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Microchip Technology for Automated and Parallel Patch-Clamp Recording

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***T**he patch-clamp technique is the state-of-the-art technology for the study of a large class of membrane proteins called ion channels. Ion channels mediate electrical current flow, have crucial roles in cellular physiology, and are important drug targets. However, patch clamping is a laborious process requiring a skilled experimenter and is, therefore, not compatible with the high throughput needed in drug development. The solution for automated and parallel patch-clamp measurements that is provided by microchip technology is presented here.*

Keywords:
- biomedicine
- cells
- drug development
- high-throughput screening
- ion channels

1. Ion Channels in Research and Drug Discovery

Ion-channel proteins reside in the lipid membrane of cells and can form a conductive path across the membrane, thereby enabling ionic current flow. Ion channels can switch conformation between open and closed states, often gated by external stimuli like membrane potential (voltage gated channels) or ligand binding. Ion channels are essential for many of the cell's functions, such as impulse conduction in the nervous system or pace-making in the cardiac system, and their dysfunction is pivotal in many diseases.[1] Hence, there is a great interest in ion channels not only from academia to understand the biophysics of their function[2] but also from the biotechnology and pharmaceutical industries to develop drugs that modulate ion channels to correct any malfunction.

Several methods are available today to test candidate ion-channel active drugs (ICADs): electrophysiology (patch clamp), binding assays, radioactive flux assays, membrane-potential-sensitive fluorescent dyes, ion-sensitive dyes, and voltage sensing based on fluorescence resonant energy transfer (FRET).

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As patch clamping has unsurpassed information content, high sensitivity, and can be applied to all types of ion channels, it clearly represents the gold standard for the evaluation of compound actions. However, conventional patch clamping requires highly trained personnel, is a laborious process, and can be used only in a sequential manner. Because of the low throughput of this technique, conventional patch clamping is only applicable as a tertiary screening method, following less sensitive and more indirect approaches. Other employed ICAD screening methods are capable of high throughput, but are based on indirect measurements such as, for example, fluorescence or binding assays; these techniques have low time resolution (in the range of seconds to minutes, as compared to sub-milliseconds for patch clamp), often require extensive assay development, and are known for their high false-negative and false-positive rates.

The patch-clamp technique was developed by E. Neher and B. Sakmann (for which they received the Nobel Prize in Physiology or Medicine in 1991).[3,4] It employs an electrolyte-filled glass pipette with a tip diameter of about a micrometer to make electrical contact with the membrane of a cell and enables the direct measurement of current flow through the ion-channel proteins. Such micropipette techniques have been used for decades and constant improvement and development has enabled routine, and electrically very tight sealing of pipette tips with the membrane to be achieved, the so-called "gigaseal" (from seal resistance $>1\,G\Omega$), which is required to avoid leakage current and

allow for high-resolution measurements. In fact, patch clamping is one of the very early single-molecule techniques, since one can detect the opening and closing of single ion-channel proteins.[5] The most widely used patch-clamp configuration is the so-called "whole-cell configuration", where the cell membrane sealed at the tip of the pipette is opened by a brief suction pulse to gain access to the cells interior. In the whole-cell configuration, all of the ion channels in the cell membrane are recorded from, which provides a signal current of up to a few nA, whereas a single ion channel typically has a conduction in the range of a few pS.

2. Limitations in Ion-Channel Screening

Its outstanding scientific success notwithstanding, conventional patch clamping still is a laborious process requiring experimental skill and patience, as well as technical means to position and visually control the pipette. Usually, a patch-clamp rig consists of a microscope on a vibration isolation table, a micromanipulator, and amplifier electronics. All of this has to be placed inside a large Faraday cage. Therefore, patch clamping traditionally has been somewhat too complicated for general use in the development of new compounds, that is, drug screening. Rather, pharmaceutical companies have relied on high-throughput-screening-(HTS-) compatible techniques such as the previously mentioned fluorescence-based or binding assays. Such techniques can generate several-hundred thousand data points per day, but deliver significantly lower quality data. Thus, the traditional view has been that there is an inevitable trade-off between high information content and high throughput.

