The following chapter 14 is a review of the development of the planar patch technique. Instrumentation based on the planar patch clamp principle is made available by Nanion Technologies GmbH.

(www.nanion.de)
1. Introduction

The technique of patch clamping can be seen in retrospect as a combination of two separate lines of development that both originated in the 1960s and 1970s. The classical biophysics of the nerve impulse had by then been established in the squid giant axon using a combination of (1) voltage clamping with axial wire electrodes and (2) internal perfusion or dialysis. This combination had given experimenters control of both the electrical and the chemical gradients governing membrane ion flux. The problem of the day was to extend this type of analysis to smaller, noncylindrical, cellular structures (such as neuronal somata) that would not allow insertion of metal wires, let alone tolerate any of the procedures used for internal perfusion or dialysis of squid axons. While intracellular glass microelectrodes (Ling and Gerard, 1949) afforded intracellular electrical access to most cellular somata, two independent electrodes for current passing and voltage recording, respectively, were initially necessary, until time-sharing systems made single-microelectrode voltage clamping possible (Wilson and Goldner, 1975). Even then, however, two severe problems remained: (1) spatially nonuniform voltage control (the so-called space-clamp problem), and (2) the lack of control over intracellular ionic composition. Each of these provided the driving force for one of the two branches of development mentioned above.

The need for perfect space clamp led to the development of patch clamping in its original sense, that is, extracellular voltage clamping of an electrically isolated patch of membrane by means of a smooth-tipped glass pipette filled with electrolyte (Huxley and Taylor, 1958; Pratt and Eisenberger, 1919) that was connected to a voltage-clamp
circuit pressed against the cell membrane (Frank and Tauc, 1963; Neher and Lux, 1969; Strickholm, 1962). The accomplishments of this original mission of the patch-clamp pipette (e.g., Eckert and Lux, 1976; Heyer and Lux, 1976), however, were soon eclipsed by the well-known scientific triumph of the first direct recordings of single-ion-channel currents through very small areas of a cellular membrane, which was enabled by a miniaturized and simplified version of the same device (Neher and Sakmann, 1976).

Solutions to the problem of internal perfusion, on the other hand, were pioneered in the Bogolometz Institute of Physiology in Kiev (Kostyuk et al., 1975; Krishtal and Pidoplichko, 1975). The first version of this internal perfusion method used 250- to 300-μm-thick sheets of polyethylene forming partitions between two electrolyte-filled compartments and containing single pores produced by thermal moulding with a hot metal microneedle (Fig. 1). These relatively large openings (10–50 μm) could be used to trap large, enzymatically isolated neurons from snail ganglia so that their membrane sealed off the opening. The membrane inside the aperture could then be destroyed by a short pressure transient in order to establish access to the whole cell. The seal (or, more appropriately, shunt) resistances thus achieved were rather low but sufficient for recording because the snail neurons had very low input resistances themselves. Internal perfusion, on the other hand, was quite efficient. However, this first appearance of the apertures in planar substrates in cellular electrophysiology remained a short episode. The Kiev group rapidly abandoned the planar geometry in favor of a pipette-like structure made from plastic tubing (Kostyuk et al., 1984), while in another laboratory, that of Arthur M. “Buzz” Brown in Galveston, TX, the internal perfusion principle was adopted for use with smaller vertebrate and mammalian cells by using carefully fire-polished, perfusable glass suction pipettes (Lee et al., 1980).

The final synthesis of both strands of development happened when the gigaohm seal and whole-cell patch clamp recording were discovered in Göttingen: applying suction instead of just pressing the pipette against the membrane did an important part of the trick for the gigaseal, and rupturing the membrane patch with further suction pulses to obtain whole cell access provided the long-sought full control of the ionic driving forces, even with very small cells.

