Ion Channels on Silicon*

S. J. Wilk,[†] L. Petrossian, M. Goryll, T. J. Thornton, and S. M. Goodnick
Arizona State University, Center for Solid State Electronics Research, Tempe, AZ 85287, USA

J. M. Tang and R. S. Eisenberg
Rush Medical College, Department of Molecular Biophysics and Physiology, Chicago, IL 60612, USA

M. Saraniti
Illinois Institute of Technology, Department of Electrical and Computer Engineering, Chicago, IL 60616, USA

D. Wong, J. J. Schmidt, and C. D. Montemagno
UCLA, Department of Bioengineering, PO Box 951600, Los Angeles, CA 90095, USA
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We present results showing that silicon substrates can be used as a universal platform for recording the electrical activity of ion channels inserted into suspended bilayer membranes. The bilayers span 150 μm apertures etched into silicon substrates using standard microelectronics processing techniques. The silicon is oxidized, patterned with a 75 μm thick SU-8 epoxy resist and then coated with a thin layer of polytetrafluoroethylene rendering the surface hydrophobic. Reversible Ag/AgCl electrodes are integrated around the circumference of the opening and provide long-term stable measurements of the ion channel currents. Characteristic measurements of OmpF porin ion channel protein in phospholipid bilayers and α -hemolysin toxin protein in triblock copolymer layers were made. Long-term measurements showed that ion channel activity could be recorded 22 hours after initial formation of a lipid bilayer. [DOI: 10.1380/ejsnt.2005.184]

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I. INTRODUCTION

Cell membranes are made up of phospholipid bilayers that serve as high resistance impermeable barriers to the flow of charged ions and small molecules. Ion channels are proteins that form a pore across the cell membrane so that specific ions can pass through the cell wall. These proteins are of considerable interest because they are used to send signals throughout the human body. The patch-clamp was first demonstrated to measure single ion channel proteins in 1976 by Neher and Sakmann [1] and is able to form giga-ohm ($\text{G}\Omega$) seals between a pipette tip and cell membrane by sucking the cell into the pipette [2]. A stable high resistance seal is crucial for low noise measurements because it reduces electrical noise due to thermal currents and isolates different sides of the membrane [3, 4].

Recently, there has been considerable effort to planarize the patch-clamp setup into a high throughput system for possible pharmaceutical applications. Apertures in different substrates have been designed for both patch-clamp type experiments and ion channel reconstitution. Ion channel reconstitution takes advantage of the natural properties of lipids to spontaneously form a lipid bilayer and then insert particular proteins of interest. Glass [5–7], Si/SiO₂ [8–11], polytetrafluoroethylene [12] and silicon elastomers [13] have been used as the substrate in these devices to span lipid bilayers and record ion channel activity. A glass aperture has been fabricated with heavy

ion irradiation and wet etching to form a low capacitance aperture suitable for low noise bilayer measurement [5–7]. Silicon has been used as a substrate and then coated with polytetrafluoroethylene (PTFE, Teflon) to form stable high resistance, repeatable seals between painted lipid bilayers and the device [9]. Silicon devices have also been integrated with polydimethylsiloxane (PDMS) microfluidic channels to help direct single cells to the aperture [10]. Measurements with single apertures are possible in these devices but there are still significant obstacles to a fully integrated device.

The surface properties of the aperture are very important because the contact between the lipid hydrocarbon chains and aperture determine the stability of the membrane and may also be involved in protein insertion. Apertures must have hydrophobic surfaces to increase contact with the lipid [14] and to 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 [8]. Teflon has also been chemically vapor deposited onto a device resulting in a hydrophobic aperture [9].

