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Robotic multi-well planar patch-clamp for native and primary mammalian cells

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Abstract

Multi-well robotic planar patch-clamp has become common in drug development and safety programmes because it enables efficient and systematic testing of compounds against ion channels during voltage-clamp. It has not, however, been adopted significantly in other important areas of ion channel research, where conventional patch-clamp remains the favoured method. Here we show the wider potential of the multi-well approach with the capability for efficient intracellular solution exchange, describing protocols and success rates for recording from a range of native and primary mammalian cells derived from blood vessels, arthritic joints, and the immune and central nervous systems. The protocol involves preparing a suspension of single cells to be dispensed robotically into 4-8 microfluidic chambers each containing a glass chip with a small aperture. Under automated control, giga-seals and whole-cell access are achieved followed by pre-programmed routines of voltage paradigms and fast extracellular or intracellular solution exchange. Recording from 48 chambers usually takes 1-6 hr depending on the experimental design and yields 16-33 cell recordings.

INTRODUCTION

In 1991 Bert Sakmann and Erwin Neher were awarded the Nobel Prize in Physiology or Medicine for their discoveries on the function of ion channels, which were made possible by

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their development of patch-clamp technology1. The technology revolutionized electrophysiological studies of cells and ion channels. It enabled resolution of unitary current events through single ion channel proteins as well as recording of macroscopic ionic currents across the membranes of a plethora of previously unexplored cell types from the animal and plant kingdoms, with options for simultaneous membrane potential control (voltage-clamp), fast kinetic resolution, and intracellular dialysis. The field of electrophysiology was transformed from one that was focused mostly on voltage-clamp recordings only from a restricted selection of large cells (i.e. that are amenable to two-electrode impalement) or to observations of membrane potential fluctuations in the absence of voltage-clamp (i.e. by cell impalement with a single sharp electrode). The vast expansion in the volume of high quality data on the properties of the many hundreds of ion channels controlling ion movement and electrical signaling in cells is testament to the seminal nature of Sakmann's and Neher's discoveries and the developments made by many scientists who worked with them or followed their example2.

However, along with recognition of the power of patch-clamp technology came recognition of the slow data output and need for highly specialized operators, aspects that have blocked or hindered implementation for a substantial body of investigators. Other techniques can be used to measure ion channel activity and may deliver higher data throughput and require less specialized operators. Rubidium is, for example, used to measure K^+ channel activity because many types of K^+ channel are permeable to this ion3. Fluorescent dyes are used to detect changes in membrane potential caused by changes in ion channel activity4-5. Nevertheless, these methods lack many important advantages of patch-clamp, including capabilities for voltage-clamp, intracellular dialysis, high kinetic resolution and quantification of ionic selectivity1. An alternative is enabled by incorporation of ion channels into artificial lipid bilayers6, permitting voltage-clamp but not providing information on ion channels within the cellular context, which is so often essential for how the ion channels behave. Driven by appreciation of patch-clamp as a gold standard for ion channel studies and the desire for greater data output in therapeutic drug discovery and safety programmes, major efforts were made to produce robotic patch-clamp systems7-9.

A particularly successful robotic approach is based on planar patch-clamp chips and this is the basis for the technology described here. With this method, the glass pipette of conventional patch-clamp recording is replaced by a thin sheet of flat glass with a small aperture in the middle (the planar chip). Conventional patch-clamp involves the maneuvering, under a microscope, of the fine tip (1-2 μm) of the patch pipette onto a cell, which commonly has a diameter in the region of 2-50 μm. In contrast, the planar method involves the robotic delivery of a suspension of cells to the chip, with one cell attaching to the aperture randomly by the application of negative pressure underneath the chip; the intracellular solution is underneath the chip, and there is no microscope or micromanipulator (e.g. Fig. 1a). Various forms of the method have been successful. In some cases, numerous apertures are made in the chip and 384-well throughput is achieved (www.moleculardevices.com), but without many of the important advantages of conventional patch-clamp. In other cases, systems have been developed for 2, 4, 8 or 16-well recordings whilst retaining advantages of the conventional system (Fig. 1a-d and see below). During this development period, interest in the multi-well systems has resided mainly in the pharmaceutical sector, with focus on cell lines induced to over-express ion channels9. While this is important, it does not affect or relate to significant elements of the large ion channel community in the basic or academic research sectors where studies of endogenous ion channels and electrical phenomena in native cells remain common and important.

We have found that robotic multi-well planar patch-clamp technology can generate high quality publishable data10-12 (and illustrative data shown in this article) from native cells and cells in primary culture, as well as confer advantages and be enabling compared with conventional patch-clamp. Systematic data production has been possible for a reasonably large number of cells on one day of recording. Therefore, comparison of two experimental conditions has been more reliable than previously, reducing problems due to day-to-day variation in cells and giving greater statistical power. In some cases, we have seen significant increase in performance and studies have been enabled that would previously have been abandoned. The micro-fluidic system uses small volumes, reducing wastage of agents that may be expensive or in limited supply. Although there is possibility for operator intervention during recordings, the reduced tendency for intervention increases the likelihood of generating reliable, objective, outputs. Routine intracellular perfusion opens possibilities for studying intracellular regulation and pharmacology of channels, studies that were previously available only to highly skilled and dedicated experimenters. Use with transiently transfected cells is possible. One potential concern is the cost of planar chips in the context of a typical research grant and during development of methods for a previously unexplored cell type. Another is that there is reduced sealing success rate in the absence of seal enhancer solutions. For a detailed comparison of multi-well planar and conventional patch-clamp methods the reader is referred to Table 1. We expect that the remaining challenges will largely be overcome or accepted. Users may decide that the lesser flexibility compared with conventional patch-clamp (e.g. absence of the outside-out patch configuration) is outweighed by the advantages, especially when the primary need is whole-cell recording. Lastly, multi-well planar technology has the potential to widen access to patch-clamp and generate a platform for achieving international agreement on the properties of ion channels.

EXPERIMENTAL DESIGN

Cell types and sample preparation

A wide range of single cells can be used for multi-well patch-clamp recording (for examples, see Reagents list). There are few specific limitations to the choice of cell, though we recommend that there is prior knowledge of the suitability of the cells for conventional whole-cell patch-clamp recording before embarking on multi-well planar patch-clamp studies. Cells that are contractile (e.g. cardiac muscle cells), very large (e.g. *Xenopus laevis* oocytes) or that have long processes (e.g. hippocampal neurones) present special challenges and we would advise first obtaining experience with other cell types (e.g. as shown by our illustrative examples). Single cell preparation techniques are similar to those generally used for many different studies of freshly-isolated cells or cells maintained in culture media. Homogeneity in the cell population would often be a feature the user would want to achieve to an acceptable level, although we suggest a method that might enable studies of heterogeneous cell populations (see below). Clumps of cells and pieces or slices of tissue are unlikely to be suitable, but we have not specifically investigated such preparations. The technique is not suitable for whole organ or *in vivo* studies.

Ionic current amplitude

Whole-cell patch-clamp recording has good signal-to-noise ratio and can resolve current amplitudes of a few pico (10^{-12}) Amperes (pA). It is, however, easier to obtain reliable data when current amplitudes are larger (e.g. 0.05-5×10^{-9} A, nA). Problems with the quality of voltage-clamp may arise with large currents and these need to be addressed by adoption of suitable compensatory circuitry, as in conventional patch-clamp recordings13. If the ionic current of interest cannot be resolved by conventional whole-cell patch-clamp recording, it is unlikely to be detected by multi-well planar patch-clamp. We have investigated multi-well

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planar patch-clamp for studies of whole-cell (macroscopic) currents but the reader should be aware that single channel events are detectable in planar cell-attached patch-clamp recordings8 and it might be possible to generate a configuration similar to the inside-out patch if the whole-cell membrane is permeabilised.

Optimisation of conditions

We recommend that users follow one of our suggested protocols at least for the first time. On the basis of data obtained, users can optimise experimental conditions. There are various aspects to optimise, including: cell preparation; aperture of the hole in the chip; ionic solutions during seal formation and sampling data; suction parameters to form seals and break-through to the whole-cell configuration; and voltage paradigms.

Design of voltage protocols and ionic solutions

We recommend that users follow published voltage protocols and ionic solutions for their ion channel of interest because these can have critical effects on the determination of ion channel properties and especially on quantitative information about such properties.

Duration of recordings

The duration of each recording depends substantially on the ion channel and cell type under investigation. In whole-cell configuration users might normally expect recordings to be up to 1 hour long.

