EXPERT OPINION

New strategies in ion channel screening for drug discovery: are there ways to improve its productivity?

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Introduction: From a drug discovery point of view, ion channels are very interesting and challenging targets. Over the past decade, great efforts have been made in developing platforms for patch clamp-based high-quality screening of ion channels in discovering new drug candidates as well for evaluating their safety profiles. Indeed, the automated patch clamp (APC) has recently reached the data throughput requirements of high-throughput screening (HTS) allowing for new screening strategies with ion channel active compounds.

Areas covered: This editorial article comments on the past and present developments of APC-based drug screening. Furthermore, it also looks at the implications of APC technology meeting HTS-standards as well as its use in compound safety evaluation.

Expert opinion: In the imminent future, we will see a paradigm shift in ion channel drug screening toward using APC-based platforms for primary drug library screens. This way, the redundancy of the drug discovery process and the risk of false-negatives could be drastically reduced. Furthermore, cardiac safety can be addressed early, avoiding late-phase withdrawals with promising drug candidates. It is our firm belief that APC-based ion channel HTS will facilitate the discovery of candidates, which otherwise would have not been found, and shorten the drug development cycle, saving time and cost.

Keywords: automated patch clamp, drug discovery, high-throughput screening, ion channel screening

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1. Introduction

Ion channels are essential for every breath, step or heartbeat, and occur in each cell of the human body. Therefore, they play an important role in pathophysiology, and consequently also as drug targets. The gold standard for direct, real-time measurements of ion channel activity is the patch clamp technique, celebrating its 33rd birthday this year [1]. Patch clamp is an extraordinary technique when you think about it: a glass tube is pulled to a tip diameter of micrometer dimensions and after filling with buffer, it is carefully pressed onto a cell's membrane. A tight interaction forms between the glass and the cellular membrane, the 'gigaseal', with giga referring to the Giga-Ohm electrical resistance between the interior of the patch clamp pipette and the cell bath. A brief suction pulse ruptures the membrane patch covering the pipette tip, offering full electrical contact and control over the cellular membrane. In this way, the ion channel activity, measured as transmembrane currents flowing over the open channels, and response to voltage pulses or added compounds can be monitored with ultra-high resolution (μs, pA).

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2. Patch clamp – the gold standard

Patch clamp yields detailed information not only about the ion channel itself, but also about the interaction between ion channel and compound, and is therefore suitable for a broad range of pharmacological experiments: kinetics, permeability, dose–response relationship, and state- and use-dependence of compound interactions. However, because of its tedious nature, manual patch clamp is light years from being adaptable for ion channel drug screening, where the throughput of 10^4 – 10^5 data points are required per day, compared with a daily throughput of roughly 10 data points for manual patch clamp. Consequently, screeners had to rely on other techniques for ion channel screening, often indirect and with drastically lower sensitivity and information content than patch clamp, and with limited or no means to investigate state- and use-dependence of ion channels and compounds, due to poor or no control of cell membrane voltage. Only far down the discovery path, promising drug candidates were analyzed using patch clamp [2].

This gap between throughput, functionality and sensitivity spurred the development of machines doing the patch clamping. The pipettes were replaced by micro-machined plates containing micron-sized apertures, where a cell was captured from solution using suction. In this way, the manual handling was reduced to preparing cells and solutions and adding them to the recording substrate or machine, described already in 2002 [3]. The first commercial products followed shortly; the PatchXpress [4] from Axon Instruments (acquired by Molecular Devices in 2005) the Ionworks from Molecular devices [5], and the Port-a-Patch [6] from Nanion Technologies. The Port-a-Patch, launched in 2003, records from only one cell at a time, and was early adopted by academic and industrial laboratories replacing conventional patch clamp setups.

Until now, about a dozen of different platforms have entered the market, with different technical solutions and standpoints regarding data quality, for excellent reviews on automated patch clamp (APC) platforms see Dunlop and Comley [7,8]. PatchXpress was the first system supporting gigaseal recordings from 16 cells in parallel, followed by the high data quality systems QPatch 16 (2004, Sophion Biosciences) and the Patchliner (2006, Nanion Technologies). The Ionworks platforms (HT, Quattro, and Barracuda from Molecular Devices) do not rely on gigaseal recordings and have in comparison with other platforms limited perfusion capabilities.

