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Microstructured glass chip for ion-channel electrophysiology

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We present a technique by which it is possible to produce a planar sensor for ion channel electrophysiology from glass substrates. Apertures with diameters in the low micrometer to submicrometer range are achieved by irradiation of a glass chip with a single heavy ion and subsequent wet track etching. The function of the device is demonstrated by recordings of single channel currents mediated by the model ion channel gramicidin A in lipid bilayers spanning the micromachined aperture.

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Ion channels play key roles in functions and dysfunctions of all cells. The most direct and accurate methods for studying ion channel behavior record the transmembrane current that results when channels open to allow ions to flow, while keeping the transmembrane voltage constant. The most precise of these voltage-clamp techniques is patch clamping [1,2], where a tight, high resistance seal is formed between the tip of an electrolyte-filled glass pipette and the cell membrane. This high resistance increases the resolution of recording so that currents mediated by few or even single open channels can be directly observed [3].

Recently, attempts are being made to replace the patch clamp pipette by planar chip-based sensors. Such an arrangement could facilitate automation and parallelization of ion channel recording when arrays of multiple sensors on a single chip are used to record from multiple membranes simultaneously. Moreover, a miniaturization of the sensor will reduce its electrical capacitance and, therefore, further increase resolution. Last, a planar geometry of the set up favors simultaneous use of optical or other techniques to study ion channels.

In order to take advantage of standard microstructuring techniques, silicon has been used as a substrate for such a device. However, this approach forgoes the superior electrical insulation provided by glass. The minimal requirement for an electrophysiological ion channel sensor is a small (micron-sized) aperture in an insulating material that separates two electrolyte-containing compartments. Drawing out a glass pipette to provide a micron-sized orifice is an elegant way of producing an aperture of small dimensions at the tip of a device that can be handled. However, this also produces a relatively long pathway through which the current must flow to the opening, leading to a considerable series resistance and capacitance of the device. It is also obvious that the fabrication procedure and the resulting geometry is unfavorable for producing arrays of such apertures.

Even before the advent of the patch clamp technique, single ion channels were studied using planar lipid bilayers [4]. The apertures for bilayers are commonly produced in a thin teflon sheet by mechanical methods such as drilling. This perforated film is mounted as a diaphragm separating two solution-containing compartments. These apertures typically have diameters ranging from a few millimeters down to about 150 μm. The bilayer is composed either of solvent containing lipids [5] or of two solvent-free lipid monolayers [6]. Due to the large area of the resulting lipid membranes, their capacitance is rather high. The membrane capacitance in conjunction with the input voltage noise of the field effect transistor (FET) of the amplifier headstage plus the thermal voltage noise of the access resistance to the bilayer is mainly responsible for the noise level in the experiment [7]. In addition, the capacitance across the sheet containing the bilayer in a series with the input voltage noise of the FET must also be considered. Low-pass filtering is applied to achieve a suitable signal-to-noise ratio that limits the time resolution of an experiment. Consequently, reducing the size of the apertures and optimizing the geometry of the whole arrangement is most important for lowering the background noise. The development of the shaved aperture technique [8] enables the fabrication of apertures with sizes down to about 25 μm and improves the performance considerably. Up to now the best approach for low noise recordings from lipid bilayers is the so-called tip-dip technique [9,10], in which a bilayer is spread across the tip of a low resistance pipette, with tip diameter about 5–10 μm.

For achieving not only very fine apertures, but also to enable array fabrication in a parallel manner, advanced processing techniques from semiconductor technology can be applied. The standard material for the production of integrated circuits is silicon. Hence, in pilot studies by Fertig *et al.* [11], a silicon chip covered with silicon-nitride insulation layers was microstructured using conventional processing techniques. In this way, small orifices were realized. Initial conductance measurements on artificial bilayers formed by vesicle spreading were performed using a very similar approach by Schmidt *et al.* [12].

However, the glass materials used for patch pipettes are superior to semiconductor materials, due to their lower charge carrier density and the lower dielectric constant $\epsilon_Q = 4$ as compared to 10 for silicon, leading to much lower total capacitances. The main problem with glass and quartz so far is the difficulty in micromachining wafer materials. We describe below, the fabrication of submicron apertures using the ion-track etching technique for on-chip single-channel recording in detail. Such an ''on-chip pore'' in glass, fulfills in particular, the desired low noise requirements. Fi-

nally, initial experiments on artificial bilayers containing the pore-forming peptide gramicidin A are presented.

