EXPERT OPINION

HTS techniques for patch clamp-based ion channel screening – advances and economy

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Introduction: Ten years ago, the first publication appeared showing patch clamp recordings performed on a planar glass chip instead of using a conventional patch clamp pipette. "Going planar" proved to revolutionize ion channel drug screening as we know it, by allowing high quality measurements of ion channels and their effectors at a higher throughput and at the same time de-skilling the highly laborious technique. Over the years, platforms evolved in response to user requirements regarding experimental features, data handling plus storage, and suitable target diversity.

Areas covered: This article gives a snapshot image of patch clamp-based ion channel screening with focus on platforms developed to meet requirements of high-throughput screening environments. The commercially available platforms are described, along with their benefits and drawbacks in ion channel drug screening.

Expert opinion: Automated patch clamp (APC) platforms allow faster investigation of a larger number of ion channel active compounds or cell clones than previously possible. Since patch clamp is the only method allowing direct, real-time measurements of ion channel activity, APC holds the promise of picking up high quality leads, where they otherwise would have been overseen using indirect methods. In addition, drug candidate safety profiling can be performed earlier in the drug discovery process, avoiding late-phase compound withdrawal due to safety liability issues, which is highly costly and inefficient.

Keywords: action potential, automated patch clamp, cell lines, current clamp, drug screening, high-throughput screening, ion channel, primary cells, stem cells, temperature control

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1. Introduction

Ion channels are transmembrane protein pores, present in all cells throughout the human body. They serve as means for intra- and intercellular communication, and are essential for every movement, thought, or sensation. Because of their pivotal role, ion channel malfunction underlie many chronic and acute disorders [1]. There are different classes of ion channels, defined by what stimuli is required to evoke a response. These are changes in membrane voltage (voltage-gated), by chemical modification (ligand-gated), or by mechanic force (mechano-sensitive) [2]. In addition, there are ion channels activated by hot or cold stimuli [3]. Ion channels are fairly easily modulated by small molecules, which make them very interesting as drug targets. An often cited reference states that 13 – 15% [4,5] of the best selling drugs today target ion channels, which reflect their importance for good health as well as their broad involvement in neuropathological states, pain, diabetes, hypertension, etc. Most of these medications were discovered by serendipity, and not due to brute force screening efforts, since direct measurements of ion channel

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Article highlights.

This article covers aspects of ion channel drug screening including the following themes:

- Automated patch clamp instrumentation has, during the past decade, become pivotal for different phases of drug discovery, since it offers unprecedented data quality and direct, real-time measurements of ion channels, and their effectors. Further development of existing and new platforms strives at providing data throughput capabilities compatible with the requirements of ion channel drug screening.
- Several factors affect the data output, not only how many recordings are done in parallel. For example, parameters such as seal quality, liquid handling capabilities, recording longevity, cycle-times, built-in redundancy, automated cell storage and preparation, can affect the throughput very positively or negatively.
- Level of automation of existing APC platforms differ. Some platforms allow many operating hours without user-intervention, and others require attention on hourly basis.
- Throughput vs. data quality – the compromise is not needed. There are platforms supporting high quality data and higher throughput.
- Some applications, for instance, the use of stem cell-derived cardiomyocytes or primary cells, are more demanding for successful recordings, and also require more sophisticated experimental features such as temperature control, current clamp recordings or perfusion capabilities. The versatility of existing APC platforms differ greatly.

This box summarizes key points contained in the article.

activity in a high-throughput screening environment still is a challenge. A handful of companies, including us, work hard to overcome this hurdle, making high fidelity ion channel drug screening compatible with the requirement of industrial HTS environments and rational drug development.

There are several reviews giving excellent historical background of ion channel screening [6-9] and the advent of the planar patch clamp technique [10]. In brief, the workhorses of the past and the present are indirect methods, for example, affinity binding (cell-free method), fluorescence-based cellular assays (Ca^{2+} chelators or voltage-sensitive dyes), or cellular efflux assays (Rb^{+}) [11]. The major benefits of named methods are their HTS compatibility and a low cost-per-data point.

