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Towards a planar sample support for in situ experiments in structural biology

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Abstract

We report about the development of a planar support for biological samples. The aim is to combine AFM and patch-clamp measurements, in order to perform simultaneous measurements of topography and electrical properties of biological membranes. Sub-micrometer apertures have been fabricated using standard MEMS techniques. The electrical quality of the support has been experimentally assessed.

Keywords: Nano; Nanotechnology; Biology; Nanobiology; Structural biology; Biological membrane; Patch-clamp; Conductive probe; Scanning electrochemical microscopy

1. Introduction

Channel proteins, which are embedded in cell membranes, are responsible for a variety of elementary cell functions. Consequently, there is a great interest in understanding the transport mechanism through these channels. The patch-clamp technique gives insight in the electrical behavior of such proteins [1]. This method uses a glass pipette onto which a patch of a cell membrane is aspirated, and a pair of electrodes positioned across the membrane, such that an ion current can be measured as function of the applied voltage. Atomic force microscopy (AFM) on the other hand, has been applied to measure the topography of membranes and to monitor topography changes as function of pH or globally applied electrical potentials [2,3]. In order to learn more about the relationship between the structure and the functionality of membrane channels, it would be advantageous to observe the electrical and topographic information simultaneously. This can be

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done by means of a setup that combines conventional patch-clamp technique and AFM [4,5]. However, operating an AFM at the end of a pipette is difficult. This problem can be overcome by a planar sample support featuring a small aperture, which allows accessing the absorbed membrane also from the backside. Fig. 1 shows the schematic representation of our setup. The biological sample is adsorbed onto a thin Si_xN_y membrane, featuring a submicron sized aperture. One electrode is embedded in the microchannel beneath the aperture and the Si_xN_y membrane; the counter electrode is implemented into the cantilever [6]. Applying an electric potential between this two electrodes enables the sensing of local variations in the electrical transfer. This report focuses on the fabrication and characterization of such planar sample supports.

1.1. Design and fabrication

The challenge in fabricating planar patch-clamp sample supports for biological applications is the tight, giga ohm seal needed between the biological membrane and the support [7–9]. In most planar supports the sample is only deposited onto the surface, compared to patch clamping, where the membrane is partially sucked into the pipette and hence, conformably covers a large surface. We assume that therefore the surface roughness of the planar support is of great importance for achieving a good seal. AFM measurements of Si_xN_y films deposited onto a Si-wafer, showed a roughness of 5 nm rms. Using however the much smoother side of the interface that is accessible after removing the Si, improved that value to 0.12 nm rms (Fig. 2). Moreover, low values of distributed RC noise are critical in obtaining highly accurate single channel recordings. Noise from parasitic capacitances can easily exceed the current generated by a single ion channel [10]. Therefore, keeping the parasitic capacitance and series resistance of our supports as low as possible is important for obtaining single channel recordings.

For fabricating our sample supports we used a two-mask process. A Si_xN_y film was deposited onto a Si (1 0 0) wafer by a low-pressure chemical vapor deposition (LPCVD) process. Micron-sized holes were patterned into the nitride film by photolithography and reactive ion etching (RIE) (Fig. 3(a)). The patterned film was released by KOH. The holes in the freestanding nitride membrane were then shrunk to a submicron-size by a second Si_xN_y LPCVD deposition (Fig. 3(b)). Alternatively, the second LPCVD step, followed by a blanked RIE, was performed before releasing the Si_xN_y membrane. This formed an annular inlay in the aperture, which reduced the aperture diameter as well (Fig. 3(c)). The optimal diameter for biological membrane-patch sites varies depending on the particular application and the size of the biological sample under investigation. Biological membrane patches such as Bacteriorhodopsin

