

The Chiptip: A Novel Tool for Automated Patch Clamp

Albrecht Lepple-Wienhues^{*}, Dirck Lassen, Alexander Hümmer, Uwe Czubayko, Martina Knirsch and Andjelko Golubovic

Flyion GmbH, Waldhäuserstr. 64, D-72076 Tübingen, Germany

Abstract: To facilitate automated patch clamp measurements of ion channels in cells, the development of an all-glass Chiptip pipette is reported that may be combined with the previously described Flip-the-Tip technology. A single measurement requires less than 50 cells, and the addition of drugs for screening can be limited to very low volumes down to 1 μL . This apparatus is suitable for the study small cells, subcellular organelles and bacteria.

Keywords: Ion channel, automated patch clamp, glass micropipettes, membrane protein, voltage gated channel, ligand gated channel.

INTRODUCTION

Ion channels are a drug target class of increasing importance. In the past decade many channel proteins have been linked to diseases. Examples include the sodium channel SCN9a playing a unique role in peripheral pain transmission [1], the infamous hERG cardiac pacemaker channel responsible for deadly toxicity effects [2,3] or the TRP channel family involved in focal segmental glomerulosclerosis (TRPC), hypomagnesemia with secondary hypocalcemia (TRPM6), Guamanian amyotrophic lateral sclerosis/Parkinsonism dementia (TRPM7) polycystic kidney disease (TRPP), and mucopolidosis type IV (TRPML) [4].

The major hurdle to develop drugs targeted at ion channels consists in the complicated procedures required to study their function. In contrast to enzymes and GPCRs, these proteins need to be examined in intact cells requiring physiological membrane orientation, millisecond voltage control and precise manipulation of chemical composition on both membrane sides. In addition, tiny currents are to be measured and are prone to artefactual change.

Gigaseal patch clamp is the far superior method providing all the aforementioned prerequisites. However, its traditional manual application is painfully slow and requires artistic micromanipulation. In the last 5 years, several approaches have been made to automate and simplify this method. However, many automated devices show unexpected problems regarding seal and whole-cell success. They can be vulnerable to subtle cell culture variations that are poorly understood and may show discrepancies in pharmacological potency when compared to manual patch clamp data.

Here we describe a unique method using specially shaped glass pipettes for a fully automated patch clamp device that maximizes success rate and seal stability while providing unique robustness of the patch clamp process and unmatched pharmacological precision.

GIGASEALS INSIDE A PATCH CLAMP PIPETTE - FLIP-THE-TIP™

We recently described a novel method [5] to obtain a true and stable gigaseal and whole-cell preparation inside a regular glass pipette by simply flushing suspended cells towards the tip as shown in Fig. (1). The gigaseal inside the pipette is extremely durable. Tapping against the pipette, flushing the pipette with saline, or even moving the pipette does not break the seal. Therefore, the method has allowed us to develop the Flyscreen robot. This machine forms seals, establishes whole-cell access, and adds compounds completely automatically in 3-6 parallel channels. Its little brother, the Patchbox, is a half-automated device that has built-in capability of creating seals and whole-cell access. The Patchbox accepts glass pipettes pulled with any puller, the liquid handling has to be performed manually by the user.

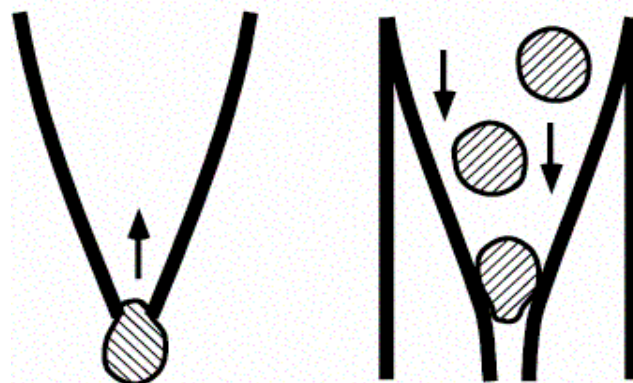


Fig. (1). (left) Conventional patch clamp: A single cell is approached with a glass micropipette and a Gigaseal between glass and cell membrane is achieved with gentle suction. (right) Flip-the-Tip: In the flyscreen robot a cell is sucked into the glass micropipette and a gigaseal is achieved inside the pipette.