3. Microtechnology as an Enabling Element

The limitations mentioned above are increasingly being overcome by the development of automated and parallel patch-clamp methods, as evidenced by a considerable number of publications on developments from academia,[6–12] as well as industrial applications.[13,14] Our group has made use of state-of-the-art microstructuring techniques to define three-dimensional (3D) micro-openings in planar substrates, with a geometry of an inverted patch-clamp pipette tip.[15,16] Borosilicate glass is used for these chip structures and cells can be positioned and electrically sealed on the apertures with great ease. Instead of manually positioning a pipette onto the cell membrane, cells in suspension are simply pipetted onto the chip and suction is applied to

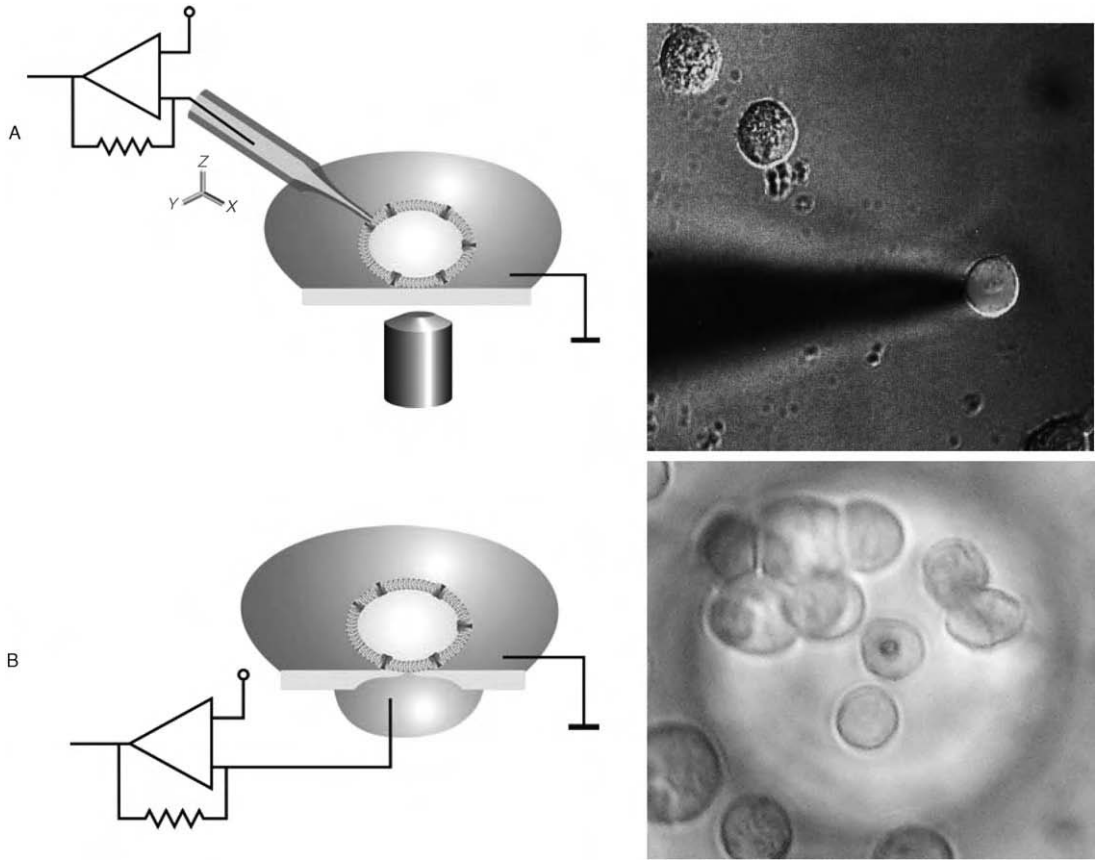

Figure 1. Replacing the patch-clamp pipette with a chip: A) Schematic of the classical patch-clamp technique (left) and a light microscopy image of a pipette on a cell (right). Using an *x,y,z* micromanipulator and a binocular microscope, the tip of a glass pipette filled with electrolyte solution is positioned onto a cell. B) Schematic and microscopy image of the planar chip device. Cells in suspension are positioned and sealed onto the aperture by software-controlled application of suction. No microscope or micromanipulator is needed.

