

Microfabricated Silicon Apertures for Ion Channel Measurement

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ABSTRACT

Well-known cleanroom microfabrication technologies have been used to etch an aperture in silicon, pattern a capacitance reducing SU-8 layer and passivate the surface with polytetrafluoroethylene to form an integrated support to study specific ion channel proteins. The silicon support has demonstrated successful lipid bilayer sealing measurements with repeatable seal resistances in the giga-ohm range. Characteristic measurements of OmpF porin ion channel proteins have been made.

Keywords: microfabrication, ion channel, sensor

1 INTRODUCTION

Cells are made up of phospholipid bilayer membranes that serve as high resistance impermeable barriers to the flow of charged ions between the intra and extra-cellular regions. Ion channels are proteins that form a pore across the cell membrane so that specific ions can pass more easily through the cell wall. The conductance of the channel towards the specific ions changes due to voltage or ligand gating events and local salt concentration. Recordings of ionic current are made by keeping the transmembrane voltage constant while observing step current fluctuations due to the gating mechanisms. Patch clamping is a technique that allows for physiological measurements of single ion channel proteins by sucking a cell into a glass pipette and forming a GΩ seal [1]. A stable high resistance GΩ seal is crucial to limit leakage current and enable low noise measurements of single proteins [2, 3].

Planar substrates with micrometer sized diameters have recently been used to span phospholipid bilayers so that ion channels can be measured in an artificial environment. This allows for the study of only the particular ion channels of interest added into the system. Glass [4, 5], plastics [6, 7] and Si/SiO₂ [8-12] have been used as the substrate to span lipid bilayers and record ion channel activity. Glass apertures have been fabricated with heavy ion irradiation and wet etching to form low capacitance devices suitable for low noise bilayer measurement [4, 5]. Small holes have also been punched in Teflon AF and polystyrene using a metal stylus so that a conical shaped aperture for planar bilayer experiments is formed [6, 7].

Current state of the art silicon technology has the advantage of precise micromachining capabilities with high throughput facilities. In previous articles we have demonstrated the fabrication of a silicon aperture that has been hydrophobically functionalized to allow for stable high resistance bilayer membranes [8]. In this article, similar techniques were used to fabricate an aperture of 150μm diameter in a thinned silicon substrate with an additional layer of SU-8 added to reduce the capacitance. The surface was rendered hydrophobic with chemical vapor deposition of a polytetrafluoroethylene (PTFE, Teflon) surface layer. The samples were then tested in a bi-chambered Teflon cell using Montal Mueller techniques [13] to form stable bilayers and insert OmpF porin ion channel proteins.

2 EXPERIMENTAL

Samples were prepared using 4", double-sided polished Si (100) wafers having a thickness of 440 microns. The aperture was designed to have a 150μm diameter similar to that currently used for Teflon devices [7]. An aspect ratio of 1:1 of the diameter to height of the aperture is desirable for planar lipid bilayer formation [14] so a central region of 1mm diameter was thinned to a final thickness of 150μm. The substrates were patterned using photolithography and standard AZ4330 resist and then etched in a deep silicon reactive ion etcher (STS Advanced Silicon Etcher) using the Bosch process. After etching of the aperture, a thermal oxidation of 200nm followed to produce an electrically insulating layer on the surface. The device was then coated with 75μm of SU-8 and patterned with conventional photolithography so resist entered the thinned region and decreased the overall capacitance of the device (Figure 1). Finally, a Teflon layer was chemically vapor deposited using the deep etcher and C₄F₈ as the gas source and measured with a Woolam ellipsometer.

Lipid bilayer experiments were performed using a Teflon bilayer chamber with a 5 mm diameter opening in between two baths of electrolyte solution. Both baths were filled with 3 ml of 1 M KCl solution, buffered with 20 mM N-(2-Hydroxyethyl) piperazine-N'- (2-ethanesulfonic acid) (HEPES) at pH 7.4. The device was sandwiched between the baths with the aperture in the center of the opening. Lipids (1,2-Dioleoyl-sn-Glycero-3-Phosphoethanolamine and 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine) (DOPE: DOPC, 4:1) were dissolved in n-Decane (10mg/ml) and

