Published in final edited form as:

Anal Chem. 2013 October 1; 85(19): . doi:10.1021/ac401639n.

Photolithographic fabrication of microapertures with well-defined, three-dimensional geometries for suspended lipid membrane studies

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Abstract

Robust and high-density biosensors incorporating suspended lipid membranes require microfabricated apertures that can be readily integrated into complex analysis systems. Apertures with well-defined, three-dimensional geometries enable the formation of suspended lipid membranes, and facilitate reduced aperture size compared to vertical-walled apertures. Unfortunately, existing methods of producing apertures with well-defined, three-dimensional geometries are based on complex and expensive fabrication procedures, some of which yield apertures in excessively fragile thin-film materials. Here, we describe a microfabrication method utilizing incline and rotate lithography that achieves sloped-wall microapertures in SU-8 polymer substrates with precision control of aperture diameter, substrate thickness, and wall angle. This approach is simple, low cost, and readily scaled up to allow highly reproducible parallel fabrication. The effect of incident angle of UV exposure and the size of photomask features on aperture geometry were investigated, yielding aperture diameters as small as 7 μm , and aperture wall angles ranging from 8° to 36° measured from the normal axis. Black lipid membranes were suspended across the apertures and showed normalized conductance values of 0.02 to 0.05 pS μm^{-2} and break down voltages of 400 to 600 mV. The functionality of the resulting sloped-wall microapertures was validated via measurement of reconstituted α -hemolysin activity and the voltage-gated channel activity of alamethicin.

Keywords

Black lipid membranes; microfabrication; planar apertures

Introduction

Suspended lipid bilayers, or black lipid membranes (BLMs), provide a valuable analytical tool in the study of transmembrane protein activity.^{1–7} Since transmembrane proteins make up a significant fraction of all known pharmaceutical targets,⁸ BLM-based measurements can play a critical role in the design and discovery of new therapeutic molecules.^{9,10} Additionally, artificial lipid membranes are increasingly utilized in a range of biomimetic chemical sensors,^{11–13} with much focus on-hemolysin nanopores for the detection and characterization of biopolymers and other biological analytes.^{14–21} The recent advent of

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Supporting Information

Images and illustrations of fabrication apparatus and components, and UV exposure dose calculations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

engineered transmembrane proteins that combine the high sensitivity of ion channel conductance measurements with the specificity of molecular recognition,^{22,23} presents a number of opportunities for novel sensor development. However, producing BLMs in a high density device format amenable to long-term monitoring applications, or to being integrated into robust and deployable analytical systems, presents a significant challenge. Apertures with diameters in the low μm regime have been formed on glass micropipettes, such as patch clamp pipettes, to enable BLM-based studies.^{24–26} While this approach is simple, it suffers from a number of drawbacks. Fabricating glass pipettes requires a great deal of manual labor, and producing pipettes with a high degree of reproducibility in aperture size and geometry in the low μm regime is challenging. The formation of stable BLMs on glass pipettes requires the additional complexity of chemically modifying the glass to produce a moderately hydrophobic surface.²⁶ Commercial systems are available that incorporate planar microapertures for membrane analyses,^{27,28} although the focus of these systems is on high throughput membrane screening rather than sensor development. Additionally, in at least one case,²⁸ BLMs are suspended over microcavities rather than microapertures, restricting flow access to one face of the BLM. Bilayers can be formed at the interface of microdroplets in lipid-containing organic solvents,^{29–31} requiring no apertures, however this approach does not lend itself to integration with flow-based processes. Next-generation integrated analysis systems for chemical sensing, drug discovery or diagnostic platforms will require low dead volume coupling of BLMs with flow-based analysis methods. Thus, methods that yield high density, easily fabricated planar microfabricated platforms for suspending BLMs are desirable.^{32–39}