An alternative processing technique had to be developed for micromachining amorphous glass substrates. The substrates have a thickness of 200 μm and are partitioned into 5×5 mm^2 chips. Circular areas with a diameter of 300–500 μm are thinned down to 80 μm by isotropic etching in hydrofluoric acid (HF, 10 vol % aqueous solution, at 60 °C). An etch mask is defined by photolithography and evaporation of a 200-nm thick layer of gold. In a second step, the prethinned glass film is exposed to a gold ion beam of 2260 MeV available at the linear accelerator UNILAC (Darmstadt, Germany). This experimental facility allowed us to penetrate each chip with precisely one ion. Heavy ions of such high energies produce so-called latent tracks characterized by a cylindrical damage zone of a few nanometers in diameter [13,14]. Tracks exhibit a much higher etching rate than the surrounding bulk material [15]. Upon etching with hydrofluoric acid from one side of the chip, a conically shaped groove forms along the track. The etching process is stopped as soon as the etching cone reaches the opposite side of the chip. Circular, smooth apertures on a submicron scale can be fabricated. Figure 1 shows scanning electron micrographs at different magnifications of such microstructured glass chips.

The geometry of aperture chips significantly reduces the capacitance of the device to well below 1 pF compared to the pipettes used previously with capacitances in the range of a few pF. Furthermore, the chip exhibits very low series resistances of about 100 kΩ for 1M CsCl solution and a micron-sized aperture. This is more than ten times less than in the case of the pipette and improves the RC behavior of the device. The diameter of the apertures etched into the chip ranges from less than a micron to about 50 μm. So far, only chips with single openings have been processed to demonstrate the feasibility of this chip-based approach, but the technique can be readily extended to micromachining arrays of apertures on a single chip. Using planar processing techniques from semiconductor technology, the fabrication can be brought to wafer-scale production, thereby minimizing costs.

In bilayer recording, most of the capacitance arises from the bilayer itself. Due to the small pore sizes, the capacitance of the lipid bilayers is also reduced significantly. With a specific capacitance of the order of 0.5 μF/cm^2 and bilayer diameters of 150–500 μm, the bilayer contribution to the capacitance is approximately 100–1000 pF. For bilayers having diameters around 1-5 μm, this can be reduced to 5–100 fF leading to a very good low noise performance.

The aperture-equipped chips are finally mounted onto a custom made holder made of polycarbonate. Different holding systems have been developed to mount the chip either horizontally or vertically. The chip is surrounded by an electrolyte solution and is connected with the headstage via Ag/AgCl electrodes. To produce bilayers, the lipid diphytanoylphosphatidylcholine (DPhPC, Avanti Polar Lipids) solved in *n*-decane (1 mg/ml) is spread over the aperture with a teflon-sheathed silver wire. Due to the small size of the opening, pretreatment of the aperture, as usually done