Drawbacks are the low fidelity, low sensitivity, and low resolution obtained using the above-mentioned techniques. In case of cell-based assays, recordings are made from a large population of cells, lacking voltage control over the cellular membrane, and with slow response times. The risk of missing out on a high quality lead is large, since mentioned methods are prone to false-negatives, due to the low sensitivity and temporal resolution. In addition, ion channels are diverse and offer different challenges in screening, where some are less difficult than others. Here, the versatility of the platform is equally important to accommodate the different ion channel requirements.

The only technique capable to monitor the actions of ion channel active compounds in real-time, with ultra-high resolution (µs, sub-pico-amperes), is the patch clamp technique. Patch clamp also goes under the term "*electrophysiology*," referring to methods able to record ionic currents passing over the membrane throughout all living organisms, even in tiny bacteria. For measurements of ionic currents in single cells, patch clamp is often denoted as the gold-standard, since it is the only technique that is able to resolve the activity and behavior of single ion channels and their minimal currents. In 2011, the patch clamp technique celebrated 30 years "giga-sealing," where "giga" refers to the highly resistive interaction, ideally > 1 giga-ohm, between the glass micro-electrode and the cellular membrane. The giga-seal proved to be essential for recordings of highly resolved currents passing over the ion channels residing in the cellular membrane [12]. The technique described by Hamill and co-authors was not straight-forward or even remotely suitable for screening purposes. Instead of 100,000+ compound screens per day, traditional patch clamping allows the profiling of 10+ compounds per week. The method is primarily employed in research laboratories, and used in end-phases of target validation and for final compound evaluation. This does not mean that the patch clamp method as such is obsolete, but due to its serious throughput restrictions, it is simply not possible to employ conventional patch clamping in most phases of ion channel drug screening.

Patch clamp is extremely laborious and work intensive, and a craft that requires years to master. Still, no other technique offers such detailed information about the ion channel, and its response to applied compound(s), in terms of function, kinetics, gating, pharmacology, and desensitization. Ten years ago, the first publication on microchip-based patch clamp appeared, describing the use of a planar, perforated glass substrate for high quality patch clamp recordings [13]. The planar glass chip replaced the traditionally used, manually manipulated, glass microelectrode for patch clamp recordings.

The use of a planar substrate proved to be a viable and successful strategy for automation of the patch clamp technique procedure (automated patch clamp, APC), proven by the fact that the majority of the commercially available platforms use this approach, although with varying materials used for the recording substrate. What all platforms have in common is that they require single-use disposables for recordings and use suction to place cells on top of micron-sized apertures. Some systems utilize pore-forming agents to gain electrical access to the cellular membrane, and others apply further suction to rupture the membrane patch covering the aperture. Some platforms rely on the population patch clamp (PPC) method [14], aiming at reducing cell-specific current variability. Here, recording wells contain *multiple* apertures, to record from a small population of cells. Either way, a pre-programmed protocol takes care of everything from priming the chip for recordings, to additions of cell-suspension,

sealing a cell to the substrate using further suction, and eventually ending up with cells ready for recordings.

The planar approach has two major benefits: it allows for automation and parallelization. Automation takes most of the pain out of patch clamping and drastically reduces the manual interactions typically required in conventional patch clamping. In this way, the start-up time is minimized for successful ion channel recordings. Still, you will not find the gold nuggets in the 100,000+ compound libraries with poorly performing cells, that is, low success rates for completed recordings, or faulty protocols for recordings or analysis. Most APC systems require more from the user than merely pressing the "Start" button. However, while having valid protocols and cell preparation procedures, compound testing is fairly straightforward and easy. Thus, a team of a few electrophysiologists and technicians can operate an entire machine park.

Parallelization allows using an array of apertures, that is, up-scaling the number of simultaneous recordings and thus increasing the data output obtained from the platform. It increases the efficiency of the platform (data points per day) and employees (data points per day and person). The existing staff can achieve more in less time, in terms of processed compounds per time unit. Everyone (or at least the senior manager) working within the pharmaceutical industry knows that the cost of the drug discovery process can be quantified by the hour. To put it short – time is money when going from target identification to the last clinical trial. Every day counts for getting a medication on the market as fast as possible, especially when the patent for the drug candidate has been filed.

There has been a paradigm shift in that sense that automated patch clamp platforms are nowadays commonplace commodities and, in most cases, part of the standard ion channel drug screening machine park. No one questions the validity or the usefulness of the platforms and methods as such. The different platforms, offered by a handful of providers, are fairly mature and quite successful at fulfilling requirements of *most* phases of drug discovery and ion channel-related research.