Controls

The following controls should be included when studying effects of compounds or other substances on channel activity: (i) Solution exchange with the solvent used to dissolve the test substance; and (ii) Solution exchange using the same solution to explore if fluid movement has any effect on channel function. When the experiment involves cell transfection, controls might include cells transfected with DNA vector or scrambled siRNA.

Multi-well patch-clamp and associated equipment

Commercial systems available for multi-well planar patch-clamp include PatchXpress 7000A (www.moleculardevices.com), QPatch (www.sophion.dk) and Patchliner (www.nanion.de). The protocol described here is for the Patchliner, which is provided with a Tecan robot (www.tecan.com) and HEKA (www.heka.com) patch-clamp amplifier and software. In principle, multi-well systems other than the Patchliner may be suitable for the types of studies we describe in this article but we have not investigated this matter directly and are not aware of published evidence to confirm use in this context. There are differences in the design, manufacturing and storage of the planar chips that may lead to differences in performance with different cell types.

Data analysis

Data analysis routines are similar to those developed for conventional patch clamp, with various software options and levels of automation available. Data can be converted for use with common analysis and presentation packages, including Excel (office.microsoft.com) and Origin (www.originlab.com).

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MATERIALS

REAGENTS

- Cells: Rat type-I cortical astrocytes14, human T lymphoblasts15, human saphenous vein smooth muscle cells12 & 16, human synoviocytes10, human neutrophils17-18, cultured Jurkat T cells (human lymphoma; DSMZ no. ACC 282)19, SH-SY5Y neuroblastoma cells20 and HEK293 cells stably transfected with human brain α (K_{Ca}1.1) and β (encoded by *KCNMB1*) subunits of maxi-K channels21, rat $P2X_7$ receptors22 or tetracycline-regulated (Invitrogen Ltd, UK) human TRPC523 or human TRPM311 (see REAGENT SETUP). Only references 10-12 contain multi-well planar patch-clamp data. Multi-well data are shown for other cells as illustrative examples in this article. All cells are maintained at 37 °C in a humidified atmosphere of 5 % CO_2 in air.
- Dulbecco's Modified Eagle's Medium (Invitrogen cat. no. 31885-023)
- RPMI 1640 (Invitrogen, cat. no. 11835-063)
- DMEM/F12 + Glutamax (Invitrogen, cat. no. 31331-028)
- F-12 nutrient mixture (Invitrogen, cat. no. 21765-029)
- MEM non-essential amino acids (Invitrogen cat. no. 11140-035)
- Fetal bovine serum (Invitrogen, cat. no. 10106-169)
- Penicillin and streptomycin (Invitrogen, cat. no. 15070-063)
- Recombinant IL-2 (Invitrogen, cat. no. PHC0021)
- Gentamicin (Invitrogen cat. no. 15750-045)
- L-Glutamine (Sigma, cat. no. G7513)
- Antibiotic antimycotic (Invitrogen, cat. no. 15240-096)
- Tetracycline hydrochloride (Sigma, cat. no. T9823)
- Blasticidin (Autogen Bioclear cat. no. ant-bl-1)
- Zeocin (Autogen Bioclear cat. no. ant-zn-1)
- Dulbecco's Phosphate Buffer Saline Ca^{2+} and Mg^{2+}-free (Invitrogen, cat. no. 14190-094)
- Trypsin EDTA 0.05% (Invitrogen, cat. no. 25300-054)
- Detachin (Gelantis Inc. USA, cat. no. T100110)
- Accutase® (Sigma, cat. no. A6964)
- Intracellular recording solutions (see REAGENT SETUP)
- Extracellular recording solutions (see REAGENT SETUP)
- Amphotericin B (Sigma, cat. no. A4888)
- Bleach (any commercially available liquid bleach is suitable)

EQUIPMENT

- NPC©-16 Patchliner Quattro/Octo (Nanion Technologies, Germany)
- Patch clamp amplifier (EPC-10 quadro, HEKA Instruments, Inc. Germany)

- Computer with 24" TFT monitor (Dell, UK)
- PatchControl©HT software (Nanion Technologies, Germany)
- Software for data acquisition (Patchmaster, HEKA Instruments, Inc. Germany) and analysis (OriginPro7.5 OriginLab Corporation, USA; IGOR-Pro WaveMetrics Inc., USA)
- NPC©-16 chips (single use disposable)
- NPC©-16 electrode set (require chloridation see EQUIPMENT SETUP)
- Cell culture flasks (175 cm^2; BD Falcon, cat. no. 353028)
- 2-, 10-, 200- and 1,000- μl Pipettes
- Fisherbrand accuSpin™ 400 centrifuge (Fisher Scientific, UK, cat. No. FB56652)

REAGENT SETUP

Culture Media—choose the medium relevant to the cell line(s) in use.

- **Rat type-I cortical astrocytes** Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % (vol/vol) fetal bovine serum (FBS) and 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin.
- **Human T lymphoblasts** RPMI 1640 supplemented with 10 % (vol/vol) FBS, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin, supplemented with 20 ng/ml recombinant IL-2.
- **Human saphenous vein smooth muscle cells** DMEM supplemented with 10 % (vol/vol) FBS, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin and L-glutamine.
- **Human synoviocytes** DMEM/F-12 + Glutamax supplemented with 10 % (vol/vol) FBS, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin.
- **Jurkat T cells (human lymphoma)** RPMI 1640 supplemented with 10% (vol/vol) FBS, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin.
- **SH-SY5Y neuroblastoma cells** 50 % (vol/vol) DMEM, 50 % (vol/vol) F-12 nutrient mixture, 10 % (vol/vol) FBS, 1% (vol/vol) MEM non-essential amino acids and gentamicin at 50 μg/ml.
- **HEK 293 cells expressing human K_{Ca}1.1** 1% (vol/vol) MEM non-essential amino acids supplemented with 10 % (vol/vol) FBS, 1 % (vol/vol) antibiotic antimycotic, and 0.2 % (vol/vol) gentamycin.
- **HEK 293 cells expressing rat $P2X_7$** DMEM/F12 medium supplemented with 10 % (vol/vol) FBS and 2 mM L-glutamine.
- **HEK 293 cells expressing human TRPC5 or TRPM3** DMEM/F12 + Glutamax supplemented with 10 % (vol/vol) FBS, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Tetracycline-regulated TRPC5 or TRPM3 were selected using 5 μg ml^{-1} blasticidin and 400 μg ml^{-1} zeocin. Expression was induced by 1 μg ml^{-1} tetracycline 24 h prior to experiments.

Intracellular recording solutions—Prepare the appropriate intracellular solutions (50 ml) in deionised water, filter (0.22 μm) and measure the osmolarity and the pH. The solutions can be aliquoted (4 ml aliquots) and stored at -20 °C.

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▲ **CRITICAL STEP**: To reduce the likelihood of degradation, ATP should be added to the internal solution only on the day of the experiment and then the osmolarity and pH re-checked.

▲ **CRITICAL STEP** All recording solutions should be filtered. The internal solution is particularly important and should be filtered with a 0.22 μm pore diameter filter. The osmolarity of recording solutions is measured directly using a freezing-point osmometer and adjusted if necessary with mannitol or sucrose, non-permeating molecules, to 290 mOsM for all internal solutions and between 290 and 310 mOsM for all external solutions, unless otherwise stated. External osmolarity should always be higher than the internal.

- **Astrocyte solution (mM)** 140 KCl, 1 $MgCl_2$, 0.5 $CaCl_2$, 5 EGTA, 10 HEPES, 3 MgATP (pH 7.25 with KOH).
- **T lymphoblast solution (mM)** 140 KCl, 10 NaCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES (pH 7.2 with KOH). For perforated patch recordings, prepare a stock solution of 40 mg ml^{-1} amphotericin B in DMSO and dilute in the intracellular solution to give a final concentration of 400 μg ml^{-1}.
- **Smooth muscle cell solution (mM)** 135 CsCl, 2 $MgCl_2$, 1 EGTA, 10 HEPES, 5 NaATP (pH 7.2 with CsOH).
- **Synoviocyte solution (mM)** 115 CsCl, 10 EGTA, 2 $MgCl_2$, 5 Na_2ATP, 0.1 NaGTP, 10 HEPES, 5.7 $CaCl_2$ (pH 7.2 with CsOH).
- **Neutrophil solution (mM)** 140 KCl, 10 NaCl, 2 $MgCl_2$, 0.7 $CaCl_2$, 1 EGTA, 10 HEPES (pH7.3 with KOH).
- **SH-SY5Y solution (mM)** 140 KCl, 0.5 $CaCl_2$, 5 EGTA, 10 HEPES, 2 KATP (pH7.2 with KOH) adjusted to 320 mOsm with sucrose.
- **HEK293 TRPM3 or TRPC5 solution (mM)** 135 CsCl, 1 EGTA, 2 $MgCl_2$ 10 HEPES, 5 NaATP, 0.1 Na_2GTP (pH 7.2 with CsOH).
- **HEK293 K_{Ca}1.1 solution (mM)** 140 KSCN, 5 EGTA, 1 $MgCl_2$, 0.5 $CaCl_2$, 10 HEPES, 3 MgATP, 0.3 NaGTP (low Ca^{2+}:15 nM free) or 140 KSCN, 4.5 EGTA, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 3 MgATP, 0.3 NaGTP (physiological Ca^{2+}: 108 nM free) or 140 KSCN, 5 EGTA, 1 $MgCl_2$, 3.5 $CaCl_2$, 10 HEPES, 3 MgATP, 0.3 NaGTP (high Ca^{2+}: 316 nM free).
- **HEK293 $P2X_7$ solution (mM)** 145 NaCl, 10 EGTA, 10 HEPES (pH 7.2 with NaOH).

