Stable integration of isolated cell membrane patches in a nanomachined aperture: a step towards a novel device for membrane physiology

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Abstract

We investigate the microscopic contact of a cell/semiconductor hybrid. The semiconductor is nanostructured with the aim of single channel recording of ion channels in cell membranes. This approach will overcome many limitations of the classical patch-clamp technique. The integration of silicon-based devices 'on-chip' promises novel types of experiments on single ion channels.

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Nanostructuring allows to build devices with dimensions similar to those of basic biological units, e.g. ion channels in cell membranes. Ion channels are proteins that are integral parts of cell membranes and act as pores. They regulate the flow of ions in- and out of the cell \[1\]. Exhibiting different kinds of gating mechanisms they act as basic excitable units in biological systems. The function of these elementary units is therefore of fundamental importance for information processing in neural systems.

For about two decades physiologists have been able to resolve ionic currents through single ion channels by using the patch-clamp technique \[2,3\]. This method relies on forming a \(\mu\)-size contact with the cell membrane by means of an electrolyte-filled glass pipette. The open tip of the pipette is pressed against the membrane, defining an isolated patch. Due to the strong glass-membrane adhesion \[4\], a \(\Omega\)-seal is obtained which allows current measurements with resolution of a few 100 fA. A basic limitation of this approach is the limited recording bandwidth \(B<100\ kHz\). This limitation arises mainly because of stray capacitances and the high access resistance of the long-tapered pipette \[5\]. In contrast, the geometry of the semiconductor based probes used in our approach should overcome these limitations. We define a nanoscale aperture located in a suspended Si\(_3\)N\(_4\) membrane on micromachined silicon substrate. This enables us to minimize the distance between the ion channel under investigation and the recording electrode. In addition, with semiconductor structuring techniques, the passive glass pipette can be replaced by a versatile probe, which can easily integrate active semiconductor elements e.g. amplifiers or electromechanical devices. Finally, due to the open geometry of the probe, imaging techniques such as fluorescence microscopy, atomic force microscopy (AFM) and scanning electron microscopy (SEM) can be applied. In Fig. 1(a) an SEM-micrograph of our device with an integrated cell membrane is depicted. A Si\(_3\)N\(_4\)-layer is suspended on a micron scale by etching a V-groove in the (100)-silicon substrate beneath. In order to build these suspended membranes we deposit a 120 nm thick Si\(_3\)N\(_4\)-layer on both sides of a (100) low n-doped silicon substrate using Low Pressure Chemical Vapor Deposition (LPCVD). Applying standard optical lithography and Reactive
Ion Etching (RIE) we define an etch mask on the backside of the samples. Subsequent anisotropic wet etching in a KOH-solution results in a V-shaped groove, where the upper Si$_3$N$_4$-layer serves as an etch stop. Adjusting the size of the etch mask we build a suspended Si$_3$N$_4$-layer with dimensions of a few ten microns side length. Both optical lithography as well as low-energy electron-beam lithography is used to define an orifice in the suspended membrane. The lithographic pattern is transferred into the membrane by an RIE process. The lower inset in Fig. 1(a) shows apertures in such a suspended membrane with sizes ranging from 500 nm down to 50 nm. Due to this nanostructuring process the geometry of the aperture can be freely chosen.

The integration of a cell membrane is achieved by positioning a cell on top of the probe. The device is installed into a classical patch-clamp setup including a remotely controlled positioning system and a microscope. The inset in Fig. 1(a) shows the schematical arrangement of the semiconductor-cell hybrid. In order to carry out electrical measurements, the ensemble is connected via electrodes in standard Ringer’s electrolyte solution (270 mOsm) forming the extra-cellular medium. Cultured embryonic cells from rat striatum or C6-glioma cells are acutely dissociated applying standard trypsin treatment and trituration. A glass suction pipette is used to move an isolated cell onto the aperture as shown in Fig. 1(b). By applying negative pressure from below, the cell’s membrane is partially sucked into the opening. This procedure is in close analogy to the standard patch-clamp technique. In order to obtain a cell-free patch, the glass pipette is used to remove the cell body, leaving an excised membrane patch in the aperture. In Fig. 2, this excised patch is shown from both sides of the device. The micrographs were taken with a low-voltage scanning electron microscope (SEM) at resolution of about 1 nm. Fig. 2(a) shows a top view of the aperture. Cellular material (presumably cytoskeletal elements) is seen to fill the entire lumen. Fig. 2(b) shows a close-up view of the cell membrane that has been dragged into the opening. Imaging cellular structures is only possible after fixatation with glutaraldehyde solution, dehydration in graded alcohol and drying in a critical point drier. This procedure is responsible for the somewhat distorted surface structure of the cell membrane. However,
the image clearly shows, that there is an extremely close association of the membrane with the silicon nitride material without any visible gaps. This finding justifies the expectation that Si$_3$N$_4$ can, in our design, substitute for glass in creating GΩ-seals. It is also in line with the glass-like properties of this material. These adhesion properties are of great importance when interfacing neurons and silicon [6]. Single channel recording is only made possible by the so-called GΩ-seal where the membrane sticks tightly to the glass of the pipette [4]. Demonstrating a GΩ-seal with the Si$_3$N$_4$ membrane is therefore the next major step towards patch clamp recording with the device presented here. Another advantage of our approach becomes obvious: due to its geometry our device lends itself to visualization techniques such as SEM, atomic force microscopy (AFM) or scanning nearfield optical microscopy (SNOM).