A BLM forms when lipid monolayers meet from both faces of the substrate material to form a bilayer over the aperture (Scheme I). Ideally, this substrate material would be an infinitely thin film, allowing monolayers to meet over the aperture with no perturbation in membrane topology where the bilayer meets the aperture wall. Since real substrate materials have thickness, there is always a perturbation in membrane topology, as monolayers deform to meet across the thickness of the substrate.³² An annulus forms where the bilayer meets the aperture wall, reducing the effective area of the bilayer. With large aperture diameters, this deformation may be small relative to the area of the bilayer; however, with decreasing aperture diameter at a fixed substrate thickness, the required deformation relative to the bilayer area likely increases, challenging bilayer formation (Scheme I). Therefore, substrate thickness is believed to be a limiting factor in decreasing aperture diameters (Scheme I).

To overcome this limitation, apertures have been prepared in vapor deposited SiN substrates of only a few hundred nm thickness.^{33, 34} While these apertures support stable lipid membranes, the materials are fragile, challenging the development of robust and deployable analytical systems. An alternative to forming apertures in ultrathin films is to produce apertures with diameters that taper as they penetrate the planar substrate (Scheme I, iii). The resulting sloped-wall geometry allows the mechanical stability of a thick substrate material while minimizing substrate thickness in the immediate region of bilayer formation. Sloped-wall apertures formed by anisotropic etching of silicon have proven useful in suspended lipid membrane studies,³⁵ but microfabrication of silicon is more complex than polymer fabrication, the substrate materials are more expensive, and silicon is a semiconductor that requires additional processing to be suitable for high-sensitivity conductance-based measurements. Many polymer materials offer improved insulating properties over silicon substrates; however, precision microfabrication of apertures in polymers is less common.^{34, 36, 37} For example, apertures with three-dimensional geometries have been produced in the negative photoresist SU-8 by overexposure lithography,³⁴ though precision control of feature sizes by this type of lithography is challenging.

Here, we describe a microfabrication method to produce apertures with well-defined three-dimensional geometries in SU-8. This approach is based on incline and rotate photolithography, with process modifications aimed at facilitating the liftoff of thin-film SU-8 from the supportive substrate used during fabrication, and at achieving fabrication with high fidelity. In this work, we describe the fabrication process as well as the characterization of resulting aperture geometries with respect to the angle of sloped aperture walls and aperture diameter as a function of variables in the fabrication process. Additionally, we demonstrate the feasibility of the resulting apertures for ion channel conductance measurements.

Materials and Methods

Materials

SU-8 3050, SU-8 developer, and Shipley S1813 were purchased from Microchem Corp. (Newton, MA). Diphytanoylphosphatidylcholine (DPhPC) was purchased from Avanti Polar Lipids Inc. (Alabaster, AL). Decane, Sigmacote, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma Aldrich (St. Louis, MO). H_2SO_4 , NH_4OH , and H_2O_2 were purchased from EMD Chemical (Billerica, MA). KCl was purchased from J.T. Baker. Ethylenediaminetetraacetic acid (EDTA) was purchased from Mallinckrodt (Hazelwood, MO).

Photomask preparation

All photolithography and microfabrication work was performed in the cleanroom facility in the Department of Chemistry and Biochemistry at the University of Arizona. Photomasks were purchased as 20000 dpi prints on acrylic film (CAD/Art Services, Bandon, OR), and were transferred to chrome-on-borosilicate photomask blanks (Telic Co., Valencia, CA) by standard photolithography using Shipley S1813 as per the manufacturer's recommended process. The photomask design consisted of circular features with diameters ranging from 10 to 700 μm in 10 μm increments, with 4 replicates of each diameter. The photomask used in this study is shown in the Supporting Information (Figure S1). Glass photomasks were cleaned for 20 min in a 5:3:1 (v:v:v) solution of $\text{H}_2\text{O}:\text{NH}_4\text{OH}:\text{H}_2\text{O}_2$ at 60 °C followed by 20 min in 3:1 (v:v) $\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2$. The cleaned masks were dried with compressed N_2 , then dehydrated on a hot plate at 150 °C for 30 min. Dried photomasks were submerged in Sigmacote for 5 min, rinsed with isopropanol, dried with compressed N_2 , and maintained on a hot plate at 95 °C until spin coated with SU-8.