Extracellular recording solutions—Prepare extracellular solutions (500 ml) in deionised water, filter and measure the osmolarity and the pH. The solutions can be stored at 4 °C for up to 5 days. Solutions should be warmed to room temperature (20±2 °C) before use.

- **Astrocyte solution (mM)** 150 NaCl, 5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 glucose (pH 7.4, adjusted to 300 mOsm with sucrose).
- **T lymphoblast solution (mM)** 160 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 HEPES (pH 7.4 with NaOH).
- **Neutrophil solution (mM)** 140 NaCl, 2.5 KCl, 0.5 $MgCl_2$, 1.2 $CaCl_2$, 10 HEPES, 5 glucose (pH 7.4 with NaOH).
- **SH-SY5Y solution (mM)** 120 NaCl, 2.5 KCl, 2 $MgCl_2$, 0.5 $CaCl_2$, 10 HEPES, 5 glucose (pH 7.4 with NaOH).

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- **HEK293 K_{Ca}1.1 solution (mM)** 150 NaCl, 5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 glucose and 10 HEPES.
- **HEK293 $P2X_7$ solution (mM)** 147 NaCl, 2 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 13 glucose (pH 7.3 with NaOH)
- **Synoviocytes, smooth muscle cells and HEK293 TRPM3 or TRPC5 solution (mM)** 130 NaCl, 5 KCl, 8 glucose, 10 HEPES, 1.2 $MgCl_2$, 1.5 $CaCl_2$, (pH 7.4 with NaOH).

Seal enhancer solutions—Prepare solutions in deionised water, filter and measure the osmolarity and the pH. The solutions can be stored at 4 °C for up to 5 days. Solutions should be warmed to room temperature (20±2 °C) before use.

- **Extracellular solution (mM)** 105 NaCl, 4.5 KCl, 1 $MgCl_2$, 40 $CaCl_2$, 5 glucose, 10 HEPES (pH 7.4 with NaOH). (Users may wish to investigate the impact of partially substituting the $CaCl_2$ by $BaCl_2$ if the high Ca^{2+} concentration is a concern).
- **Intracellular solution (mM)** 75 KCl, 10 NaCl, 70 KF, 2 $MgCl_2$, 10 EGTA, 10 HEPES (pH 7.2 with KOH; 320 mOsm). (Inclusion of fluoride has long been known to improve patch-clamp sealing and stabilises the cell membrane, resulting in longer, more stable patch-clamp recordings24-26; the mechanism of this effect is unknown).

EQUIPMENT SETUP

PatchControl©HT and Patchmaster software setup—Pre-programmed protocols (*Trees*, e.g. Fig 1e) and pulse generator files are available. The Editor window allows the user to load, create and modify *Trees* for sealing a cell, achieving and maintaining the patch clamp configuration and performing patch clamp experiments.

Chloridating electrodes—The electrodes are manufactured from Ag/AgCl coated steel and need to be regularly chloridated in bleach, controlled via a pre-programmed *Tree* that takes 45 min to complete. Electrodes need to be re-chloridated about once a week and are generally replaced every 2 months.

Solutions—At the start of all experiments, planar chips are filled with intracellular seal enhancer solution and the extracellular solution that will be used later during data collection. Cells are dispensed into the extracellular chamber and giga-seals formed, usually with the aid of the extracellular seal enhancer solution. Typically, following seal formation and before establishment of the whole-cell, solutions are exchanged for those appropriate to the cell type under investigation (Fig 1e).

Analysis programs—The Patchmaster software has integral online analysis capabilities allowing user-defined levels of automation.

PROCEDURE

Typical modification of a pre-programmed experimental routine (PatchControl©HT Tree) · TIMING ~ 30 min—1| Load a pre-programmed PatchControl©HT *Tree*. A simplified *Tree* is shown for illustrative purposes (Fig. 1e).

2| Select Edit Mode to access COMMAND folders that can be edited and inserted in the *Tree* using the generic drag-and-drop function.

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3| To program the robot to collect solutions at the desired volume, dispense them at the selected speed and activate the appropriate pulse generator file in Patchmaster, the 'define experiment sequence' of the *Tree* is selected and edited (Fig. 1e, Step 1). Using the dialogue box, compound positions are set (e.g. position C2 seen in Fig 1d). Possible speeds of solution application range from 1 to 857 μl/s, with speeds of 4 μl/s (intracellular solution) and 10 μl/s (extracellular solution) being typical. For ligand-gated ion channels, ≥30 μl/s is advised for extracellular solution application.

4 | Highlight the 'set compound positions' branch of the *Tree* (Fig. 1e) to select the rack settings for solutions/compounds, specify the compound name and stipulate the concentration. Define these in the drop down menu using the dialogue option. Once the data acquisition is activated (see step 9) an Excel file is generated which logs the compound used, its concentration and the time of application, and allows for subsequent automated analysis (e.g. IC_{50} determination).

5| Select the 'add cells' folder and set the pressure (for attracting the cell) to between -50 and -150 mB depending on the characteristics of the cell membrane. Adjust the pressure to be maintained to -50 mB once a cell seals on to the aperture of the chip chamber. Pressure is applied from below the chip to bring a cell onto the aperture.

? TROUBLESHOOTING

6| Within the 'check for cell contact' folder, adjust the pressure settings in the drop down menu to improve the seal or encourage a cell onto the aperture. Set the pressure to a desired value for a given time (e.g. -100mB for durations such as 10 s) and then program it to return to atmospheric pressure for the same duration. Adjust the settings to repeat this process multiple times with increasing pressure (e.g. -10 mB incremental increase) up to a maximum -200 mB or until the desired seal resistance is obtained. Pressure settings in this folder will need to be optimised by the operator to suit the cell type depending on the type and/or characteristics of the cell membrane.

? TROUBLESHOOTING

7| To improve the seal further, highlight the 'monitor seal' branch command within the 'add seal enhancer solution' folder and in the drop down menu adjust the pressure and voltage settings and also the condition parameters to give an increase-release pressure cycle. These settings will need to be optimised for different cell types/characteristics. Pressure pulses are typically applied every 5 s, with incremental increases in pressure. Once the desired seal is attained, the pressure is typically maintained at -50 mB. The condition for a high-quality seal is typically set to ≥1 GΩ and, if 1 GΩ is not achieved, recordings are continued if the resistance is >0.6 GΩ, but otherwise timed out and the chamber disabled. The operator defines the thresholds for proceeding with data sampling.

▲ **CRITICAL STEP** It is helpful to use seal enhancer solutions to increase the chance of giga-seal formation. The extracellular seal enhancer solution is a high Ca^{2+}-containing solution, whereas the intracellular solution contains fluoride (see REAGENT SETUP). Seal enhancer solutions are usually replaced once a giga-seal is achieved and before gaining whole cell access (Fig 1e *Tree* 'exchange solutions').

? TROUBLESHOOTING

8| Select the 'check for whole cell' folder (Fig 1e) and set the pressure in the drop down menu to apply suction pulses, typically from -50 mB up to -350 mB in -50 mB increments. These pressure changes are applied individually to the different cells and the settings depend

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on the seal resistance, membrane capacity and series resistance. In addition, adjust the size of the high voltage pulses to 600-800 mV ("zap"), which can be applied to help rupture the patch of membrane, thus establishing the whole-cell configuration. Chip chambers where cells have not sealed or achieved the whole cell configuration are disabled at this stage. The operator can adjust pressure, voltage and condition settings according to the cell type/ characteristics.

