Highly passivated silicon chips with micron aperture for the pA measurements of single ion channels

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ABSTRACT

Detection of single channel activities in the pA range on a high throughput screening (HTS) device is of major interest for pharmaceutical industries developing new drugs targeting ion channels. A silicon chip, with 9 silicon membranes having a micron aperture each, was functionalized and characterized with ion channels. The reduced size of the aperture has the advantage to be compatible with the formation of a solvent-free bilayer, thus protecting the functional activity of the integrated membrane proteins. The highly passivated silicon chip with a 7 µm thick SiO_2 TEOS and a 1 µm SiO_2 PECVD reduced the intrinsic capacitive noise of the material, resulting in a measured current noise of 0.56 pA rms at 1 kHz low pass filter. The sensitivity of the recordings on the chips was validated by the detection of small conductance ion channels.

Keywords: silicon, ion channel, bilayer, gramicidin, capacitance

1 INTRODUCTION

Ion channels are high potential drug targets [1] and biosensors as they naturally act as specific transducers of molecular recognition involved in cell signalling. Patch clamp is a powerful technique that has participated in unravelling the intricacies of ion channel function [2]. For about ten years, extensive efforts have been made to parallelize and automate electrophysiological measurements [3], resulting in important advances in the development of electrophysiological recordings on planar substrates. Two main approaches are investigated: planar patch clamp of cells enriched in a specific receptor [4], and electrical characterization of channels reconstituted in model membranes such as black lipid membranes (BLM). Thus, several reports of ion channel recordings using planar substrates have been published, using different materials to support the lipid membrane. Polymers and glass materials have intrinsic low capacitance, and have been extensively used as low capacitive noise allows fine pA current measurements [5-7]. Silicon is a semiconductor material widely used in the production of Application Specific Integrated circuits. The manufacturing processes well established for the microelectronic industry are declined for MEMS having the benefit of the submicronic control of the processes, and large scale production. But the intrinsic high capacitance of the silicon was the major drawback for the use of this material in detecting single channel activity. Recently, efforts were put on reducing the capacitance. Passivation with SU-8 layers [8], addition of a 1 µm thick SiO2 layer [9, 10] significantly reduced the capacity of the chip. We show here that passivation with an additional 7 µm SiO_2 TEOS was compatible with the low-noise recording of low conductance single channel events in a solvent-free lipid bilayer suspended over a 2 µm diameter size aperture.

2 MATERIALS AND METHODS

2.1 Chemicals

Asolectin lipids were purchased from Sigma-Aldrich (St Louis, MO) and a stock solution of 5 mg/ml lipids in hexane was used. A fluorescently labelled lipid, N-(6-tetramethylrhodaminethiocarbamoyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (TRITC-DHPE) (Invitrogen, Carlsbad, CA) was added to a final concentration of 0.05% of the lipid solution. Gramicidin A from *Bacillus brevis* was purchased from Sigma-Aldrich (St Louis, MO). A buffer solution of 5 mM HEPES pH 7.4, 100 mM KCl and 5 mM $CaCl_2$ was used throughout the electrophysiological measurements.

2.2 Chip processing and device setup

Chips with nine silicon nitride membranes bearing each an aperture of 1.8-2 micron in diameter were prepared (see Fig. 1). Micropore fabrication was achieved by dry etching [11]. To minimize the current noise due to the interaction of the preamplifier with the total capacitance, the chip was designed with a thick SiO2 layers (7 µm TEOS and 1 µm PECVD) (see Fig.2). With this configuration, a chip capacitance of 15 pF was estimated. Chips were positioned between two structured PDMS pads, forming 5 µl volume wells on each side of the nine silicon membranes. The wet contact area was confined to 4 mm^2. A printed circuit board with nine Ag/AgCl wire electrodes was placed on the lower side of the chip, and a single Ag/AgCl electrode was placed

on the upper side of the measuring site. The device was placed in a Faraday cage on a mechanically isolated support. Electrical currents were recorded with an Axopatch 200B patch clamp amplifier (Axon Instruments). The analog output signal was digitized with an A/D converter (Digidata 1322A, Axon Instruments). Data processing was done with a pClamp9.2 software (Axon Instruments).