Incline, rotate and liftoff fabrication

Photomasks were cooled to room temperature and spin coated with SU-8 3050 (25 s @ 500 rpm, 25 s @ 2000 rpm), followed by a 15 minute rest at room temperature prior to soft baking. Soft baking was performed on a hot plate at 65 °C for 15 min. The soft baked substrates were placed on the stage of a lab-built turntable and rotated at > 60 rpm while subjected to collimated UV radiation at incident angles of 15°, 30°, 45°, 60°, and 80° (measured from the normal axis) using a lab-built UV exposure system based on a UV-LED (model LZ4-40U600, LEDEngin Inc., San Jose, CA) (Supporting Information, Figure S2). Exposure times are given in Table 1 and discussed below. Following exposure, substrates were hard baked at 95 °C for 5 min. To prevent fractures in the SU-8 film induced by thermal stress, the hot plate was turned off and substrates were allowed to return to room temperature at the rate of the cooling hot plate. Substrates were submerged in SU-8 developer solution, sonicated for 7 min to remove unexposed SU-8, rinsed with isopropanol, and dried under compressed N_2 . The polymerized SU-8 was covered with a piece of low tack wafer-dicing tape (Semiconductor Equipment Corp., Moore Park, CA) to help prevent fracturing during lift-off (Supporting Information, Figure S3). The edge bead was cut from

the polymerized SU-8 film using a razor blade, and the remaining SU-8 was lifted from the photomask in a single sheet by carefully sliding a razor blade between the SU-8 film and the photomask.

Geometric characterization

Profilometry was performed at the Micro / Nano Fabrication Center in the University of Arizona College of Engineering. SEM imaging was performed at the W.M. Keck Center for Surface and Interface Imaging in the Department of Chemistry and Biochemistry at the University of Arizona. The thicknesses of polymerized SU-8 films were measured using a stylus profilometer (model P-15, KLA Tencor, Milpitas, CA). Transmitted light microscope images of the apertures were obtained via a 4X or 20X objective on a Nikon Eclipse TE300 microscope (Nikon Instruments Inc., Melville, NY) and a Photometrics Quantix camera (Photometrics, Tucson, AZ). Aperture diameter was measured using ImageJ⁴⁰ by scaling against a stage micrometer with 10 μm gradings. Direct measurements of aperture wall angles were not possible by profilometry because the slope of the aperture walls exceeded that of the profilometer stylus tip. Measurements of wall angle by SEM were impractical, since an accurate measure required a perfectly bisected aperture. Thus, wall angles were calculated according to:

$$\theta_w = \tan^{-1} \frac{m - a}{2t} \quad (1)$$

Where θ_w is the wall angle, m is the diameter of the photomask feature, a is the aperture diameter and t is the thickness of the SU-8 film (Figure 1). To ensure durable SU-8 films, t was held constant in this work at $75 \pm 4 \mu\text{m}$.

Pipette assembly

To interface the planar apertures with a patch clamp amplifier, the apertures were mounted to the opening of 100 μL plastic pipette tips (Supporting Information, Figure S4). The lower 4 mm were cut from standard disposable pipette tips (VWR International, Radnor, PA) to give an opening of approximately 2 mm. The pipette opening was heated over a flame and pressed flat against a glass microscope slide which formed a smaller opening ($< 1 \text{ mm}$) surrounded by a flat rim. Adhesive o-rings were punched from VHB tape (3M, St. Paul, MN) and affixed to the flat rim surrounding the pipette tip opening. Individual apertures were cut from a sheet of polymerized SU-8 and mounted to the adhesive o-ring under a stereoscope. Apertures were mounted with the narrow aperture opening facing outward, and care was taken to ensure that the aperture was positioned to avoid clogging by the small amount of adhesive that reflowed from the o-ring while curing. The pipette tip/o-ring/aperture assembly was placed in a 70°C oven to cure overnight.