? TROUBLESHOOTING

9| Insert the appropriate commands into the 'whole-cell experiment' folder in the correct order to correspond to the experiment sequence defined at the beginning of the *Tree*. These commands will activate Patchmaster data acquisition software and direct the robot to apply compounds according to the experimental protocol. Also adjust the time in the 'time step' branch of this folder to control the duration of data acquisition and the exposure time of the cells to different compounds (Fig. 1e).

▲ **CRITICAL STEP** The 'maintenance' command (Fig. 1e) is routinely inserted in the *Tree*, immediately following any extracellular solution application command, to minimise risk of solutions leaking into the electrical hardware and to wash the robot pipette arm. These features also mean that there are no external solution volume constraints due to limited waste chamber size and no mixing of compounds due to contamination by the pipette. The maintenance commands are especially important when applying several compounds consecutively (e.g. when constructing dose-response curves).

10| Highlight the 'End' folder and adjust the settings in the drop down menu to move the chip wagon to the next chamber and then, if desired, to the next chip. Furthermore, set the program here to re-activate any chip chambers disabled in the previous run. The chip cartridges contain 16 chambers each with a planar glass chip embedded in it. These chambers are arranged in two rows of eight (Fig. 1b, c). For Patchliner Quattro, the first chip site consists of the first four chambers; two in row one and two in row two (i.e. there are four sets of four chambers in one cartridge). For Patchliner Octo there are 2 chip sites (i.e. there are two sets of eight chambers in one cartridge).

11| Save *Trees* in a user-defined folder. Once a *Tree* is optimised for a particular cell/ channel type it is suitable for further use with the same cell/channel type without additional modification.

■ **PAUSE POINT** The user can pause at this point before proceeding with cell preparation and recording.

Preparation of cells · TIMING ~ 20-30 min—12| To prepare cell suspensions of T lymphoblasts, neutrophils and Jurkat cells (i.e. non-adherent cells), follow option A. For adherent cell types, follow option B.

▲ **CRITICAL STEP** Cell confluency of 60-80 % is critical. If cells are allowed to grow to a higher confluency, widespread contact among cells occurs and cells cluster and form unhealthy cytoplasmic granules.

▲ **CRITICAL STEP** Use only healthy cells. The health of the cell impacts on the quality of the seal formed between the cell membrane and the planar chip, which ultimately influences the quality of the recording. Once cells are prepared in suspension there is no opportunity to select the healthiest cells unless fluorescent cell-selection is incorporated (Anticipated Results).

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Option A: Preparing cell suspensions of T lymphoblasts, neutrophils and Jurkat cells:

- **i.** Transfer the cells to 15-ml conical centrifuge tubes and centrifuge at 100 *g* for 2 min at room temperature (20±2 °C) and discard the supernatants by decanting.
- **ii.** Suspend the cell pellets in 1 ml Dulbecco's Phosphate Buffer Saline (D-PBS; Ca^{2+} and Mg^{2+}-free), centrifuge at 100 *g* for 2 min and discard the supernatant by decanting. This will wash the cells to remove the culture medium which may otherwise affect the recording solutions.
- **iii.** Re-suspend cell pellets in appropriate extracellular recording solution (see REAGENT SETUP) at a density of 1×10^6-5×10^7/ml (cells can be counted using a haemocytometer).

Option B: Preparing suspensions of adherent cells:

- **i.** Remove culture media from 75 cm^2 culture flasks and discard.
- **ii.** Wash the cells in 5 ml D-PBS and then add 2 ml of 0.05 % Trypsin/EDTA solution and incubate the cells at 37 °C until the cell layer is dispersed (usually 3-8 min). Washing the cells with D-PBS not only removes the serum-containing culture media, but also dead cells.

  ▲ **CRITICAL STEP:** Do not hit the flask to make the cells detach from the bottom as they will come off without this intervention. Cells must be handled gently at all times.
- **iii.** Add 5 ml of appropriate serum-containing culture media (see REAGENT SETUP) to each flask (to inactivate the trypsin), aspirate gently (to separate cell clusters) and transfer each cell culture to a 15-ml conical centrifuge tube.
- **iv.** Centrifuge cells at 100 *g* for 2 min at room temperature and discard the supernatant by decanting.
- **v.** Suspend the cell pellets in 1 ml D-PBS and centrifuge at 100 *g* for 2 min and discard the supernatant by decanting.
- **vi.** Suspend cell pellets in appropriate extracellular recording solution (see REAGENT SETUP) at a density of 1×10^6-5×10^7/ml. The final suspension volume should be 1-3 ml.

13| View cells under a light microscope to ensure they are single, non-clustered, cells with smooth membrane edges (e.g. Fig. 2).

Commencing the experiment · TIMING ~ 10 min—14| Select the appropriate NPC©-16 chips based on our experience or user optimisation. Typically, our chip (aperture) resistances are 1-2 MΩ (smooth muscle cells), 2-3 MΩ (astrocytes), 3-5 MΩ (lymphoblasts, Jurkat cells, synoviocyte, SH-SY5Y cells and HEK 293 cells) or 5-8 MΩ (neutrophils). NPC©-16 chip resistance can be manufactured within a range from 1-8 MΩ with an accuracy of ±0.5 MΩ for resistances between 1-4 MΩ and ±1 MΩ for resistances between 5-8 MΩ, making them suitable for a variety of cell types of different sizes. Place three chips on the chip wagon (Fig 1d).

15| Place the suspended cells from step 12 in the cell hotel (Fig 1d) and activate the hotel using the robot drop down menu option in the software. Set the cell hotel pipette to aspirate the cells every 30 s not only to avoid clumping and sedimentation, but to increase the length of time that the cells remain viable once prepared in suspension. Based on trypan blue exclusion and ionic current recording we find that cells retain good viability for at least 3 h

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after they have been prepared in suspension, although some deterioration in success rates has been observed after 2 h.

16| Place the solutions in their appropriate positions according to the 'define experiment sequence' settings of the chosen *Tree*.

Patching · TIMING Varies from 1-6 h—17| Select the 'initialise robot and electronics' folder (Fig 1e) and activate initialisation by clicking the Start arrow at the bottom of the Editor Window. This folder only needs to be activated once at the beginning of each day of experiments. It generates a new data file within the HEKA software and sets all amplifier and robot parameters to reasonable starting values. The robot is initialised and the pipette is washed.

18| Activate a newly modified *Tree* by highlighting the 'start' folder (Fig 1e) and clicking the start arrow at the bottom of the Editor Window. This will activate the robot and the *Tree*, loop back to the start at the end of each run and continue this process until all NPC©-16 chips on the chip wagon have been used. At this point the robot will stop.

▲ CRITICAL STEP Whole-cell patch-clamp experiments are performed at room temperature (20±2 °C).

? TROUBLESHOOTING

19| Close the PatchControl©HT software by selecting 'quit' from the menu in the *Editor* window. Select the 'save and exit' option when closing down Patchmaster to ensure that data files are saved properly.

■ **PAUSE POINT** Data can be analyzed anytime after recording.

Data analysis · TIMING ~1-10 h—20| For data analysis, use the standard export and import functions of the HEKA software (www.heka.com). The data analysis is as time-consuming as for conventional experiments, although for standard IC_{50} fits an automated export function (e.g. IGOR-Pro software) can be activated during data acquisition.

21| To display the data for immediate presentation, set up the online analysis using instructions in the Patchmaster manual and open the online analysis. Highlight a sweep or a series of sweeps in the replay window. Raw data traces will be displayed in the oscilloscope window. Select the overlay sweep and overlay series functions in the oscilloscope window and activate the scan option. The scan function generates a digital display of amplitude, time or voltage measurements from the raw data trace. At the same time, time-course or current-voltage plots can be displayed in the online analysis window. Create a screen shot of windows of interest to provide an easy way of presenting data visually without first undertaking lengthy analysis.

TROUBLESHOOTING

Troubleshooting advice can be found in Table 2.

TIMING

Steps 1-11, typical modification of pre-programmed experimental routine: ~30 min

Step 12-13, preparation of cells: ~ 20-30 min

Step 14-16, commencing the experiment: ~10 min

Step 17-19, patching: ~ 1-6 h

Step 20-21, data analysis: ~ 1-10 h

ANTICIPATED RESULTS

Experiments have been performed using a planar system with capacity for up to 8 simultaneous recordings and incorporating micro-fluidics for rapid, unlimited, extracellular and intracellular solution exchange (Fig. 1). Experiments were performed alongside conventional patch-clamp experiments that have been routine in our labs for up to 20 years. Validation of the system was achieved with cell lines over-expressing ligand-gated, receptor-operated and voltage-gated ion channels (Fig. 3a-d). Although the cells need to be released into suspension to make the recordings, the data are similar to those obtained by conventional patch-clamp when cells are often attached to a glass coverslip (Fig 3d).

