest features that we can achieve are \( \sim 10 \) μm with a mask aligner and \( \sim 200 \) μm with UV handheld UV lamp.

D. Criteria for substrate selecting

The choice of substrate is critical in the two strategies presented in this note. We have identified three criteria for selecting an appropriate substrate. (1) The surface of the substrate does not strongly adhere to the formed membrane so that the membrane can be separated after polymerization. (2) The hardness of the substrate should be appropriate, for example, a hard SU-8 membrane requires a soft and elastic substrate such as PDMS, which facilitates easy removal of the membrane from the substrate and prevents cracking of the membrane. (3) The top substrate (other than the photomask) should be transparent to allow light pass for UV exposure.

E. Micropatterning

Both SU-8 and NOA microperforated membranes can be used as masks for patterning various materials. We patterned proteins on PDMS using an SU-8 membrane, thin metal films on glass using both SU-8 and NOA membranes, and cells on a commercial Petri dish using a NOA membrane (Fig. 3). The fidelity of the pattern produced by metal sputtering is acceptable for feature sizes greater than \( 200 \) μm with SU-8 membranes; however, for smaller feature sizes, we experience difficulties in producing high-quality patterns with sharp edges. These problems are caused by the stiffness of SU-8 membranes, which are unable to conform to substrates. In contrast, NOA membranes adhere to surfaces better than SU-8 membranes, which improves the range of feature sizes that can be produced using the method described [Fig. 3(e)]. The same strategy can be used to pattern cells; compared to seeding cells followed by selectively surface modification,\(^3,16,4,22,23\) the use of perforated membranes as a mask to pattern cells is more straightforward.\(^1,12\) The ability of NOA membranes to act as templates for the fabrication of patterns of metal and cells [Fig. 3(d)] makes it a viable alternative to PDMS and parylene for these
particular applications. One drawback of NOA membranes is that, being soft, the membrane can deform to some extent when they contain certain perforations with large length-to-width ratio, e.g., long lines. For these types of perforations, our experience using several photosensitive polymers indicates that SU-8 (the hardest) is most appropriate, NOA is less appropriate, and PDMS (the least hard) is least appropriate for the fabrication of membranes.

F. Fabrication of 3D microfluidic devices

A 3D channel structure can be topologically mapped onto a flat surface so that it only contains channels and crossings where channels at different levels overlap on the 2D projection. To reconstruct any 3D channel structure, we can mathematically decompose it into three layers: The top and bottom layers containing fluidic channels that are connected by a middle membrane with perforated holes. The middle perforated membrane is important because it enables two channels at different levels to cross each other without connecting them. Because the surfaces of our perforated membranes are flat, they are ideal components for the construction of 3D microfluidic devices. Figure 4(a) shows an example of fabricating a microfluidic device that contains a double-helix structure with an SU-8 perforated membrane. We have demonstrated the operation of the 3D structures with red and blue inks. We also fabricate other types of 3D channel structures without channel crossings. In Fig. 4(b), a 3D micromixer is generated with a thin perforated SU-8 membrane sandwiched between two PDMS channel pieces. We use a syringe pump to fill the channels with blue ink and water, and this microfluidic device is able to operate well at tested flow rates (1–100 μl/min).

All the formed membranes have two flat surfaces and through holes. However, we can also generate membranes with both through holes and channels on two surfaces. The fabrication process is similar to the membranes shown before; the difference is that the flat PDMS substrates are replaced with patterned PDMS pieces [Fig. 5(a)]. The patterns on the PDMS substrates have been transferred into the membranes after UV exposure and development. In this manner, the 3D channel crossings are realized in the perforated membrane; to complete the desired 3D microfluidic structure, only two flat PDMS slabs are required to seal the top and bottom of the channels in the membrane. Figures 5(b)–5(d) show an example of a 3D channel structure that contains a basket-weave pattern using a NOA membrane sandwiched between two PDMS slabs that contain a multilayer structure.

IV. CONCLUSIONS

In this report, we have demonstrated a convenient method that can fabricate freestanding, perforated membranes in photocurable materials, such as SU-8 and NOA polymers. Membranes having 3D microstructures can also be formed with similar procedures. These formed membranes can be used to pattern surfaces and to construct 3D microfluidic structures. Because SU-8 membranes cannot conformally seal to hard materials, such as glass and polystyrene, it can be used to
pattern certain substrates, such as PDMS to which SU-8 can seal conformally. In contrast, NOA membranes can seal conformally to both hard and soft substrates including glass, PDMS, and polystyrene. Noting that conventional photolithography is limited to the fabrication of SU-8 perforated membranes, our method is advantageous in making perforated membranes in various materials with extended applications. In addition, our method for fabricating membranes presents several other advantages over existing methods:

1. Only one step of photolithography is required;
2. the surfaces of the membranes are flat;
3. little treatment of substrate surfaces is needed;
4. the thickness of the membrane can be easily controlled ranging from tens of microns to millimeters. We believe that our method will be of interest to people working in microfluidics and bioengineering.

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