Electrophysiological measurements

BLM formation and ion channel activity were measured using a patch clamp amplifier (EPC-8, HEKA Instruments Inc., Bellmore, NY). All membrane experiments were conducted using a solution of 1M KCl, 5mM HEPES, 1mM EDTA, $\text{pH} = 7.5$ on both sides of the aperture. BLMs were manually painted over the aperture using a solution of 10 mg mL^{-1} DPhPC in *n*-decane.⁴¹ Membrane conductance was determined for each BLM by a linear fit to current vs. voltage plots from -100 mV to 100 mV in 10 mV increments. Breakdown potentials were measured by applying bias potential across the membrane from 0 mV to 1100 mV in 10 mV steps of 50 ms duration. Breakdown was assessed as a large magnitude, non-linear increase in ion conductance. Ion channel activity was measured following addition of $3 \mu\text{L}$ of an ion channel solution (either $0.5 \mu\text{g mL}^{-1}$ -hemolysin in water, or $1 \mu\text{g mL}^{-1}$ alamethicin in ethanol) to the bath solution ($400 \mu\text{L}$) in the vicinity of

the aperture. Ion channel activity was measured by applying a bias potential (40 mV for - hemolysin, 100 mV for alamethicin) to the membrane, and monitoring ion current over time. For alamethicin insertions, once ion conductance was observed, the bias potential was varied from 0 mV – 60 mV to verify voltage-gated ion channel activity.

Results

Preparation of apertures that support BLMs requires consideration of aperture size, geometry and substrate material. Further, the ability to reproducibly fabricate apertures that support BLM formation in a rapid and high-throughput manner would enable the widespread utilization of this technique for analytical measurements. Specifically, we sought to prepare apertures with conical geometries to yield aperture substrates that have a lower effective thickness than straight wall apertures (Scheme I) thus allowing allow smaller diameter apertures to be fabricated in substrates that retained sufficient thickness and rigidity to be robust enough for use in integrated analytical devices. To achieve this goal, we explored the fabrication of apertures with sloped walls in the common MEMS and microfluidics polymer material SU-8. SU-8 was chosen for its ease of fabrication, hydrophobic surface chemistry, and dielectric properties which are superior to those of silicon-based substrate materials.⁴²

Incline, rotate, and lift-off fabrication

Incline and rotate photolithography has been utilized to prepare three-dimensional structures in SU-8.^{43, 44} We modified this approach to produce geometrically well-defined voids in a thin film of SU-8 to serve as apertures for BLM functionalization. Utilization of BLM-functionalized microapertures for analytical measurements requires electronic and fluidic access to both sides of the membrane, and therefore both sides of the thin film material. Thus, a method was needed to release the aperture-containing thin film from the handle material. In tandem to forming sloped wall apertures, we addressed the need to remove the thin films from their supportive substrates by developing an SU-8 lift-off process. Since SU-8 exhibits poor adhesion to glass,⁴⁵ we utilized the glass photomask directly as the handle material during photolithographic patterning of SU-8 (Figure 1A), which facilitated lift-off and ensured hard-contact between the SU-8 film and the photomask during UV irradiation, thereby eliminating the possibility of misalignment during rotation and UV exposure. While characteristically poor SU-8/glass adhesion was observed when SU-8 films were coated directly on the photomasks, the adhesion was sufficient to cause fracturing when polymerized SU-8 films were lifted from the photomask. Treatment of the photomask with Sigmacote prior to spin coating with SU-8 resulted in polymerized SU-8 films that readily lifted from the glass photomask with little or no fracturing. Sigmacote treated glass was optically clear, and no differences were observed either in required UV dose or in the geometry of the resulting apertures when SU-8 was spin coated on Sigmacote treated photomasks versus untreated masks.