Furthermore, we applied confocal fluorescence microscopy for imaging the hybrid in the ionic solution i.e. in a situation where the membrane proteins and their functions are intact. In order to visualize the membrane, we incubated isolated cells with a solution containing the fluorescent marker bis-oxanol prior to integrating the membrane into the probe. The fluorophore is excited by blue light (488 nm) emitted from a Ar-ion laser. In Fig. 3(a) a scanning micrograph of the membrane-semiconductor hybrid taken with a confocal fluorescence microscopy is shown. On the suspended Si$_3$N$_4$-layer fluorescent cell debris is found in the environment of the aperture. A structure of more regular, round shape can be discerned near the center of the image and represents fluorescent cellular membrane incorporated in the aperture. As shown in Fig. 3(b), using a z-scan series, this structure can be definitely distinguished from the surrounding debris: The graph shows a plot of the fluorescence intensity as a function of the distance of the confocal plane from the probe surface. Thus, successive optical sections parallel to the probe surface ranging from about $-4 \mu m$ to $4 \mu m$ with zero set at the Si$_3$N$_4$-membrane level are taken. The three curves correspond to the normalized fluorescence light intensity emitted from the clean Si$_3$N$_4$-film, the debris on top of the film and the membrane in the aperture, respectively. Obviously, the fluorescence of the fractured cell material is emitted starting from a z-position higher than that of the
Si$_3$N$_4$-film. In contrast, the fluorescence of the incorporated membrane displays a z-range on the same level or even lower than the reference Si$_3$N$_4$-film. The increase of fluorescence intensity of the debris in the negative z-range is related to the backscattering of excitation light from the Si$_3$N$_4$-layer acting as a bifringent mirror. Since the integrated cell-membrane is freely suspended, this effect is not seen in the aperture.

In conclusion, we have shown a first realization of a cell membrane patch integrated into a nanostructured semiconductor device verified by fluorescence and SEM-micrographs. Attaching native cell membranes to nanostructured probes is the first step towards patch clamp recording with semiconductor or silicon-on-insulator (SOI) devices. In addition, the geometry of our hybrid enables various methods of microscopy such as confocal fluorescence or atomic force microscopy to be applied in situ. The application of the device presented for patch clamp recording will be discussed elsewhere [7]. Furthermore, it has to be noted, that the principles of processing such a nano patch clamp (NPC) chip can easily be transferred to Silicon-on-Quartz or other material classes. Combining the patch-clamp technique with semiconductor devices allows the integration of active amplifying devices, e.g. field effect transistors. By using lithographic methods, these active elements can be positioned in the immediate vicinity of the ion channel. The noise level of the measurement can thus be lowered dramatically due to an ‘on chip’ amplification, leading to a highly improved resolution.

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Fig. 1: (a) SEM micrograph of V-groove in (100)-silicon with a suspended Si$_3$N$_4$-layer on top. In the suspended Si$_3$N$_4$-layer a small aperture is nanostructured by optical or electron-beam-lithography and RIE. In the aperture cell material is incorporated. The upper inset depicts the schematical arrangement of the semiconductor-cell hybrid. The lower inset shows a series of holes in a suspended membrane with dimensions down to 50 nm.

(b) Photograph of the probe with cell positioned on top of the aperture. The glass pipette on the right is used to manipulate the cell.

Fig. 2: (a) Top-view of the aperture with incorporated cell material. The arrows indicate the circumference of the opening.

(b) Close-up of the cell membrane taken from the backside protruding from the opening. The membrane is sealed tightly to the Si$_3$N$_4$-layer with no remaining cleft in between.

Fig. 3: (a) Fluorescence scanning micrograph taken with a confocal microscope. The cell-membrane is labeled with fluorescent marker (thick arrow). Some cell debris is also visible (thin arrows).

(b) Z-series taken from the fluorescence of the cell-membrane, the Si$_3$N$_4$-layer and the debris on top of it (for details see text).
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