1 In fact, in Hamill et al., 1981, the reader is introduced to the whole-cell configuration of the patch-clamp technique: “The technique to be described can be viewed as a microversion of the internal dialysis techniques originally developed for molluscan giant neurons.”
Planar Patch Clamping

1 Whole-cell patch clamping, in particular, developed from a niche method into a mass movement and the pipette became the natural tool of the electrophysiologist. The alternative structure, the aperture in a planar partition, however, lay unused for almost two decades. In retrospect, the main reason for this is that there was no good method to make suitably small openings in planar substrates. Clearly, diameters need to be in the low micrometer range in order to be useful for small cells, and the elegance and ease of micropipette fabrication by pulling heated glass tubes is such that one tends to eschew a search for alternative designs.

It is therefore logical that the “pore in a planar partition” resurfaced more or less simultaneously in a number of laboratories when solid-state microstructuring technologies became more generally known and available to scientists outside industrial
microchip fabrication. While the true primary motive for the attempt to produce a planar version of gigaseal patch clamping probably—in our case, certainly—was the presentiment that it could be done and that it would be both entertaining and informative to try, several advantages of the planar design over the pipette were envisaged, of which a potential for automation, parallelization, and high throughput of cellular electrophysiology was almost immediately realized by all groups involved. The renaissance of the planar partition in the late 1990s coincided with a heightened focus on ion channels as pharmacological targets mediating desirable as well as undesirable effects (e.g., acquired LQT syndrome), which prompted the Food and Drug Administration (FDA) and other regulators to stipulate, for example, human Ether-A-Go-Go Related Gene (hERG) testing for all candidate drugs. Thus, for instance, a pharmaceutical company funded what were probably among the earliest attempts at reviving the planar concept at the Institute for Natural and Medical Sciences Institute (NMI) of Tübingen University in Germany. This work, made public only much later, started out from plastic (polyimide) films into which holes were drilled using focused ion beam milling. The results of these first trials, however, were not particularly encouraging with regard to formation of gigaohm seals (Stett et al., 2003). The same has obviously to be said of several other first attempts, including our own using apertures formed by reactive ion etching in free-standing silicon-nitride/silicon-oxide layers (Fertig et al., 2000). It seems remarkable, therefore, that, less than a decade later, planar patch clamping is an established technology with approximately five different commercially available devices producing useful data in a growing number of industrial and academic laboratories worldwide.

2. Designs and Concepts

2.1. Planar Patch Clamp Chips Based on Polymer Substrates

The laboratory of Fred Sigworth, one of the original Göttingen pioneers of patch clamping, brought forward the idea of using the

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2 This situation at the end of the 1990s also produced technologies to automate pipette-based patch clamping such as the Apatchi by Sophion SA (a spin-off from Denmark’s Neurosearch) and the Interface patch clamp invented by the late David Owen of CeNeS in England, a great pioneer in the field (see review by Mathes, 2006).
heat-curable polymer polydimethylsiloxane (PDMS), better known to electrophysiologists under its trade name of Sylgard™ (Dow Corning, Midland, MI) to fabricate planar patch clamp partitions. Inspired by the work of Whitesides and others (1998), they teamed up with a neighboring solid-state physics lab to apply PDMS-micro-molding technology to the problem. Micromolding either from a micromachined silicon master or from a quartz rod pulled into the shape of a patch pipette and subsequent serial sectioning of the molded polymer was used (Klemic et al., 2002). In this way, apertures with diameters down to about 4 μm could be produced. After oxidation in a plasma oven, the PDMS assumes glass-like surface properties and becomes sufficiently hydrophilic to allow filling of the aperture with salt solutions. These apertures were used to produce gigaohm seals on Xenopus oocytes with a success rate of 13% (Klemic et al., 2002). The technique using pipette-shaped rods was expanded for volume production of apertures by the company Axon Instruments Inc. (Molecular Devices, Sunnyvale, CA) in order to produce the planar patch clamp substrate for their automated patch-clamp project, and whole-cell recordings from rat neuroblastoma cells were obtained using such devices (Osipchuk et al., 2001).