By introducing a novel bowl shaped tip – the Chiptip – to the patch pipette, we were able to overcome some intrinsic limitations and to further improve the method (Fig. 2). The

^{*}Address correspondence to this author at the Flyion GmbH, Waldhäuserstr. 64, D-72076 Tübingen, Germany; E-mail: info@flyion.com

combination of the Flip-the-Tip technology with the new Chiptip pipettes now presents a solution to a whole variety of problems associated with most other automated patch clamp methods.

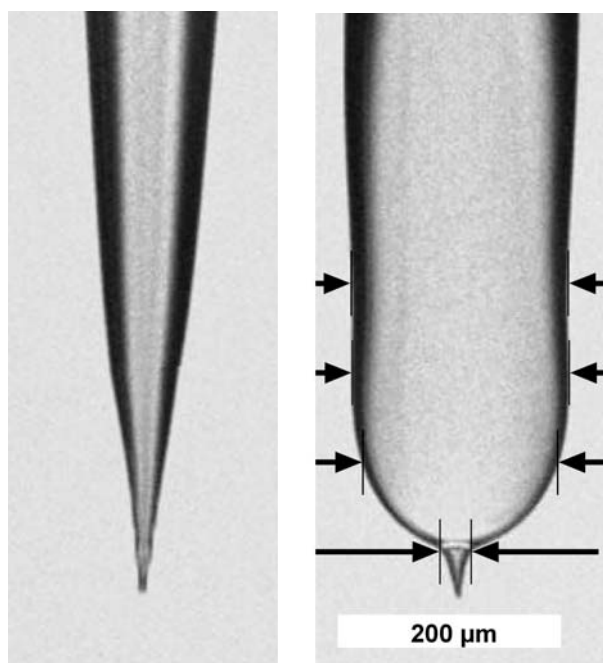


Fig. (2). (left) A fliptip is a conical micropipette pulled out of borosilicate glass capillaries. (right) The Chiptip is manufactured using an optically controlled glass blowing step, where the indicated diameters are measured continuously and the tips are reshaped with micrometer precision.

GLASS TIPS AVOID NONSPECIFIC BINDING

For pharmacological studies in small volumes, drug binding to the liquid container wall material has proven critical. Small volumes are desirable in order to minimize consumption of synthesized compounds available only at small quantities. However, the smaller the volume the more critical nonspecific binding to containing surfaces becomes. Many plastic materials used in planar chip approaches to automated patch clamp show undesirable nonspecific binding especially of lipophilic drugs. Therefore, we have developed a microchamber that consists entirely of glass: the Chiptip. The compounds to be tested contact only glass surfaces, a superior material with minimal adsorption properties. Small volumes down to 1 μl are entirely sufficient to safely flush the entire extracellular compartment without any apparent potency shifts due to nonspecific binding.

SEALING WITH FEW CELLS

Undesirable binding of drugs can also occur when small volumes contain large numbers of cells expressing the target protein on their surface. The scavenging effect of these bystander cells becomes more critical in small unstirred volumes close to the single cell under scrutiny. In most automated approaches, the footprint of the extracellular com-

partment is typically in the square mm range, whereas the cells have diameters close to 20 μm and must settle on a 2 μm aperture. Typically thousands of cells are added to the patch chamber in order to achieve a high probability of one single cell arbitrarily sealing the patch clamp aperture. The remaining cells settle onto the bottom of the chamber located closely to the cell being examined. However, these extra cells also express the ion channel protein of interest and can provide a formidable drug sink. A single cell patch chamber design must therefore aim at using as few cells as possible.

In Chiptips, only 30-50 cells are being added to the Chiptip "bowl" as close as 200 μm above the orifice of the tip. With mild suction being applied subsequently, single cells are drawn into the centered liquid stream and transferred into the seal area gently, quickly and reliably, avoiding the arbitrary "settlement" step required in other chamber types. The liquid stream stops as soon as the first cell reaches the seal area. Only few extra cells bind to the glass surface minimizing the presence of lipid membranes and drug binding proteins in the vicinity of the recorded cell.

AUTO-CENTERING LIQUID HANDLER TOOLS

The rotational symmetry of the Chiptip forces the capillary to the center of the Chiptip. Therefore, cells and drugs are delivered in a focused way directly onto the patch-clamp aperture (Fig. 3). This "auto-centering" property of the Chiptip allows using extremely low cell numbers at extraordinarily high seal rates (80-90%). The released cloud of suspended cells is aligned in the middle of the liquid stream generated by suction on the 1 μm orifice of the tip.



Fig. (3). A hollow quartz capillary with 170 μm outer diameter is inserted and centered into a Chiptip.

