Novel screening techniques for ion channel targeting drugs

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Ion channels are integral membrane proteins that regulate the flux of ions across the cell membrane. They are involved in nearly all physiological processes, and malfunction of ion channels has been linked to many diseases. Until recently, high-throughput screening of ion channels was limited to indirect, e.g. fluorescence-based, readout technologies. In the past years, direct label-free biophysical readout technologies by means of electrophysiology have been developed. Planar patch-clamp electrophysiology provides a direct functional label-free readout of ion channel function in medium to high throughput. Further electrophysiology features, including temperature control and higher-throughput instruments, are continually being developed. Electrophysiological screening in a 384-well format has recently become possible. Advances in chip and microfluidic design, as well as in cell preparation and handling, have allowed challenging cell types to be studied by automated patch clamp. Assays measuring action potentials in stem cell-derived cardiomyocytes, relevant for cardiac safety screening, and neuronal cells, as well as a large number of different ion channels, including fast ligand-gated ion channels, have successfully been established by automated patch clamp. Impedance and multi-electrode array measurements are particularly suitable for studying cardiomyocytes and neuronal cells within their physiological network, and to address more complex physiological questions. This article discusses recent advances in electrophysiological technologies available for screening ion channel function and regulation.

Introduction

Each cell is separated from its environment by a lipid membrane. Ion channels embedded in the membrane regulate the flux of ions across the cell membrane. They are involved in nearly all physiological processes, and malfunction of ion channels has been linked to many diseases. Therefore, ion channels have been extensively studied as drug targets, and owing to their potential role in drug discovery they have been termed the “next GPCRs.”\textsuperscript{1} In addition, ion channels are responsible for a number of drug side effects, most prominently cardiac arrhythmia, potentially induced by effects of drugs on cardiac ion channels, in particular the hERG ion channel. The importance of investigating these side effects is emphasized by regulatory guidelines and documents currently in development.\textsuperscript{2} High-throughput screening assays for ion channels have been established in pharmaceutical companies using indirect readout technologies, often with fluorescent assays, e.g., by monitoring the change of the cellular potential with potentiometric dyes, or by fluorescence-labeling known ligands.\textsuperscript{3} These methods provide an indirect measure of ion channel function. In addition, over the past years direct label-free biophysical technologies for screening ion channels by means of electrophysiology have been developed. These enable functional high throughput screening of ion channels and provide insight into function and regulation of ion channels, even within their cellular environment.

The Patch Clamp Technique

The patch clamp technique first described in the 1970s by Neher and Sakmann\textsuperscript{4} remains the gold standard for studying ion channel function. The technique involves bringing a fine glass pipette in close contact with the cell membrane under the control of a skilled operator using a microscope and fine micromanipulator. A tight seal, in the GΩ range, is then formed between the glass of the pipette and the cell membrane. Once a tight seal is formed, the small patch of membrane which plugs the end of the pipette can be ruptured, forming a continuous electrical circuit between the internal solution and electrode contained within the patch clamp pipette, and the inside of the cell, a configuration known as the whole cell patch clamp technique (Fig. 1A). In this way, openings and closings of ion channels can be monitored in terms of electrical current. The ion channel activity which occurs over the whole of the cell membrane is measured in response to external stimuli, typically either voltage or ligand, but also temperature or mechanical stress. The technique
yields data which is extremely information rich but is notoriously low throughput and requires skilled personnel with years of training to perform the experiments. As well as a microscope and micromanipulator, other pieces of equipment to minimise electrical interference and vibration, in the form of a Faraday cage and anti-vibration table, respectively, are also essential parts of a conventional patch clamp rig.

**Automation of the Patch Clamp Technique**

Soon after the introduction of the patch clamp technique, the race to automate the technique began. The goal of automation was to improve ease of use and increase throughput by parallelization. One of the most successful methods for automation involves replacing the patch clamp pipette with a planar glass chip perforated with one (or, recently, a number of) aperture (Fig. 1B). The first successful recordings of cells and bilayers on a planar glass chip were described in 2002.\(^5\) In these initial recordings, quartz glass was used for the glass substrate because of its dielectric properties. Nowadays, in common with the majority of conventional patch clamp experiments, borosilicate glass is used for its low capacitance, and, therefore, low noise. A cell is attracted to the patch clamp aperture of the glass chip by the use of suction from underneath. Following this, a tight seal is formed between the glass chip and the cell membrane in the same way as in the conventional patch clamp technique. Similarly, the cell membrane is ruptured and electrical access gained to the inside of the cell. The planar design and the use of suction negate the need for a microscope, micromanipulator and anti-vibration table, making these devices smaller and more compact. Incorporating the glass chip into a robotic environment provides a means to fully automate and parallelize the experiments to increase throughput.