While the adhesion-inhibiting treatment facilitated liftoff of the polymerized SU-8, it introduced a complication during soft baking. Although thin, uniform films of SU-8 were observed following spin coating, upon soft baking, the films receded from the perimeter of the photomask and pooled to form an uneven distribution of thicknesses across the SU-8 film. The thickness of the SU-8 film is a key variable in controlling aperture geometry (Figure 1B), necessitating a reduction of the observed pooling effect. A 15 minute rest step was introduced between spin coating and soft baking to allow solvent evaporation from the SU-8 film at ambient temperature, thus increasing SU-8 viscosity prior to soft baking. Additionally, the soft bake temperature was reduced from the conventional 95 °C to 65 °C, which further minimized the reduction in SU-8 viscosity that is observed upon heating. These process modifications were effective at reducing the pooling effect to achieve SU-8

films with uniform thicknesses, which agrees with previous work demonstrating that decreased soft bake temperatures improved fidelity in the photolithography process.⁴⁶

UV exposure was performed on a lab-built lamp and turntable apparatus illustrated in the Supporting Information (Figure S2). The lamp allowed control of the incident angle of UV exposure (θ_{UV}), with an output power density of 1 mW cm^{-2} , which is significantly lower than that of conventional photolithography exposure systems (ca. 20 mW cm^{-2}). The low power density of UV exposure was further complicated by reflection of incident UV radiation at the surface of the glass photomask, which increased with increasing θ_{UV} . The increased reflection, and corresponding decreased transmission, as a function of θ_{UV} is defined by Fresnel's equations, and is discussed in the Supporting Information. Predicted exposure times as a function of θ_{UV} were calculated based on the manufacturer's recommended UV dose of 375 mJ cm^{-2} . The required UV dose for effective SU-8 polymerization depends on a number of factors, including SU-8 film thickness and substrate material.⁴⁷ The current process is unique from conventional SU-8 processing in that it involves no supportive substrate other than the glass photomask. Therefore, preliminary tests were performed to determine appropriate exposure doses using the predicted exposure times as a first approximation. Table 1 shows the fraction of transmitted light, predicted exposure times, actual exposure times required to achieve effective SU-8 polymerization, and actual exposure doses for each of the 5 values of θ_{UV} tested.

The SU-8 film was supported on the glass photomask and exposed to UV radiation from above, thus the photomasks were positioned with the SU-8 film facing down on the stage of the turntable. To avoid damage to the unpolymersized SU-8 film, a sample holder was designed to support the photomask at the corners, minimally affecting the SU-8 thin film, and only in regions isolated from photomask features. The sample holder suspended the photomask approximately 1 cm above the surface of the turntable, preventing the degradation of features that might arise from reflection of UV radiation at the surface beneath the SU-8 film, a problem seen in previous implementations of inclined photolithography.⁴³

Liftoff of the exposed aperture was performed using a removable adhesive material. A supportive layer of low-tack dicing tape was applied to the SU-8 thin film prior to removal from the photomask. This supportive layer was necessary to prevent fracturing in the SU-8 thin film during lift-off, and was removed by rinsing with acetone prior to further utilization of SU-8 apertures. Incomplete polymerization of SU-8 (e.g. from insufficient UV dose) resulted in significant fracturing during lift-off due to increased adhesion between the SU-8 film and the photomask. When SU-8 was sufficiently polymerized, lift-off occurred readily and rapidly with little or no damage to the SU-8 film. Images of SU-8 films after successful and unsuccessful lift-off are shown in the Supporting Information (Figure S3).

