Whole Cell Patch Clamp Recording Performed on a Planar Glass Chip

Niels Fertig,*†‡ Robert H. Blick,*‡ and Jan C. Behrends†‡

*Center for NanoScience and Sektion Physik, Ludwig-Maximilians-Universität, Geschwister-Scholl-Platz 1, 80539 Munich, Germany; †Center for NanoScience and Physiologisches Institut, Ludwig-Maximilians-Universität, Pettenkoferstr. 12, 80336 Munich, Germany; and ‡Nanion Technologies GmbH, Schellingstr. 4/IV, 80799 Munich, Germany

ABSTRACT The state of the art technology for the study of ion channels is the patch clamp technique. Ion channels mediate electrical current flow, have crucial roles in cellular physiology, and are important drug targets. The most popular (whole cell) variant of the technique detects the ensemble current over the entire cell membrane. Patch clamping is still a laborious process, requiring a skilled experimenter to micromanipulate a glass pipette under a microscope to record from one cell at a time. Here we report on a planar, microstructured quartz chip for whole cell patch clamp measurements without micromanipulation or visual control. A quartz substrate of 200 μm thickness is perforated by wet etching techniques resulting in apertures with diameters of $\sim$1 μm. The apertures replace the tip of glass pipettes commonly used for patch clamp recording. Cells are positioned onto the apertures from suspension by application of suction. Whole cell recordings from different cell types (CHO, N1E-115 neuroblastoma) are performed with microstructured chips studying K^+ channels and voltage gated Ca^{2+} channels.

INTRODUCTION

Ion channels have crucial roles in physiology and pathophysiology and are important drug targets (Hille, 1992). Electrophysiological techniques (known as voltage clamp) using microelectrodes, which access the interior of the cell can directly measure the ionic currents these proteins carry over a cell membrane. The most successful of these is the patch clamp technique (Sakmann and Neher, 1995) in its whole cell configuration (Hamill et al., 1981), where the cell membrane is partially aspirated into a glass pipette to form a tight electrical seal and then ruptured to provide intracellular access (Fig. 1 *a*). Ionic current flow can then be measured over the whole cell membrane.

Patch clamping has rapidly become the "gold standard" in studying ion channel function but is still a laborious process requiring precision micromanipulation under high power visual magnification, vibration damping, and last but not least, an experienced and skillful experimenter. Because of this, high-throughput studies required in proteomics as well as drug development have to rely on less valuable methods such as fluorescence-based measurement of intracellular ion concentrations or membrane voltage (Denyer et al., 1998; Gonzalez et al. 1999; Xu et al., 2001). There is, consequently, considerable interest in an automated version of the whole cell patch clamp principle, preferably one that has the potential to be used in parallel on a number of cells. Such a device would vastly increase throughput and make electrophysiological testing with its many advantages, the option of choice in early screening for ion channel active drugs.

Additionally, in pipette-based patch clamping the cell and its membrane are not easily accessible by other physical means. This is a major difficulty in combining patch clamp experiments with optical, fluorescence, or scanning probe methods. A planar patch clamp device with an accessible aperture is ideally suited for these kinds of combined experiments by which new insights on ion channel behavior can be gained.

We here report the development of an automatic device for whole cell patch clamping that can easily be scaled up into a parallel array. The device consists of a planar chip made from fused quartz. Due to its dielectric properties quartz is the almost ideal material for patch clamp pipettes (Rae and Levis, 1992; Levis and Rae, 1993) and is thus a very suitable substrate for planar patch clamp chips. Into the chip an aperture with submicron diameter is defined by irradiation of a prethinned area of the chip with a single heavy ion and subsequent wet track etching (Spohr, 1990). The highly accelerated ion locally damages the electronic structure in the quartz, leaving a latent track that is etched open to achieve small apertures (Fertig et al., 2001).

We here present whole cell recordings from different cell types performed with the microstructured chip. Cell suspension is given onto the patch clamp chip, and using a simple pressure/suction protocol a single cell is automatically positioned onto the aperture. To achieve the good cell adhesion necessary for an electrically high resistance seal it is of prime importance to have smooth, preferably round apertures absolutely free from organic or other contamination. The microscopic nature of the seal is not understood in great detail (Corey and Stevens, 1983; Opsahl and Webb, 1994) and sealability of different cell types as well as for different geometries of the aperture varies significantly. In earlier work anisotropic etching techniques were used to microper-

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Address reprint requests to Niels Fertig, Center for NanoScience and Physiologisches Institut, Ludwig-Maximilians-Universität, Pettenkoferstr. 12, 80336 Munich, Germany. Tel.: 49-89-599-6248; Fax: 49-89-599-6250; E-mail: niels.fertig@physik.uni-muenchen.de.