While commercial development of the PDMS technology at Axon was later discontinued in favor of a glass substrate (see below), the Sigworth group found yet another ingenious way of making small apertures in flexible patch partitions made from this cheap and easy to use material; they used a stream of pressurized nitrogen made to flow through a 2-μm opening in a steel plate supporting a thin layer of PDMS during its curing process (Klemic et al., 2005). With these apertures, which have also been obtained in array format (Li et al., 2006), whole-cell and cell-attached measurements from rat basophilic leukemia (RBL) cells have been performed. It is tempting to follow the author’s suggestion that using this approach, planar patch-clamp apertures could be made in the lab in the image of the traditional “electrophysiologist who makes things himself” using relatively inexpensive machinery, much like classical patch pipettes are made with a puller standing next to the patch rig. Because the effects of plasma treatment wear off relatively quickly and shelf life is limited even under water, local production of the devices is clearly mandatory.

Recently, Luke Lee and colleagues at Berkeley published the first promising experiments with a device where the patch aperture is not a through-hole in the substrate but is formed where
a micrometer-sized horizontally oriented channel joins a larger compartment, thus forming what the authors call a “lateral cell trapping junction” (Ionescu-Zanetti et al., 2005; Seo et al., 2004). The device is made by combination of lithographic microstructuring of the photosensitive polymer SU8 and PDMS micromolding from a silicon master. This lateral-patch principle makes it possible to accommodate multiple, electrically independent apertures within a few hundreds of micrometers from each other. Even if only 5% of the seals obtained in the most recent study were gigaohm seals, this technique certainly holds great promise for the future, because it opens the way for a very close integration of microfluidics and automated patch clamping.

Polyimide films, which had performed unpromisingly at the NMI, were nevertheless used in a first commercial product, the Ionworks platform originally developed by Kirk Schroeder and Brad Neagle at Essen Instruments in Ann Arbor, MI (Kiss et al., 2003; Schroeder et al., 2003). Here, arrays of micrometer-sized apertures produced in polyimide films by laser irradiation were arranged in a 384-well format. Seal resistances obtained are in the range of 100 MΩ, which agrees well with the NMI experience. Nevertheless, in the true spirit of our Kievan forebears, even with high leak conductances, useful whole-cell recordings from mammalian cell lines can be obtained using leak current compensation circuitry, and several further studies using the Ionworks device have been published. Recently, a version of the substrate containing 64 apertures per well has been introduced. Here, the recorded membrane current is summed over multiple cells and the relative importance of the large shunt conductance reduced (Finkel et al., 2006). The device is today considered a useful screening tool, even if the recordings obtained are not of the highest quality.

### 2.2. Planar Patch-Clamp Designs Based on Silicon Microstructuring

Silicon micromachining was a well-established technology and had relatively recently become available in academic settings when the planar partition was rediscovered for electrophysiology. It is natural, therefore, that when the search began for a suitable technology to produce an insulating partition with a micrometer-sized orifice, silicon micromachining suggested itself.

Two generally different approaches emerged. In the first, the aperture is formed by electron beam or ultraviolet (UV) litho-
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graphy and reactive ion etching in a thin (100–200 nm) suspended silicon-nitride membrane, which has been obtained by a one-sided KOH-etch of the underlying silicon wafer. The thin layer facilitates the production of very small apertures even well into the submicrometer range. We attempted to use such structures to form gigaseals on cells by suction and, while we were able to trap cells in these apertures (Fertig et al., 2000), obtained very little shunt resistance increase. However, Christian Schmidt, Michael Mayer, and Horst Vogel in Lausanne, using essentially the same structure plus an additional layer of SiO$_2$ and polylysine treatment or silanization, were able to form suspended bilayers from giant unilamellar vesicles on these apertures using electrophoretic positioning (Schmidt et al., 2000). This procedure gave very tight electrical seals (from 1 to 200 GΩ), enabling the recording of ionic current through, for example, channels formed by the peptibol alamethicin. Later on, this design was used by a start-up company called Cytion SA in Lausanne to form gigaohm seals on cells. The company’s procedure to form seals did not rely on suction, but rather on a proprietary coating of the chip surface and promotion of strong surface adhesion of the cell membrane. Whole-cell access could be obtained by adding pore formers to the lower compartment to obtain a perforated patch configuration (Horn and Marty, 1988). Cytion developed a single-channel planar patch-clamp automat that is reported to have worked quite well. Cytion was acquired by Molecular Devices Inc. (Sunnyvale, CA) in 2001, but shut down shortly after.

