Activity of single ion channel proteins detected with a planar microstructure

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We present recordings of currents mediated by single ion channel proteins obtained using planar, microstructured glass chips. In these chips, pores with diameters of 1–2 μm are produced by ion track etching and are used for patch clamping instead of using the classical micropipette. Our results represent success in using such devices to record from single channels in cell membranes. The planar chip greatly enhances the accessibility of the ion channel containing membrane and can serve as a workbench for experiments on single ion channels using combinations of patch clamp current recording with other single molecule techniques. © 2002 American Institute of Physics.

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Ion channels are proteins in cell membranes and act as pores which can adopt closed and open states, thus gating the flow of ions in and out of the cell. The first technique available to directly study gating of ionic currents on the single protein level was patch clamping, where an electrolyte-filled glass pipette connected to an amplifier via a silver wire establishes electrical contact with a cell membrane and measures ionic current. Controlled by different mechanisms such as membrane potential or binding of a ligand, ion channels are of central importance for many cellular functions. They essentially determine the ways cells exchange information in all tissues of the human and animal body, including those of the nervous, the cardiovascular, the intestinal, and the reproductive systems.

While the major part of the current interest in chip based patch clamping is owed to the need in the pharmaceutical industry for a high throughput ion channel screening method, other considerations—directed more towards basic questions—provide considerable motivation as well. The change in geometry from the glass pipette to an aperture in a planar device improves the $RC$ characteristics (lower series resistance, lower capacitance), thus lowering background noise. Furthermore, the planar geometry of the “patch on a chip” arrangement greatly facilitates concomitant application of scanning probe techniques, high-resolution fluorescence microscopy, and simultaneous electrical recording. Such combined experiments will be important in furthering our understanding of the gating mechanism in ion channel proteins.

Here, we demonstrate how to integrate single ion channels into a planar glass microstructure, enabling chip-based single channel recording. In an earlier attempt we have used silicon as a substrate which allows chip fabrication by standard planar processing techniques. Recordings from pore-forming peptides and ion channels proteins reconstituted into planar lipid bilayers spanning apertures in silicon chips have also been reported. But due to the rather small specific resistance, silicon is not the best suited material for electrophysiological measurements. In a related approach polydimethyl-siloxane (PDMS), a silicone polymer, was used in a micromolding process to achieve small apertures in a planar substrate to enable patch clamp recording from cells. However, compared to silicon or PDMS, glass has superior dielectrical and mechanical properties. As glass is also the material from which patch pipettes are made, it obviously is a good material choice for a biochip.

We have previously shown that planar glass substrates containing a single microaperture produced by ion track etching can be used to record currents through single artificial ion channels in bilipid membranes and ensemble currents through large populations of ion channels in living mammalian cells. Currents through single cellular ion channels could not be resolved during those studies because we were not routinely able to achieve high enough seal resistances (>1 GΩ) between cell membrane and glass. Now, we report recordings with single channel resolution on planar...
substrates. These have become possible through continuous improvement of the technique employed.

The focus in this letter is on recordings on single ion channels with GΩ-seal resistances on a mammalian cell membrane. We study a BK-type potassium channel\(^{1,2}\) genetically expressed in a Chinese hamster ovary (CHO) cell.\(^{12}\) The recordings were performed in a cell-attached configuration, where the cell was positioned onto the aperture of the microstructured glass-chip as seen in Fig. 1.

The details of the structuring technique have been presented previously. Briefly, for processing of the chips, borosilicate glass substrate of 200 μm thickness is selected. Using optical lithography and wet etching with fluoridic acid, circular areas of 500 μm diameter are prethinned into the substrate down to about 20 μm of remaining glass. Total dimensions of the glass chips used finally are 5×5 mm\(^2\). An aperture with submicron diameter is defined within the prethinned area by a single heavy ion penetrating the material. For this purpose a highly accelerated gold ion (~11 MeV/nucleon) is shot through the glass substrates which leads to the formation of a latent track with diameter of about 10 nm along the passage path of the ion. Such latent tracks are then etched open to achieve small apertures of different shapes and geometries. A more detailed description of the processing can be found in Refs. 8 and 9.

The recordings are performed by a commercially available amplifier and data acquisition software (Axopatch 200B, Axon Instruments, CA). The recorded data is commonly filtered at 1 kHz and sampled at 12 kHz. CHO cells are grown in Dulbecco’s modified Eagle medium (Gibco), supplemented with 10% fetal calf serum (Gibco) at 37 °C in humidified atmosphere of 5% CO\(_2\) in air. Electrolyte solutions used have the following ionic compositions (mM): extracellular (top of chip): Na–Aspartate (160), KAsp (4.5), HEPES (5), CaCl\(_2\) (1), MgCl\(_2\) (1), pH=7.4; intracellular (underside of chip): K–Aspartate (135), EGTA (10), HEPES (10), CaCl\(_2\) (8.5), MgCl\(_2\) (2.1), pH=7.2, the resulting free Ca\(^{2+}\) concentration was 1 μM.