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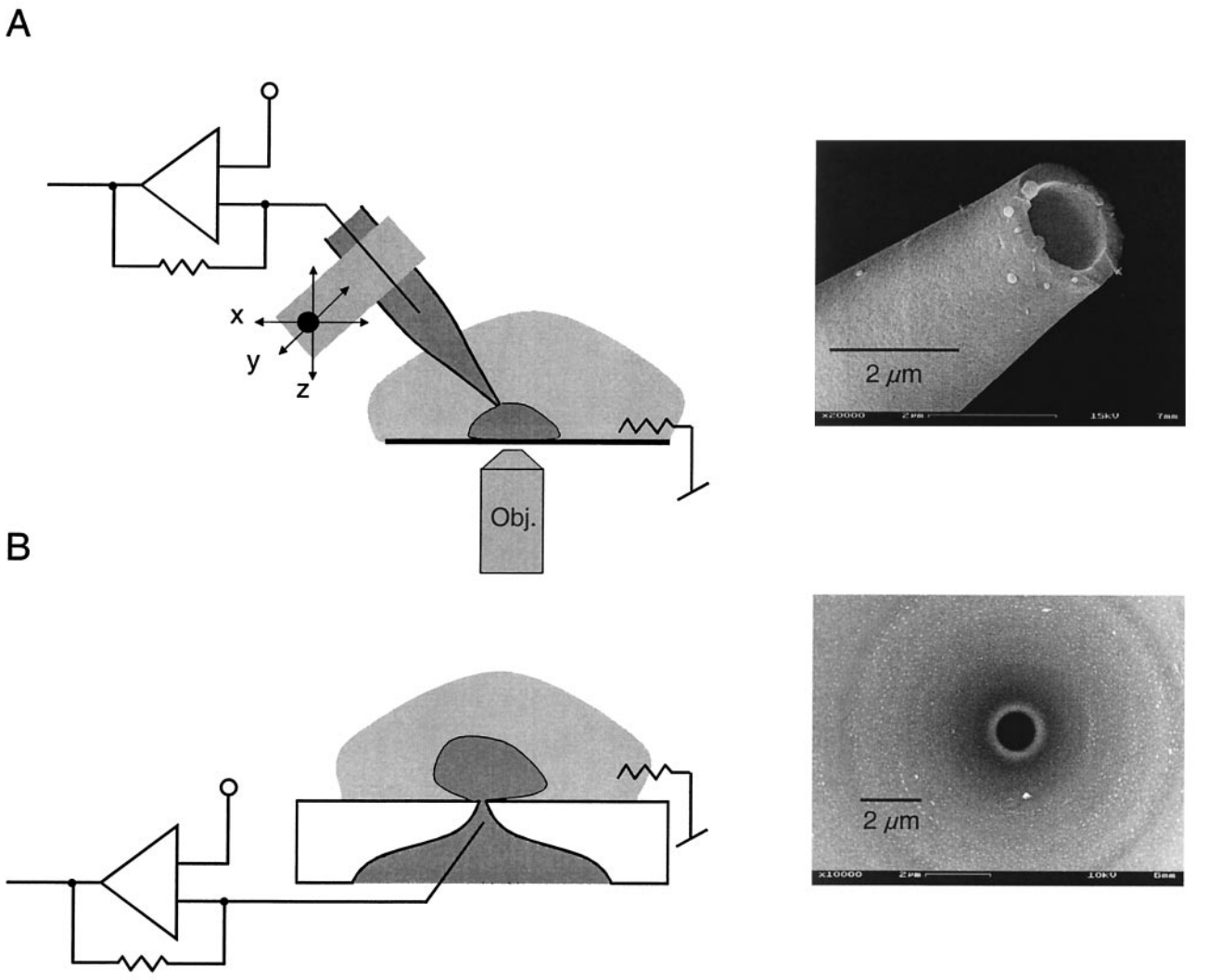