There is now a general consensus in the field that forming high-resistance seals requires sufficient contact area between the membrane and the wall of a pore. Apertures in very thin (<1–2 μm) diaphragms such as a passivation layer on a silicon wafer, therefore, are not suitable for this traditional seal formation strategy, while, as shown by Schmidt et al. (2000), promoting strong membrane adhesion to the surface of the chip can yield good results. However, the problem might in principle be overcome by thickening the diaphragm with deposition of suitable materials, for example, SiO$_2$. This strategy is currently being pursued by a group in Grenoble, France, and has led to a 16% success rate in forming seals >1 GΩ (Picollet-D’hahan et al., 2004; Sordel et al., 2006).

The second approach based on silicon micromachining starts out with a thin layer of “naked” silicon, into which an aperture is etched by deep reactive ion (DRI) etching of silicon, the so-called Bosch process, which enables high aspect ratios. In a second step,
the silicon is covered with an insulating layer of SiO$_2$ to provide electrical insulation and reduce the size of the aperture. In the first version of these devices, the insulating layer was built using plasma-enhanced chemical vapor deposition (Pantoja et al., 2001, 2004). These devices were very suitable for recording from suspended lipid bilayers (Pantoja et al., 2001), while the probability of forming gigaohm seals with cells was low, probably due to a considerable roughness of the pore’s inner walls. Recently, Matthews and Judy (2006) described a more involved process for making the insulating layer inside and around DRI-etched pores, which consists of thermal wet oxidation (heating to 1100ºC in the presence of saturated water vapor) to form a first SiO$_2$ layer that carries all the original roughness of the parent silicon surface, then removing this layer with hydrofluoric acid to expose the underlying smooth crystalline silicon surface, low-pressure chemical vapor deposition of amorphous silicon and a second thermal wet oxidation step to produce smooth, round openings. Seal resistances exceeding 1 GΩ have been obtained using this aperture.

The great advantage of silicon as a starting substrate is the fact that one can draw from the well-stocked toolbox of an established and mature industry for structuring it and modifying its surface properties. On the other hand, no amount of machining can remove the fact that silicon itself is not an insulator but a semiconductor. Therefore, any silicon-based strategy for making a patch partition has to grapple with the problem of high density of free charge carriers that leads to large capacitances, slow voltage transients, and high noise, as well as that of the photoelectric effect by which light induces even more charge carriers and can give rise to capacitive current flow across the partition.

Nevertheless, careful minimization of fluid contact area (Matthews and Judy, 2006) and packaging of the chips can be used to reduce these problems significantly. Proof of this is the silicon-based planar patch-clamp chip used in the QPatch, a commercial 16-channel planar patch-clamp automat (Kutchinsky et al., 2003; Mathes, 2006) that was successfully introduced into the market by Sophion SA of Ballerup in Denmark, in 2004. This chip device is also produced starting from silicon substrates. Another company, Cytocentrics AG, has been spun off from the NMI to commercially develop a highly ambitious concept called “cytocentering” (Stett et al., 2003), where, in order to imitate the sequence of the separate steps of contacting and sealing that are characteristic of classic
pipette-based patch clamping, the cell is first positioned and held in place by suction through a larger, outer aperture before being electrically contacted by separately controlled negative pressure through a second aperture inside the first. The complex structure required for this approach has been realized in a thick (15-μm) SiO₂ layer on top of a silicon substrate. While the commercial product is not yet available, 10 of 11 chips used were reported to have formed a gigaseal in one test run (Van Stiphout et al., 2005).