The Ionworks Barracuda has proven to work excellently for diverse ion channel targets, but can be a limitation when it comes to more challenging targets, for example, ligand-gated ion channels exhibiting rapid desensitization kinetics, or investigations of drug use-dependency of voltage-gated ion channels. For studies on drug use-dependence, precise control of the membrane potential is essential for accurate results, where a high-quality seal in combination with a low series resistance allow for a good voltage-clamp of the membrane. Relying on a low seal resistance, 'loose patch,' in combination with high series resistance conferred by perforated patch recording, makes a good voltage control of the membrane difficult, if not impossible.

Ionworks HT and Barracuda were the first platforms supporting recordings from cellular populations, that is, substrates with multiple apertures instead of only one, in this case 64, and 384 parallel recordings, respectively, and have provided significant input on screening broad range of ion channels. Population patch clamp has proven very helpful with cell lines exhibiting a highly variable protein expression rate or if the ion channel has a low conductance.

A screening assay is never better than the cell line, meaning that care needs to be taken to find a reasonable protein expression level, and to optimize parameters affecting gigaseal formation and stability. There is a fairly great variation between existing APC platforms in how much assay development is required to get acceptable success rates for a given cell line. Putting it simple, some platforms are more forgiving than others.

3. Development of APC platforms

Over the past decade, the increases in obtainable throughput in patch clamping or the numbers of parallel recording channels of the instruments have steadily scaled upward. This somewhat reminds of the scaling in semiconductor technology, nicely described by the famous law of the co-founder of Intel, Gordon E. Moore: Moore's law is the observation that, over the history of computing hardware, the number of transistors on integrated circuits doubles approximately every 2 years. However, for our APC devices the number of parallel recording channels increased with one order of magnitude every 4 years (**Figure 1**). We started with one recording channel in 2003 with the Port-a-Patch, and in 2013 we introduced the SyncroPatch 384/768PE platform capable of 768 parallel whole-cell, gigaseal recordings [9]. In 2014, Sophion introduced the Qube, also capable of 384 parallel gigaseal recordings [10].

At this point, a throughput has been reached that fulfills the requirements of high-throughput screening (HTS) with a machine setup and design allowing full integration into HTS-environments and processes. For example, the SyncroPatch 384PE employs automated liquid handling using a 384 channel pipettor head, a 384 channel parallel amplifier and offers a daily throughput of 20,000 data points per day [9], while still maintaining high-quality, gigaseal recordings and fast fluidics suitable for both voltage- and ligand-gated targets. One unique property of this system is the open design, allowing for full integration into automated drug screening environments, both regarding hardware and software. It remains to be seen how far this will push the boundaries of APC in HTS.