FIGURE 1 Replacing the patch clamp pipette with a microstructured chip. (*A*) Whole cell configuration of the classical patch clamp technique. Using an *x-y-z* micromanipulator and a binocular microscope, the tip (diameter 1–2 μm) of a glass pipette filled with electrolyte solution is positioned onto a cell. Suction is applied to the pipette interior to seal the cell membrane onto the tip. Another suction pulse then breaks the membrane, establishing electrical access into the interior of the cell and replacing diffusible substances inside the cell with those contained in the pipette. (*Inset to right*) Scanning electron (SE) micrograph of the tip of a typical borosilicate pipette. (*B*) Whole cell recording using a planar chip device. The quartz chip of thickness; 200 μm contains a region thinned to 20 μm by chemical wet etching. Into this region, an aperture of micrometer dimensions is produced using ion track etching (see text). The back cavity of the chip is filled with electrolyte. Extracellular solution is given onto the chip surface where a droplet is formed due to surface tension. Cells in suspension are positioned and sealed onto the aperture by brief suction. Further suction establishes electrical contact as in *A*. No microscope or micromanipulator is needed.

forate crystalline quartz substrates resulting in triangular shaped apertures. High resistance seals were not obtained with these triangular openings.

The chip device is also applicable for recordings from artificial lipid bilayers. Bilayers are prepared by the painting method (Müller et al., 1962) and due to the small diameter of the apertures, the lipid membranes have low capacitance. This is desirable for low noise or high bandwidth experiments (Levis and Rae, 1998), as the bilayer capacitance is among the dominant noise sources in bilayer recording (Wonderlin et al., 1990). Different approaches have been reported using microfabricated silicon chips for bilayer recordings, either using an suspended, microperforated Si_3N_4-layer (Schmidt et al., 2000), or a somewhat larger hole machined into silicon substrate with a subsequently deposited SiO_2-layer for insulation (Pantoja et al., 2001). Extraordinarily small openings were defined by ion beam sculpting (Li et al., 2001), also using suspended Si_3N_4-layers. The use of quartz a substrate bears the intrinsic advantage of having an insulating bulk material, which is favorable for electrical recording. Chips made from silicon, being a semiconducting material, always introduce a certain amount of capacitance

due to the free charge carrier density in the substrate, which leads to transient, parasitic currents upon voltage steps applied. Also the light transmitting properties of the transparent quartz can be advantageous for optical or spectroscopical experiments.

Due to its planar surface, the chip device is ideally suited for the application of scanning probe techniques (Gimzewski and Joachim, 1999) like scanning force microscopy or scanning near field optical microscopy (Lewis et al., 1999). As this can be done with concomitant electrical recording, new kinds of experiments to elucidate the structure function-relation of ion channels become feasible (MacDonald and Wraight, 1995).

From a more applied point of view the advantages of the chip approach are even more obvious, as the automation and parallelization of patch-clamp recording is a long sought goal of the pharmaceutical industry for drug screening of ion channel active compounds.

MATERIALS AND METHODS

Chip fabrication

Amorphous quartz with a thickness of 200 μm was used as a substrate for the chips. The quartz was locally thinned to ~20-μm remaining thickness, applying standard planar processing techniques. A 200-nm-thick Au layer was deposited on both sides of the substrate using a thermal evaporation chamber. As an adhesive layer, 5 nm of NiCr were deposited below the Au mask. A thin (1 μm) layer of photoresist (Microposit S1813, Shipley, U.K.) was deposited on the quartz with a programmable spin coater operated at 3500 rpm. The photo resist was baked at 90°C for 20 min. The lithographic step defining round etch masks with 500-μm diameter was performed with a mask aligner (Karl Suess, Munich, Germany) using a mercury lamp (350 W, 365 nm). The sample was then developed using a premixed developer (Shipley). The etch masks in the photo resist were transferred into the Au layer by a wet etching step in $HCl:HNO_3$ (1:2). The thinning of the quartz was also done chemically using fluoridic acid (10% distilled water room temperature) in which the structured Au layer served as the etch mask.