2.3. Planar Patch-Clamp Chips Based on Glass Microstructures

Our initial unsuccessful trials with silicon-nitride membranes had also acquainted us with the disadvantages of the silicon as bulk material (see above), which led us toward the one material that is a good insulator and known to seal with membranes: glass. This idea, however, engendered a somewhat lengthy search for ways of making a micrometer-sized aperture into a glass substrate layer. After visiting several dead ends, a cooperation with the Gesellschaft für Schwerionenforschung (Society for Heavy Ion Research) in Darmstadt, resulted in a viable method: after locally thinning a glass or quartz wafer by a one-sided hydrofluidic acid (HF) etch, a single heavy ion can be shot through and leave a so-called ion track, a local structural disturbance that is etched much more quickly by HF than the undisturbed material. Thereby, a further, carefully timed one-sided etching step results in a conical pore with a clean, smooth-rimmed opening (Fertig et al., 2001). With these structures, we were able to perform the first whole-cell (Fertig et al., 2002a) and, a little later, single-channel recordings (Fertig et al., 2002b) on a planar chip device.

Today, glass-based planar patch-clamp chips are commercially used by Nanion Technologies GmbH, our own spinoff, in their semiautomated single-recording device (the Port-a-Patch) as well as in their fully automatic, multirecording patch-clamp robot (the Patchliner) and by Aviva Biosciences Inc., of San Diego, CA, which produce the chips for the Axon Instrument (now part of Molecular Devices Corp.) PatchXpress, another multirecording robot. The Aviva Sealchip™ is chemically treated using a proprietary process and is delivered and stored in a liquid of equally proprietary composition, which has to be removed by washing before use, while Nanion’s borosilicate Nano-Patch-Clamp (NPC) chips are shipped
dry and are used as delivered. They do not degrade even over long periods of time (>1 year) as long as they are protected against moisture and contamination. We typically vacuum package the chips and store them dry at room temperature.

3. Experimental Considerations

In conventional patch clamping, the patch pipette is manually maneuvered under optical control via a microscope, and a specific cell is chosen to place the pipette tip onto the surface of the cell membrane. Then a tight seal is established by gentle suction and an omega-shaped protrusion of membrane is drawn into the patch pipette.

The procedure is very similar but still somewhat different in the case of planar patch-clamp chips. Here, a suspension of cells is placed on top of the chip. Then a single cell is positioned onto the aperture in the chip by application of suction, typically by execution of an automated, software-controlled suction protocol. In contrast to the classic patch-clamp technique, it is the cell that is moved to the aperture and not the pipette that is moved to the cell. As in conventional patch clamping, a seal is obtained by application of suction and the membrane can then be ruptured for whole-cell access with suction or voltage pulses. The result is an electrical connection to the inside of the cell allowing for current recording.

3.1. Cell Culture

As with planar patch clamping, a single cell is randomly chosen from a suspension by application of suction (essentially a blind patch approach!), and good cell quality and viability are mandatory for obtaining optimal results. Hence, the requirements for cell culture and preparation in planar patch clamp tend to be somewhat greater than for the conventional technique. The cells provided in suspension must also be well isolated, as cell clusters are detrimental for the success rate of automated gigaseal formation.

Most experiments to date have been made using Chinese hamster ovary (CHO), human embryonic kidney (HEK), Jurkat, and rat basophilic leukemia (RBL) cells. The cells are either used as wild-type cells or stably transfected with various types of ion channels. The culture conditions used were the standard conditions described in the American Type Culture Collection (ATCC) catalogue for each cell line.
Optimal results were obtained by splitting the cells every second to third day, avoiding confluent cells. Cells such as HEK293 often build clusters, when they are left for longer than 3 days on the plate or in the flask. For splitting it is advisable to use a long trypsin treatment to obtain single cells. Cells should be uniformly distributed throughout the dish. For cell harvesting, a short treatment with a cell detacher such as trypsin or various substitutes (see below) is sufficient and results in healthier cells. While both HEK293 and CHO cells are adherent cells, the CHO are more strongly attached to the substrate than the HEK293 cells. With RBL cells, typically one half of the cells is attached to the plastic while the other half is growing in suspension, which makes them very amenable to cell suspension preparation for automated patch-clamp applications.