move a cell onto the aperture. Further suction pulses are then used to break open the cell membrane across the aperture, allowing the formation of the whole-cell configuration. Cells can then be electrophysiologically characterized as with a pipette by applying voltage protocols and perfusion of compounds to study the ion channels in the cell membrane. The recording situation for the chips is contrasted with that for the classical pipette in the sketch shown in Figure 1.

4. Patch-Clamp-on-a-Chip: Automation and Parallelization

The greatly simplified method of contacting a cell renders the patch-clamp chips well suited for automation of electrophysiological measurements. As apertures can be processed in arrays, the planar-patch-clamp approach also enables parallel investigations of many cells on a single chip. Since the initial developments, this technology has been made available for other researchers and drug-developing companies by Nanion Technologies (www.nanion.de), a spin-off company from LMU, Munich. One measurement platform (called the Port-a-Patch) focuses on miniaturization of the actual setup and ease of use for the experimenter. With this instrument, experiments are run on a single cell at a time in a semi-automated process (see Figure 2). Cells are added to the chip manually and are positioned and sealed in a software-controlled manner. As standard protocols can be defined in the software, the experiments are straightforward to carry out, very reproducible, and do not require specific skills. The quality of patch-clamp recordings performed with the chips is comparable to a conventional patch clamp. Due to the fact that the cell is firmly attached to the chip, there are no relative movements between the cell and aperture, which in pipette-based patch clamping is one of the main sources for fragility of the recording situation. Therefore, the recordings with a chip are very stable and long-lasting, even improving on the traditional approach.

Application of compound-containing solutions can be achieved with a standard pipette and due to the small volumes required (a few μL), very fast perfusion time constants of less than 10 ms can be achieved. We have also developed a laminar flow chamber, which can be used together with a manifold connected to a software-controlled perfusion system, as shown in Figure 3. This system allows the experimenter to apply a series of different solutions in a very well defined manner according to predefined protocols. This is of great interest for work on ligand-gated channels, where compound application should be triggered. To demonstrate the capabilities and performance of the perfusion system, we have made recordings from a human embryonic kidney (HEK) cell expressing a GABA receptor. Upon application of different concentrations of GABA, these channels are activated as displayed in Figure 3.