The quartz membranes were penetrated by a single, highly accelerated gold ion (11.5 MeV/nucleon, available at the linear accelerator UNILAC, Darmstadt, Germany), which leaves a cylindrical damage zone in the substrate, the so-called ion track (Toulemonde, 1990). To avoid exposure of the quartz membrane with multiple ions, a detector monitored the penetrating ion and activated a shutter to shield the sample accordingly. The process is described in detail elsewhere (Fertig et al., 2001). Briefly, the latent track in the quartz produced by the swift ion was etched open using fluoridic acid, resulting in a small aperture. The etching was done only from the prethinned side of the chip, whereas the chip surface was protected by an etch mask. With this approach, a conical-shaped etch groove is formed along the latent track. The etching is performed under temperature control, and fresh etchant with well-defined concentration was used to maintain a consistent etch rate in between different batches. With this process stability control, the etch time can be estimated for a given quartz membrane thickness, which is sufficient to reproducibly achieve micron-size apertures. The remaining Au layer from the etch mask was then stripped off the chips using $HCl:HNO_3$ (1:2).

Cellular electrophysiology

For patch clamp recordings, a commercially available amplifier and data acquisition software (Axopatch 200B, Axon Instruments, CA) was used. The recorded data were filtered at 5 kHz and sampled at 12 kHz. Cells of mouse neuroblastoma clone N1E-115 (Amano et al., 1972) as well as Chinese hamster ovary (CHO) cells (Zhou et al., 1998) were grown in Dulbecco's modified Eagle medium (Life Technologies/Gibco-BRL, Cleveland, OH), supplemented with 10% fetal calf serum (Life Technologies/Gibco-BRL) at 37°C in humidified atmosphere of 5% CO_2 in air. For the preparation of cell suspensions the CHO cells were acutely dissociated applying standard trypsin treatment and trituration, whereas the N1E-115 cells were simply scraped of the petri dish. In both cases the solution containing the cells from a standard round petri dish (3.5-cm diameter) was centrifuged at 120 $\times$ *g* for 5 min and the resulting cell pellets were resuspended in 300 to 500 μL of the appropriate electrolyte solution. Electrolyte solutions used had the following ionic compositions: N1E-115 cells, extracellular (top of chip), 125 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 12 mM $CaCl_2$, 20 mM HEPES, 10 mM glucose, pH 7.35, 270 mM mOsm; intracellular (underside of chip), 110 mM CsCl, 1 mM $CaCl_2$, 10 mM HEPES, 3.45 mM BAPTA, 5 mM MgCl, pH 7.28, 240 mOsm; CHO cells, extracellular (top of chip), 160 mM sodium aspartate, 4.5 mM K-Asp, 5 mM HEPES, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4; intracellular (underside of chip), 135 mM K-Asp, 10 mM EGTA, 10 mM HEPES, 8.5 mM $CaCl_2$, 2.1 mM $MgCl_2$, pH 7.2, the resulting free Ca^{2+} concentration was 1 μM.

Lipid bilayers

The lipid used for painting the bilayers was diphytanoyl-phosphatidylcholine (DPhPC) purchased from Avanti Polar Lipids (Alabaster, AL). Lipid was dissolved in *n*-decane at a concentration of 1 mg/mL. The bilayers were painted onto the aperture in the chip using a teflon sheathed silver wire. The surface of the chip was not chemically pretreated as commonly done in bilayer recording. The formation of bilayers was monitored by voltage pulses and corresponding capacitive currents. Alamethicin purchased from Sigma Aldrich was given to the *cis* side of the chip from a methanol stock solution in appropriate concentration to observe single channel activity. The bilayers in the chip were voltage clamped at 100 mV, and the ionic currents were amplified with a gain of 10 mV/pA. The data were filtered at 10 kHz and sampled at 22 kHz. The solution used for bilayer recording was 1 M NaCl in 120 $\times$ *g* (MilliPore).

RESULTS AND DISCUSSION

Whole cell recordings

For the patch clamp experiment, the quartz chip is mounted in a recording set up and is covered with electrolyte solution on both sides (Fig. 1 *b*). The chip is glued onto a custom made holder, which allows the application of suction/pressure via a small tube. The aperture in the planar quartz membrane thus replaces the pipette tip commonly used to contact the cell membrane. To carry out electrical measurements, the ensemble was connected to an amplifier via $Ag/AgCl_2$-electrodes in the electrolyte. Due to its geometry, series resistance and capacitance associated with the chip are somewhat reduced compared with the patch clamp pipette. The series resistance of chips containing a 1 μm aperture is ~4 MΩ in standard Ringer solution. The capacitance of the whole chip in electrolyte solution is less than 1 pF.