Aperture geometry

Aperture geometry is defined by two metrics: 1) the slope of the aperture wall (Figure 1B, θ_w), and 2) the diameter of the aperture opening (Figure 1B, a). θ_w is a function of θ_{UV} , and depends on refraction in the SU-8 medium as defined by Snell's law. Figure 2 shows SEM images of apertures fabricated with θ_{UV} of 15° and 60° (A – C and D – F, respectively) in top-down (A and D), off-axis (B and E), and cross sectional (C and F) views. Although SEM images were needed to observe changes in θ_w corresponding to changes in θ_{UV} in apertures with diameters less than ca. $150 \mu\text{m}$, for larger apertures this phenomenon could be observed by close inspection with the naked eye. The ability to control θ_w is essential to designing apertures with desired geometry, therefore we characterized refraction in the UV exposure process. Figure 3 shows the predicted (dashed line) and experimental (circles) values of θ_w as a function of θ_{UV} . θ_w was predicted by Snell's law using an index of refraction for SU-8

of 1.7,⁴⁸ though this value has only been described for polymerized SU-8. The agreement between observed and predicted n_w suggests that the refractive index of polymerized SU-8 is appropriate for a first approximation of refraction in actively polymerizing SU-8. As predicted, increasing θ_{UV} from 60° to 80° yielded only a 6° increase in n_w ; however, a dramatic increase in the variation of the aperture diameter (10.3% RSD to 16.4% RSD) was observed.

Direct measurement of a was possible from both SEM and transmitted light micrographs (Figure 4A). In transmitted light, the sloped aperture wall reflected incident light, resulting in a dark ring that gave way to transmission at the aperture opening. Measurements of the outer diameter of the dark ring could be used to approximate m ; although, in this study, m was inferred by the position of the aperture on the SU-8 film, since the photomask used in this work (Supporting Information, Figure S1) correlated position with feature size. Figures 4B–F show a as a function of m at θ_{UV} ranging from 15° – 80° ($n_w = 8^\circ - 36^\circ$), respectively. At low θ_{UV} (Figure 4B) low RMS error (RMSE = 2.6 μm) is observed between measured aperture diameters (squares) and those predicted by equation 1 (dashed line). The correlation deteriorates with increasing θ_{UV} to a maximum error (RMSE = 25 μm) at $\theta_{UV} = 80^\circ$. Combined with the trends observed for refraction (Figure 3), the practical limit for θ_{UV} was determined to be 60°.

The data shown in Figure 4 represent the aperture diameters for which fabrication was 100% successful ($n = 4$ or greater). Apertures with diameters as small as 7 μm were prepared, albeit with lower fabrication success rates (typically 25%).

Poor correlation with predictions, and poor reproducibility at high θ_{UV} may result from lengthy exposure times required by the low power UV exposure system. Increased exposure times reduce the fidelity of fabrication due to acid diffusion within the SU-8 film during polymerization.⁴⁹ We predict that increased power density at the site of UV exposure will improve fabrication fidelity at all values of θ_{UV} , leading to increased fabrication success at lower aperture diameters. While future work will address this limitation, the present study demonstrates that n_w can be predicted by Snell's law using the refractive index of polymerized SU-8, and that the current fabrication process yields apertures with 100% success at diameters as low as $22 \pm 1.6 \mu\text{m}$.

BLM formation

Based on the hypothesis that a high n_w would facilitate formation of stable, high-quality BLMs, apertures with $n_w = 30^\circ$ and a ranging from 50 to 100 μm were utilized to investigate the electrical properties of BLMs. Apertures were mounted on 100 μL plastic pipette tips and connected to a patch clamp amplifier (Figure 5A, i) to measure membrane and ion channel conductance. BLMs were formed across microfabricated apertures by manually painting a 10 mg mL^{-1} solution of DPhPC in *n*-decane over the aperture (Figure 5A, ii) using a disposable pipette tip. BLMs formed readily, with area-normalized conductance ranging from 0.02 to 0.05 $\text{pS } \mu\text{m}^{-2}$, and breakdown voltage ranging from 400 to 600 mV, values that agree favorably with previous reports.^{24, 50} BLMs were formed with a 100% success rate ($n=5$ apertures), and were formed more rapidly than those formed on control glass micropipettes (< 15 min vs. ca. 30 min). BLM lifetimes greater than 2 hours on 50 μm diameter apertures ($n=3$) were observed, and were comparable to BLMs formed on modified micropipettes.²⁴ Apertures mounted on plastic pipette tips could be reused daily for more than two weeks with no adverse effects. Device failure was only observed when mechanical failure of the SU-8 film was evident, such as from fracturing by mishandling.