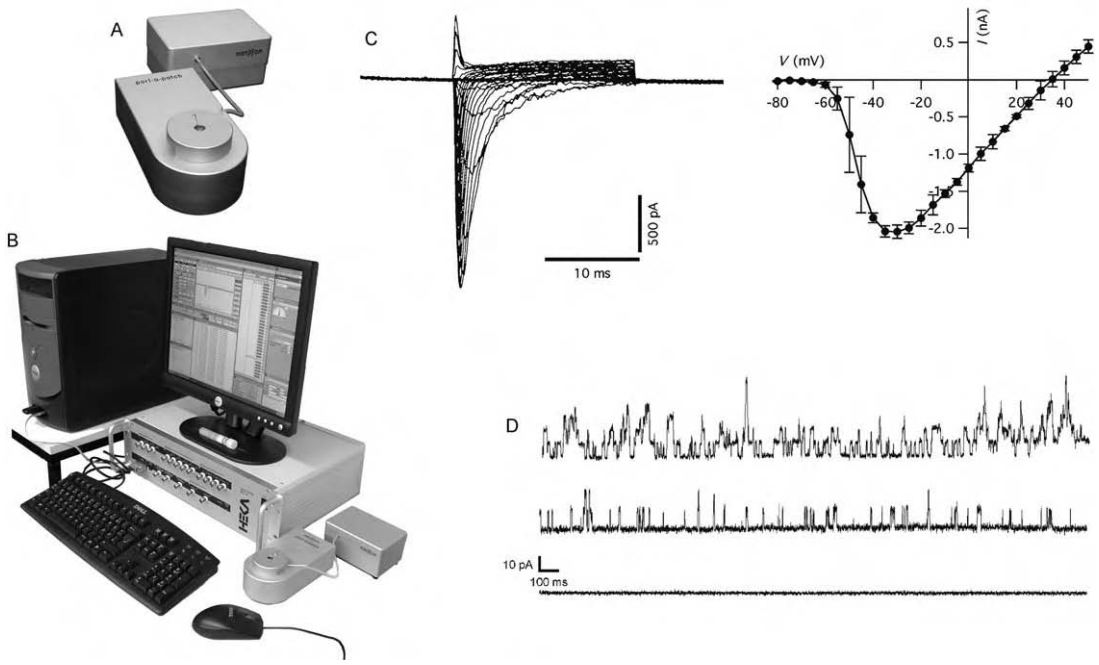

Figure 2. Chip-based patch clamp with the Port-a-Patch: A) The Port-a-Patch workstation together with its software-controlled pump unit for suction application. B) A complete Port-a-Patch setup including amplifier and PC. C) Whole-cell recording from a human embryonic kidney (HEK) cell. A voltage protocol with increasing amplitudes is applied to activate the sodium channels expressed by this cell line (left). The corresponding current–voltage relation is shown on the right. The cell line was kindly provided by Cytomyx, UK. D) Single-channel recording with the chip from a potassium channel (Bk) expressed in a Chinese hamster ovary (CHO) cell. The investigated membrane patch contains multiple channels, and, due to the high resolution, the opening and closing of the individual channel proteins is easily resolved. The cell was voltage-clamped to increase membrane potential, which correlates with the increased open-channel probability and single-channel conductance.