2. Automated patch clamp platforms in brief

The PatchXpress was the first automated patch clamp platform on the market, launched in 2003 by Axon Instruments, Inc. (later acquired by Molecular Devices Corp. (MDC, now MD)) [15]. The PatchXpress utilizes glass substrates for parallel recordings from 16 cells, supporting giga-seals and multiple solution additions. MDC has two further automated patch clamp platforms, the IonWorks Quattro [16] and the IonWorks Barracuda [17]. These platforms utilize plastic recording substrates and the population patch clamp technique, that is, multiple holes per recording well, and in the case of the IonWorks, this is 64 cells per recording well. The IonWorks product family does not support giga-seal recordings; rather, it relies on mega-ohm seals, which has proven sufficient for various assays. The IonWorks Quattro records from 48 recording wells at a time, whereas the IonWorks Barracuda allows recordings from 384 wells at a time. The IonWorks Barracuda supports recordings during compound addition, but does not allow wash out of for instance ligands, which reduces the efficiency for investigating ligand-gated ion channels, since only one concentration per recording well can be obtained.

Nanion Technologies launched the Port-a-Patch [18] in 2003, a miniaturized patch clamp rig recording from one cell at a time. The Port-a-Path makes patch clamp accessible to non-experts, and is greatly appreciated in academia because of its great versatility and user-friendliness. The Patchliner followed in 2006, recording from eight cells in parallel, with versatile experimental features such as temperature control, heatable pipette, current clamp recordings, internal solution exchange, fast solution switch times, high success rates with primary cells, and stem cell-derived cardiomyocytes and neurons [19,20]. In 2010, the SyncroPatch 96 was introduced, a screening platform recording from 96 cells in parallel. As with all Nanion's platforms, the SyncroPatch 96 supports giga-seals, and uses a glass substrate for the patch clamp recordings. It has been validated with a wide variety of cells, including stem cell-derived cardiomyocytes and neurons, as well as voltage- and ligand-gated channels, including advanced targets such as nicotinic and purinergic channels (nAChR α7, P2X3, etc.) [21]. The SyncroPatch 96 has a fully open design that allows for integration in robotic avenues and supports a data throughput of 6000 data points per day.

The QPatch [22], developed by Sophion Bioscience, was launched in 2004, and has had a successful market entry worldwide. It records from 16 cells at a time, using silicon oxide-coated silicon nitride structures for high quality giga-seal recordings. The QPatch recording cartridge utilizes microfluidics for rapid solution exchange, and the platform was the first system to encompass a "cell hotel" on-board for cell-plate storage and automated cell preparations. Sophion claims 10 h of unattended operation, thanks to compound plate storage and the automated cell preparation facilities hosted by the QPatch platforms. Later to follow was the QPatchHT [22], recording from 48 cells at a time, and the QPatch HTX [23] employing the population patch clamp approach. The QPatch is a much appreciated platform useful for ligand- and voltage-gated ion channels. The QPatch HTX supports a data throughput of 7000 data points per day.

Two more recent platforms, which are using a similar microfluidic-based approach, are the Dynaflow HT [24] platform from Cellectricon and the IonFlux [25] from Fluxion Biosciences. These platforms utilize a silicone rubber-based (PDMS) microfluidic structure with micro-channels used for sealing the cells, although in different fashions. The platforms do not support high quality recordings, since the seals are in the mega-ohm range. Both systems utilize sophisticated microfluidic structures for fast and brief compound application.

The Dynaflow HT records from 96 cells at a time. However, the cells are divided into "six-packs," where the individual six

cells are exposed to the same solutions, that is, the system has a built-in redundancy. The Dynaflow HT hosts a cell hotel on-board, and claims to require very small amounts of cells for completed recordings, which could make it suitable for primary cell recordings, since these often come in small numbers.