Another huge advantage of this "auto-centering" assembly lies in the rapid application of drugs and / or ligands. Since the sealed cell is centered in the liquid stream out of an inserted capillary, drug-containing solutions can be applied at high speed. The shear forces on the seal are minimal, and the cell membrane is pushed into the seal. Therefore, the unstirred liquid layer on the extracellular surface is mini-

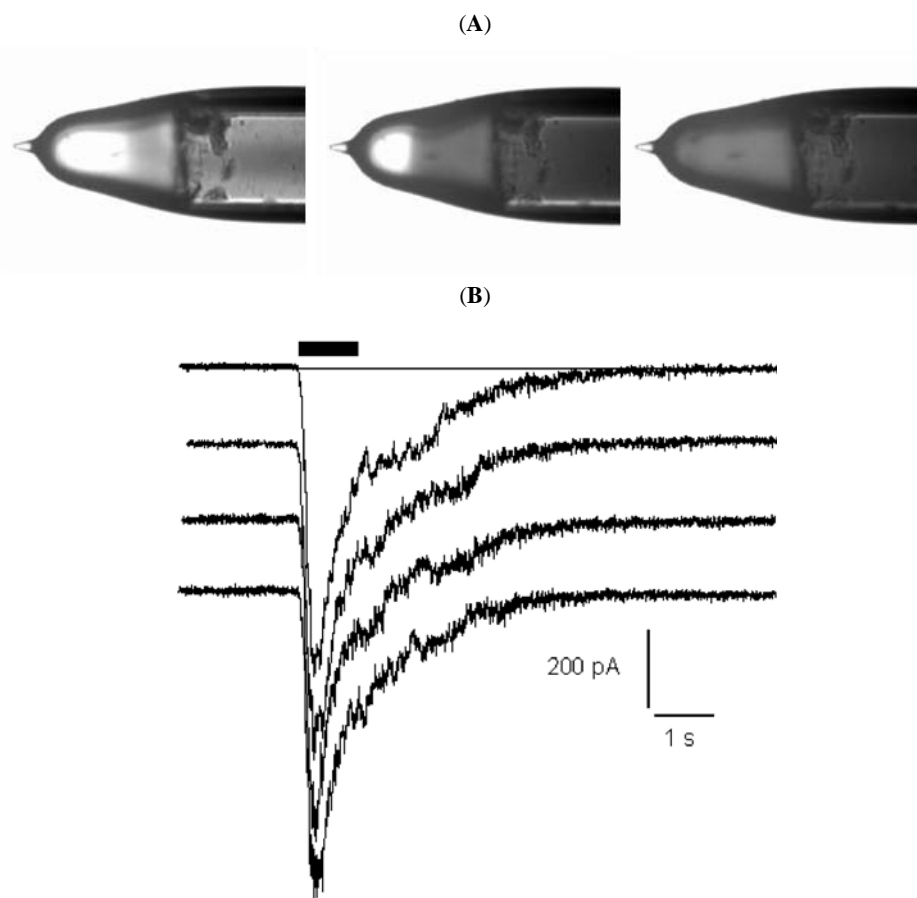


Fig. (4). (A) Videoframes taken 100ms apart showing the border between two liquids moving towards the cell-containing tip. (B) Four repetitive applications of 1 μ L solution with 100 μ M acetylcholine at 1 μ L/s are indicated by the black bar, followed by 10 μ L wash on the endogenous nicotinic receptor in TE671 cells.

mized. Solution exchange is complete in less than 50 ms comparable to the switching of cell position between solution streams when using conventional patch clamp. This allows mimicking ligand concentration kinetics approaching the kinetics observed in a natural synapse (Fig. 4).

LIQUID STREAMS PUSH CELLS INTO SEAL RATHER THAN DISRUPTING SEAL

The application of solutions to a whole-cell configuration traditionally posed a threat of losing the seal due to shear forces. This has posed a problem in conventional patch-clamp as well as with planar patch-clamp approaches. In contrast to the Chiptip, all of the planar patch clamp approaches perform solution exchange perpendicular to the sealing orifice creating shear forces that risk ripping the cell off the seal with increasing fluid speeds (Fig. 5).

An intrinsic benefit of the Chiptip geometry is the application of solutions axially towards the sealing orifice. A fast solution application results in a stream pushing the cells even tighter into their seal. As a consequence hardly any seals are lost during solution application which in turn results in overall success rates of >70% for individual measurements. This is extremely important in large primary screening campaigns, where every compound is typically measured only once and lost seals lead to a missing result. The respective compounds must then be tediously retrieved from the original array and re-tested.