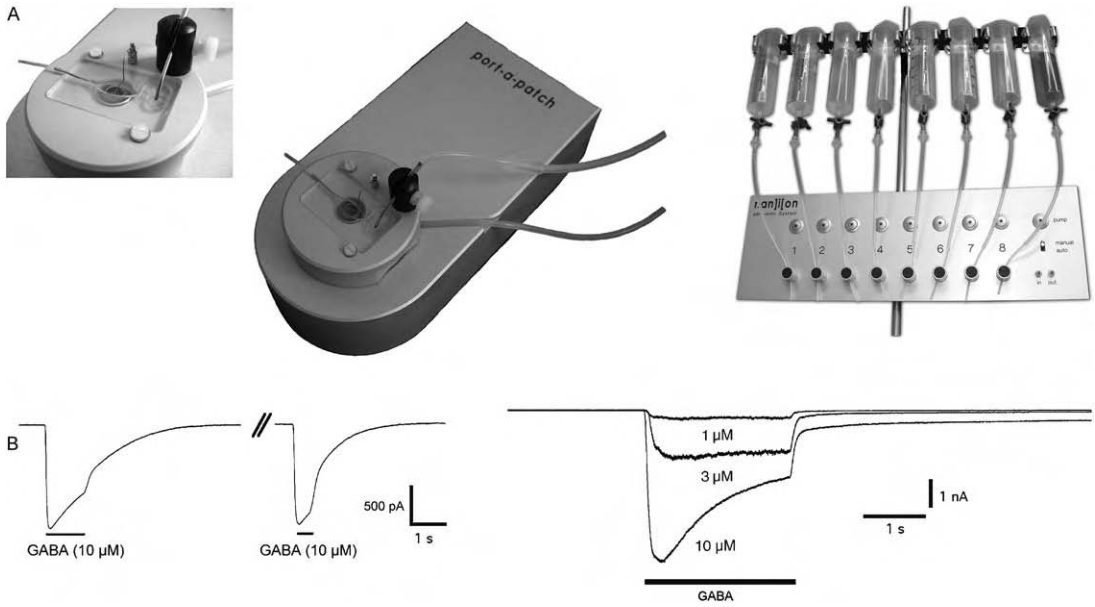

Figure 3. Perfusion system for ligand-gated ion channels: A) On the left the laminar perfusion chamber is shown, which fits onto the Port-a-Patch apparatus. On the right is an eight-channel perfusion system, which is steered via software and is gravity-flow based. B) For activation of the GABA receptor, the ligand GABA is applied for 0.5 and 1.2 seconds to the flow chamber during a whole-cell recording. One can clearly see the desensitization and the closing of the channels after washout with control solution. On the right, different GABA concentrations are applied and an increasing signal is recorded.

