Teflon™-coated silicon apertures for supported lipid bilayer membranes

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We present a method for microfabricating apertures in a silicon substrate using well-known cleanroom technologies resulting in highly reproducible giga-seal resistance bilayer formations. Using a plasma etcher, 150 μm apertures have been etched through a silicon wafer. Teflon™ has been chemically vapor deposited so that the surface resembles bulk Teflon and is hydrophobic. After fabrication, reproducible high resistance bilayers were formed and characteristic measurements of a self-inserted single OmpF porin ion channel protein were made. © 2004 American Institute of Physics. [DOI: 10.1063/1.1805712]

Ion channels are transport proteins that form an ion specific pore across lipid bilayer membranes. Precise electrophysiological techniques allow for the measurement of ion currents by keeping transmembrane voltages constant. The patch clamp is one such technique where a cell is sucked into a glass pipette forming a stable high resistance GΩ seal. This stable seal is crucial for the recording of few to single ion channel protein activities.

Recently, progress has been made to transform the patch clamp methodology to a planar microfabricated aperture. Lipid bilayers have been spanned across glass, polytetrafluoroethylene (PTFE), and polystyrene.5–6 One such recent advance is the etching of a glass aperture using irradiation with a single heavy ion and then wet track etching a final hole for bilayer measurements. Another is the fabrication of 5–200 μm punched holes in amorphous Teflon (Teflon AF) and using the device in a Teflon fluid cell for higher frequency measurements. This method allows for a more precise control over the aperture than the now common methods of burning or drilling a hole in a thin sheet of Teflon.

One important issue is the surface properties of the device in the regions interacting with the lipid bilayer. Modern methods of ion channel electrophysiology use Teflon substrates with machined apertures for artificial bilayer formation with either painting or Montal Mueller techniques. Hydrophobic surfaces are required for bilayer formation because they favor contact with the lipid hydrocarbon chains. Recently, apertures have been formed in a silicon substrate and have been hydrophobically functionalized using self-assembled monolayers. Functionalization of the surface enhances the attraction between the substrate and n-decane solvent to help in tight seal formation.

Teflon has been chemically vapor deposited (CVD) on substrates and measured for surface property agreement with bulk Teflon material. PTFE has been shown to be a highly stable passivating layer with contact angles of 108°. The high surface energy of the PTFE makes it ideal for lipid bilayer experiments because it enhances the attraction of the tails of the lipids and enables easier formation of a GΩ seal between the bilayer and aperture. Adhesion of CVD films is much better then spin coated or evaporated films. In this letter we report the results of a PTFE coated microfabricated aperture of 150 μm diameter in a thinned silicon substrate. Emphasis was put on using common silicon processing techniques to fabricate a hydrophobic aperture. Samples were prepared and an ellipsometer was used to check for similar surface properties to that of bulk Teflon. The samples were then tested in a bi-chambered Teflon cell using Montal Mueller techniques to form stable bilayers and a porin OmpF protein was inserted.

Common silicon processing techniques allow for precise control of device parameters on the micron level, which makes silicon an ideal substrate for fabricating reproducible apertures. Samples were prepared using 4in., single sided polished Si (001) wafers having a thickness of 500 μm. The substrates were patterned using photolithography and etched in a deep Si reactive ion etcher (STS Advanced Silicon Etcher) using the Bosch process. For bilayer attachment, the aspect ratio of aperture diameter to sidewall length should be approximately 1:1. In order to achieve this aspect ratio, the wafer was thinned to 150 μm thickness in the area where the aperture was formed using the deep silicon etcher. Once the wafer was thinned, an aperture of 150 μm was etched though the center of the thinning region from the back side [Fig. 1(a)]. After etching, a thermal oxidation of 200 nm followed to produce an electrically insulating layer on the surface. A Teflon (PTFE) layer was then chemically vapor deposited using the STS deep etcher [Fig. 1(b)] and characterized with a Woolam ellipsometer.

A unique feature of this design is the PTFE layer (deposited from chemical vapor) with very similar properties to bulk Teflon. This layer is critical to bilayer formation because it renders the surface hydrophobic, which enables routine giga-seal formation. An ellipsometer was used to characterize the Teflon coating deposited on the surface of the device. The resulting measurements were compared to that of published deposited and bulk Teflon material prop-

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erties. Using an isolated Cauchy model, a very good ellipsometric fit of the change in the polarization state of the beam upon reflection from the sample \( \Psi, \Delta \) (degrees) was obtained.\(^{13}\) The refractive index of the deposited Teflon \( n = 1.36 \) compared very closely to that of bulk Teflon \( n = 1.40 \).\(^{11}\) The reason that it was slightly lower is because the deposited Teflon is more amorphous in nature than bulk Teflon.\(^{16}\) A contact angle of 110° was measured showing the hydrophobicity of the device after surface preparation and compared closely to measured values of such films.\(^{11}\)

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 \( \text{N-(2-Hydroxyethyl)piperazine-N'-}(2\text{-}\text{ethanesulfonic acid}) \) (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 hexane and used to form a bilayer with the techniques of Montal and Mueller. Current and bilayer capacitance were measured using an Axon Instruments Axopatch 200B amplifier in conjunction with the pClamp software.\(^{17}\) Recordings were performed at a sampling rate of 5 kHz and filtered with a four-pole lowpass Bessel filter with a cutoff frequency of 2 kHz. The bilayer resistance was derived from the slope of the current trace and the capacitance from the charging transient.

After initial formation using the painting technique, the bilayers were ruptured with a short voltage pulse of \( V_{\text{pulse}} \) greater than 0.5 V and then reformed using the same method. The device was cleaned with ethanol and reused to demonstrate robustness of the PTFE layer and reusability of the device. In order to determine the seal resistance of formed bilayers, current–voltage measurements were made and the seal resistance was extrapolated from their slopes. Initial re-

![FIG. 1. Conceptual drawing of fabricated aperture and surface treatment of device for lipid bilayer measurements. (a) 150 \( \mu \)m aperture etched through thinned silicon. (b) Surface treatment showing 200 nm thermal oxide and 60 nm Teflon layer on device surface.](image1)

![FIG. 2. Current–voltage traces of bilayers formed and reformed on silicon substrate aperture. Initial formation on fresh sample (bottom plot) followed by bilayers reformed after rupturing using the painting method.](image2)

![FIG. 3. Histogram showing repeated formation of bilayer \( \Omega \) seals on reusable Teflon coated silicon apertures. These measurements were made on one device after multiple cleaning washes with ethanol.](image3)
Once a bilayer was formed and measured for giga-seal resistance, an OmpF porin protein was self-inserted into the membrane by introducing it to the trans bath. Figure 4 shows the current recording of characteristic single channel properties of the protein. The recordings were performed with a holding potential of 120 mV while changes in current were measured. Using the height of the current change, the average conductance of a single channel was measured to be 1.1 nS, which compared closely to measurements of OmpF channels.\textsuperscript{18,19}

In summary, Teflon surface modifications (PTFE deposited from chemical vapor) of a silicon substrate based device allows formation of stable bilayers across its aperture. The surface properties of the device were measured with an ellipsometer to show they were comparable to bulk Teflon. Artificial bilayers showed sealing resistance in the G\Omega range, which made for repeatable bilayer formation and measurement of an OmpF porin ion channel proteins. This device can be easily scaled and manufactured using high throughput silicon technologies and integrated with microelectronics to form ion channel sensors.

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