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Silicon-based ion channel sensor

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Abstract

In this paper we present a method to fabricate an aperture in a silicon wafer that can be used to suspend a freestanding lipid bilayer membrane. The design offers the feature of scalability of the aperture size into the submicron range. Lipid bilayer membranes formed across the aperture in the oxidized silicon substrate show a gigaohm sealing resistance. The stability of these membranes allowed the insertion of a nanometer-sized ion channel protein (OmpF porin) and the measurement of voltage dependent gating that can be expected from a working porin ion channel.

Keywords: Biosensors; Lipid bilayers; Silicon devices

1. Introduction

The integration of biosensors with silicon-based readout circuits is a field of current interest. By combining detection of biochemical reagents and signal processing on a monolithic chip, sensors can be made much more versatile and robust. The crucial point in the fabrication of such a sensor is the biochemical detection mechanism. Here, gated ion channel proteins inserted in lipid bilayer membranes could serve as signal transducers that allow the detection of a change in ionic current when a biochemical agent is present. Ion channels have the advantage of being highly selective while being extremely sensitive. Atomic events (binding of an ion) are converted into a macroscopic signal, and a current of picoamps flows from one macroscopic reservoir to another, just as field effect transistors convert the ‘binding’ of a small charge on a gate into a macroscopic current from

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source to drain. Ion channels inserted in suspended artificial lipid bilayer membranes are already used in a variety of biological and chemical detection applications [1]. However, measurements using ion channel gating still require a rather sophisticated and delicate laboratory setup. To make ion channel sensors more easy and practical to use, while providing reliable and reproducible performance, the problem of suspending the host lipid bilayer membrane on a suitable substrate must be addressed [2–15].

Currently, lipid bilayer supports are usually apertures in Teflon (PTFE) films that have been created either mechanically [2] or by electrical discharge: the reproducibility of these apertures is limited as well as their minimum size.

In this paper we demonstrate a process using only well-established microfabrication tools such as UV photolithography, deep silicon reactive ion etching using a cyclic etch/passivation process (Bosch process) [16] and thermal oxidation to create a support for lipid bilayers. This approach allows a significant downscaling of the bilayer area and the integration of reversible electrodes and even of detection electronics on the same chip. The latter point is the reason why silicon was chosen as the substrate over glass [12–14]. While similar approaches use a silicon nitride layer as support [8–10], our process provides electrical isolation using only silicon that is thermally oxidized after the aperture has been etched. We will show that a lipid bilayer suspended across such an aperture forms a gigaseal even without the necessity for specific surface treatment of the silicon [10, 11]. These bilayers allow the measurement of the behavior of an OmpF porin ion channel inserted into the membrane.

2. Experimental

Samples were prepared using 2″ Si(001) substrates having a thickness of 270 μm. The substrates were patterned using photolithography. To protect the silicon during the subsequent etch process, a 2.4 μm thick positive photoresist (AZ 4330) was used. The resist was exposed by a UV laser-based direct writing system (Heidelberg Instruments DWL66) that allows backside substrate alignment. The samples were etched in a deep Si reactive ion etcher (STS Advanced Silicon Etcher) using the Bosch process [16]. After etching, a thermal oxidation step followed to produce an electrically insulating layer on the surface. To decrease the capacitance of the samples in the measurement setup, a 50 μm thick SU-8 photoresist (epoxy) layer was spun on the back side of the chip.

Lipid bilayer experiments were performed using a Teflon bilayer chamber with a 5 mm diameter opening between two baths of electrolyte solution. The oxidized silicon wafer with SU-8 on the back side was inserted in between the baths separating the individual solution chambers. The polished front side of the wafer faced the side of the bath connected to ground and the SU-8 covered (back) side of the wafer faced the side of the bath used to record potential. (This bath is isolated from ground by an impedance of many gigaohms.) Both baths were filled with 3 ml of 1 molar potassium chloride (KCl) solution, buffered with 20 mM N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES) at pH 7.4. 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 by painting over the hole of the wafer. The bilayer resistance was measured from