Cells are grown in a petri dish and isolated by a brief enzyme treatment using trypsin. The solution containing the isolated cells has to be free from membrane fragments or other cell debris to avoid contamination of the chip aperture. Therefore, the cell suspension is centrifuged twice at 1000 rpm for 2–5 min and the obtained pellet of cells is resolved in extracellular electrolyte solution. The cells are then pipetted onto the chip, suction is applied to the chip-aperture to position a single cell on the opening as indicated in the sequence of Fig. 1: the number of cells given onto the chip is a few hundred to thousands, but only a single cell is selected for the transport experiment. After positioning of the cell, very gentle suction is used to facilitate the formation of an GΩ-seal resistance between the cell membrane and the chip [Fig. 1(c)]. Once a cell is sealed onto the aperture, the command voltage of the electrode inside the chip is clamped to a predefined holding potential. This is done via the feedback loop of the preamplifier of the headstage. By clamping the holding potential to different values, the open probability of the ion channels can be varied. By depolarizing the potential the open probability increases. Also the driving force for the ionic current passing through the channel protein is varied by the applied holding potential.

Minimal seal resistances required for single channel recording are in excess of 1 GΩ. In our experiments with the chip seals exceeding a GΩ were sometimes formed spontaneously when positioning the cell on the aperture, but usually some suction was necessary. We routinely obtained seal resistances ranging from 2 to 5 GΩ and occasionally up to 25 GΩ.

The microfabricated chip replaces the glass pipette and improves the experimental situation and greatly facilitates the experimental procedure. The positioning of cells for patch clamp recording with suction applied to the chip works reliably without use of micromanipulators or visual control. No moving parts are needed and the setup is insensitive to vibration, as no relative movements of the recording probe and the cell are possible. Typically, only a few seconds are necessary to position a cell onto the aperture after suction is applied.

In Fig. 2 single channel recordings from potassium channels of a CHO cell are displayed. The CHO cells have almost no endogenous ion channels, so that the over-expressed BK-type channel can be investigated without interference from other ionic current sources. The recordings were performed with the patch clamp chip (a) and for comparison with a classical patch pipette\(^{1,2}\) (b), both in cell attached configuration with a holding potential of ~70 mV. The aperture in the chip has a diameter of 1 μm which is the same as the tip diameter. The measured current response to a voltage pulse of 10 mV applied to the chip is shown, from which the series resistance across the aperture in the chip can be calculated to 4 MΩ. In (b) the same voltage pulse is applied after a cell is sealed onto the aperture by suction, increasing the resistance to more than 5 GΩ. The close up in (c) shows the mechanically and electrically tight contact of the cell membrane and the chip in cell attached mode. By voltage clamping the membrane patch defined by the aperture in this cell attached configuration it is then possible to directly record current that is flowing through single ion channels in the membrane.
opening of the pipette. The series resistance of the chip, e.g., the resistance across the aperture in electrolyte solution without a cell on the opening, is about 4–6 MΩ for the chip as well as for the pipette. The capacitance of the pipette is on the order of a few picofarads and well below 1 pF in case of the chip. The cells and electrolyte solutions used in these comparison experiments are the same. Typical seal resistances of the cells with either chip or pipette are in the range of 1–10 GΩ. The capacitance of the pipette is still higher with the pipette. Electrophysiological characterization of the channels yields identical results and quality of data obtained with both approaches is very similar. Both recordings are low-pass filtered at 1 kHz and the rms current noise level is about 160 fA for the pipette recordings and 270 fA for the patch clamp chip.

In the measurements shown in Figs. 2 and 3, potassium ions are the predominant current carriers, as only these ions permeate the BK-type channel. The concentration gradient of potassium across the membrane can be predefined by selecting the appropriate electrolyte solution for the inside of the chip with respect to the intracellular solution composition. In Fig. 3, single channel currents recorded with the patch clamp chip at different holding potentials from a CHO cell in cell attached configuration are shown. The corresponding current–voltage relation is linear at the moderate potentials applied. The reversal potential is around ~45 mV which reflects the cell membrane potential.

In summary, recordings from single ion channel proteins conducted with a microstructured glass chip were performed. The presented development demonstrates a successful combination of nanotechnology/microelectromechanical systems and cell biology. The broad interest in this advance not only comes from the fact that this novel device has important applications in proteomics and pharmacology, but also because the approach facilitates biophysical experiments on single ion channel proteins, using a combination of electrophysiology with other physical techniques.

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