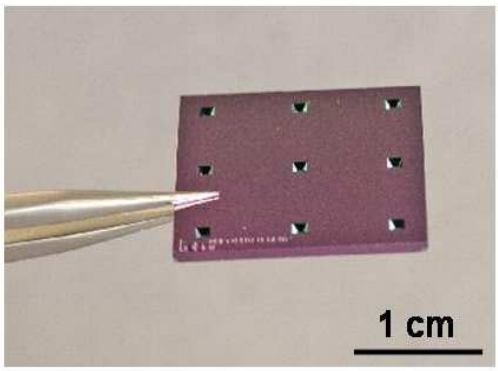

Figure 1: Silicon chip with nine SiO2 passivated membranes.

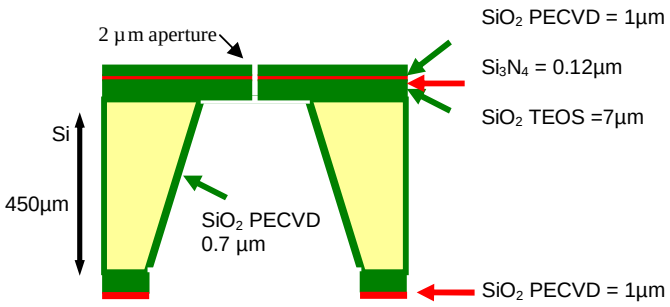

Figure 2: Structural characteristics of one of the nine measuring sites on the silicon chip.

2.3 Formation of suspended lipid bilayers

Prior to use, the surface of the chips were negatively charged by oxygen plasma treatment and functionalized with lipid bilayers using the giant unilamellar vesicle fusion technique: Briefly, giant unilamellar vesicles (GUVs) made of asolectin were produced by electroformation in a sucrose solution. The largest vesicles were then collected after sedimentation in glucose, and 0.5 µl of the solution was deposited on the chip. The presence of divalent cations in the ionic buffer induced the electrostatic interactions between the silicon oxide surface and the negatively charged vesicles. Thus, sedimentation and spontaneous burst of giant unilamellar vesicles over the apertures formed patches of lipid bilayers with size varying from 10 to over 50 µm diameter (see Fig.3). Self-inserting channel forming proteins were added after electrical characterisation of the pure lipid spanning membrane.

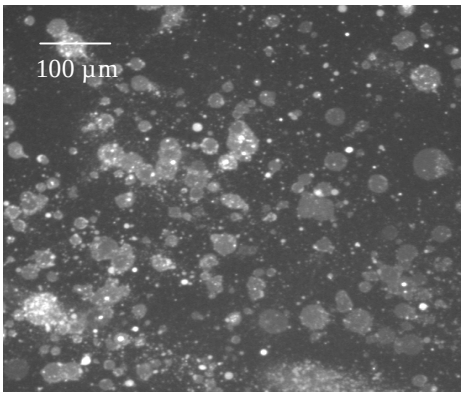

Figure 3: Patchs of lipid bilayers on a SiO2 surface

3 RESULTS AND DISCUSSION

3.1 Characteristics of the suspended lipid bilayer

Success rate of the deposition of a lipid bilayer over the pore depended strongly on the homogeneity of the GUV solution and on the time elapsed after the surface treatment of the chip. The presence of small vesicles impaired the adhesion of GUVs over the aperture. After removal of small vesicles from the solution of liposomes, formation of a lipid bilayer with a seal resistance comprised between 1 and 20 GΩ was obtained in a few seconds (see Fig. 4) with a success rate decreasing from over 70% down to 50 % during the two and half hours following surface treatment of the chip (see Fig. 5). The formation of a high resistance seal is necessary to detect pA currents. Furthermore, the lipid membranes were stable for 60-90min. After deposition of a lipid bilayer over the aperture, measurement of the total capacitance of the system was done by applying a triangular wave potential. A total capacitance of 30 pF and a current noise of 0.56 pA rms at 1 kHz Bessel filter were measured. The total capacitance comprises the chip capacitance and the lipid bilayer capacitance. As the bilayer capacitance is around 1 µF/cm^2, the contribution to the total capacitance of a bilayer over a 2 µm diameter aperture is around 30 fF and is negligible. The total capacitance is expected to be identical to the chip capacitance. The difference between the measured capacitance (30 pF) and the theoretical capacitance of the chip (15 pF) probably arises from a thinner PECVD silicon oxide layer inside the back side pyramidal opening.