Fig. 2 shows on-chip whole cell recordings from N1E-115 neuroblastoma cells (Amano et al., 1972) of Ca^{2+} currents induced by a series of depolarizing voltage pulses. For an experiment, 5 to 10 μL of the cell suspension is

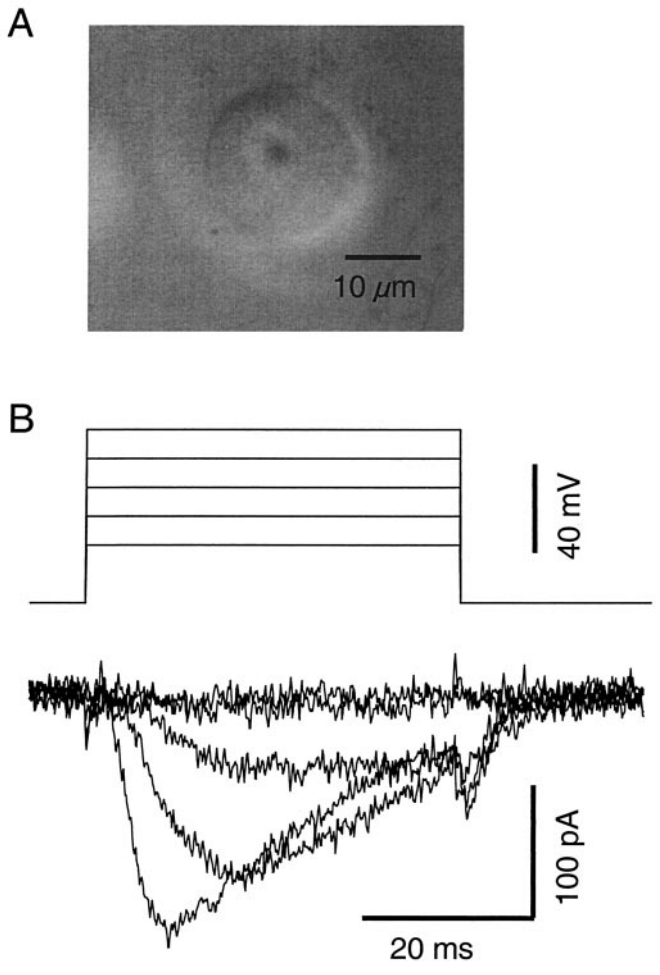

FIGURE 2 Whole cell Ca^{2+}-currents from a neuroblastoma cell recorded with the chip device. (*A*) Video-micrograph of a N1E115 neuroblastoma cell positioned on top of the quartz chip device. The aperture is discernible below the cell. (*B*) Ca^{2+}-currents were elicited in response to voltage steps from a holding potential of −70 mV.