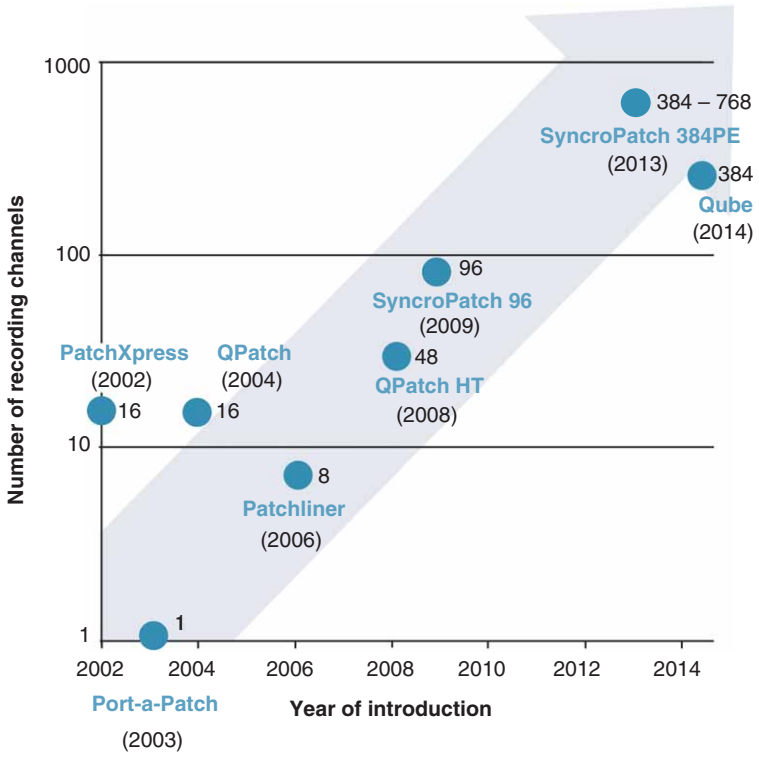

Figure 1. The progression of automated patch clamp devices supporting Giga-Ohm seals and the number of parallel recording channels.

4. Implications of using patch clamp-based platforms in ion channel HTS

Using HTS-compatible APC devices in ion channel drug screening has a number of benefits. First, the increased number of channels in combination with efficient robotic platforms has increased the data throughput now reaching HTS levels. This means that it would be possible to perform primary screens directly on a high-quality APC system. Second, by omitting the use of indirect and low-quality workhorses of the past, the hit-to-lead process becomes substantially faster, since the APC-based screen will directly provide detailed information on the compound interaction. Third, and also very important, is the high reliability of the outcome of APC screens in contrast to its successors. Previously, false-positives were caught in the APC re-screens, but the false-negatives were lost forever, meaning hot drug candidates could be sitting idle in a drug library. This risk is clearly reduced by using APC in primary screens, because of its high information content, high data quality and reliability. In this way, HTS-compatible APC platforms have tremendous potential to reduce redundancy in the ion channel screening process to a minimum, and finding (all)promising drug candidates at a faster pace.

On the other hand, APC screens are substantially more expensive compared with fluorescence-based cellular assays, where, in the latter case, the cost per data point is a couple of cents, in comparison with 1 – 10 USD per data point depending on APC platforms. The cost of the patch clamp substrate is obviously essential for keeping the running cost low, especially when expanding the scope of APC screens. However, competitive substrate pricing in combination with multiple solution additions to the recording wells, with intermittent wash out and control steps, have allowed the price per data point to drop to < 25 cents, for some APC platforms. This is still more expensive than fluorescence-based methods, but considering the possible increase in efficiency and predictability combined with the historically poor outcome of rational screening toward ion channel targets, APC can prove a viable route for new and efficient screening strategies with substantially shortened cycle time going from hit to a successful drug candidate.

5. View on cardiac safety screening is about to change

Another important area of use for APC-based screening is cardiac safety testing [11,12]. Increased throughput means that safety liability issues can be addressed early in the drug discovery process, preventing costly late-phase withdrawal of drug candidates. Today, all compounds have to undergo rigorous testing before release to market, and it has become apparent that safety screening has to go beyond the cardiac hERG-channel, which together with Nav1.5 typically received most of the

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attention in early cardiac safety screens. Drug discovery industry now has to prepare for the new safety toxicity profiling and screening guidelines suggested from Food and Drug Administration (FDA), Cardiac Safety Research Consortium (CSRC), and Health and Environmental Sciences Institute (HESI), aiming at standardizing tests and methods for compound safety evaluation [13]. FDA/CSRS/HESI suggest in their synopsis to expand the number of relevant safety targets to a handful of cardiac channels, including hERG, Nav1.5, Cav1.2 and KvLQT1, Kir2.1. In this way, the panel of cardiac ion channels to consider in a safety screen has expanded, consequently requiring more throughput to keep up the safety screening efforts. Recently, the Cytopatch workstation was introduced by Cytocentrics. This is not an HTS system, but what makes it interesting in this context is its claimed compatibility with good laboratory practice (GLP), allowing samples to be taken from tested compound solutions [14].