The Fluxion's IonFlux is the first "mix-and-read"-type automated patch clamp system on the market. It has a small footprint, and can be integrated in a robotic environment. The IonFlux comes in two versions, IonFlux 16 and IonFlux HT. The IonFlux HT has 64 individual recording sites per substrate, with a built-in redundancy of two recording sites per solution set, and claims to achieve 10,000 data points per day. The IonFlux instruments employ the Population Patch Clamp principle and average over 20 different cells per recording site. At the end of 2011, Fluxion announced a new version of the recording cartridge containing one single aperture per recording zone, compared with 20 in their standard chips, for single cell, giga-seal recordings. At this moment, limited data are available on sealing success rates, compatibility with cell assays and obtainable throughput, etc., and so, this platform has therefore been left out in the comparisons.

In terms of throughput, the platforms most suited for screening purposes based on claimed throughput are (in alphabetical order): Dynaflow HT, IonFlux HT, IonWorks Barracuda, SyncroPatch 96, and the QPatch HTX.

3. Ion channel screening considerations

It is important to remember that there are more considerations to take into account when screening a large number of compounds, than only throughput capabilities. However, starting with throughput in a primary screen, the platform has to offer the ability to process 100,000 – 1,000,000 compounds in a short time (weeks). This in turn requires the infrastructure for liquid-, cell-, recording substrate-, compound-, and waste-handling. If the APC platforms can be integrated into robotic environments, robots would serve the patch clamp machine with solutions, cells and compounds, just as with the low-fidelity screen workhorses, and thus allowing extended work hours (for the machine).

Several of the APC providers claim a data throughput of 10,000 data points per day. According to users, this is enough for focused screens of 10,000+ compound libraries. This approach is often used in combination with either primary screens using in-direct methods or in-silico estimations of the structure–activity relationship of a given compound. When considering APC platforms for primary screens, there are several important considerations to take into account. First, is it worth the effort? Is it plausible that a primary wet screen would pick up high quality leads that would have been missed by a primary screen using in-direct methods, or the computational approach followed by the focused library APC screen? If yes, is the throughput offered by current APC systems enough for a primary screen? The first impulse is "No." 10,000 data points per day and three machines give 30,000 data points per day. Operated 4 days a week, saving 1 day for maintenance, and a screen redundancy of 30%, gives 84,000 data points per week. Screening a 1 million compound library would then take about 12 weeks. How much consumables would be required for such a screen? That depends how picky the screeners are. Here, electrophysiologists are much more difficult to deal with, when it comes to adding multiple compounds to a cell. Multiple compound additions together with a reasonable use of control compounds and preset success criteria, and a de-convoluting approach, including some extent of redundancy in the screen, would allow the use of current platforms in primary screens. An estimate of the consumable cost of a primary APC screen of about a million compounds would roughly correspond to $ 0.5 million USD. Considering the question whether this screen would have the potential to pick up drug candidates that otherwise would have been missed using traditional screening methods, we return to the question: is it worth it? The future will prove to what extent primary screens will be conducted using automated patch clamp platforms, or if they will be used for this purpose at all. The technology as such is here to stay, and is essential for secondary screening, lead optimization, and cardiac safety testing.

4. APC platforms – how automatic does it get? How fast is automatic?

Automation should mean a reduction in manual labor and an efficiency increase in the daily work. However, how automatic are automated patch clamp devices? Some platforms host a "cell hotel," where the cells are freshly prepared for each run (DynaflowHT/QPatch). The providers claim up to 10-h walk-away-time, provided that solutions and recording substrates are at hand (QPatch). Other platforms require new cells and recording plates after each (IonFlux) or a couple of runs (IonWorks Barracuda, SyncroPatch 96), where some platforms allows full integration into robotic environments, where they are served by robots feeding them with cells, compound plates, and recording substrates (IonFluxHT, SyncroPatch 96). This means that the requirement for human interaction varies quite a bit between the platforms, and can become a limiting factor for the successful HTS screen.

Parallelization should mean an increased throughput due to massively parallel recording wells. Of course, the numbers of parallel recordings are pivotal for the throughput, but it is not the only factor. For example, does the recording substrate have a built-in redundancy, only allowing one experiment for a set number of cells (Dynaflow HT, IonFlux HT)? Are multiple additions possible to the individual recording wells? Are wash-out steps between compound applications allowed (Dynaflow HT, IonFlux HT, SyncroPatch 96, QPatch)? All these factors can affect the obtainable throughput very