Microfabrication of the aperture allows precise control of the diameter and high volume throughput. Using silicon as the substrate enables the use of cleanroom microfabrication techniques already well characterized for this material. In this article, such techniques were used to fabricate an aperture of 150 μm diameter in a thinned silicon substrate, coat the surface with $\sim 75 \mu\text{m}$ of SU-8 epoxy resist and fabricate electrodes on the surface of the device. The surface was then made hydrophobic with chemical vapor deposition of a PTFE surface layer. The samples were tested in a bi-chambered Teflon cell using Montal-Mueller techniques [14] to form stable bilayers and insert OmpF porin ion channel proteins. The samples were then used to test the formation of a biomimetic membrane and

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[†]Corresponding author: seth.wilk@asu.edu

FIG. 1: Conceptual drawing of the completed device showing the capacitance reducing SU-8 layer and integrated silver/silver chloride electrodes.

insert an α -hemolysin protein [15].

II. EXPERIMENTAL

Samples were prepared using 4", double-sided polished Si (100) wafers having a thickness of 440 μm . The aperture was designed to have a 150 μm diameter similar to that currently used for Teflon and commercial devices. An aspect ratio of 1:1 (diameter to height of the aperture) is desirable for planar lipid bilayer formation so a central region of 1mm diameter was thinned to a final thickness of 150 μm . 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 200 nm followed to produce an electrically insulating layer on the surface. The device was then coated with 75 μm of SU-8 and patterned with conventional photolithography, so that resist entered the thinned region thus decreasing the overall capacitance of the device (Fig. 1). Next, 8000 Å of silver was evaporated onto the surface of both sides of the wafer with a CHA 600-SE electron beam evaporator. The silver layer was patterned using conventional lithography and then etched with a 1:1:20 mixture of sodium hydroxide, hydrogen peroxide and water. On the side of the device with oxide exposed, a 20 nm adhesion layer of titanium was deposited prior to silver deposition. A Teflon layer was then chemically vapor deposited using the deep etcher and C_4F_8 as the gas source. Finally, the electrodes were chloridized in 5% NaOCl before experimental measurements were taken. All fabrication was performed at Arizona State University in the Center for Solid State Electronics Research cleanroom.

Lipid bilayer experiments were performed using a Teflon bilayer chamber with a 5 mm diameter opening between two baths of electrolyte solution. Both baths were filled with 3 ml of 0.5M KCl solution, buffered with 20 mM N-(2-Hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid) (HEPES) at pH 7.4. The de-

vice 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 (10 mg/ml) and used to form a bilayer with the techniques of Montal and Mueller [14]. Current and bilayer capacitance were measured using an Axon Instruments Axopatch amplifier, a Stanford Research Systems SR 830 lock-in amplifier and a National Instruments DAQ PCI card programmed with LabView software. Recordings were performed at a sampling rate of 5 kHz and filtered with a four-pole low-pass Bessel filter with cutoff frequency of 2 kHz. The bilayer resistance was derived from the slope of the current trace. It is important to test that the membrane was a bilayer that could be broken by short voltage pulses with $V_{\text{pulse}} > 0.5$ V. Thick 'membranes' of lipid cannot be broken by such pulses. Ion channels were inserted into the membrane by adding OmpF porin to the trans (ground side) bath.

Copolymer experiments were performed with polymethyloxazoline-polydimethylsiloxane-polymethyloxazoline (PMOXA-PDMS-PMOXA) [15]. The copolymer solution was prepared by initially diluting the copolymer molecules in chloroform (2 wt% polymer). This solution was then diluted further with toluene (1 wt% polymer) [16]. Measurements were performed using the same Teflon holder as described above with 1 M KCl solution buffered with HEPES at pH 7.4. A sawtooth waveform at a known frequency and voltage was applied to determine the membrane capacitance. α -hemolysin (αHL) membrane protein was added to the trans bath and self inserted into the membrane. Polymer membrane preparations and corresponding measurements were performed at the University of California, Los Angeles [15].

III. RESULTS AND DISCUSSION

Common silicon processing techniques allow for precise control of device parameters on the micron level. For bilayer attachment, the aspect ratio of aperture diameter to sidewall length should be 1:1 [17]. The aspect ratio is important because it determines the shape of the region where the bilayer meets the support, the 'torus region'. In order to achieve this aspect ratio, a 1mm circular area of the wafer was thinned to ~ 150 μm final thickness in the area where the aperture was formed. Once the wafer was thinned, an aperture was etched through the center of the thinned region using backside alignment lithography and the reactive ion etcher. SU-8 was then patterned into the thinned region and onto the surface of the device in order to reduce the capacitance. SU-8 is a thick, photolithography patternable, epoxy resist with dielectric constant of ~ 3 that is commonly used for MEMS applications because it can withstand many different solutions.