3.2. Cell Harvesting

The confluency of the cells should be in the range of 50% to 80%. For harvesting of the cells for an experiment, no significant difference in percentage of gigaseals could be found for using trypsin, Accutase, enzyme-free solution, or phosphate-buffered saline ethylenediaminetetraacetic acid (PBS-EDTA) for lifting the cells. In some cases, treatment with trypsin made the cells more fragile, and the cells sometimes needed a recovery time of approximately 30 minutes.

3.2.1. Harvesting Protocol

Using T75 flasks or plates with diameter = 96 mm and a surface of 60 cm$^2$):

- Wash two times with 10 mL PBS (without Ca$^{2+}$ and Mg$^{2+}$).
- Add 2 mL of detacher (PBS-EDTA 2 mM, trypsin/EDTA, Accutase, . . .).
- Incubate for 3 minutes at 37°C and in 5% CO$_2$ for detaching of the cells.
- Check the detachment of cells under a microscope. Move the plate or flask gently to detach all cells from the bottom (do not hit the flask to detach cells).
- Add 10 mL of HEK medium and FCS.
- Pipette the cells gently up and down with a 10-mL pipette.
After pipetting five times, look at the cells under a microscope. If the cells are already single (~80–90%), no further pipetting is needed.

If cells still form clusters, gently pipette cells another 10 times. Repeat this step until cells are single (~80–90%).

Perform 2 minutes of centrifugation (1000 U/min, 100 g).

Discard the supernatant.

Resuspend the cells in 10 mL medium with fetal calf serum (FCS).

Perform 2 minutes of centrifugation (1000 U/min, 100 g).

Discard the supernatant.

Resuspend the cells in ~200 μL of external recording solution (resulting in a cell density of approximately $1 \times 10^6 – 5 \times 10^7$/mL medium).

An optical control of the cells under the microscope should then reveal single, round cells with smooth membrane edges and no cell clusters.

3.3. Cell Application and Positioning on the Chip

In a very simple manner, the actual aperture containing the partition is mounted onto a twist cap, which is placed onto a holder that contains the reference electrode and a suction line (Fig. 2). Before placing the chip-containing cap onto the holder, a droplet of intracellular electrolyte solution is pipetted to the bottom part of the chip. Another droplet of extracellular electrolyte solution is pipetted onto the top part of the chip, where in both cases about 5 μL of a sterile filtered solution is sufficient. This approach allows for fast exchange of chips after each experiment and enables experiments with low volume consumption.

Fig. 2. The chip is filled with saline solutions and mounted in a holder that allows applying pressure/suction. The electrolyte solution on both sides of the chip is electrically contacted via Ag/AgCl electrodes.
For making electrical contact, an Ag/AgCl electrode inside the holder reaches into the electrolyte solution and another silver wire is placed in the saline on top of the chip, serving as the ground electrode. Due to the miniaturized arrangement, a small metallic container can be used as a Faraday cage for very effective shielding (Fig. 3).

Before the delivery of cell suspension, slightly positive pressure is applied to the chip to prevent contamination of the aperture.

Fig. 3. Schematic of the chip and the procedure of cell contacting. (A) The measured current response to a voltage pulse is shown and again (B) after a cell is sealed onto the aperture by suction. (C) Close-up view of the mechanically and electrically tight contact of the cell membrane and the chip in cell attached mode. (Fertig et al., 2002, with permission. Copyright 2002, American Institute of Physics.)
Then, about 5μL of cell suspension are added to the saline on top of the chip, and a single cell is positioned onto the aperture by suction. The resistance across the aperture in the chip is continuously monitored by application of small voltage pulses and is used as feedback parameter for suction application. After cell application, the positive pressure is released and suction slowly increased. Once a cell is positioned, the resistance increases and continuous suction is applied to form a gigaseal. After gigaseal formation, either single-channel recordings can be obtained in cell-attached mode or brief suction pulses are used to obtain whole cell access.

In a conventional patch-clamp experiment, there often is a significant drop of the seal resistance when breaking the membrane to obtain whole-cell access. This is usually not the case with planar patch-clamp chips. The resistance remains more constant during the process of accessing the whole cell or often even increases further. Due to the planar geometry a very short electrical path is provided to the inside of the cell, allowing for very low access resistances.