While validating the planar system we were engaged in a separate project using conventional patch-clamp to study ion channels of rabbit and human fibroblast-like synoviocytes, which are relevant to arthritis. The success rate in making these conventional recordings from the cells was poor (e.g. Table 3). The cells attach to coverslips, spread out as thin structures and show irregular shapes (Fig. 2a). Despite preconception that such cells would not perform well with planar technology, the success rates were high, both in terms of giga-seal formation and whole-cell recording (Table 3). Whole-cell series resistance values were remarkably consistent, suitably low compared with membrane resistance, and independent of cell size (Fig 4a, Table 3). Similar success occurred with primary-cultured proliferating human vascular smooth muscle cells (Fig 4b), which are also troublesome in conventional studies (Table 3). An illustrative recording shows reversible block of constitutive non-selective cationic current by gadolinium ions, and subsequent block by 2-aminoethoxydiphenylborate (2-APB) (Fig 4c). Recordings were long in duration and stable during solution exchange (Fig 4c). We were encouraged to explore other cell types. Similar high performance occurred with rat astrocytes and, albeit at lower success rates, human lymphoblasts or neutrophils (Table 3). Current-voltage relationships (I-Vs) were similar to those observed during conventional recordings (Fig 4d). Original traces show the signal-to-noise ratio and characteristics expected of whole-cell current recordings (Fig 4e).

Whole-cell access could be attained by physical rupture of the cell-attached patch or by permeabilisation with amphotericin B (Table 3, Fig 4e). Amphotericin B limits intracellular dialysis to small ions, making recordings more physiological and reducing run-down of channel activity. Physical rupture allows intracellular application of exogenous substances. With the robotic system, not only could substances be applied intracellularly, but complete intracellular solution exchange was easy and efficient (Fig 4f). Multiple successive exchanges could be achieved (e.g. Fig. 3c).

The planar method of patch-clamp has special features that need to be handled by the investigator. For example, some membrane processes may be adversely affected by the protocol required to produce the cell suspension, or by seal enhancer solutions. Therefore, independent assessment of key ion channel properties should initially be obtained for comparative purposes, either from the published literature or new conventional patch-clamp recordings. Secondly, if transient transfection is important for the experiments (e.g. for RNAi studies), high efficiency of the transfection may be necessary because of the random process by which a cell reaches the aperture in the chip. Several electroporation or lipid transfection methods provide such high efficiency. Contamination of transfected cell populations with non-transfected cells will tend to increase the variance within the test data

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group, increasing the need for greater numbers of cell recordings prior to statistical analysis; that is, transfection efficiency less than 100 % may be acceptable if the recording success rate is high enough. Sorting of transfected cells before recording (e.g. by FACS) is an option for increasing the proportion of transfected cells, but may carry with it disadvantages such as reduced total cell count and expense. Users should be aware that a fluorescent cell (e.g. expressing green fluorescent protein) can be detected on the aperture of the chip by viewing the underside of the cartridge on a fluorescence microscope after ionic current recording. By this means, ionic current data can be categorized into test (transfected cells) and control (untransfected cells) groups, enabling all ionic current data to be used and avoiding wastage of chips. Such an approach also has potential to eliminate data from unhealthy cells if a fluorescent live-dead cell indicator is incorporated into the experiment, or to categorize data from heterogenous cell populations.

Acknowledgments

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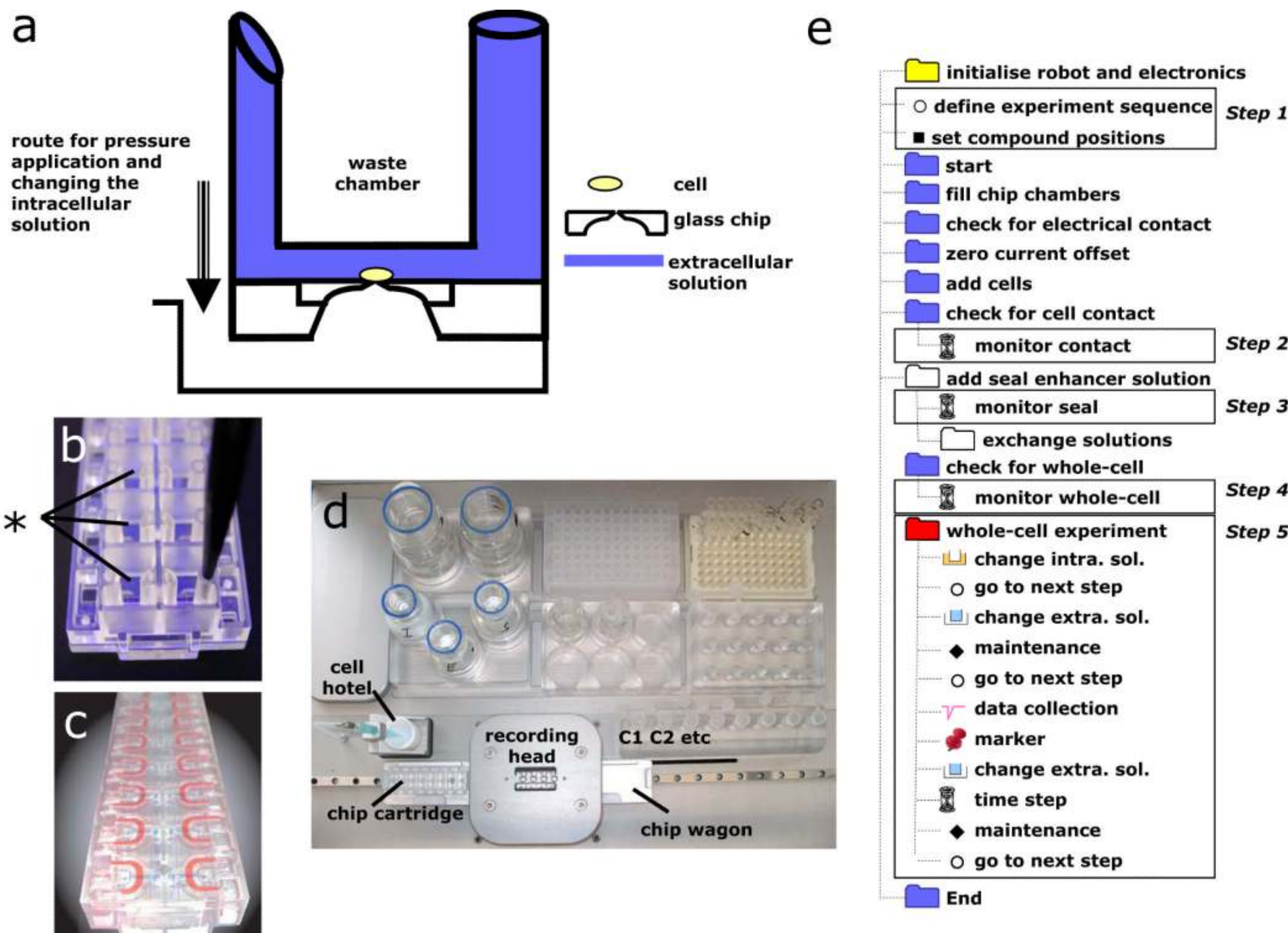

Figure 1.

Planar chip design and the robotic platform. (**a**) Schematic of a cross section of one chip chamber illustrating the arrangement of solutions, chip and cell. (**b**) Picture of part of the 16-well micro-fluidic cartridge with a solution delivery pipette inserted into the extracellular inlet of one chamber. *Chambers. (**c**) Image of the under side of the cartridge in which the intracellular solution compartments are highlighted with red dye. (**d**) Picture of the work area, showing a typical arrangement. At the front is the recording (or measurement) head, which contains the pneumatic and electric contacts and moves up and down to address the patch-clamp chip cartridges. The chip cartridges are placed on a motorized stage (chip wagon), which takes up to three cartridges, hence allowing for 48 recordings. The recording head can accommodate up to 8 chip chambers. Cells and compounds are placed in small vials or microtiter plates and are sampled by the pipetting robot into the microfluidic chambers of the chip cartridge. For more information, see http://www.nanion.de/. (**e**) A typical experimental procedure defined by the software *Tree*. Step 1 enables the user to define the job sequence and compound positions. Steps 2-4 are critical steps for capturing cells on the aperture, forming seals and establishing the required patch configuration (cell attached, whole cell or perforated whole cell). Step 5 shows an example experiment that can be modified according to operator requirements.