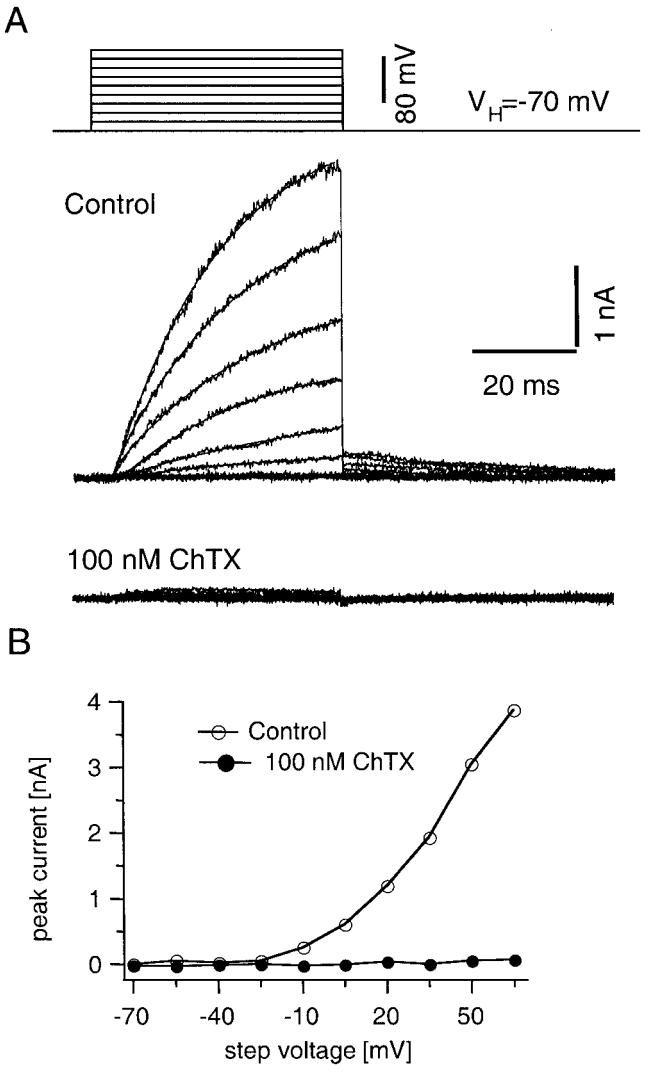

FIGURE 3 Pharmacological characterization of K-channels using the chip device. (*A*) CHO cells overexpressing a BK-type Ca2+-activated potassium channel were recorded. Outward currents were blocked by the selective blocker charybdotoxin (100 nM). (*B*) Current-voltage relations from the experiment shown in *A*.

given to ~30 to 50 μL of the extracellular solution on top of the chip. Whereas the cells in suspension are settling for ~30 s, pressure (250 mbar) is applied to the chip. The outstreaming fluid prevents contamination of the aperture with cell debris. After switching to suction (200–600 mbar), a cell is moved onto the aperture. Depending on the distance of the nearest cell to the aperture, e.g., the cell density and settling time, this process takes place within a few seconds. Once a cell is on the aperture, suction is reduced to enable seal formation. The magnitude of suction applied in this approach also depends on cell type, for example the larger N1E-115 cells needed somewhat more suction than the small CHO cells. After automatically positioning and sealing a cell onto the aperture via suction, a short, more intense suction pulse is applied to break open the cell membrane for whole cell recordings. The whole procedure is performed without use of a microscope or micromanipulator normally used in patch clamp experiments for positioning the recording pipette. To avoid any contact of the aperture with cell debris during suction pulses, a very clean cell suspension is necessary. Whole cell recordings with the quartz chip as shown here were successfully recorded in ~30% of the trials. The Ca^{2+} currents show the known characteristics (Moolenaar and Spector, 1978) and demonstrate the functionality of the patch clamp chip.

Fig. 3 illustrates a similar recording of currents through Ca^{2+}-activated K^+-channels expressed in CHO cells (Zhou et al. 1998). For CHO cells in more than 50% of the trials, whole cell recordings were obtained. Application of the selective antagonist charybdotoxin to the upper side of the

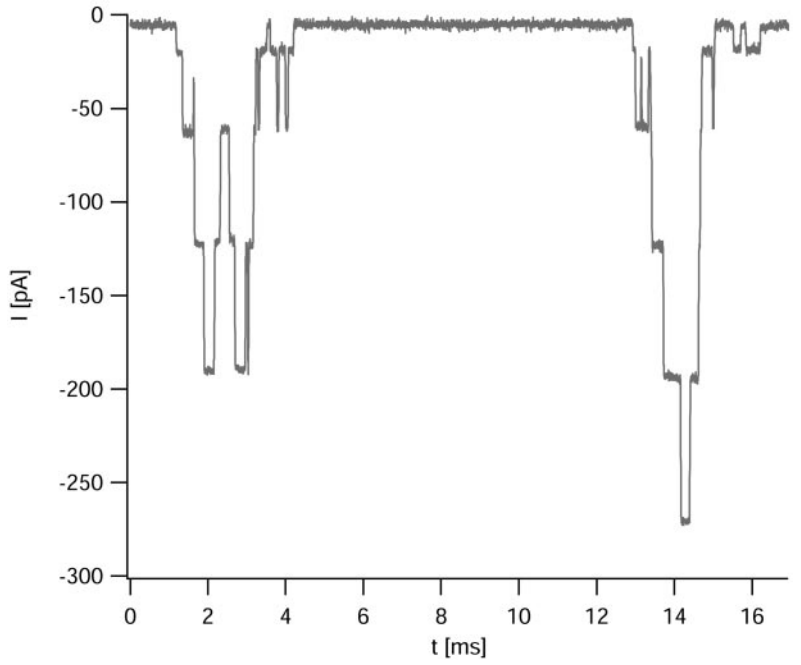

FIGURE 4 Single channel recordings from alamethicin in a lipid bilayer. Alamethicin was given to the electrolyte solution (1 M NaCl) from an methanol stock solution. Apertures in the chips used for bilayers recordings had diameters of 10 μm. A potential of 100 mV was applied and the recording was low-pass filtered at 10 kHz.