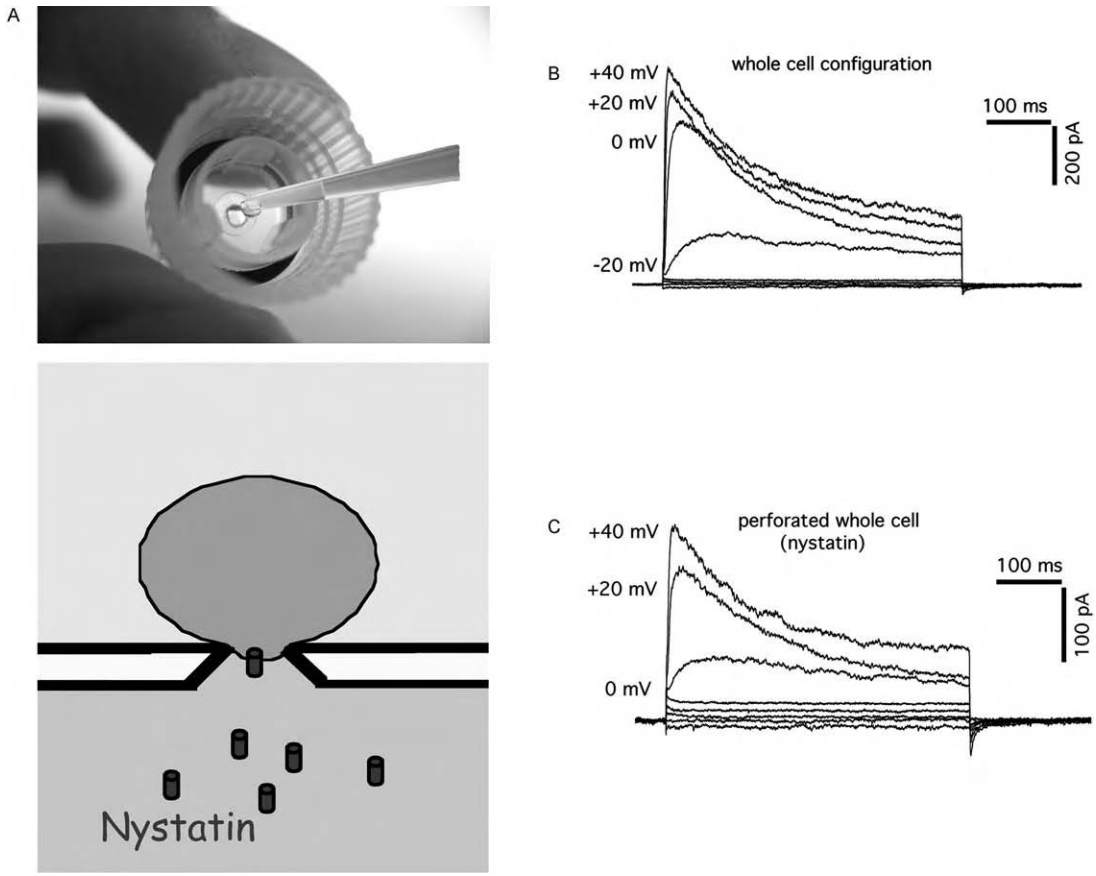

Figure 4. Perforated patch recording with internal perfusion: A) The perforated patch configuration: A cell is sealed on the chip, but the membrane is not broken open by suction, therefore it remains in the so-called "cell-attached configuration". The pore former nystatin is then applied to the internal side of the chip as depicted in the upper part. Nystatin inserts into the membrane across the aperture in the chip, making it electrically permeable without rupturing it, as sketched out in the lower part. B) Standard whole-cell recording from a Jurkat cell expressing a endogenous potassium channel (as control). C) Perforated patch recording with nystatin on the same cell type. The same voltage protocol with increasing amplitudes is applied to activate the potassium channels in both cases, showing effectively the same result.

5. Enabling Intracellular Perfusion

Another interesting opportunity is the application of different solutions not only from the extracellular side but also from the intracellular side. For the case of recordings 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 are 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, which can be helpful in preventing rundown or kinetic variances during the recordings. In Figure 4 such a perforated patch recording is shown and compared with a conventional whole-cell recording.