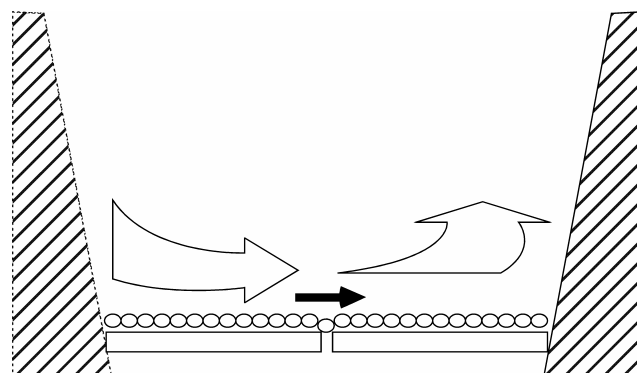


Fig. (5). Schematic drawing showing typical flow direction in a planar patch system. The flow direction perpendicular to the patch orifice creates mechanical stress on the seal.

STABLE GIGASEALS USING PHYSIOLOGICAL SALINES

For decades it has been acknowledged by the patch clamp community that the pipette material with the most inert surfaces which will form the best gigaseals is borosilicate glass. The seal formation and stability can be even further improved by the addition of fluoride to the extracellular solution. Fluoride etches the glass surface by chelating polyvalent cations like aluminum out of its surface. However, fluoride also has huge disadvantages. It tends to chelate

calcium and magnesium and in the worst case it will precipitate as calcium or magnesium fluoride. It also binds to G-proteins, phosphatases and kinases, mostly in a poorly reversible way [6]. It easily permeates amphothericin pores and - once binding to proteins in the cytosol - can hardly be washed away. Therefore, many ion channels can not be studied at all using fluoride containing solutions.

In order to avoid fluoride, we decided to make the Chip-tip pipettes from borosilicate glass. We were able to show that the Chiptips form stable true gigaseals when using only strictly physiological solutions. Under a microscope, it can be seen that the seal forms between the outer circumference of the rounded cell and the smooth inner surface of the glass pipette in much the same way as it would in traditional manual patch clamp. It is therefore not surprising that the seal is forming with a seal resistance and quality comparable to the conventional technique. Seal resistances in Chiptips typically average 10 GOhm or more.

TIP GEOMETRY GUARANTEES STABLE ACCESS

Using the traditional manual patch clamp, one has to deal with a significant series resistance after the cell is ripped open due to the adherent membrane flap, which may even seal the cell again. The inverse setup in the Flip-the-Tip method resealing never happens. The series resistance is

below 5 MOhm in open whole cell configuration and stable throughout the experiment. One other intrinsic advantage of the inverted setup is the extremely fast perforation in perforated patch experiments. During the seal process a small pressure gradient prevents the intracellular solution from contaminating the seal area. As soon as a cell forms a seal, the perforating chemical reaches and opens the membrane. Series resistance with perforated patches is typically in the 10-15 MOhm range and stabilizes within 100 s.

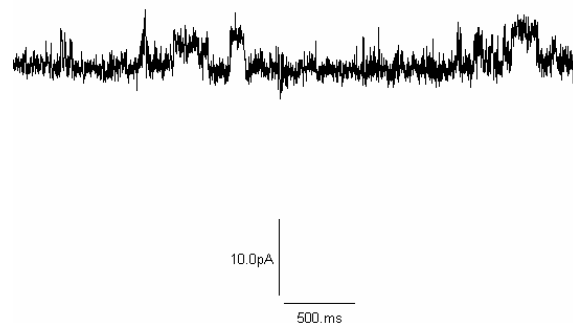


Fig. (6). Single channel gating in a mitochondria-attached recording from the outer membrane of a mitochondrion prepared from HEK cells. The open pipette resistance was 12 MOhm in 145 mM NaCl.