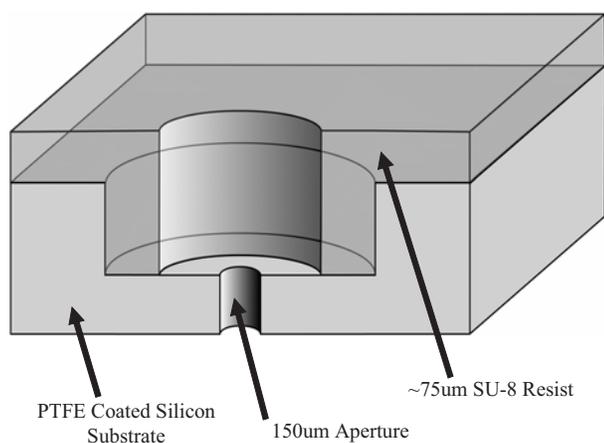


Figure 1: Conceptual drawing of silicon substrate device for transmembrane protein characterization where 75µm of SU-8 has been patterned into a thinned aperture and then coated with Teflon.

used to form a bilayer with the techniques of Montal and Mueller [13]. Current and bilayer capacitance were measured using an Axon Instruments Axopatch amplifier [15], a Stanford Research Systems SRS 830 lock-in amplifier and a National Instruments DAQ PCI card programmed with Labview software. Recordings were performed at a sampling rate of 5kHz and filtered with a four-pole low pass filter. The bilayer resistance was derived from the slope of the current trace. Additionally, it was checked if the layer formed could be broken by the application of a short voltage pulse with $V_{\text{pulse}} > 0.5 \text{ V}$. Ion channels were inserted into the membrane by adding OmpF porin to the trans (ground side) bath.

3 DISCUSSION AND RESULTS

Devices were fabricated such that the aperture closely resembles those found in current planar device geometries with a 1:1 aspect ratio. The aspect ratio is important because it determines the shape of the torus and affects the formation and stability of the final bilayer membrane. A 75µm layer of SU-8 resist, dielectric constant of ~ 3 , was then patterned over the thinning recess to reduce the capacitance of the device. SU-8 is a chemically amplified epoxy based negative resist that allows for high aspect ratios and smooth sidewall features. Figure 2 shows the final device with SU-8 patterned in the etched recess surrounding the 150µm aperture.

It is important to reduce the capacitance of the device in order to decrease the noise of the recordings and increase the possible recording bandwidth. The interaction of the input voltage noise of the amplifier headstage with the input capacitance of the device (septum capacitance coupled with the electrode and membrane capacitance) as well as the dielectric noise due to thermal fluctuations in lossy

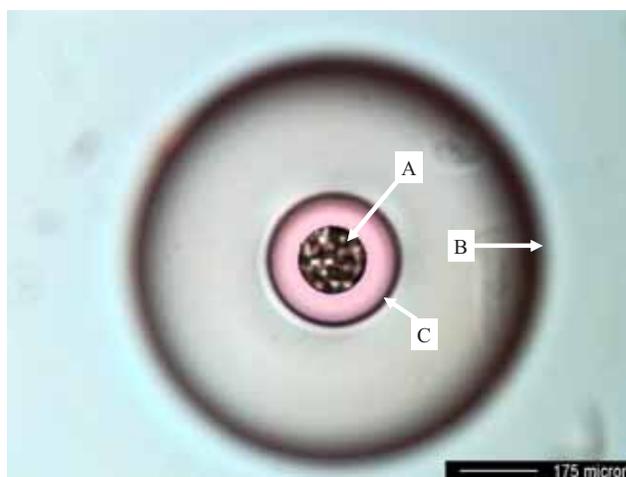


Figure 2: Optical micrograph of device showing (A) the inner aperture of 150µm, (B) the outer thinned region and (C) the SU-8 layer for capacitance reduction.

dielectric materials limits the minimum total rms noise of the measurements. A more in-depth discussion of the noise factors in such measurements can be found in references [2, 6, 7]. The recording bandwidth is proportional to the inverse of the input capacitance, so a decrease in device capacitance will increase the overall bandwidth of the device. High bandwidth is desirable because it enables the recording of fast channel gating events. The capacitance of the devices was found to be $20 \pm 5 \text{ pF}$ using the lock-in amplifier or by applying a triangular waveform and measuring the current response [15].

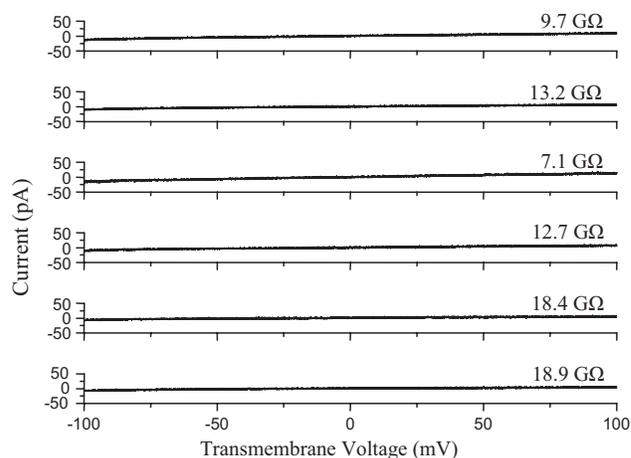


Figure 3: Current-voltage traces of bilayers formed and reformed on silicon substrate device with capacitance reducing SU-8 layer. Initial formation on a fresh sample (bottom plot) followed by bilayers reformation after rupturing using Montal Mueller techniques.