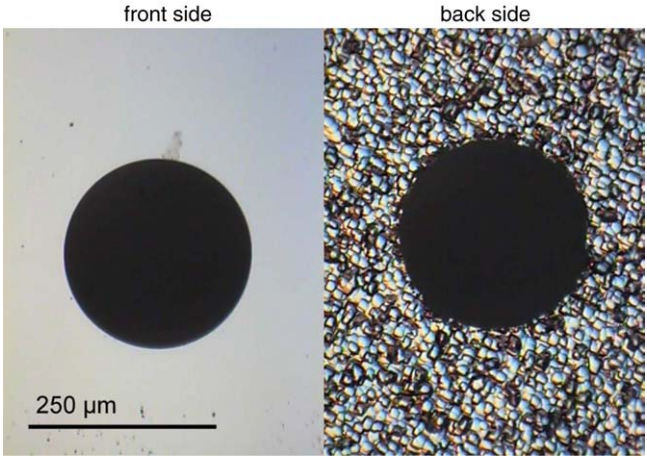

Fig. 1. Optical micrograph of the polished front and non-polished (rough) back side of a wafer with a hole etched using the deep Si reactive ion etch process. This process exhibits a good uniformity and no size enlargement during the etch process. This aperture has been used for lipid bilayer experiments.

the slope of the current trace. Current and bilayer capacitance were measured using an Axon Instruments Axopatch amplifier in connection with the pClamp software. The formation of a lipid bilayer was checked by comparing the data on resistance and capacitance with previously recorded values from bilayer chamber measurements. We also checked to see if the layer formed could be broken by the application of a short voltage pulse with $V_{\text{pulse}} > 0.2$ V. If the bilayer could not be broken by a voltage pulse and the capacitance was lower than expected, we rejected the preparation, thinking a bimolecular lipid layer had not been formed. The lipid blob was removed and another attempt was made. Ion channels were inserted into the membrane by adding OmpF porin to the ground bath.

3. Results and discussion

First samples were prepared with an aperture of 250 μm diameter etched all the way through the silicon substrate. The aperture diameter was chosen to be large and resemble the size of commercially available bilayer chambers made out of PTFE or similar plastic material [17]. Because the aspect ratio of the aperture affects the formation probability as well as the stability of the bilayer film [18], an aspect ratio close to 1 was our goal. Using 2″ wafers with a thickness of 275 μm allowed the 250 μm hole to be etched without the need to thin down the substrate using either dry or wet chemical silicon etching in a separate process step. Fig. 1 shows the polished front side as well as the non-polished back side of an etched sample. Fig. 1 shows that the hole exhibits an excellent uniformity and smooth vertical sidewalls. Concerning the surface roughness, no influence on bilayer formation is expected.

Fig. 2(a) shows the I–V characteristics of a bilayer formed across a 250 μm wide aperture in silicon as shown in Fig. 1. The bilayer resistance measured is 10.2 GΩ which