It is important to reduce the capacitance of the device in order to decrease the noise of the recordings and increase the 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

FIG. 2: Micrograph showing the final aperture from the SU-8 side of the device. The aperture (A) is surrounded by a capacitance reducing SU-8 layer (B) with integrated silver/silver chloride electrodes on the surface of the device (C).

lossy dielectric materials limits the minimum total rms noise of the measurements [3, 12, 18]. 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 needed to record fast channel gating events. The capacitance of the device was found to be 20 ± 5 pF as measured by the lock-in amplifier, or by applying a triangular waveform and measuring the current response [15].

Measurements of the lipid bilayer and ion channel electrical properties require reference electrodes in the bath solutions. Commonly, silver/silver chloride (Ag/AgCl) electrodes are made by inserting silver wire into 5% NaOCl and chloridizing the surface. It is important to maintain an adequate layer of AgCl on the electrode so the electrode potential does not drift and invalidate the measurement [19, 20]. Electron beam evaporation was used to deposit 8000 Å of silver onto both sides of the device. The deposited layer of silver must be thick enough so that the electrodes can be adequately chloridized and still have a remaining silver layer under the AgCl. Thin silver layers would be consumed during chloridization and the resulting silver chloride layer would lift off during measurements. After silver evaporation, the electrodes were patterned around the aperture using conventional lithography and wet etching techniques. Figure 2 shows an optical micrograph of the final device.

Teflon has been chemically vapor deposited (CVD) on substrates [9, 11, 21, 22] and has been shown to have better adhesion than spin coated or evaporated films [21]. Teflon serves as a highly passivating layer with water contact angles of 108° [21]. The hydrophobic properties of Teflon make it ideal for lipid bilayer experiments because it enables formation of a GΩ seal probably because of interaction of lipid tails [14] and substrate. We have previously demonstrated the fabrication of a Teflon coated device with repeatable high resistance lipid bilayer sealing resistances [9]. Similar methods have been used here for surface modification of the device.

FIG. 3: Current recordings of OmpF porin protein inserted into a lipid bilayer suspended on a commercial bilayer cuvette (Warner Instruments). The plot shows single channels opening and closing due to an applied potential of -200 mV in 0.5 M KCl solution.

In order to compare the measurements of the silicon based device to commercially available apertures, porin ion channels have been measured using a standard commercial polystyrene bilayer cuvette from Warner Instruments (Fig. 3). After formation of a stable lipid bilayer, OmpF porin ion channel protein was added to the trans bath and then self-inserted into the membrane after several minutes of stirring. Figure 3 shows porin gating characteristics measured at a holding potential of negative 150 mV where the steps represent changes in conductance due to opening and closing of single channels in the protein. The porin is a trimer with three individual channels that open or close due to an applied potential across the membrane. As the potential increases, the channels are more likely to close and reduce the total current through the system. The conductance of the channels is also determined by the concentration and pH of the solution used for experimentation.

For measurements with integrated electrodes, the silicon device was sandwiched between the two cells of the Teflon chamber so that the etched silicon aperture formed a central hole between both baths. Small leads were attached to the electrodes on the device using conductive silver epoxy. The painting method allowed repeatable seal resistances in the GΩ range.

After bilayer formation on the silicon device, OmpF porin was introduced to the trans bath and self inserted. Figure 4 shows a current recording from the bilayer and subsequent open trimer channel properties of the OmpF porin channel protein in the silicon device. Figures 3 and 4 show that the measurements made with the silicon device were very similar to that of the commercial bilayer cup. The average conductance of a single channel (measured from the size of the step current) was ~ 0.7 nS which is very similar to other measurements of OmpF porin channels in 0.5M KCl solution [23, 24].