To date, a number of planar patch clamp systems have been developed, ranging from small devices recording from a single cell at a time, e.g. the Port-a-Patch (Nanion Technologies), through medium throughput devices, e.g., the Patchliner (Nanion Technologies), PatchXpress (Molecular Devices) and QPatch (Sophion A/S), up to high throughput devices such as the SyncroPatch 96 (Nanion Technologies), IonFlux (Fluxion), Qube (Sophion A/S), IonWorks Barracuda (Molecular Devices) and, most recently, the SyncroPatch 384PE (Nanion Technologies). These devices differ in their flexibility, diversity of experimental protocols, quality of data and throughput. Table 1 provides a comparison of available automated patch clamp systems. The first part of this review focuses on recent advances on the devices Port-a-Patch, Patchliner and SyncroPatch 384PE from Nanion Technologies. For a more comprehensive review of available systems, please see Dunlop et al.\(^6\) and Bebarova.\(^7\)

In the case of semi-automated devices such as the Port-a-Patch, operation offers a similar experimental flexibility to conventional patch clamp, with the added advantage of easy exchange of the internal solution\(^8\) so that actions of compounds on the inside of the cell membrane can be investigated with relative ease. The Port-a-Patch is used in academia and industry alike for basic ion channel research and drug discovery. Cell lines such as HEK and CHO cells are used with equal success rates and experiments can be performed at room or physiological temperature.\(^8\) Additionally, primary cells such as human corneal epithelial cells,\(^9\) stem cell-derived cardiomyocytes\(^10\) and lysosomes\(^11\)
have been used successfully. As the data recorded is low noise and high resolution, single channel recordings in the cell attached mode on the cell membrane, and experiments recording ion channels reconstituted into lipid bilayers, are also routinely performed using the Port-a-Patch. Recently, the flexibility of the Port-a-Patch has been further emphasized by its application to measuring the translocation of antibiotic drugs through single pores reconstituted into bilayers, thereby paving the way to studying one mechanism of bacterial antibiotic resistance.

### Flexibility and Throughput

In order to increase throughput, robotic systems have been introduced which record from multiple cells in parallel. This can be done using a chip with a single hole per well such as the Patchliner, SyncroPatch 96, SyncroPatch 384PE, PatchXpress, and QPatch, or multiple holes per well as in the IonWorks Quattro and IonWorks Barracuda. The Patchliner is a fully automated planar patch clamp robotic system recording from up to 8 cells simultaneously. Its medium throughput properties are complemented with useful features such as internal solution exchange, temperature control and current clamp. The chip design is such that micro-fluidic channels are formed. The exchange time is fast (< 10 ms) and compound volume requirements are low (< 25 μl per well to completely exchange the solution). Additionally, solutions can be stacked inside the pipette of the Patchliner to minimise compound exposure time. In this way, challenging ligand-gated ion channel targets such as the nicotinic acetylcholine α7 receptor (nAChR) can be reliably and reproducibly recorded on the Patchliner and the Patchliner to minimise compound exposure time. In this way, challenging ligand-gated ion channel targets such as the nicotinic acetylcholine α7 receptor (nAChR) can be reliably and reproducibly recorded on the Patchliner (Fig. 1E), and on the QPatch. The temperature control feature on instruments such as the Patchliner and Ionflux can be used to record ion channel activity at physiological temperature, and on the Patchliner, fast changes in temperature can be used to activate temperature regulated ion channels such as TRPV1 and TRPV3 (Fig. 1F). A number of cell lines including HEK293, CHO, LTK, RBL, SHSY5Y, GH4C1, ND7–23 and Jurkat are routinely used on the Patchliner with equally high success rates. In addition, currents from primary cells such as rat cortical astrocytes, human fibroblast-like synoviocytes, red blood cells and vascular smooth muscle cells have been successfully recorded.