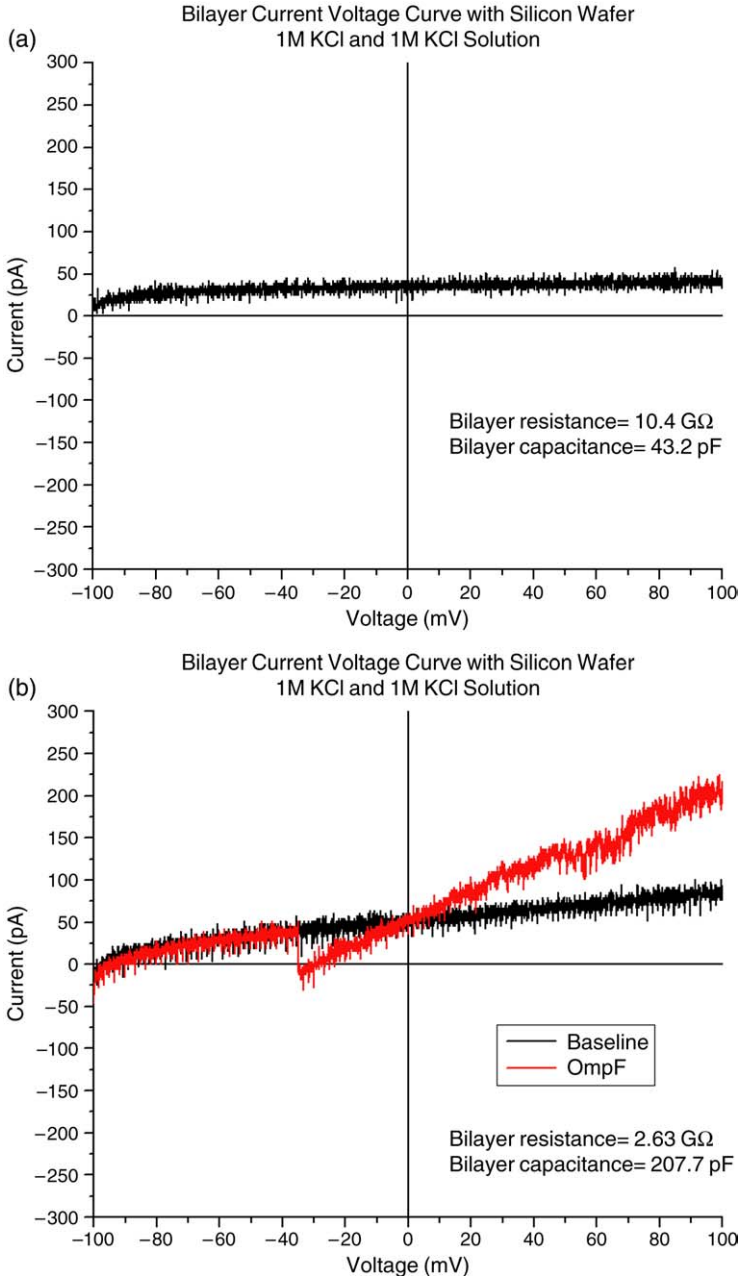

Fig. 2. (a) Determination of the electrical properties of a bilayer that has been formed by painting lipids over the silicon hole. The resistance was derived from the slope of the current trace and the bilayer capacitance was measured using the pClamp software. (b) Insertion of an OmpF porin channel into the lipid bilayer. The channel shows the expected voltage gating action of a working channel in a host membrane.

clearly indicates that a gigaseal between the lipid bilayer and the silicon support could be established. The capacitance associated with the lipid layer suggests that in this case a true bilayer was formed. After the measurement the bilayer could be broken by a brief high voltage pulse. The rise of the I–V curve at low voltage and the current offset at $V = 0$ are due to capacitive charging of the bilayer which leads to a voltage offset shown 'raw'. No compensation has been used.

The bilayers formed across these apertures were stable enough to allow the measurement of the insertion of an OmpF porin ion channel into the bilayer. Fig. 2(b) shows the current response of this voltage gated ion channel inserted into the bilayer membrane. This response is what is expected from the channel being inserted into a bilayer suspended across an aperture. Thus we were able to show that a micrometer sized aperture in silicon is capable of suspending a lipid bilayer as a host membrane for the insertion of a nanometer-scale ionic channel protein showing its regular properties.

Our procedure is successful but not efficient, or perfect. Several painting attempts had to be made until the desired result could be achieved. Also the bilayer resistance measured on the membrane reported in Fig. 2(b) is significantly lower than that measured in Fig. 2(a). We imagine that these problems are associated with the surface energy of the oxidized silicon used as the substrate material. An indirect measure of the surface energy of a material is the contact angle between a water droplet and the surface of the respective substrate [19]. PTFE and the plastic materials used for traditional bilayer chambers have hydrophobic surfaces, i.e. large water contact angles. The silicon dioxide used here, however, is not hydrophobic. It is easily wet by water and has a small water contact angle. The contact angle between the bilayer torus region and the substrate is sigificantly different from that in a bilayer in PTFE. The probability of bilayer formation is thought to be sensitive to wetting properties. Other laboratories even report that they were unable to form bilayers across silicon dioxide supports without changing the surface properties [10, 11]. Thus we will further investigate modification of the surface in the bilayer attachment region of the substrate.