Also preferred by FDA is to not only work with cellular expression systems, but also with relevant and hopefully more predictive models such as stem cell-derived cardiomyocytes. Using cardiomyocytes would allow for investigations on compound impact on the whole ensemble of cardiac ion channels, for example, through action potential recordings [15,16]. Another important safety aspect to consider is ion channels with a high probability of causing neurotoxic side effects, which can be addressed by APC recordings from, for example, induced pluripotent stem cell-derived neurons [17]. Thus, APC platforms supporting current clamp might be increasingly important for the future. Medium and high-throughput platforms supporting current clamp are Patchliner, QPatch 16 and the SyncroPatch 384/768PE.

6. Conclusion

Historically seen, drugs targeting ion channels have been notoriously difficult to screen and have mostly been found by coincidence rather than by rational screening. The traditional screening cascade has proven inefficient for finding new compounds targeting ion channels. However, using APC as secondary, and recently also primary screening tool, allows for a direct, highly resolved and direct detection of compound effects. The benefits of APC-based screens are the possible detection of otherwise overseen compounds, and the reduced redundancy in the screening process, saving time and, therefore, also costs.

7. Expert opinion

The future of ion channel screening for drug discovery is most surely APC-based and it is our belief that APC will gradually replace other screening methods because of the increased information content and sensitivity offered by the method. Moreover, the number of recording channels of the APC platforms, interesting ion channel targets and demands on experimental sophistication of the HTS machines will increase. Ion channels as drug targets are extremely diverse and some are very challenging in terms of finding stable assays and recording conditions. Platforms supporting HTS, high-quality data, versatile experimental protocols, sophisticated perfusion capabilities and the use of primary cells or stem cell-derived cardiomyocytes of neurons, allow the screeners gaining the upper hand in the hit-to-lead and safety testing race. We are therefore convinced that data quality, sufficient throughput and experimental sophistication become increasingly important to fish out the potential block buster drugs in library screens, since the 'easy' ion channel targets are long gone and the old workhorses for ion channels screening methods have proven ineffective.

Therefore, APC-based screening has great potential for finding the gold nuggets in 100.000 + compound drug libraries, by allowing faster discovery cycles and increased throughput capabilities also for evaluation of drug safety. Ideally, better drugs would be developed much faster, and at a lower cost. However, it remains to be shown how well APC platforms can be integrated in fully automated ion channel drug screening environments, and the outcome of APC as primary screening methods in terms of successfully developed and launched drugs targeting ion channels.

Additionally, the versatility of APC are increased by experimental features like temperature control, automated action potential recordings, or the possibility to record from scarce or costly cells such as primary cells and stem cell-derived cardiomyocytes or neurons. There are platforms on the market supporting these features [15-17], including compound testing using automated action potential recordings; however, not at an HTS-compatible throughput. Recordings from primary cells [18] and stem cell-derived cardiomyocytes [16] and neurons [17] have successfully been made on medium to high-throughput systems. Here, low-density cell suspensions are required to make the experiments feasible, a severe problem for some, but not all, platforms. It remains to be shown to what extent stem cell-derived cells will be used for routine safety projects, since these cells are typically quite costly.

New platforms using impedance- and/or electric field potential-based recordings from beating networks or three-dimensional clusters of stem cell-derived cardiomyocytes can in combination with APC be used for better predictions of neuro- and cardiac safety and toxicity [19]. The combination of techniques would allow for a paradigm shift from highly focused screening (hERG, Nav1.5) to a 'cloud' of high-information content data obtained from a panel of cardiac ion channels present in stem cell-derived cardiomyocytes. This allows a profound knowledge based on compound safety at an early stage in the discovery process, saving time, costs and efforts by focusing on the most suitable candidates.

The year 2013 was when APC met HTS standards, and in a couple of years we will see the real effects and outcome of using proficient, reliable and cost-efficient ion channel screening platforms in ion channel screening and safety profiling.

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Declaration of interest

N Fertig is the CEO of Nanion Technologies, a provider of automated patch clamp platforms. C Farre is a senior scientist and marketing director of Nanion Technologies. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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