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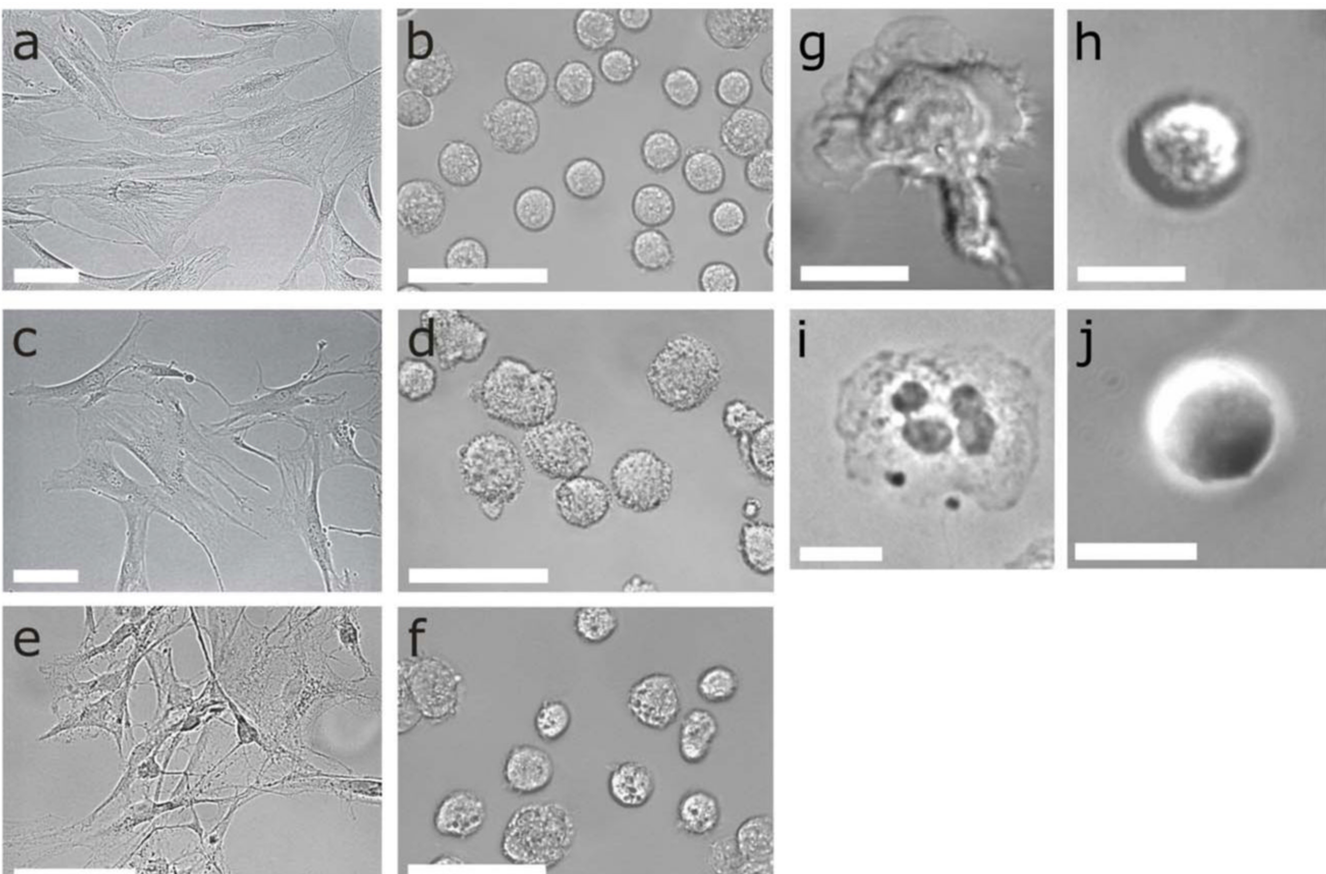

Figure 2.

Images of cells. (**a, b**) Synoviocytes. (**c, d**) Smooth muscle cells. (**e**, **f**) Astrocytes. (**g**, **h**) Lymphoblasts. **(i**, **j)** Neutrophils. In each case the image on the left is the cell(s) attached to a cover-slip, and on the right in suspension. Scale bars: 40 μm (**a**, **c**, **e**); 30 μm (**b, d, f**); 10 μm (**g-j**).

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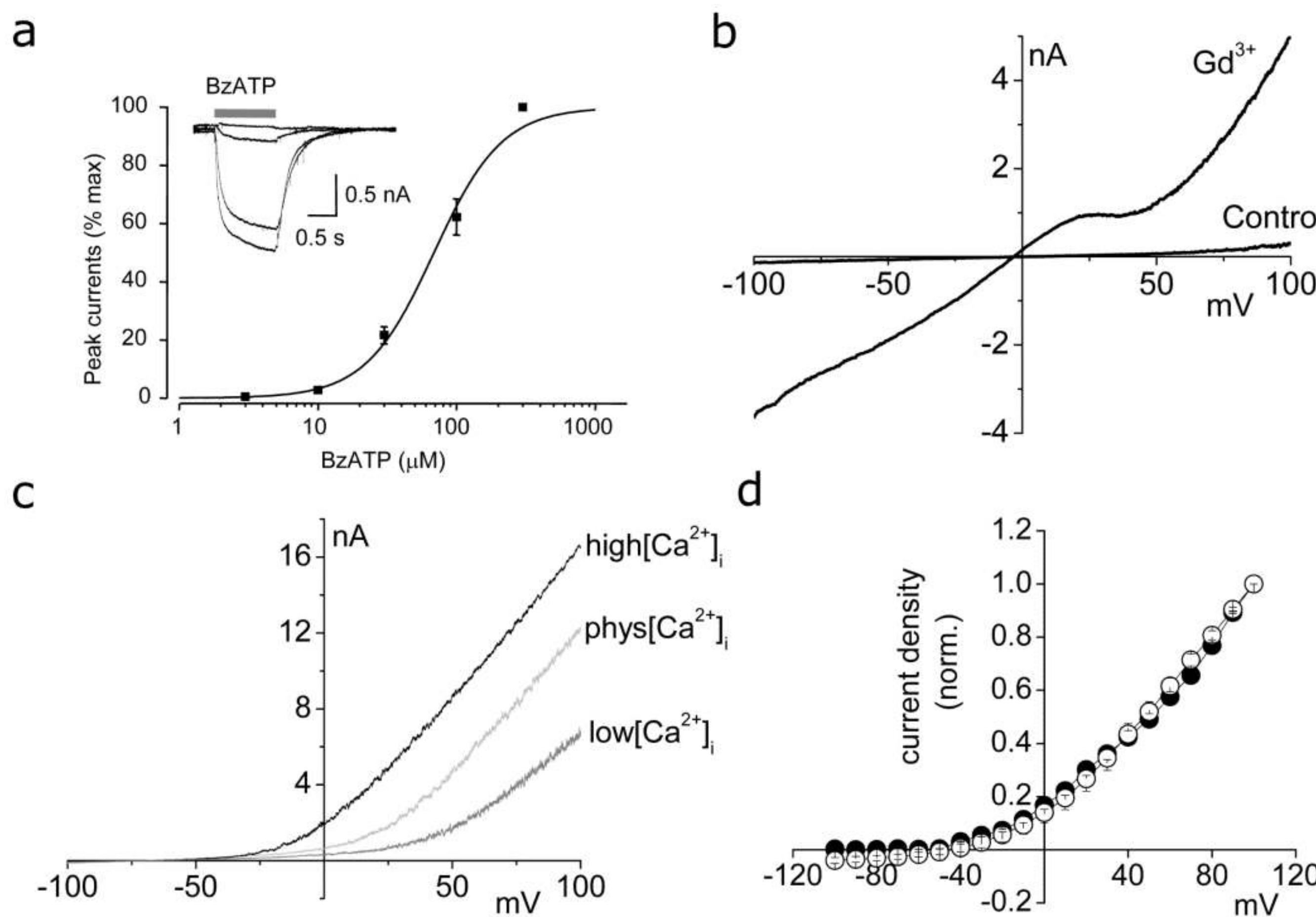

Figure 3.