For some cell lines it tends to be difficult to rupture the membrane to gain whole-cell access. This is often the case when the cells were grown for more than 3 days on the plate. In this case, it appears to be helpful to use a zapping pulse in addition to some suction. This method works with the patch-clamp chips more reliably than with a conventional patch pipette. Different amplifiers allow different voltage pulses for zapping. In our hands, it works best to use the highest voltage the amplifier can deliver and a short duration.

Typical success rates in obtaining gigaseals and stable whole-cell recordings are in the range of 60% to 90%. Generally, the recordings are somewhat more stable and long-lasting as compared to conventional patch clamping. Any relative movements of the patch pipette with regard to the cell are detrimental to the seal and overall stability; hence, a solid vibration isolation is required in conventional patch clamping. In cases where a chip is used, there are no relative movements and the whole arrangement is mechanically more insensitive, requiring no vibration isolation or suspension.

Pharmacological experiments are carried out by simply adding compounds to the patch clamped cell on the chip either by a pipetting step or by means of a laminar flow chamber for perfusing the cell. Depending on the holder/cartridge design of the chip, microfluidic means can be used for compound applications. We have, for
example, developed a microfluidic cartridge that contains a glass substrate with 16 patch-clamp apertures, each of which is individually addressable by microfluidic channels on the intra- as well as on the extracellular side. This design allows perfusion of cells and compounds by robotic pipetting means, making the whole approach very suitable for automation. In this case of multiple apertures on a single chip, individual, feedback-controlled, suction lines are required for positioning and sealing of the cells.

In addition to scaling up the number of recording channels, the throughput capability is increased by automated application of drugs by a pipetting robot. This is shown in Fig. 4, where a con-

![Graph showing experimental stability](https://example.com/graph.png)

**Fig. 4.** Experimental stability. The data show the maximum current amplitude elicited with a voltage pulse to a holding potential of 0 mV as recorded in Nav1.5 expressing CHO cells. Five concentrations of tetrodotoxin (TTX) (0.3, 1, 3, 10, 30 μM) have been applied, then washed out and applied again to demonstrate the stability of whole-cell recordings. The different current amplitude plateaus correspond to the different TTX concentrations applied, whereby the current is completely blocked at 30 μM, the highest concentration. (Source: Small, 2006, with permission. Copyright 2006, Wiley-VCH Verlag GmbH & Co KG.)
centration series of a compound (tetrodotoxin, TTX) is applied to a whole-cell recording from a cell expressing a sodium channel to generate a dose–response curve and determine the concentration that inhibits 50% (IC$_{50}$) for the compound. In these recordings, a standard voltage pulse is repetitively applied to activate the sodium current such that the blocking action of TTX can be observed as downward steps in the overall sodium current amplitude of the cell. With increasing concentrations of TTX, the amplitude diminishes further and is finally completely blocked. A washout step with control solution shows the complete recovery of the original current amplitude. Following this, the entire protocol is repeated, showing the stability and reproducibility of the experiments.

A further interesting capability of the planar approach is the application of different solutions not only from the extracellular side but also from the intracellular side. For recording with a patch pipette, this is not really possible, due to the long and thin shaft of the pipette, which leads to a diffusion-limited and hence very slow solution exchange. Intracellular perfusion is easily achieved with the patch-clamp chip, as both sides of the cell are accessible due to the flat geometry of the chip. This possibility permits the application of drugs on the intracellular side for the investigation of ligand-dependent ion channels or signaling pathways. As many ion channels are regulated via internal binding sites for second messengers, this is a valuable tool.

Another example of a useful application for internal perfusion capabilities is perforated patches. Here, instead of breaking open the cell membrane for whole-cell access, a pore-forming compound (such as nystatin or amphotericin) is applied internally to render the membrane electrically permeable. In this way, whole-cell recordings can be performed without rupturing the membrane; this can be helpful in preventing rundown or kinetic variances during the recordings.