While the large aperture size was chosen to resemble geometries currently used in channology laboratories, it would be very interesting to reduce the size of the bilayer area, both to reduce noise and (presumably) increase mechanical stability. We imagine that the nanometer-sized ion channel protein does not need a large bilayer area for insertion. Varying the hole size (and thus the area of the lipid bilayer membrane) allows us to study the stability of the membrane; the attachment to the silicon support; and the mechanisms governing the noise from a lipid bilayer in future experiments. In Fig. 3(a) we show a schematic of a down-scalable aperture in a silicon wafer. Because the aspect ratio of the aperture has to be kept constant, the substrate has to be thinned down in the area where the aperture will be etched. This recess has to be larger than the aperture itself so it does not influence the process of bilayer formation. Recess and aperture can both be etched using deep Si reactive ion etching. Because the reactive ion etching process is common in the semiconductor industry [16], the process flow described here is compatible with current device fabrication technology. So there is no necessity e.g. for wet chemical silicon etch steps [9, 10] that require dedicated process equipment, which would lead to an increase in the associated costs. The only additional step is a second photolithography step involving back side substrate alignment. Fig. 3(b) shows this concept in practice. It displays an

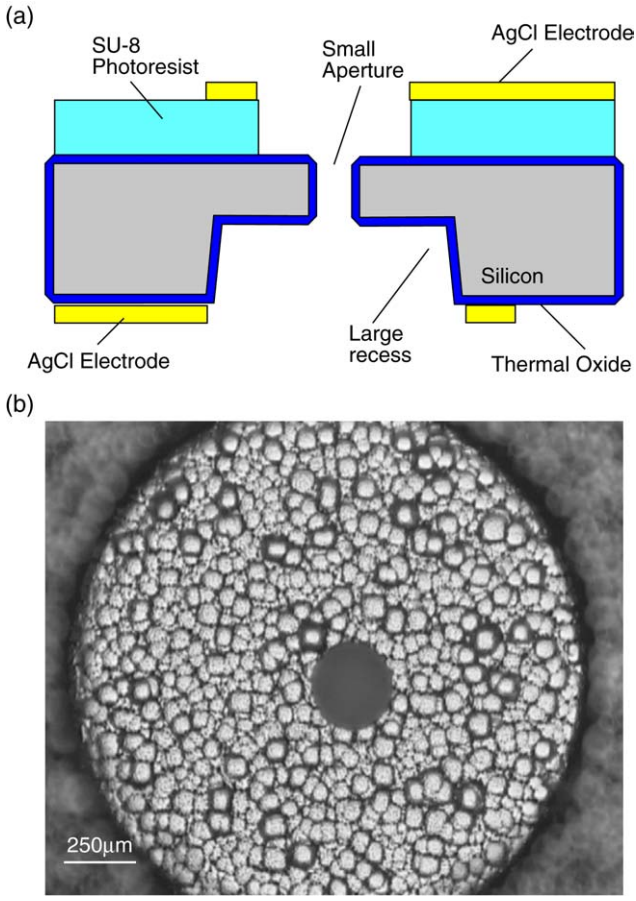

Fig. 3. (a) Schematic cross section through the aperture. (b) Small hole (diameter 150 μm) etched inside a thinned recess. Both recess and small hole have been prepared by reactive ion etching. The visible roughness is due to the non-polished back side of the wafer. The original structure gets transferred during the deep etch process.

optical micrograph of the wafer back side with a 150 μm aperture etched inside a 1 mm wide recess. The substrate has been thinned down to 170 μm inside that recess. Future downscaling of the bilayer support into the submicron range seems feasible.

4. Conclusions

In this work we show how to fabricate an aperture in a silicon wafer that can be used as a support for a functional lipid bilayer membrane. We used only standard semiconductor fabrication technology. Samples were prepared with a hole of 250 μm diameter etched all the way through a wafer of 275 μm thickness. The large diameter had an aspect ratio of 1 between aperture size and height of the support and was chosen to resemble plastic bilayer chambers used by channologists to provide stable bilayers.

Measurements of the sealing resistance of a lipid bilayer attached to a 250 μm wide silicon aperture show that a gigaseal could be achieved. Subsequently an OmpF channel protein could be inserted into this membrane. The corresponding I–V curve shows the expected voltage dependent gating action from a working porin ion channel.

While the initial aperture size is still in the micrometer range, the process used here allows a reduction of the aperture size even to submicron diameter. To preserve an aspect ratio of 1 the substrate around the aperture has to be thinned down. We present a design and proof of concept of the feasibility of this fabrication process. Varying hole size will allow study of the stability of the membrane and of the mechanisms governing the noise from a lipid bilayer. As the lipid bilayer acts itself as an inert substrate, the response of single nanometer-size ion channels can be detected, thus making this sensor a true nanoscale device.

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