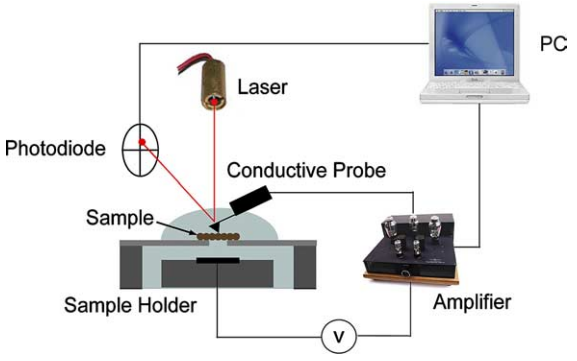

Fig. 1. Schematic representation of the setup. The biological sample is adsorbed onto the patch-clamp cell. One electrode is embedded in the microchannel beneath the aperture; the counter electrode is implemented into the cantilever.

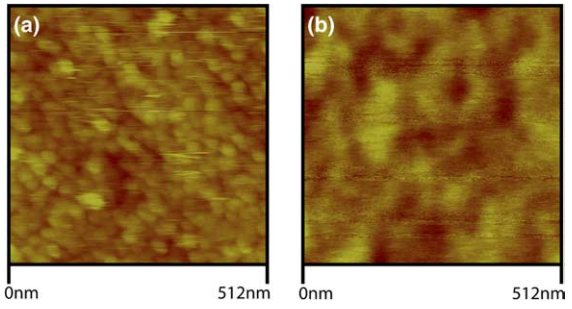

Fig. 2. (a) AFM measurement of the front side surface of the Si_xN_y membrane. The z-range is 6 nm and the rms roughness is 5 nm. (b) AFM image of the released backside surface of the Si_xN_y membrane. The z-range is 1.2 nm and the rms roughness has improved to 0.113 nm.

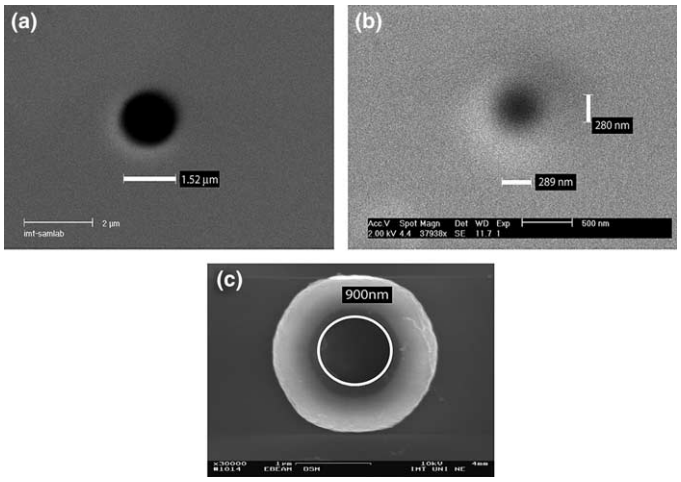

Fig. 3. (a) SEM image of the aperture in the freestanding Si_xN_y membrane realized by photolithography and RIE. The diameter of the aperture is 1.5 µm. (b) The same aperture after a deposition of 650 nm of Si_xN_y by low-pressure chemical vapor deposition (LPCVD). The diameter of the aperture has shrunken to 290 nm. (c) SEM image of an aperture after a second LPCVD of 600 nm Si_xN_y and a blank RIE before releasing the Si_xN_y membrane. The annular inlet reduced the aperture diameter by 1 µm.

have generally diameters up to 1 µm. Acceptable aperture diameters are 0.1–0.5 µm. The process presented in the fabrication section is designed to be flexible enough, so that apertures can be fabricated in this size range, without changing the mask set, simply by varying the thickness of the second LPCVD deposited Si_xN_y film. The Si wafer was then anodically bonded onto a pyrex wafer, featuring predrilled trough holes connected by a microchannel, which provides access to the backside of the biological sample. This channel was defined by lithography and etched in 20% HF.