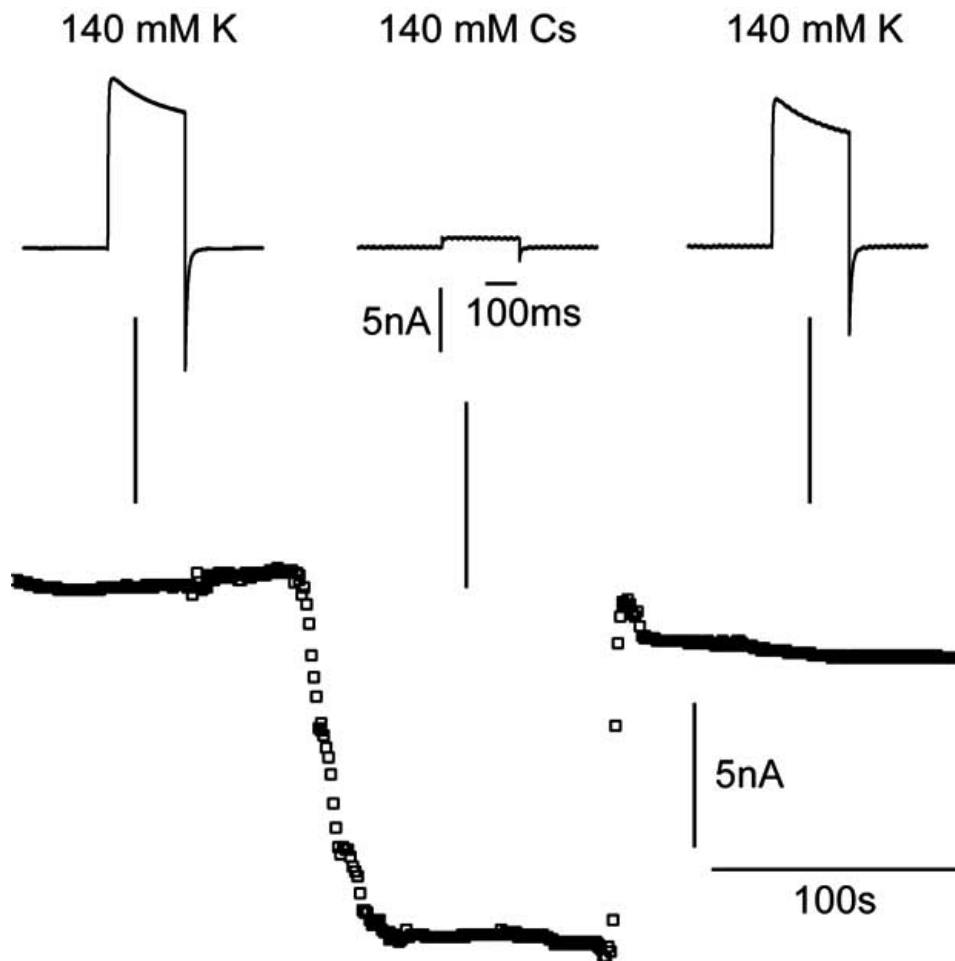


Fig. (7). Intracellular solution was changed twice, replacing potassium with cesium and back. Time course of hKv1.5 expressed in LTK cells. Oscilloscope traces show currents elicited by a voltage jump from -80 mV to 0 mV.

SCALABLE APERTURE

Most automated methods require standard cell lines with average sizes well above 10 μm diameter. In contrast, the use of glass pipettes has allowed us to tailor the diameter of the aperture down to 100 nm and less. Preliminary results were obtained for recordings of single chloride channels in outer mitochondrial membranes (Fig. 6).

RAPID INTRACELLULAR PERFUSION

The sealed cell in a Chiptip is located close to the aperture of the tip. Together with the relatively large opening in the cell membrane this makes an ideal preparation for rapid intracellular solution exchange. Intracellular application of compounds can be used to block undesired ion channels, to apply intracellular messengers and even to test compound binding to the cytosolic domains of a channel protein (Fig. 7).

TEMPERATURE CONTROL

Temperature control with the Flip-the-Tip approach is easy, stable and efficient. Using a water jacket around the recording chambers the recording temperature can be controlled over a wide range. Cooling or heating can both be easily achieved. The temperatures are stable within $\pm 0.2^\circ\text{C}$. The small volumes applied during solution exchanges makes the preheating of solutions applied onto the cells unnecessary.

SUMMARY

The novel Chiptip pipettes combined with the previously described Flip-the-Tip technology offer a superior solution to automate patch clamp measurements. This unique combination generates stable gigaseals and whole-cell access. Nonspecific binding of compounds is minimized by the exclusive contact to glass surfaces. A single measurement requires less than 50 cells and the addition of drugs can be limited to very low volumes down to 1 μL . The intrinsic benefits of the experimental setup result in extremely rapid solution exchange of < 50 ms enabling the analysis even of rapidly desensitizing ligand-gated channels. The flexibility to tailor the pipettes geometry and aperture diameters enables the study of small cells, subcellular organelles and even bacteria.

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