chip, e.g., the extra-cellular face of the membrane, blocks the current (Hanner et al., 1997). This experiment shows that it is possible to conduct pharmacological experiments using this device. Because the electrolyte volume on top of the chip is typically only 30 to 50 μL and can easily be further reduced, rapid exchange of solution is possible.

The characteristics of ionic currents were identical in all experiments using the chip device and a conventional patch clamp set-up operated in the same laboratory. The obtained seal resistances with the pipette are a factor 3 to 5 higher than those obtained with the chip. The quality of recordings taken with the pipette are therefore somewhat better compared with those from the chip. Still, there are no major differences in the quality of data obtained and improvement of seal resistances achieved with the chip device will further decrease these differences. For standard whole cell recordings, e.g., to obtain a dose/action relation of a compound on an ion channel protein, the quality of data obtained with a chip-based recording is satisfactory.

Single channel experiments

The patch clamp chips presented here also allow the probing of single ion channels as shown in Fig. 4. Here we spread a lipid bilayer across the aperture in the chip, applying the method of Müller et al. (1962). Alamethicin (Woolley and Wallace, 1992) was incorporated into the bilayer and ionic currents mediated by single alamethicin channels were recorded with a high fidelity bandwidth. This approach can be combined with scanning probe techniques, as we have shown in earlier work (Fertig et al., 2000), where cell membrane adhesion on micron-sized apertures was monitored by confocal microscopy. The application of electrophysiological techniques concomitant with other physical methods, e.g., optical (Ide and Yanagida, 1999), spectroscopical (Mannuzzu and Isacoff, 2000), or mechanical (Zhang et al., 2001), are greatly facilitated by the chip approach compared with the use of pipettes. Specifically the combination with fluorescence resonance energy transfer experiments (Selvin, 1995), which have proven very helpful in ensemble studies on ion channel protein dynamics (Cha et al., 1999; Glauner et al., 1999), bear the potential of being extended to the single molecule level (Weiss, 1999; Ha et al., 1999; Schütz et al., 2000; Lougheed et al., 2001). As for whole cell measurements, single channel measurements can be performed in parallel.

CONCLUSION

In summary, we show for the first time whole cell recordings conducted with a microstructured chip, which bears the potential of performing a great number of whole cell recordings in parallel. For drug screening applications it would be favorable to have an array of microstructured apertures on a single chip and to perform multiple patch clamp experiments simultaneously (Denyer et al., 1998;

Gonzalez et al., 1999; Xu et al., 2001). We are currently focusing on a parallel format of such patch clamp chips. Prototypes of chips containing 16 apertures have been processed successfully. The chip electrodes have also been proven suitable for single channel recording from lipid bilayers, enabling measurements with low background noise. Furthermore, there is a host of novel, emerging biotechnological applications of the patch clamp techniques that would also profit greatly by a parallel array format (Meller et al., 2000; Howorka et al., 2001). However, already in its present single aperture format, the chip-based approach presented here greatly facilitates electrophysiological experiments as the complex procedure of contacting and sealing the cell is automated and can be performed by untrained personnel.

Finally, transferring the patch clamp technique onto a planar device enables a variety of new kinds of experiments on ion channels. For instance, scanning probe techniques such as force microscopy or near field optical microscopy can easily be performed on the planar patch clamp chips presented here. The pipette is simply a passive device that enables the recording, whereas a planar electrode offers the opportunity to further integrate devices on chip. For example, electrodes can be evaporated onto the chip surface to be in the close vicinity of the ion channels and ultimately active elements like field effect transistors for an on-chip preamplification may further reduce the noise level. In this sense, the presented chip serves as the basic building block to form a workbench for probing ion channels with a variety of physical techniques.

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