Cardiomyocytes derived from pluripotent stem cells (iPSCs) are receiving increasing attention as a potential model for safety screening and toxicology for early screening of lead compounds. Recently the Food and Drug Administration (FDA) and the Health and Environmental Sciences Institute (HESI) started a new initiative to evaluate the potential for integrated non-clinical cardiac ion channel activity assessment to predict the clinical pro-arrhythmic risk of drugs. Included in this is the evaluation of the use of pluripotent stem cell applications for cardiovascular risk assessment. So far, the Patchliner has been used successfully to record from mouse embryonic stem cells (CorArt, AxioGenesis), human iPSCs from CDI, Axiogenesis (Cor.4U), and Cellectis. Importantly, individual ion channel currents relevant for safety testing such as Nav1.5 can be recorded in the voltage clamp mode, and the effect of compounds on the action potential in the current clamp mode can be measured. Figure 2A shows the current-voltage plot of an average of 4 iPSC cardiac cells (Cellectis) and the current responses to increasing voltage in the voltage clamp mode of an exemplar cell recorded on the Patchliner. The currents recorded are characteristic of a voltage-gated Na+ channel. Additionally, Figure 2B shows block of the current in the voltage clamp mode by tetcaine and the effect of tetcaine on the action potentials elicited in the same cell. Assays for cardiac safety evaluations are also developed on other automated patch clamp devices, and are increasingly combined with additional assays improving an integrated cardiac risk assessment.

Stem cell-derived neurons could provide important models for diseases such as Alzheimer. Recently, iPSC neurons (CDI) have been used successfully in both conventional electrophysiology and on the Patchliner. In these cells, a voltage-gated Na+ channel, K+ channel and the ligand-gated ion channel, the GABA<sub>A</sub> receptor, could be recorded. Additionally, action potentials could be generated in some cells.
Table 1. Comparison of automated patch clamp devices available on the market. Information contained within the table was collected from relevant company websites: www.nanion.de; www.moleculardevices.com; www.sophion.com and www.fluxionbio.com. * 16 amplifier channels and a multiplexer are used. ** 10 ms for Fast Perfusion Kit for the Port-a-Patch and 100 ms for standard External Perfusion System. *** rough estimates from the manufacturers and vendors, depending on cells, protocols etc.

| Instrument | Port-a-Patch | Patchliner | PatchXpress | QPatch | SyncroPatch 96 | IonFlux | Qube | IonWorks | SyncroPatch 384PE |
|------------|--------------|------------|-------------|--------|---------------|--------|-----|----------|------------------|
| Company    | Nanion       | Nanion     | MDS         | Nanion | Nanion        | Fluoix | MDS | Nanion   | Nanion           |
| Recording substrate | Glass, single hole or multiple holes per well | Glass, single hole or multiple holes per well | Glass, single hole or multiple holes per well | Glass, single hole or multiple holes per well | Glass, single hole or multiple holes per well | PDMS, Single hole or 20 holes per well | Polymer, single hole or 10 holes per well | Polymer, single hole or population patch with 64 holes per well | Glass, single hole or multiple holes per well |
| Recording configurations | Whole cell, cell attached, perforated patch, bilayer recordings | Whole cell, cell attached, perforated patch, bilayer recordings | Whole cell | Whole cell, perforated patch | Whole cell, perforated patch | Whole cell | Whole cell | Perforated patch (loose patch) | Whole cell, perforated patch |
| No. parallel recordings | 1 | 4 or 8 | 16 | 8, 16 or 48 | 96* | 16 or 64 | 384 | 384 | 384/768 |
| Throughput*** | 50 data points/day | 250-500 data points/day | 500 compounds per 8 hour day | 250–3000 data points/day | 6000 data points/day | 2500–4000 data points/day | 30,000 compounds per 24 hours | 1100–6000 data points/hour | 20,000–38,000 data points/day |
| Seal resistance G | GΩ | GΩ | GΩ | GΩ | GΩ | GΩ | GΩ | GΩ | GΩ |
| Compatible cells | Cell lines, primary cells, stem cells | Cell lines, primary cells, stem cells | Cell lines, stem cells | Cell lines, stem cells | Cell lines, primary cells | Cell lines, stem cells | Cell lines | Cell lines, stem cells | Cell lines, stem cells |
| Temperature control | Optional | Optional | No | Optional | No | Yes (up to 40°C) | Not known | No | Optional |
| Current clamp | Yes | Yes | Yes | No | No | No | Yes | Yes | Yes |
| Number of pipettes | N/A | 1 | 16 | 2, 4 or 8 | 16 | N/A | 384 | 384 | 384 |
| External solution exchange time | 10 - 100 ms** | 10 ms | 10 - 15 ms | 10 ms | 100 ms | 100 ms | 100 ms | 40 ms (single hole per well) to 80 ms (Population patch) | 50 ms |
| Internal solution exchange | Yes | Yes | No | No | Yes | No | No | No | Yes |
High throughput and High Fidelity