Illustrative planar whole-cell patch-clamp data from HEK 293 cells stably expressing different exogenous ion channels. (**a**) BzATP concentration-response curve obtained from wild-type $P2X_7$ receptors. Data are fit to the Hill equation with the following EC_{50} value: 69.7 ± 7.5 μM and Hill coefficient: 2.3 ± 0.5 (*n* = 8 for each data point). Inset shows representative currents evoked by BzATP (3-100 μM). Holding potential was -60 mV. (**b**) I-Vs showing activation of TRPC5 by extracellular application of Gd^{3+} (100 μM). Voltage ramps were applied at a frequency of 0.1 Hz from -100 mV to +100 mV for 1 s from a holding potential of 0 mV. Current signals were sampled at 3 kHz and filtered at 1 kHz. (**c**) $K_{Ca}1.1$ channel I-Vs in a single cell showing effects of changing the intracellular free Ca^{2+} concentration (15 nM, *n* = 9; 108 nM, *n* = 11; 316 nM, *n* = 11). (**d**) Comparison of $K_{Ca}1.1$ I-Vs using conventional (black circles, *n* = 10) or planar (open circles; n = 11) recording with high intracellular Ca^{2+}. Voltage ramps were applied at a frequency of 0.2 Hz from -100 mV to +100 mV for 500 ms from a holding potential of -70 mV. Current signals were sampled at 1 kHz and filtered at 500 Hz.

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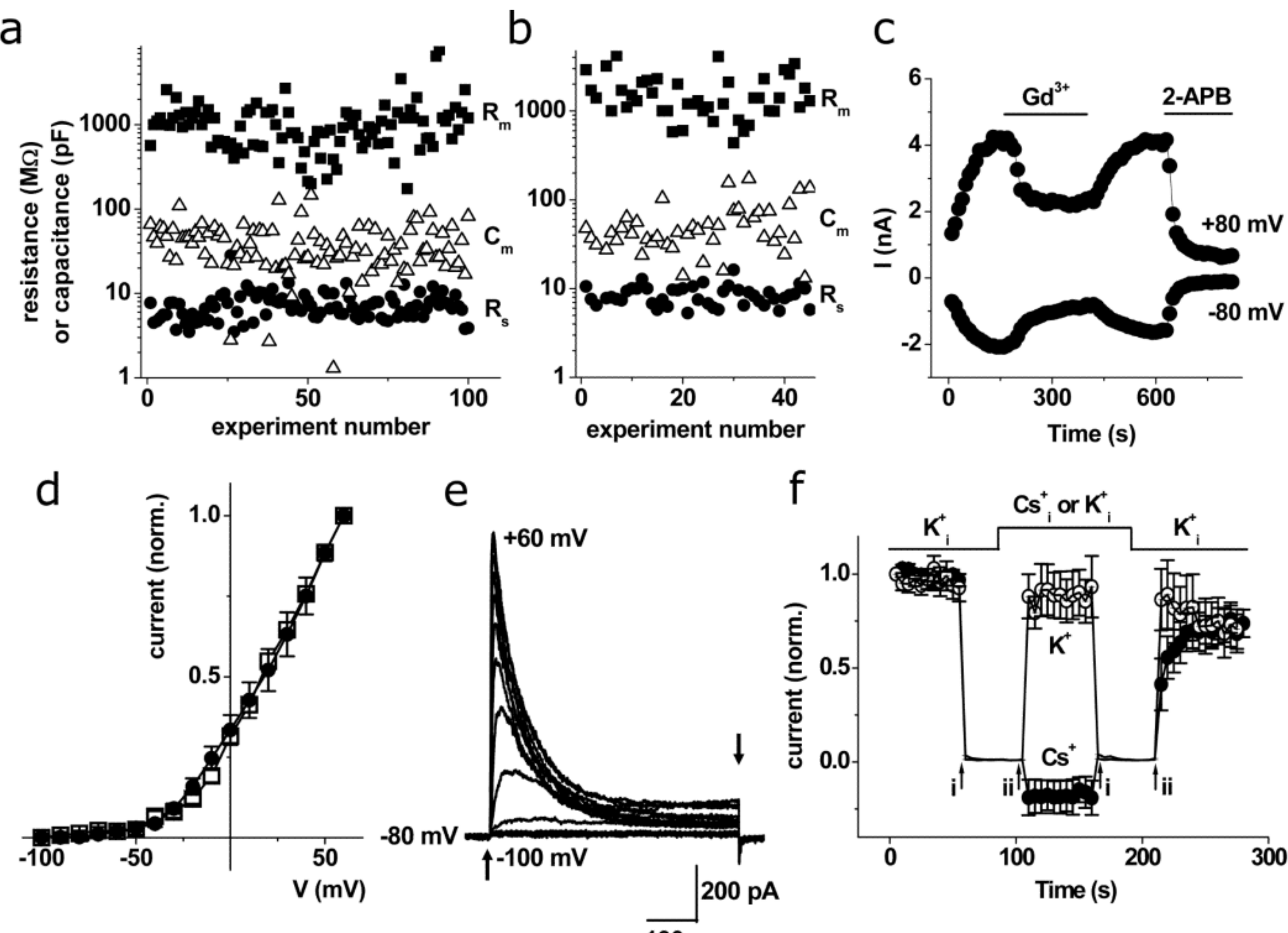

Figure 4.

Planar whole-cell patch-clamp data from native and primary cells. (**a**, **b**) Membrane resistance (R_m), series resistance (R_s) and membrane capacitance (C_m) values for successful recordings from human synoviocytes (**a**) and smooth muscle cells (**b**). The *y*-axis for (**b**) is the same as (**a**). The order of the experiments is the order of execution and does not reflect an attempt to make a correlation with recording parameters. (**c**) Illustrative time-series showing currents at two voltages in a smooth muscle cell exposed to extracellular Gd^{3+} (100 μM) and then 2-APB (75 μM). Voltage ramps were applied at a frequency of 0.1 Hz from -100 mV to +100 mV for 1 s from a holding potential of 0 mV. Current signals were sampled at 3 kHz and filtered at 1 kHz. (**d**) Comparison of K^+ current-voltage relationships (I-Vs) for rat astrocytes studied by planar (black circles, $n = 19$) or conventional (open squares, $n = 10$) patch-clamp. Currents were evoked by 500 ms voltage steps at a frequency of 0.2 Hz from -100 mV to +60 mV from a holding potential of -70 mV in 10 mV increments. (**e**) An illustrative typical family of traces obtained during construction of an I-V from a human lymphoblast recording using amphotericin B to access the whole-cell. Currents were evoked by 500 ms voltage steps at a frequency of 0.06 Hz from -100 mV to +60 mV in 10 mV increments. Arrows indicate the start and end time points of each square voltage step applied from a holding potential of -80 mV. Data were sampled at 6.67 kHz and filtered at 2.2 kHz. (**f**) Normalised K^+-current amplitudes in rat astrocytes. The intracellular solution was changed to the same solution (K^+, open circles, $n = 7$) or one where Cs^+ was substituted for K^+ (Cs^+, black circles, $n = 7$). Between (i) and (ii) there was electrical

disconnection to allow for pipetting of the new intracellular ionic solution. 500 ms depolarizing voltage steps were applied at a frequency of 0.2 Hz from -100 mV to +40 mV from a holding potential of -70 mV. Data were sampled at 5 kHz and filtered at 2 kHz. Averaged data are presented as mean±s.e.mean. Current measurements were performed using the Fitmaster software (HEKA). Data were further manipulated using Origin software.

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TABLE 1

Comparison of multi-well planar and conventional patch-clamp techniques. Investigators currently engaged in conventional patch-clamp recording, or considering adopting patch-clamp recording for the first time, may wish to consider robotic multi-well planar patch-clamp, either to complement existing conventional patch-clamp equipment or as stand-alone technology. The purpose of this table is to help investigators decide whether to adopt the new technology. The table lists strengths and weaknesses of the conventional and multi-well systems based on our experiences of various conventional patch-clamp systems and the one multi-well planar system described in this article. The costs involved are not trivial and so we provide a rough guide, but potential users should contact suppliers to obtain accurate information on prices and take into account the employment and training costs of conventional and multi-well patch-clamp operators. Local consortia or service facilities are an option for implementation of a multi-well system, but users should recognise that multi-well patch-clamp is currently quite far from the simplicity equivalent to that of, for example, the ELISA and plate-reader