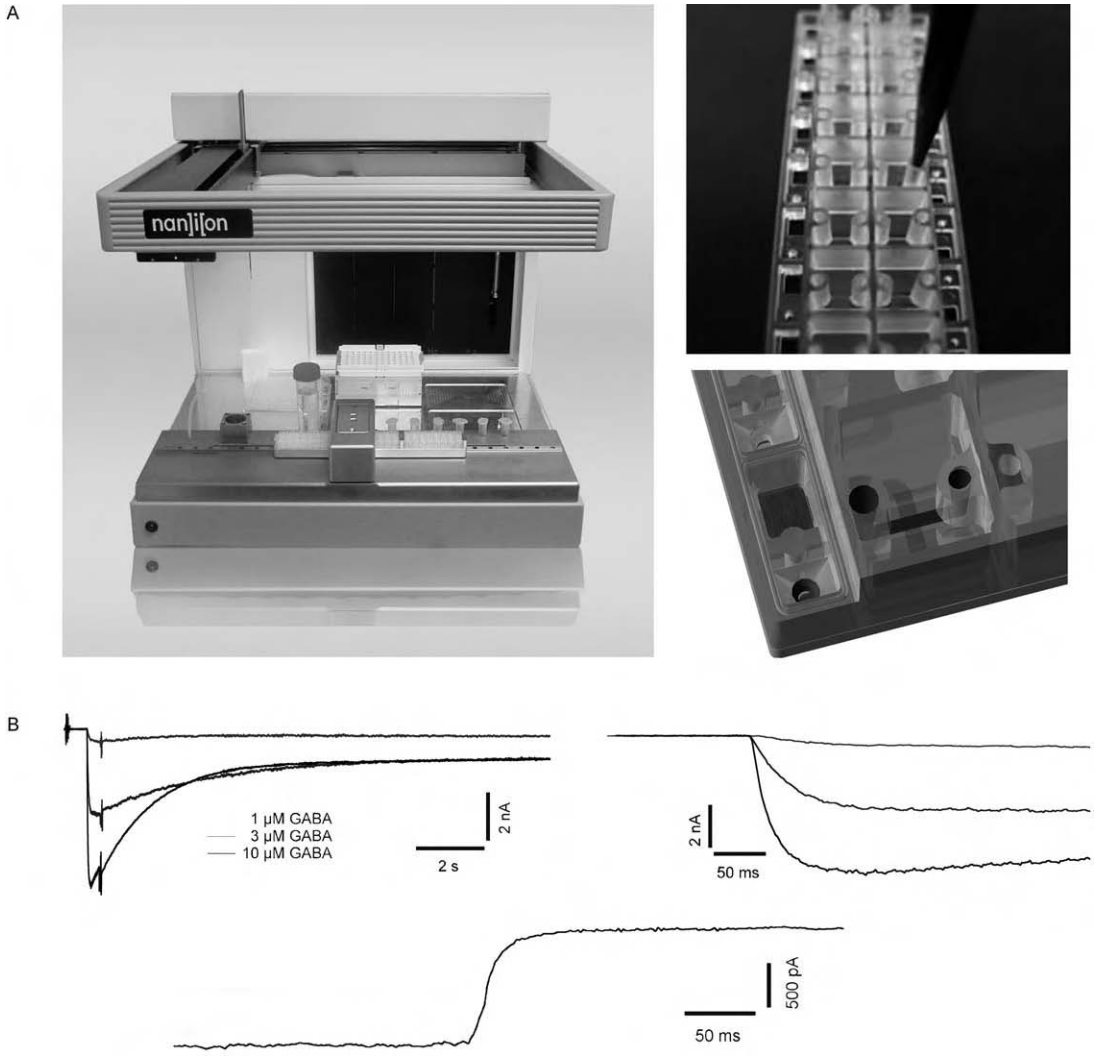

Figure 5. The robotic platform for higher throughput with fully automated and parallel measurements: A) On the left, the patchliner is shown. In the front is the measurement head, which contains the pneumatic and electric contacts and moves up and down to address the patch-clamp chips. The chips are placed on a motorized stage, which takes up to three NPC-16 chips, hence allowing for 48 recordings. The measurement head can hold up to 16 recording channels. Cells and compounds are placed in small vials or microtiter plates and are sampled by the pipetting robot into the microfluidic cartridge of the chip. On the right, a close-up of the microfluidic cartridge containing the microstructured chip with an inserted pipette is shown, as well as a technical drawing of one patch site with the microfluidic cross-channel geometry. B) Whole-cell recording from a ligand-gated ion channel. The data show the activation by 1, 3, and 10 µM GABA from a $GABA_A$ (α1, β2, γ2) expressing HEK293 cell, also at a blown-up time scale, showing the fast perfusion capabilities. In the lower part, an example of current decrease of the endogenous K^+-permeable channels in RBL cells upon changing to a solution containing a low potassium concentration is displayed. Here, the external K^+ concentration was decreased form 140 mM to 5 mM (from left to right). The time constant is approximately 50 ms.

6. Higher Throughput

In another measurement platform, the whole experimental protocol execution (including all liquid handling) is automated (see Figure 5). This instrument (the patchliner) uses patch-clamp chips containing 16 apertures for parallel measurements. The apertures are micromachined into a rectangular glass slide, which is sandwiched between the upper and lower part of a microfluidic cartridge. The cartridge contains microfluidic channels, which are addressable from the top part by means of a pipetting robot to fill in and perfuse cell suspension or compound-containing solutions. For each aperture, there are two fluidic channels that form a cross geometry at the patch side. One channel is above the glass substrate (the extracellular side) and one is below (the intracellular side). Cell suspension is added to the upper channel and suction can be applied to the lower channel to position the cell on the opening in the glass chip. The microfluidic channels enable rapid and precise compound application to the external and internal side of the cells. The channels have an outlet, which leads into a waste reservoir, so that with each perfusion step the old solution in the channel is decanted. For each perfusion step, only a few μL of solution is required.

Due to the possibility for fast solution exchange with the cartridge, this chip format is particularly suited for the study of ligand-gated ion channels. As ligand-gated channels tend to desensitize rapidly, it is important to perfuse the ligand-containing solution for channel activation very rapidly and in a well-defined manner. In Figure 5, perfusion experiments with the NPC-16 chips used in the patchliner are also shown.

With the patchliner, intracellular perfusion is possible as described above. In Figure 6, experiments on Jurkat cells expressing a potassium channel are shown. Current flow in these channels is known to be blocked by cesium ions; in this experiment, a Cs^+-containing solution is applied internally, which effectively blocks 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.