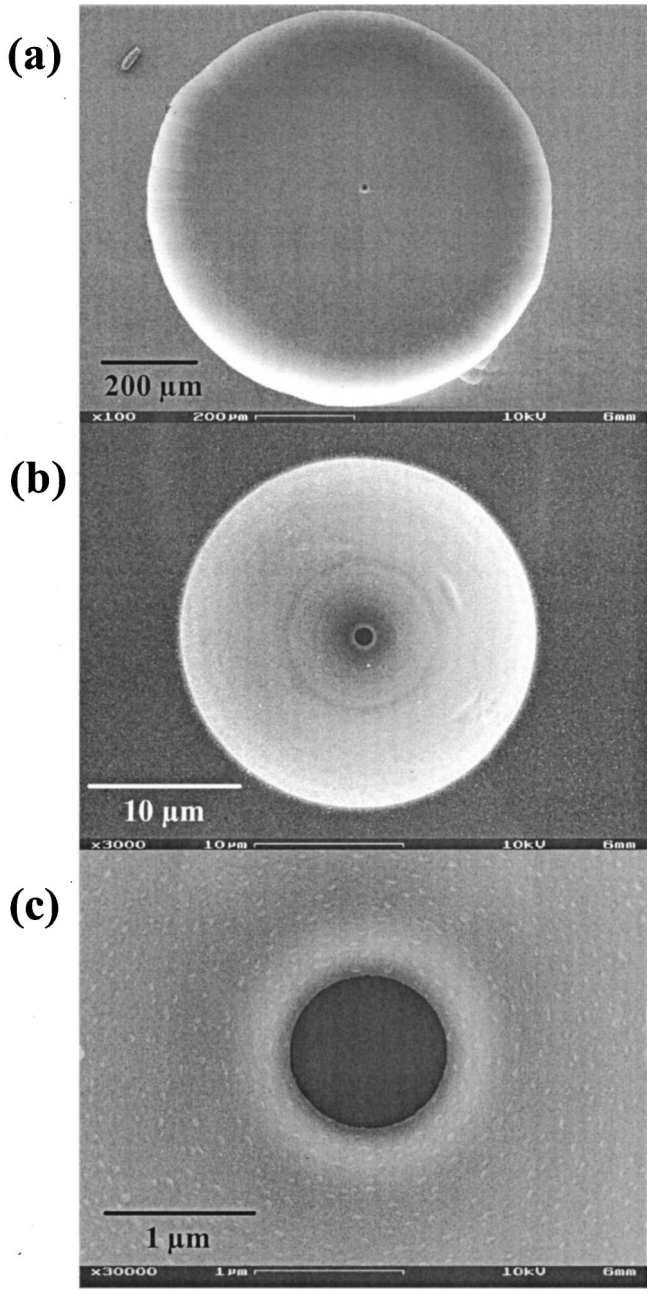

FIG. 1. Scanning electron micrographs of a glass chip microstructured with the ion track technique. The sequence shows the complete pre-etched groove (a), defined by the Au etch mask, the etched ion track in the glass (b), and its small, round aperture (c).

with bilayers in teflon sheets, is not necessary here. The standard pretreatment with a lipid containing chloroform solution actually has a deleterious effect, as it often leads to clogging of the aperture. The bilayer forms spontanously after a few seconds, monitored with a test voltage pulse at seal resistances in the range of 1–100 GΩ. Bilayers prepared by this method are stable for hours, even if high potentials (200 mV) are applied.

To test our device, ionic currents through gramicidin A channels were recorded as shown in Fig. 2. Gramicidin is a low molecular weight peptide, well characterized and widely