2. Experiments

In patch-clamp recordings the additional parasitic capacitance of the system should preferably be less than the capacitance of the membrane patch, such that the membrane is the dominant noise factor [11]. In order to extract the series resistance and capacitance of our supports, a phase gain analyzer with Ag/AgCl electrodes was used. To perform measurements the samples were placed in a testing apparatus and electrically isolated. Fifty micromole KCl electrolyte solution was filled into the microchannel, and on the front side of the support. The Ag/AgCl electrodes were then immersed into the electrolyte on both sides of the membrane. The impedance spectra were measured using a 200 mV excitation voltage with a 1 Hz–1 kHz frequency range. The equivalent circuit used to model the impedance spectra, consists of a resistance and a capacitor connected in parallel. The resistance of the aperture and the support capacitance in the model circuit were then fitted to the measured impedance spectra. The overall system resistance is dominated by the conductance of the aperture because of its small dimension compared to the rest of the conducting path. Therefore, the systems resistance can be acceptably modeled by only taking into account the resistance of the aperture. The later can be modeled as a conductor with a resistivity matching that of the KCl solution [12]. The resistances of supports with three different aperture radii were measured. Fig. 4 shows good agreement between the calculated resistance and the measured aperture resistance. The capacitance of the system is formed by the conducting parallel plates of KCl solution separated by the Si_xN_y dielectric and is given by

$$C = \frac{\varepsilon_r \varepsilon_0 A}{d}$$

where C is the capacitance, ε_r is the relative permeability of the Si_xN_y, ε_0 is the vacuum permeability,

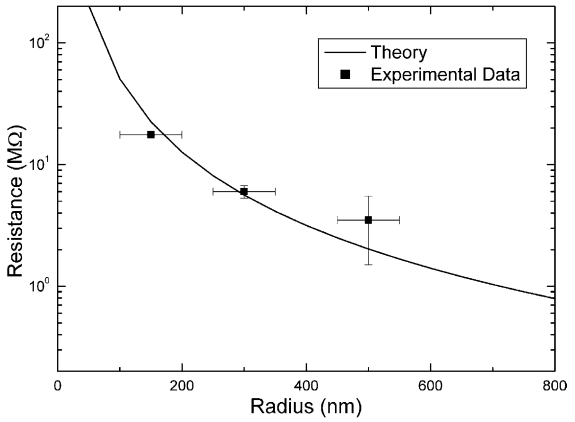

Fig. 4. Measurement of the ionic resistance through the aperture vs. the radius of the aperture. The line represents the theoretical values and the points represent the values measured for the three hole sizes fabricated. The holes with the radii 150, 300 and 500 nm showed an ionic resistance of 3.5, 6 and 17.6 MΩ, respectively.

d the thickness of the Si_xN_y membrane (1.2 μm) and A is the area of the suspended Si_xN_y membrane (3.1 mm^2). In this model we overestimate the capacitance, as there will be an additional straight capacitance from the non-suspended Si_xN_y membrane. This additional capacitance is in parallel with our model capacitance and will thus increase the overall capacitance. The capacitances of supports with three different aperture radii were measured. We measured five devices for each hole size and the mean capacitance was found to be 167 pF with a variation of 63 pF. The measured capacitance shows a reasonable agreement with the calculated capacitance (191 pF). The high variation in the experimental value is due to the different sizes of the microchannel and, hence, the different areas of fluid contact.

3. Conclusion

We developed and fabricated a planar sample support to combine patch-clamp and AFM measurements on biological membranes. In order to make single channel detection possible, the capacitance of our supports need to be smaller than the capacitance of the biological membrane confined my the aperture. The capacitance of a membrane patch is generally about 1.3 μF/cm^2 [10]. The corresponding capacitance for biological membrane patches with an area corresponding to our three different aperture radii (150, 300, 500 nm) is 0.9, 3.7 and 10 fF, respectively. Therefore, we conclude that the current device will hardly allow single channel measurements. Increasing the Si_xN_y membrane thickness and reducing the surface of the membrane that is exposed to the liquid on both sides could mitigate these effects. However, it will be possible to visualize conformal changes of the membrane protein channels.

Acknowledgement

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