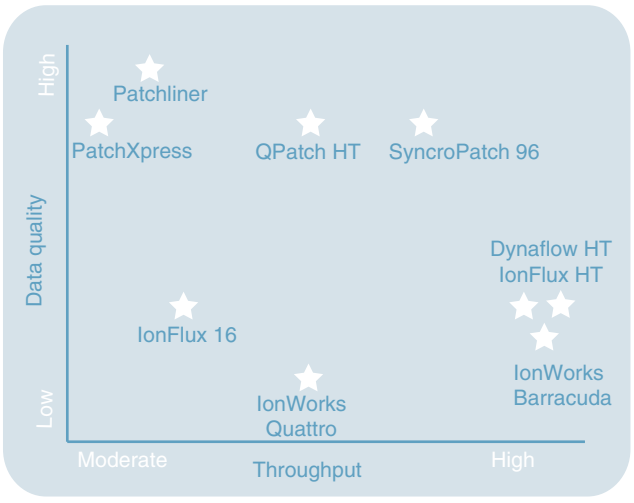

Figure 1. The graphic image illustrates how data throughput relates to data quality for the respective automated patch clamp platform. Several aspects are taken into account regarding "Data quality" including seal resistance, voltage-control over the cellular membrane, solution switch times, and the experimental diversity offered by the platform.

negatively or positively. Other important factors affecting the throughput are:

Cycle times – what cycle times are required between recordings? Cycle time includes everything from discarding the old recording chip, flushing the system if needed, getting a new recording chip, priming it with solution, adding, and sealing the cells. Here, there is a huge difference between platforms. Some platforms require 10 min (SyncroPatch 96) to be ready with sealed cells waiting for compound, whereas others need more than 30 min to start the actual screen.

Level of automation – as already mentioned – the level of platform independency determines how occupied the employees are with adding cells, compounds, solutions, etc. Integration in robotic environments maximizes efficiency and throughput, since the machine can be used for night shifts as well (IonFlux HT, SyncroPatch 96).

5. Throughput does not have to compromise the data quality

There are several important differences between the platforms on the market in terms of what data quality they offer. The "HTS"-platforms are summarized in **Tables 1**,**2**, and in **Figure 1**, illustrating recording throughput vs. quality. What is then considered high quality? Most agree that the tight interaction between the recording substrate and the cell, the so-called giga-seal, is important, since it drastically improves the resolution of the recordings, minimizes baseline drift, and enables accurate recordings without voltage errors of small currents even from individual cells (given that the noise levels of the used platform allows it) (Patchliner, SyncroPatch 96, QPatch). Another aspect of quality is the longevity of the recording, since this, together with fluidics, determines if the complete compound pharmacology can be extracted from the individual cells. Because of cell-to-cell variation, it is preferable to record a full dose response curve from one cell, as opposed to using different cells in the same curve (Dynaflow HT, IonFluxHT, Patchliner, SyncroPatch 96, QPatch). Population patch clamp, that is using multiple apertures in one recording chamber, has its benefits when the current expression among cells is low or inhomogeneous, since it evens out and amplifies the response (DynflowHT, IonFluxHT, IonWorks Barracuda, QPatch HTX). One negative aspect of PPC is that some apertures might not get a cell or a seal, or change in access resistance that results in drifting baselines. Although, that most platforms have the option to remove bad recordings before generating the average current, this approach may still not be ideal for ion channels requiring an excellent and stable voltage-clamp of the membrane (for example, Nav1.2, Nav1.7, etc.) or ion channels with current responses resembling the non-specific currents leaking between the membrane and the patch clamp substrate, often referred to as "leak currents," such as the transient receptor potential channels (TRPs). Putting it shortly, the highest recording quality is obtained while using a platform supporting giga-seals, single cell recordings, with fluidics sophisticated enough to achieve full dose response curves from the individual cells, and low-noise electronic components of the platform (PatchXpress, Patchliner, SyncroPatch 96, QPatch).