In general, BLMs suspended on planar SU-8 apertures were similar to those formed on glass micropipettes with cyanopropylsilane-modified surfaces.²⁴ Water contact angles measured

by the sessile droplet method reveal that SU-8 surfaces have increased hydrophobicity relative to cyano-modified glass surfaces ($92 \pm 3^\circ$ vs. $72 \pm 2^\circ$, respectively) which may lead to enhanced bilayer stability due to increased lipid tail-substrate interactions at the aperture wall. A comprehensive comparison of bilayer stability in these systems is needed to determine whether SU-8 offers a performance advantage over modified glass pipettes, but the present study reveals that SU-8 apertures offer, at least, comparable performance with the advantage of high-precision, high-reproducibility microfabrication.

Ion channel recordings

Adverse substrate-lipid interactions may lead to formation of multilayer membrane structures,⁵¹ thereby limiting the utility of apertures and the resulting BLMs for studies involving transmembrane proteins. To evaluate the biological function of BLMs formed on SU-8 apertures, ion channel insertion and activity were investigated using α -hemolysin and alamethicin. Insertion of single α -hemolysin channels resulted in an increase in membrane conductance of 0.9 nS (Figure 5, B and C), agreeing well with previous observations.⁵⁰ Fourier analysis of current noise showed decreased spectral intensity and a narrowed distribution of spectral density for α -hemolysin recordings made with SU-8 apertures as compared to recordings made with glass pipette apertures (data not shown). Thus, improved dielectric properties of the polymer materials relative to borosilicate glass pipettes may lead to improvements in noise performance; however, more study is needed to fully characterize noise performance and leakage current mechanisms in SU-8 apertures. The ability to measure single channel insertion of α -hemolysin with characteristic conductance suggests that a lipid bilayer, rather than a multilayer structure, is suspended across the planar aperture. Normal α -hemolysin activity supports the application of microfabricated apertures for IC-based sensors, since a number of sensing technologies utilize chemically modified α -hemolysin.^{52, 53} However, α -hemolysin channels demonstrate predominantly a single static conductance state after insertion into the membrane. The development of sensing technologies based on ligand-gated ion channels will require BLMs with sufficient fluidity to support dynamic protein activity within the membrane. Since lipid-substrate interactions can influence membrane physiology, BLM function for support of dynamic channels was investigated.

Alamethicin is a voltage-gated ion channel that requires a fluid membrane to facilitate ion channel formation by peptide diffusion within the membrane.⁵⁴ Upon insertion into DPhPC bilayers suspended from planar SU-8 apertures, multiple conductance states were observed (Figure 6, A and B) similar to previous reports.⁵⁵ Following further accumulation of monomers, voltage-gated activity (Figure 6C) was observed, which agreed well with previous observations.⁵⁶ The observation of voltage-gated alamethicin activity further supported the presence of a lipid bilayer, and demonstrated that the bilayer was sufficiently fluid to allow normal alamethicin activity. These results suggest that the physiology of bilayers formed on planar SU-8 apertures will prove suitable for studies of other dynamic transmembrane protein systems, and will facilitate the development of new ligand-gated ion channel-based sensing technologies.