In Fig. 5, experiments on Jurkat cells expressing a potassium channel (Kv1.3) are shown. Current flow through these channels is known to be blocked by cesium ions, and in the experiment Cs$^+$-containing solution is applied internally, effectively blocking the current through the potassium channel as expected. Two recordings are performed in parallel, demonstrating the scalability of the chip-based approach by simultaneous measurements from different cells on a single chip.
Fig. 5. Internal solution exchange. A nice feature of the planar patch clamp chips is the internal solution exchange during the experiment. The figure shows two simultaneously recorded Jurkat cells in the presence of a control internal solution (A), after the exchange of the internal solution with a Cs\(^+\)-containing solution (B) and a subsequent washout step (C). The complete block of current by cesium and the recovery after washout with control solution well documents the internal perfusion possibility. *(Source: Small, 2006, with permission. Copyright 2006, Wiley-VCH Verlag GmbH & Co KG.)*
4. Outlook

The planar patch-clamp principle is at present used mainly for automated whole-cell patch-clamp recordings. It may, however, have other useful applications. For example, planar lipid bilayers can be formed on these apertures, facilitating the study of reconstituted ion channels and possibly transporters. Schmidt et al. (2000) formed bilayers on their SiN₃/SiO₂ partitions from giant unilamellar vesicles that were positioned using an electric field gradient, while we initially used the painting method to produce bilayers (Fertig et al., 2001, 2002a). Recently, we have used the Porta-Patch and NPC-1 chips to obtain suspended bilayers from giant unilamellar vesicles (GUVs) formed by electroswelling (Sondermann et al., 2006). The GUVs were positioned on the aperture by suction exactly like the cells were. When they rupture, they form a suspended bilayer. With this method, bilayers can be formed on very small apertures (e.g., 1 μm in diameter), where painted bilayers do not form readily.

Besides automation of patch clamping and throughput, advantages of the planar geometry also include the potential for more sensitive recordings of ionic currents and an increased accessibility of the membrane for optical and mechanical detection techniques.

To make more sensitive current recordings, noise needs to be minimized. Given high seal resistances, one of the main sources of noise is any random voltage fluctuation in the voltage clamp amplifier, which forces an equally random current to flow across the total capacitance of the input circuit, including the recording electrode. By reducing the capacitance of the electrode, considerable reduction in noise should be possible. In planar patch clamping, the geometry of the electrode can be chosen more freely than with pulling pipettes and adapted to minimize capacitance. In particular, with pulling a pipette, the wall becomes inevitably thinner as the dimensions get smaller, increasing electrical capacitance. This is obviously not the case when apertures are etched or otherwise machined into bulk material. In fact, optimists have voiced the hope that planar patch-clamp chips may provide a way of approaching the “holy grail” of current measurement: the resolution of elementary charges (Klemic and Sigworth, 2005). The NPC glass chip, when contacted with recording solution on both sides in the way described above, contributes approximately 0.5 pF to the total input capacitance (Sondermann et al., 2006). Using a capacitive feedback
amplifier and without optimizing any other parameters, we have recently performed single-channel recordings with <2 pA root mean square (rms) noise at 30 kHz cutoff frequency (Fig. 6).

For several years, simultaneous electrophysiological and fluorescence measurements have been used to obtain information about state-dependent conformational changes in ion channel proteins. These measurements were performed on large ensembles of
channels expressed in cells (Bezanilla, 2005), where only average properties of, for example, open and closed states can be detected. It would be highly desirable to perform such combined measurements with single in channel resolution to obtain information on individual state—dependent transitions (Selvin, 2002). Pilot studies have been performed using the model channel gramicidin in painted lipid bilayers using a planar patch-clamp–like configuration (Borisenko et al., 2003; Harms et al., 2003) and it seems that similar experiments should be feasible on protein ion channels, either in native cell membranes or reconstituted in planar lipid bilayers.

Finally, the planar patch-clamp situation might be amenable to simultaneous use of electrophysiology and atomic force microscopy or some other scanning probe technique to study movement and shape changes in membrane proteins that accompany their functions (e.g., Gullo et al., 2005).

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