There are, however, other considerations and requirements on platform capabilities affecting quality, for example, experimental versatility. There is a growing interest in the use of primary cell and, more recently, stem cell-derived cardiomyocytes and neurons for safety testing and screening purposes. Primary cells and stem cell-derived cells represent a more relevant expression system for the ion channels, since the ion channel reside in an authentic cellular environment. However, these cells require a high recording sensitivity of the platform, since the ion channel density is smaller in these cells compared with over-expressed cell lines normally used by the pharmaceutical industry for screening and compound safety testing. Another factor that can be limiting when working with primary cells or stem cell derivates is the low cell count, and thus low density and suspension volumes. Several platforms can, however, accommodate low volumes and low cell densities. For example, the Dynaflow HT requires only microliter volumes of cell suspension per recording well, the Patchliner adds 10 – 20 microliters of cell suspension per recording well, and the Patchliner "cell hotel" (which does not include automated cell preparation) can accommodate cell suspension volumes down to 150 – 200 microliters.

Enabling the use of other cells for screening than cell lines confer the following requirements on the APC platforms:

Table 1. Technical comparisons of the current ion channel screening platforms 2012.

Feature	Dynaflow HT	IonFlux HT	IonWorks Barracuda	SyncroPatch 96	QPatch HT/ HTX
Company	Cellectricon	Fluxion Bioscience	MDC/MDS	Nanion	Sophion
Throughput/day	10.000	10.000	10.000	6.000	3.000/ 7.000
Success rates	60%	Not stated	50 – 85%	60 – 90%	50 – 80%
Substrate material	Silicone rubber	Silicone rubber	Plastic	Glass	Silicon oxide/ Silicon nitride
Seal resistance	50 – 100 MΩ	50 – 100 MΩ	100 MΩ	> 1 GΩ	> 1 GΩ
Access resistance	Not known	> 10 MΩ	10 – 15 MΩ	2 – 10 MΩ	< 10 MΩ
Parallel recordings	96	64	384	96	48
Unique recording sites/chip	16	32	384	96	48
Amplifier channels	96	64	384	16	48
Number of pipettes	16	n.a.	384	16	8
Solution switch time	30 ms (10 – 90%)	50 ms (0 – 90%)	Not stated	< 50 ms (0 – 100%)	80 ms
Compound wash out?	Yes	Yes	No	Yes	Yes
Unlimited compound additions	Yes	Yes	No	Yes	No
Internal perfusion	No	No	No	Yes	No
Rs, Cslow compensation?	Yes	Yes	No	Yes	Yes
Single cell recordings	Yes	No	No	Yes	Yes
PPC recordings	No	Yes	Yes	Yes	Yes

Cell compatibility – Can seals and stable electrical access be obtained from primary cells, and stem-cell derivates (Patchliner, SyncroPatch 96)? Here, there are quite some differences between the platforms considering recording success rates and cell type used.

Sensitivity – primary cells and stem cells have smaller currents that require a high signal-to-noise, for obtaining reliable pharmacology data (Patchliner, SyncroPatch 96, QPatch).

Action potentials – It is known that merely investigating the individual cardiac channels does not necessarily correspond to the situation *in vivo*, where the ion channels work as an ensemble to form action potentials. It therefore makes more sense to investigate the cardiac profile on the entire action potential, to get a more relevant answer to, whether or not the compound might pose a threat to cardiac safety [26] (Patchliner, QPatch).

Physiological temperature – Another known fact is that some compounds affecting the cardiac channel coded for by the hERG gene can have different pharmacology depending on the temperature during analysis [27,28]. For safety testing, platforms allowing temperature control can therefore be very useful (Patchliner, IonFluxHT).

Heat activation – Some of the transient receptor potential (TRP) channels are activated by heat as well as by ligands. Here, it is important to distinguish between chemical and heat activation and determine possible differences in antagonism of responses evoked either by heat or ligand (Patchliner). An example is the TRPV1 channel, a considered pain target, which also is involved in regulating the core temperature of the body. Compounds with effect on TRPV1-mediated pain, also altered the body temperature, in an undesired manner [29]. Here, the ability to screen for antagonism on the temperature vs. the chemical response would have been useful.

Platforms that to date have been used with stem cell-derived cardiomyocytes are PatchXpress, QPatch, SyncroPatch 96, and Patchliner. In addition, the Patchliner and the SyncroPatch have been used with stem cell-derived neurons [30], and the Patchliner has been successfully validated with a wide variety of primary cells [19]. A very interesting result of the published work of Milligan *et al.* [19] were the success rates obtained for human smooth muscle cells and human synoviocytes on the Patchliner compared with conventional patch clamping. For the smooth muscle cells, Milligan *et al.* reports a success rate of 62.5% (n = 72) (Patchliner) compared with < 10% (n = 403) (conventional patch clamp). The success rates for human synoviocytes were 70% (n = 144) Patchliner and 8.3% (n = 24) (conventional patch clamp). Furthermore, human neutrophils could be recorded on the Patchliner (success rate 35%, n = 20), which previously was impossible with conventional patch clamp. This shows that APC can be an enabling system for the investigations of primary cells.