The further development of automated patch clamp devices is ongoing. For high-throughput screening efforts, indirect read-out technologies such as fluorescence assays are still used, and combined with electrophysiological patch clamp compound characterization at later stages of compound development.29,30 The demand for increasing throughput in electrophysiological screening, while maintaining high resolution data acquisition, however, calls for the introduction of new patch clamp devices with higher throughput. The SyncroPatch 384PE is an example of such a device. The chip of the SyncroPatch 384PE is a 384 well micro-titer-plate format with a borosilicate glass bottom. In each well is a single patch clamp aperture. A 384 channel amplifier guarantees that all cells are continuously voltage clamped during the recordings and this, coupled with a 384-pipettor head, ensures that all 384 cells are recorded truly in parallel. This results in an immense increase in throughput, in the order of 20,000 data points per day. Ease of use and powerful data handling software are paramount to the success of such a high throughput device. The data acquisition and analysis software for the SyncroPatch 384PE provides a color-coded, user-friendly interface where the user gains an impression of the success of the experiment at a glance. A screenshot of the software during an experiment recording simultaneously 6 different cardiac channels in 6 separate cells lines is shown in Figure 3. In this experiment, CHO cells expressing either CaV1.2, NaV1.5, Kv4.3 or Kv7.1, or HEK cells expressing hERG or Kir2.1 (all cell lines provided by ChanTest, a Charles River Company) were captured to the patch clamp chip. Four columns of the patch clamp chip received the same cell line giving n = 64 for each cell line. A specially designed voltage protocol (shown at the top of Fig. 3) was used to activate all voltage-gated currents simultaneously.

Impedance Measurements and Multi-Electrode Arrays

As noted above, the patch clamp technique is widely used to measure ion channel

Figure 2. (A) Current-voltage plot of an average of 4 iPS cardiac cells (Cellectis) recorded on one run of the Patchliner. The currents were normalized to the maximum peak amplitude. The inset shows raw traces from an exemplar cell in response to voltage steps from −80 mV to 40 mV. Currents started to activate about −50 mV and peak response was elicited at around −20 mV. Bi Current responses of an iPS cardiac cell to a voltage protocol to −20 mV from a holding potential of −120 mV in control conditions and in the presence of 100 μM tetracaine. Tetracaine almost completely blocked the current response at this concentration, an effect that was completely reversible upon washout (trace not shown). Bii Action potentials were elicited in the current clamp mode using a 1 ms depolarizing current pulse. A holding current of −185 pA was used to maintain a baseline voltage of −96 mV. The action potential in control conditions is shown in black. The sodium channel blocker, tetracaine, at 100 μM was applied and this inhibited the action potentials (blue). The effect could be reversed upon washout (gray). (C) Current-voltage relationship of an average of 54 iPS neurons (CDI) recorded on the Patchliner. The inset shows raw traces from an exemplary cell showing NaV and Kv currents present in the cell. Fast, transient inward NaV currents started to activate at about −40 mV, and peak currents were elicited at −10 mV or −20 mV. (D) Activation of currents in iPS neurons (CDI) by the ligand GABA (30 μM). GABA was applied for approximately 600 ms before washout with external solution using a stacked solutions approach. Cells were held at a constant holding potential of −70 mV. (E) Action potential elicited from an iPS neuron (CDI) recorded on the Patchliner. Action potentials were elicited using a 2 ms depolarizing pulse. Panels (C-E) reproduced with permission from Haythornthwaite et al.28 © SAGE. Reproduced by permission of Michelle Binur. Permission to reuse must be obtained from the rightsholder.
Figure 3. Screenshot of PatchControl 384, the software for the SyncroPatch 384PE, during a recording of 6 cardiac ion channels expressed in different cell lines. Four columns of the patch clamp chip (64 wells) received one cell line expressing either hERG (HEK), K\textsubscript{v}4.3 (CHO), K\textsubscript{v}7.1 (CHO), Kir2.1 (HEK), Na\textsubscript{v}1.5 (CHO) or Ca\textsubscript{v}1.2 (CHO). All cell lines were provided by ChanTest (a Charles River Company). A voltage protocol was designed to activate all channels simultaneously, shown at the top of the figure. The first step to 0 mV (1) was used to activate Na\textsubscript{v}1.5 and Ca\textsubscript{v}1.2, the second part of the protocol to 60 mV followed by a step to −40 mV (2) was used to activate K\textsubscript{v}4.3, hERG and K\textsubscript{v}7.1 and the final ramp (3) was used to activate Kir2.1. The columns are scaled individually to expand the relevant current and an example is shown at the top of each set of columns.
function in different cell preparations. When automated patch clamp systems are used, this is typically performed on individual cells that have been isolated. To address the next, more complex, hierarchical level of tissue organization, electrophysiological impedance measurements and multi-electrode arrays have proven particularly useful. These techniques allow cells to be studied within their physiological network of cells.