In addition to scaling up the number of recording channels, the throughput capability of the system is greatly increased by the fully automated application of drugs by the pipetting robot. This is also shown in Figure 6, where a concentration 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 so-called IC_{50} value for the compound. TTX blocks the sodium current in a concentration-dependent manner. 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. Thus, with this robotic system a large number of data points can be generated in an automated manner in a way suitable for drug-development tasks and screening purposes.

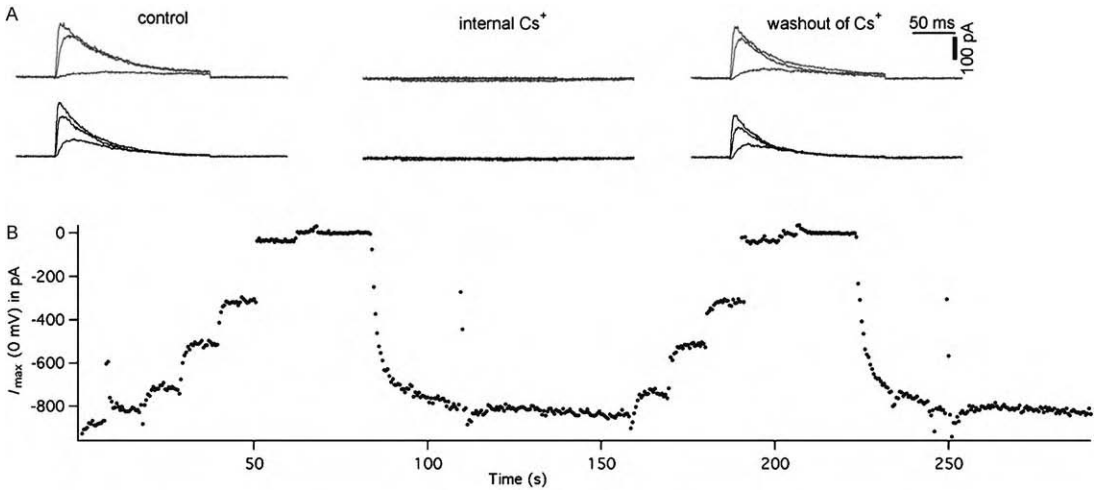

Figure 6. Internal solution exchange with the patchliner: A unique feature of the patchliner is the internal solution exchange during the experiment at both recording channels at the same time. A) Two simultaneously recorded Jurkat cells in the presence of a control internal solution (left), after the exchange of the internal solution with a Cs^+-containing solution (middle), and a subsequent washout step (right). The complete blockage of current by cesium and the recovery after washout with the control solution clearly documents the internal perfusion possibilities. B) The NPC-16 chips provide stable whole-cell recordings. Therefore, a series of drug concentrations can be applied to one cell. The data shows 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 TTX (0.3, 1, 3, 10, 30 μM) have been applied, then washed out and applied again to demonstrate the stability of the 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.

The typical success rate in obtaining gigaseals and stable whole-cell recordings is in the range of 60–80% with durations of the recordings of more than half an hour. Both systems, the Port-a-Patch and the patchliner, are very robust in handling and enable reliable patch-clamp recording with high data quality. The possibility to change the internal solution several times together with long-lasting and stable whole-cell recordings opens up a broad range of new experiments, that may prove valuable in drug discovery as well as in academic research.

7. Summary and Outlook

Microtechnology allows for the integration of a large number of functional units on a very small footprint, which can be an enabling step for many technologies. The chip-based patch-clamp technique presented here broadens the possibilities of ion-channel analysis by transferring the classical patch-clamp principle onto a microstructured chip format. The planar patch-clamp technique enables the automation and parallelization of ion-channel measurements. This is an example of the beneficial integration of microtechnological processes in the field of cellular electrophysiology. The chip-based patch-clamp technique not only improves ease of use as well as throughput capability compared to conventional patch clamping but furthermore opens new experimental possibilities for the biophysical characterization of ion-channel proteins such as intracellular perfusion or optical accessibility of the chips for combined optical and electrical recordings from ion channels.

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