Long-term stability of the lipid bilayer suspended across

FIG. 4: Current recordings of OmpF porin protein inserted into a lipid bilayer suspended on a microfabricated silicon device. The plot shows single channels opening and closing in 0.5 M KCl due to an applied potential of -200 mV measured with silver/silver chloride electrodes integrated into the device.

FIG. 5: Current-voltage traces of OmpF porin measured with silver/silver chloride electrodes integrated into silicon device 22 hours after initial bilayer formation and protein insertion. Insets show single exploded region of the trace with steps corresponding to the single channel conductance of the protein.

the integrated device was checked in preliminary experiments measuring channel activity 22 hours after initial lipid bilayer formation. Figure 5 shows a large increase in the conductance of the bilayer that is attributed to several different factors. The standard method for inserting ion channels into the bilayer requires the addition of ion channel proteins into the bath solutions. Over time, more proteins insert into the membrane and increase the overall conductance. Detergent is also added to the system during protein insertion and changes the interaction between the lipids and substrate surface. The detergent tends to reduce the long-term seal resistance between the

FIG. 6: Current recordings of α -hemolysin toxin inserted into a triblock copolymer membrane suspended on a microfabricated silicon device. The plot shows single channels opening and closing due to an applied potential of 80 mV measured with silver/silver chloride electrodes integrated into the device. Inset shows structural formula of polymer (PMOXA-PDMS-PMOXA) [15].

lipids and substrate and increase the overall conductance of the system. Even though the overall conductance has increased, it is still possible to reliably measure single channels opening and closing in response to an applied potential (Insets in Fig. 5). These steps correspond to single channels with conductance ~ 0.7 nS and is the same measured value seen in Figs. 3 and 4.

Membranes have also been formed with a polyoxazoline based ABA triblock copolymer on the silicon microfabricated aperture. The copolymer forms a membrane with a thickness of approximately 4 nm [15]. The center of the copolymer consists of a flexible and hydrophobic PDMS block with two water soluble PMOXA side blocks [25]. Stable $G\Omega$ seals were formed with resistances as high as 67.1 G Ω .

The transmembrane protein α -hemolysin was successfully inserted into planar polymer membranes formed using the triblock copolymer. α HL is a mushroom-shaped homo-oligomeric heptamer containing a channel with a length of approximately 100 Å [26]. The overall protein conductance is determined by the opening and closing of the seven monomers that conduct either simultaneously or separately and changes linearly with the conductivity of the bath solutions [27].

Following membrane formation, α HL was introduced into the trans bath and stirred to increase the probability of protein-membrane interaction. After several minutes α HL successfully self inserted into the membrane and stirring was discontinued. Characteristic measurements of the α HL were made by applying a holding potential of 80 mV, and can be seen in Fig. 6. The conductance of the protein was determined to be ~ 770 pS and is similar to values reported in current literature [27].

IV. CONCLUSION

We have demonstrated the fabrication of a silicon based device with integrated electrodes to measure conductance changes of single ion channels. The Bosch process was used to etch an aperture of 150 μm diameter in a silicon substrate and silver/silver chloride electrodes were fabricated on the surface. Teflon was then chemically vapor deposited on the surface to help ensure a high resistance seal with a lipid bilayer. Repeatable formations of a lipid bilayer across the hole were achieved and seal resistances in the $\text{G}\Omega$ range were measured. Characteristic measurements of OmpF porin channels were made on the silicon device using the integrated electrodes and found to be similar to those made on a commercial bilayer cup. Measurements of ion channel activity 22 hrs after initial formation were made using the silicon substrate device.

The device was also used to measure α -hemolysin toxin that was inserted into a biomimetic triblock copolymer membrane. Using silicon as the substrate has the added advantage of easily scaling the aperture to a much smaller diameter and integrating microelectronics into the device. With further investigation the device could be applied as an integrated biosensor used to measure conductance changes due to interactions between transmembrane proteins and local environmental changes.

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