The xCELLigence RTCA Cardio-96 (ACEA Biosciences and Roche Applied Sciences) and the CardioExcyte 96 (Nanion Technologies) are commercially available systems that have been applied successfully to cardiomyocytes and stem cell-derived cardiomyocytes. Measurements are performed by non-invasive read-out of impedance and extracellular field potentials (EFP) for continuously monitoring beating myocytes that are placed in specifically designed wells including the measurement microelectrodes (Fig. 4A). Both systems operate using a 96-well format. A low alternating voltage signal (less than 20 mV) can be applied to the microelectrodes at the bottom of each well, to generate an oscillating electric field. The substantial mechanical modulation of cell morphology and adhesion that accompanies cardiomyocyte contraction is then monitored in the impedance signal, which in turn provides a dynamic and continuous readout of cardiac cell function. The EFP signal is passively recorded by differential voltage recordings (one recording, one reference electrode; Fig. 4A). The systems can be placed in a tissue culture incubator to provide the cells with an atmosphere that facilitates long-term (several days) of measurement time to study both short- and long-term effects of compounds on cardiomyocyte function. The CardioExcyte 96 can be alternatively used in combination with a climate chamber which controls temperature, humidity and gas mix similar to that of an incubator. The xCELLigence System acquires data at 12.9 ms,31 while the CardioExcyte 96 allows sampling down to 1 ms data acquisition for the entire 96-well plate.32 Both systems are capable of cardiac pacing in the impedance mode, controlling the beat rate of the cardiomyocytes by applying electrical stimuli.

With directly providing readout for the mechanical beating of cardiomyocytes, the label-free impedance technology has the potential to bridge the gap between direct cardiac ion channel patch clamp measurements on the one hand, and animal models on the other. This is especially relevant for cardiac safety assessments that are an important part of the drug discovery and development process. Impedance measurements have been validated for mouse embryonic stem cell-derived cardiomyocytes,32 human iPS cardiomyocytes,32 rat neonatal primary cardiomyocytes31,33 and further cell lines, e.g. cardiac muscle cells (HL-1) and 3D cell clusters (hES-CMCTM).32 It has been recognized that, while the interpretation of impedance data from cardiac cells is complex, these have the potential to provide relevant information on compound action, particularly for compounds that have more than one isolated ion channel effect. The schematic setup of impedance measurement electrodes in the CardioExcyte 96 is displayed in Figure 4A. Figure 4B shows an EFP trace and the corresponding impedance trace representing the contraction. Figure 4C shows a screenshot of the data acquisition software for the

![Figure 4](image-url)
CardioExcyte 96 during an experiment. Shown is the impedance view after application of 6 different compounds (plus control).

Interestingly, impedance measurements have been shown to be applicable to cells beyond cardiomyocytes. Recently, the technique has been applied to set up an assay for determining virucidal activity of a chemical disinfectant, negating the necessity for subjective visual interpretation of light microscopy images of established protocols. Also, proliferation of PC12 cells has been monitored by impedance technology to establish an assay profiled with respect to their differential regulation by the proliferation and motility of the cells in real time. Adding to the induced apoptosis in PC12 cells is assessed by monitoring the growing numbers of assays set up with impedance measurements.

Also, proliferation of PC12 cells has been shown to be applicable to cells beyond cardiomyocytes. Recently, the tech-



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