The Patchliner and the IonFlux support recordings at physiological temperatures, whereas Patchliner is the only platform on the market that can supply the cells with heated solution, with the possibility to construct temperature dose–response curves. Action potential recordings are supported by the Patchliner, and recently also by the QPatch and PatchXpress.

Table 2. Versatility differences between the APC platforms.

Feature	Dynaflow HT	IonFlux HT	IonWorks Barracuda	SyncroPatch 96	QPatch HT/ HTX
Main application	Screening, safety testing	Screening, safety testing	Screening, safety testing	Screening, safety testing, lead optimization	Screening, safety testing, lead optimization
Recording configurations	Whole cell, loose patch	Whole cell, loose patch, PPC	Perforated, loose patch, PPC	Whole cell, perforated patch	Whole cell
Compatible cells	Cell lines	Cell lines	Cell lines	Cell lines, primary cells, stem cells	Cell lines, stem cells
Ion channels	Voltage- and ligand-gated channels	Voltage- and ligand-gated channels	Voltage- and ligand-gated channels	Voltage- and ligand-gated channels	Voltage- and ligand-gated channels
User intervention during run?	No	No	No	Yes	No
Consumable shelf life	Not known	Not known	6 months	24 months	1 month
Data fidelity - Fluidics	Excellent	Excellent	Fair	Excellent	Excellent
Data fidelity Voltage Clamp	Fair	Fair	Poor	Excellent	Excellent

6. Conclusion

Successful APC platforms for primary and secondary drug screening require a high data throughput to be able to process large compound libraries. Data output is not merely influenced by the parallelism of the platform, but also by level of automation, cycle times between recordings, built-in redundancy of the screening substrates, perfusion capabilities, as well as the data quality. There are platforms capable of high quality recordings at a higher throughput, so that the user does not have to compromise in this aspect. Automated patch clamp platforms are pivotal for safety testing, and are commonly used in secondary screening, lead optimization. The throughput capabilities of the discussed platforms are approaching HTS levels. However, the price-per-data point is being debated, and is still considered too expensive for screening efforts. Considering the different recording plates, the cost per well ranges between circa 0.5 and 10 USD. This is a great span, but does not mean that the least expensive recording plate (cost per recording well) is the most cost-efficient. Here, data reliability and recording success rates are just as decisive.

Our view is that the paradigm-shift of going toward planar patch clamp screening of ion channels has reached the machine park, in terms of technology acceptance, but has *not* reached the conception of what a reasonable cost per data point is. Furthermore, it is our firm belief that APC-based screening has the potential to find high quality leads that would not have been captured using the traditional screening approach, that is, indirect methods prior to evaluation of the hits by APC. Historically, it is just to conclude that this approach has not had much success, or any, for bringing new ion channel active medications to the market. It is obvious that a change is needed for successful ion channel drug discovery, and one of them could be the use of APC platforms in primary screening. This would entail increased consumable costs compared with using in-direct methods for primary screening, which in most cases is rejected by screeners. On the contrary, screeners require higher throughput and lower costs per data point, and with these requirements fulfilled, they would use automated patch clamp in early wet-screen campaigns [31], ideally replacing the low fidelity work horses. Cutting cost per data point is often calculated merely based on how many data points can be obtained per recording well. Neither the costs for full-time-employees (FTE), nor the monetary value conferred by possible cuts during development time are taken into account. It is a fact that some APC platforms allow more "walk-away" time than others. This means that a single FTE can serve multiple machines, and thus also increase the throughput and efficiency of the employees. In the long run, we will see that the cost per data point of APC instrumentation decrease further, as has been the case with new machines becoming available in recent years. Still, it will not reach the level of fluorescent-based platforms, which simply is due to the fact that higher quality data with more predictive value is only accessible with more sophisticated and hence more costly technology. It is still worth mentioning again that the output data obtained using APC platforms has the potential of finding compounds that otherwise would have been overseen. How much is a successful drug candidate worth? The future will tell, if and how successful APC platforms are for primary screens. We are awaiting the answer with great anticipation.