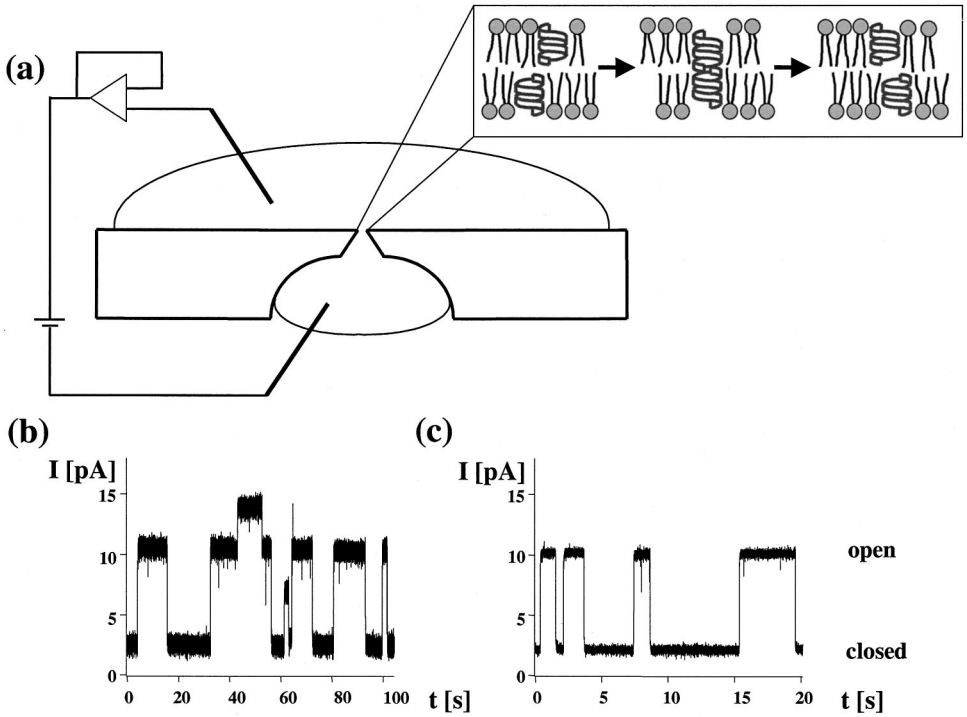

FIG. 2. (a) Schematic of the chip and the recording setup. The inset shows the gramicidin monomers in an artificial bilayer and the formation and dissociation of an ion conducting dimer. Current vs time recordings from gramicidin A channels in the CsCl solution (3M) with different filter cutoff frequencies [3 kHz (b), 1 kHz (c)] are performed with a 200-mV potential applied.

applied as a model pore [16,17]. The gramicidin monomer is barrel-shaped and has a length of about 1.5 nm. It is readily incorporated into lipid bilayers from a solution and diffuses freely in the membrane monolayers. When gramicidin is added to the electrolyte solution on both sides of the membrane, monomers diffuse in both monolayers that make up the membrane. Eventually, two monomers meet and form a metastable dimer. This dimer spans the whole bilayer and provides an aqueous diffusion path for ions.

Figure 2 shows current versus time recordings from gramicidin A channels at different bandwidths. Here, a bilayer from DPhPC is spread over a glass chip with an aperture of 2-μm diameter. The actual size of the bilayer is expected to be somewhat smaller than the aperture diameter, due to the lipid reservoir that builds a torus at the edge of the orifice. Gramicidin is added to both sides of the membrane in an appropriate amount for single channel observation. To provide a driving force for ion flux, a voltage typically around 200 mV is applied across the bilayer. Gramicidin is selective for monovalent cations and when CsCl solution is used, Cs^+ currents are recorded. The total rms current noise at a 3-kHz bandwidth is about 320 fA, which can be reduced to 260 fA using a band pass filter ranging from 0.3–3 kHz. The single channel conductance steps corresponding to the formation and/or dissociation of a dimer are clearly resolved, even at a 3-kHz bandwidth. The noise level in these experiments is very good by bilayer standards, but is certainly not the best possible by our chip approach. Best noise levels achieved in pipette patch clamping are on the order of 60–70 fA in a 5-kHz bandwidth [7]. In our experiments, besides the bilayer capacitance, other relevant noise sources are the capacitance of the entire chip device in series with the input voltage noise of the FET and the thermal voltage noise of the reference electrodes, of which the former is likely to be dominant. The prethinned round area of a 500-μm diameter on the backside of the chip is filled with solution, which also completely covers the upper face of the chip (see Fig. 2). The area of the chip exposed to the solution is about 0.3–0.8 mm^2 and has a thickness of 20–30 μm, the arising transchip capacitance is therefore about 0.5–1 pF. This is the probable main source of noise, which can be further reduced by minimizing the transchip capacitance, e.g., reducing the area of the chip exposed to solution and increasing its thickness. Although there is room for further improvement, these experiments already demonstrate the good low-noise performance of the chip device presented here.

In conclusion, we have developed a technique for microstructuring single pores in glass chips. Based on the low background noise, ionic currents through single ion channels in lipid bilayers can be recorded at a high bandwidth. Due to the planar geometry of the device, scanning near-field optical microscopy or confocal fluorescence microscopy are easily applied, thereby enabling fluorescence experiments at the single molecule level [18]. Hence, this chip is a key device for a combined spectroscopic and electrical recording setup. The opportunity of paralleling electrophysiological characterization of drug candidates and drug target channels makes our approach attractive for high throughput drug screening (HTS) applications. Making advanced electrophysiological techniques such as patch clamping compatible with HTS requirements is a goal long sought by the pharmaceutical industry [19,20]. Highly integrated chip-based electrophysiology might provide a solution for this pressing need.

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