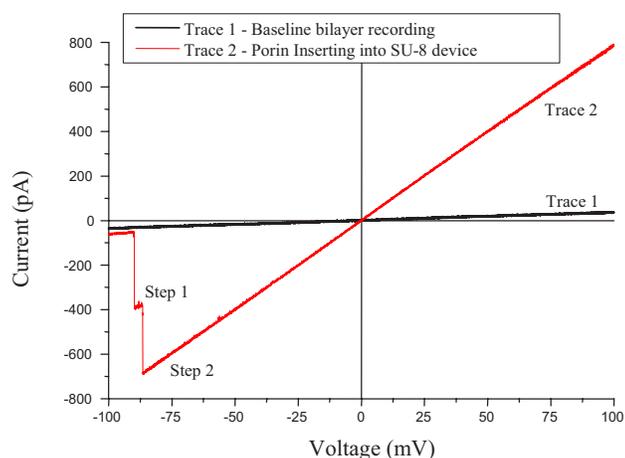


Figure 4: Current-voltage plots of the insertion of two trimers of OmpF porin into lipid bilayer membranes. Trace one shows the baseline before protein insertion with a seal resistance of 2.7 G Ω . Trace two shows the insertion of a single OmpF porin ion channel protein (step 1) followed by a subsequent insertion of a second protein (step 2).

In order to form high resistance stable bilayer membranes the surface of the device must be rendered hydrophobic. A hydrophobic surface lowers the surface energy helping to increase contact with the lipid hydrocarbon chains [13] and allow formation of a high resistance seal. Recently, apertures have been functionalized using self-assembled monolayers to enhance attraction between the substrate and n-decane lipid solvent [10].

After patterning the SU-8 layer, Teflon was vapor deposited on the surface of the device. Teflon has been chemically vapor deposited on substrates [16-18] and has been shown to have better adhesion than spin coated or evaporated films [17]. It serves as a hydrophobic passivation layer with contact angles of 108° [17]. The hydrophobic properties of Teflon make it ideal for lipid bilayer experiments because it enhances the attraction between the lipid tails and substrate and enables formation of a G Ω seal. The additional Teflon layer has previously been demonstrated to enable reproducible formation of a high resistance seal between the bilayer and substrate for ion channel measurements [8].

After device fabrication lipid bilayers were formed across the aperture using Montal Mueller techniques and the OmpF porin ion channel protein was inserted into the membrane by adding it to the trans bath. Multiple bilayers were formed and ruptured on one device with a mean resistance of 9.6 G Ω before addition of the protein. Reformation of the bilayer after rupturing with a short voltage pulse helps ensure phospholipids have not blocked the aperture and have formed a true lipid bilayer. Figure 3 shows the initial bilayer followed by bilayers formed after

rupturing. These bilayers were formed over the span of several hours and showed reproducible G Ω seal formations.

After formation of several bilayers, OmpF porin was added to the trans bath of the experimental setup and the bath was stirred until protein insertion occurred. Figure 4 shows the baseline current recording of a bilayer with resistance of 2.7 G Ω (Trace 1) and then the insertion of two OmpF porin proteins (Trace 2). OmpF porin ion channel proteins are trimers with three channels and in 1M KCl solution the conductance of a single channel is about 1.2nS [19]. The first step in the graph corresponds to the conductance of one porin protein (three open channels) and the second step corresponds to the insertion of a second protein. The lower seal resistance of the bilayer can be attributed to the addition of detergent during protein insertion. The detergent changes the molecular orientation of the lipids in the bilayer and helps to facilitate protein insertion.

4 CONCLUSION

Silicon apertures have been fabricated using well-known cleanroom technologies for the measurement of ion channel proteins. Microfabrication of the aperture offers the advantage of precise control of the diameter and high volume throughput over the common method of drilling or burning a hole in a thin sheet of Teflon. After etching the aperture a capacitance reducing layer of SU-8 was patterned and the surface was hydrophobically modified with Teflon. The ion channel protein porin OmpF was inserted into lipid bilayer membranes formed across the aperture and characteristic current voltage measurements of the protein were made. Using microfabrication techniques and silicon as a substrate offers the advantage of integrating microelectronics onto the device and the fabrication of parallel apertures for high throughput screening methodologies.

This work was supported by the Defense Advanced Research Projects Agency as part of the MOLDICE program.

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