7. Expert opinion

Patch clamp is the gold standard for obtaining highly resolved information on ion channel activity and their effectors, but the technique suffers from serious throughput restrictions. Development of automated patch clamp methods is aimed at removing this bottle neck and enabling the processing of a large number of compounds and cells in less time, primarily to facilitate the drug screening of ion channels active compounds. The platforms, introduced almost a decade ago, were gradually accepted and became highly appreciated tools for ion channel research and screening. The first generation tools made ion channels more accessible and interesting as drug targets, reflected in the following large initiatives and investments within the pharmaceutical industry. Now, almost 10 years later, it, however, remains unclear as to how much this has brought in terms of new drug leads in the pipeline. Screeners express the intent to replace in-direct methods with APC, with the potential to find qualified drug leads that would have been overseen with less sensitive methods. Still, higher throughput is required from current APC platforms, to match full compound library screens. This implies further parallelization, as well as economical considerations, since all current platforms require single-use disposables for recordings.

During the past couple of years, there has been major restructuring within pharmaceutical ion channel screening groups. Companies merge, and/or close down sites. An increasing trend has been to outsource ion channel screening efforts instead of having the facilities for that in-house. According to a recent review by John Comley [31], the trend toward more outsourcing of ion channel screening is increasing. This means that the screening environment moves from pharmaceutical companies to often smaller companies offering contract research and screening services, as well as to dedicated biotechnology companies with an ion channel focus. So, in this regard, we see more "core" ion channel screening facilities in contract research organizations (CROs) and focused biotechnology companies, where many of the different APC platforms, if not all, are available and are run by dedicated and highly experienced people. This confers high (cost-) efficiency in screening capabilities and data generation due to focus and specialization.

Drug developing companies also have to clear possible drug safety liabilities as stipulated by FDA, and part of that is safety screening of possible drug action on cardiac ion channels. Here, the APC platforms have a clear and pivotal role, for obtaining a preliminary safety profile of the compound early in the drug discovery process [32]. Companies either do this in-house or purchase the services from contract research organizations, but still, it is a task that has to be well done, since there have been several examples previously, where block-buster selling drugs had to be removed from the market, since they in worst case, were able to cause cardiac death. In this regard, we not only see the ever increasing demand in throughput and decrease in cost per data point for future APC employment but also the trend toward more specialized, sophisticated applications of APC instrumentation in dedicated tasks such as cardiac safety assays. More demanding cells and applications are being called for increased throughput, and we see a general trend that different laboratories use platforms from several of the providers to extract the core benefits of the individual platforms.

We do not foresee the use of more advanced screening applications such as the use of automated current clamp recordings early in the drug discovery phases, since these measurements often entail the use of either primary cells or stem cell-derived cardiomyocytes or neurons. These cells are scarce and/or not directly inexpensive. However, in cardiac safety testing, it is definitively possible to use automated action potential recordings, to determine how the ion channel ensemble response is affected by the added compound. This is a relevant approach more consistent with the *in vivo* situation, compared with looking at individual cardiac channels overexpressed in cell lines. Here, temperature control also is a relevant feature, since the inactivation kinetics of the hERG channel is affected by temperature, and that this in turn also results in different compound pharmacology depending on the actual temperature during the experiments.

Indeed, the APC users have gotten more viable options over the years, with regard to throughput and experimental versatility, which we foresee to continue in the next decade. For instance, ion channel recordings in cell-free membranes are gaining more and more interest, since it allows investigating ion channels residing in, for example, inaccessible organelle membranes within a cell. Here, new highly parallel products are required to efficiently screen such ion channels. Advances in the near term future will include the ability to form and record from 16 individual lipid bilayers for ion channel reconstitution [33,34].

Taken together, we not only see further increase in demand for throughput with lower cost per data point for screening purposes on one hand but also for more sophistication on the other hand to cover the requirements of the different industrial drug development phases of drugs targeting a diverse range of ion channels.

Declaration of interest

N Fertig is the CEO, whereas C Farre is a Senior Scientist and Marketing Director, of Nanion Technologies, a provider of automated patch clamp platforms.

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