Feature	Conventional	Planar
Enables high quality and stable whole-cell voltage-clamp	Usually routine for small cells and if used by a skilled operator	Usually routine for small cells and if supported by a technologist
Enables membrane potential (and thus action potential) recording	Yes	In principle yes, but not validated
High seal resistances are achievable	Yes: Usually >1 GΩ	Yes: Usually >1 GΩ
Enables amphotericin- or nystatin perforated patch whole-cell recording	Yes, but success in achieving high resistance seals may be compromised	Routinely possible
Enables cell-attached membrane patch recording	Achievable with high signal-to-noise ratio, but requiring additional skills and knowledge by the operator	Achievable, but requiring additional knowledge for the experimental design
Enables excised membrane patch recording	Achievable with high signal-to-noise ratio in inside-out and outside-out configurations, but requiring additional skills by the operator	Not established
Operator intervention during recordings	The operator commonly modifies the protocol during recording, depending on responses of the cell. Pre-programmable options are, however, available.	Usually the protocol is pre-determined and occurs robotically, but operator intervention during experiments is possible
Level of operator skill required	High levels of operator skill, knowledge, manual dexterity, dedication and patience are usually required	With support from the system-provider, a competent high-grade technician can perform the experiments, acting as the interface between multiple scientific investigators and the equipment. Scientific investigators will often be required to design novel protocols and perform data analysis.
Vulnerable to unwanted electrical noise (50-60 cycle mains interference)	Yes, unless competently addressed by the operator.	No.
Data output rate	Usually 5-10 cell recordings per day depending on the operator skill and experiment type.	Usually 16-33 cell recordings per day depending on experiment type and based on 3 cartridges. (We have used 9 cartridges in a day, raising the number of cell recordings to >50).
Operator-independent functionality	Constant involvement of the operator is required, so data will only be obtained while the operator is at the equipment.	Because of the robotic functionality, data can be generated from 3 cartridges in the absence of the operator, enabling extra recording time after the operator has left the lab.
Sensitive to physical disturbance	Yes: Requires a vibration isolation table, a low-drift micromanipulator and a relatively quiet environment.	No: Requires only a standard laboratory bench.
Requires special ionic solutions during the membrane sealing process	Physiological salt solution is suitable; Absence of Ca^{2+} may decrease performance.	Success rate may be unacceptably low without supra-physiological Ca^{2+}

Feature	Conventional	Planar
		concentrations or fluoride in the intracellular solution.
Fast extracellular solution exchange	Possible if a suitable exchange system is attached to the microscope.	Exchange in less than 1 s is standard and faster times are achievable.
Liquid volume for extracellular solution exchange	Commonly a few millilitres but may be as low as a few microlitres with some specialised application systems.	10-50 microlitres is routine.
Chemical reaction with the ground (Earth) wire	An agar bridge can be incorporated to avoid contact with a reactive chemical.	Because of the confines of space inside the chip an agar bridge has not been incorporated, but it should be possible to make this addition.
Vulnerable to contamination between intracellular and extracellular solutions.	Yes. As the patch pipette approaches the cell its contents may leak out and affect cell function (ATP may be a problem in this regard), or bath solution may enter the patch pipette and then later enter the cell during whole-cell recording (Ca^{2+} may be a problem in this regard).	No. Intracellular solution can be readily exchanged, enabling intracellular factors to be added after seal or whole-cell formation.
Limit to the number of compound applications and wash-outs	None other than the duration of the cell recording and stability of the ion channel activity.	As per conventional patch-clamp.
Intracellular solution exchange	Possible but extremely difficult and rarely used.	Routinely possible and repeatable.
Problems due to liquid-liquid junction potentials	Not a problem for many common ionic solutions but can be a problem when physiological ions are replaced by foreign ions. Correction can be made if the operator has the necessary skill and knowledge.	As per conventional patch-clamp but needing special knowledge of the multi-well system.
Recording at temperatures above or below room temperature	Possible if a suitable control system is incorporated in the perfusion system.	Not currently possible, but introduction is anticipated shortly.
Applicable to cells attached to coverslips or surrounding tissue	Possible, depending on the cell type and stability of the micromanipulator.	Not established and may not be possible even with system modification.
Applicable to heterogenous cell populations	Cells of interest may be selected by visual appearance under the microscope.	After ionic current recording, it can be determined if the cell is fluorescent, enabling categorisation of data for analysis. Alternatively, cells of interest may be pre-selected by a sorting technique that would not affect seal formation.
Complications relating to cell density	Factors released from other cells in the recording chamber may affect the behaviour of the patched cell unless steps are taken to avoid it.	The patched cell is relatively isolated and so factors from other cells would not usually be relevant.
Widely accepted as a gold standard for ion channel studies	Yes.	Not at this stage and thus validation by conventional patch-clamp recordings may be required initially.
Generating standard data in different laboratories	Without concerted effort it tends not to, because there is commonly variety in the equipment features and because operator skills and choices can vary substantially.	An opportunity for greater standardisation is presented, but coordination on experimental conditions and cells would be required.
Space occupation	Usually requires about 2.5 × 2.5 m of dedicated floor space	Requires 1.5 m of standard lab bench
Approximate cost for the primary recording equipment	In the region of £50,000	In the region of £150,000
Approximate consumable costs	In the region of £25 per day (estimate based on the purchase cost for a patch pipette fabrication system, operator time to custom-make pipettes in the lab, and the cost of commercial capillary glass).	In the region of £150 per day (estimate based on current purchase prices for 3 custom-made planar chip cartridges).

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TABLE 2

Troubleshooting

Step	Problem	Possible reason	Solution
5	Cells are only attracted to the apertures in first few chambers. A chip cartridge has 16 chambers with a single planar glass chip in each chamber (see Fig. 1b)	The pipette arm is set to take enough cell suspension to supply all the chip chambers in a single go (e.g. 100 μl) and so the cells tend to sediment in the pipette during the dispensing process	Change dispense setting so the pipetting arm will only take enough suspension for one chamber (e.g. 25 μl), dispense it and then return to the cell hotel for more cells
6	No cells are attracted to the aperture	The cell density is either too high or too low No pressure is delivered to the aperture	Minimum and maximum cell densities: 1×10^6 and $5{\times}10^7$/ml. Check the pump pressures meet the programmed values. If the programmed values are not met, calibrate the pumps.
7	Whole cell access is achieved before a giga seal is established	The initial pressure applied for seal formation is too high The cell membrane is fragile. This can occur within the first two weeks following thawing cells or one to two days after plating. It can also be caused by trypsin treatment	Reduce the pressure applied when forming the seal Adjust the time frame of cell use after thawing and plating accordingly Try more gentle dispersion treatments e.g. Ca^{2+} free D-PBS/ EDTA, Detachin or Accutase®
8	Gaining whole cell access is difficult	The cell membranes are stiff	Passage the cells more frequently (e.g. every two to three days) Increase the suction pressure.
18	No channel activity	Trypsin may have digested parts of the channel protein, disabling it functionally	Use Ca^{2+} free D-PBS/EDTA to detach the cells or another detachment method.
Equipment Setup: Robot.	The robot pipette arm no longer hits the correct location	The settings for the pipette arm or chip wagon have become misaligned	The robot settings need re-teaching.
Equipment Setup: Electrodes.	Recordings become noisy or drift, or there are high or unstable offsets.	Chloride coating on the electrodes is no longer intact.	Chloridate or replace electrodes.

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TABLE 3

Average recording parameters and success rates. R_m (membrane resistance), R_s (series resistance), C_m (membrane capacitance) and minimum whole-cell recording durations are shown for planar studies. Lymphoblast recordings were made using amphotericin B, applied after giga-seal formation, and all other recordings were made by physical rupture of the cell-attached patch

Cell type	R_m (GΩ)	R_s (MΩ)	C_m (pF)	Duration (min)	% success (planar)	n	% success (conven.)	n
Human vascular smooth muscle	1.6 ± 0.13	8.5 ± 0.3	53.7 ± 5.4	30	62.5	72	<10	403
Human synoviocytes	1.1 ± 0.1	7.4 ± 0.3	40.6 ± 2.4	11	70.0	144	8.3	24
Human T-lympoblasts	2.4 ± 0.3	6.9 ± 0.3	3.3 ± 0.2	30	33.3	216	-	-
Human neutrophils	2.4 ± 0.7	7.3 ± 0.9	1.6 ± 0.2	10	35.0	20	-	-
Rat astrocytes	1.3 ± 0.1	7.6 ± 0.6	18.3 ± 2.5	17	68.1	88	55	350
HEK 293 cells (TRPM3)	1.6 ± 0.2	6.0 ± 0.6	8.3 ± 0.9	35	48.9	90	-	-
HEK 293 cells (KCa1.1)	2.0 ± 0.4	6.7 ± 0.5	12.3 ± 1.2	12	44.0	109	60	300
SH-SY5Y cell-line	2.3 ± 0.1	7.7 ± 0.3	4.8 ± 0.2	20	43.0	456	20	120
Jurkat cell-line	1.3 ± 0.2	8.2 ± 0.7	5.3 ± 0.5	30	46.5	116	-	-