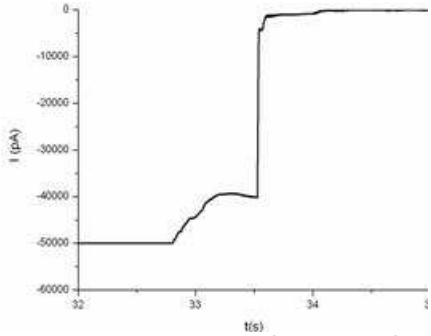

Figure 4: Spontaneous formation of a gigaseal upon addition of GUVs made of asolectin on a O2 plasma treated surface, in presence of Ca2+ ions: The current intensity decreased abruptly over a few seconds after addition of the vesicles.

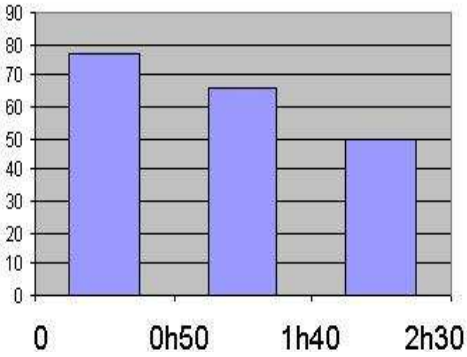

Figure 5: Success rate of seal formation as function of time elapsed after surface treatment.

3.2 Recording of ion currents

Gramicidin A, a 1.9kDa channel forming peptide, was added at 1 nM final concentration in the upper compartment. The peptide facilitates transmembrane flux of monovalent cations through the lipid bilayer. The association of two monomers, each spanning one lipid membrane leafllet, forms a dimeric transmembrane channel. A voltage was applied at 80 mV and the time evolution of the current was recorded. Characteristic signal currents with a single channel current activity of 1.2 pA were detected (see Fig. 6 and Fig.7), corresponding to a conductivity of 15 pS as already reported [12]. The detection of gramicidin channel events confirms the presence of a single lipid bilayer spanning the silicon aperture.

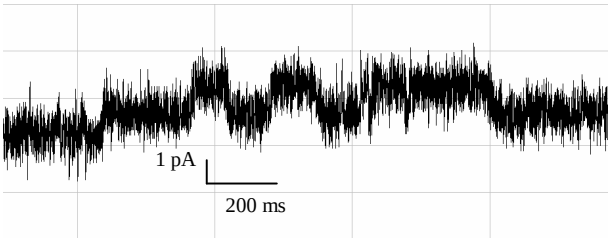

Figure 6: Unfiltered current induced by 1 nM Gramicidin in a bilayer made of asolectin, in buffer solution (100 mM KCl, 5 mM CaCl2, 5 mM HEPES pH 7.4). Applied voltage: + 80 mV.

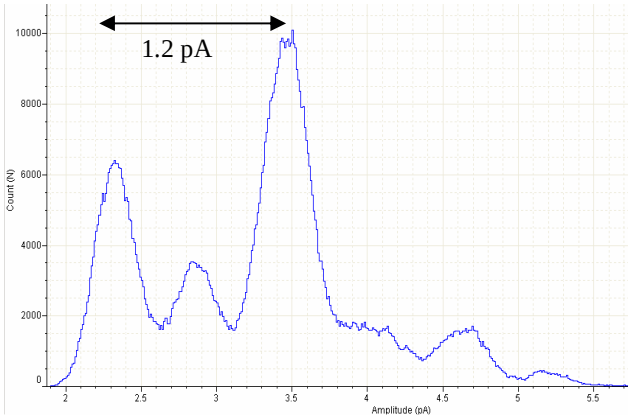

Figure 7: Histogram of the filtered signal showing a main conductance at 15 pS characteristic of Gramicidin pores.

4 CONCLUSION

The channel used for this study is an aqueous soluble peptide which spontaneously inserts into lipid bilayers. But most of ion channels of interest are large insoluble membrane proteins that may only be incorporated by vesicle fusion on the preformed suspended bilayer [10] or by the direct fusion of giant proteoliposomes [13] over the aperture. We have shown here the possibility to use silicon chips in detecting small conductance single channel currents at physiologically relevant salt concentrations. As seal resistance and wet contact area contribute to the total current noise, further improvement can be achieved by increasing the gigaseal resistance by specific surface treatment and by reducing the wet contact area of the chip. Sensitivity conferred to the silicon chips opens new fields in HTS devices requiring exquisite low noise recordings, such as the use of synthetically tailored peptides in biosensing [14], or the investigation of single ion channel coupled receptors [15].

5 ACKNOWLEDGEMENTS

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