Conclusion

Here, we described a new approach for preparing microfabricated planar apertures with well-defined geometries, based on incline and rotate photolithography. This method reduces the complexity and expense involved in previous microaperture fabrication processes, such as silicon-based fabrication, and produces apertures in the common MEMS and microfluidics material, SU-8. Precision control of wall angles and aperture diameters was accomplished by modulating UV incident angle and photomask feature size. BLMs were readily formed on planar SU-8 apertures and demonstrated characteristic conductance,

breakdown voltage, and lifetime. The resulting suspended membranes yielded sufficient membrane fluidity to support peptide diffusion and ion channel activity. The ability to readily form BLMs on microfabricated, sloped wall apertures will contribute significantly to the development of new analytical platforms that integrate membranes and transmembrane proteins for drug discovery, biosensing, and a range of other applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by the National Institutes of Health under grant numbers GM095763 and EB007047. We would like to thank Paul Lee, Michael Heien, Richard Vreeland, and Nick Laude for their assistance and cooperation regarding microfabrication facilities and materials, and Alexandra Petlick for her assistance with SEM imaging.

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Figure 1. Schematic of aperture fabrication by incline and rotate photolithography

A. In the incline, rotate, and lift-off fabrication protocol, sloped-wall apertures are formed by UV exposure of SU-8 to collimated UV radiation at an incident angle of θ_{UV} while the photomask/SU-8 substrate is rotated in-plane through greater than 360° . **B.** Aperture wall angle (θ_w) results from refraction of θ_{UV} . Aperture diameter (a) is determined by photomask feature diameter (m), SU-8 film thickness (t), and θ_w , as defined by equation 1.

Figure 2. SEM images of aperture geometry

Apertures were fabricated with $\theta_{UV} = 15^\circ$ (**A – C**) and $\theta_{UV} = 60^\circ$ (**D – F**) resulting in θ_w of 8° and 30° , respectively. Images are shown from above (**A** and **D**), 30° and 45° off-axis (**B** and **E**, respectively), and in cross-section at 70° off-axis (**C** and **F**).

Figure 3. θ_W as a function of θ_{UV} . θ_W vs. θ_{UV} was predicted by Snell's Law (dashed line), based on a refractive index of 1.7 for the SU-8 film. Observed values of θ_W (circles), were calculated using equation 1 from direct measurements of m , a , and t .

Figure 4. Aperture diameter as a function of photomask feature diameter and UV incident angle
A. SEM images and transmitted light micrographs (inset) allowed for the direct measurement of aperture diameter. The relationship for apertures fabricated with $\theta_{UV} = 15^\circ$, 30° , 45° , 60° , and 80° (**B – F**, respectively) are shown, along with the trend predicted by equation 1 (dashed lines) using the Snell's Law prediction for w .

Figure 5. BLM formation and α -hemolysin insertion on planar SU-8 apertures

A. Apertures were mounted on a modified plastic pipette tip, and connected to a patch clamp amplifier (i). BLMs were formed by manual painting with a lipid:decane solution (ii), and insertion of α -hemolysin (iii) was measured by conductance. **B.** Current was monitored at a bias potential of 40 mV; insertion of α -hemolysin resulted in an increase in ion current across the BLM of 36 pA, corresponding to a conductance increase of 0.9 nS. **C.** Single channel insertions were readily resolved from background conductance, resulting in well resolved populations in the all-points histogram of the current distribution.

Figure 6. Alamethicin activity in aperture-suspended BLMs

A. At incubation times < 1 min, and thus low concentration of inserted alamethicin, multiple conductance states were observed at a bias potential of 100 mV. **B.** The all-points histogram of the current distribution shown in **A** revealed six distinct conductance states, each resolved by 2 pA (20 pS). **C.** Voltage-gated activity was observed as the bias potential was increased from 0 mV (i) to 20 mV (ii), 40 mV (iii), 60 mV (iv), and subsequently returned to 0 mV (v). Current traces are offset for clarity.

Scheme I. Effects of aperture geometry on effective BLM area

Table 1

UV exposure time and dose.

UV	Transmitted fraction	Predicted exposure time (min)	Actual exposure time (min)	Actual exposure dose (mJ cm ⁻²)
15°	0.953	6.5	10	574
30°	0.951	6.5	10	574
45°	0.931	6.7	10.5	589
60°	0.902	6.9	11	598
80°	0.604	10.3	15	547