2 Advancing Ion Channel Research with Automated Patch Clamp (APC) Electrophysiology Platforms

Damian C. Bell and Mark L. Dallas

Abstract

Since its development on the cusp of the new millennium, automated patch clamp (APC) technology has matured over the last two decades. The increased throughput it afforded promised a new paradigm in ion channel recordings: It offered the potential to overcome the time-consuming, low-throughput bottleneck arising from manual patch clamp (MPC) investigations. This chapter highlights the advances in technology, showing how APC platforms have 'democratised' ion channel recordings, lowering the technical bar whilst substantially raising throughput. It will describe the background of the seminal first-generation and updates on advances in second-generation platforms. Furthermore, the chapter summarises the advances APC has made in ion channel studies, including finding new tool compounds and medicines. New functionality and applications on APC platforms give ion channel researchers flexible tools to study ion channels with high quality and high throughput.

Keywords

Electrophysiology · Ion channels · Automated patch clamp · Manual patch clamp

Abbreviations

APC	Automated patch clamp
CHO	Chinese hamster ovary
CRO	Contract research organisation
d.p.	Data points
HEK293	Human embryonic kidney 293
hERG	Human ether-a-go-go-related gene
HTS	High throughput screen
MPC	Manual patch clamp
MTS	Medium throughput screen

2.1 Introduction

The biggest advances in recording ion channel currents occurred in the 1970–1980s, a decade in which the godfathers of electrophysiology formalized the methods and recording hardware that allowed measurement of ion currents from single cells via a technique dubbed whole-cell patch clamp [1, 2], subsequently referred to as manual patch clamp (MPC). MPC has maintained its place in the electrophysiologists' armoury and continues to contribute to our understanding of ion channels but does have downsides. It requires substantial technical training and teaching

D. C. Bell (✉)
Sophion Bioscience A/S, Ballerup, Denmark
e-mail: dbe@sophion.com

M. L. Dallas
School of Pharmacy, University of Reading, Reading, UK

L. Zhou (ed.), *Ion Channels in Biophysics and Physiology*, Advances in Experimental Medicine and Biology 1349, https://doi.org/10.1007/978-981-16-4254-8_2

resources to become competent, a growing problem in the competitive and time-starved modern-day academia [3]. As an highly labour-intensive and very low-throughput technique (~10–30 data points, d.p./day, where a d.p. is defined as a current recording under different test conditions e.g. a four-point consecutive drug concentration response would be five d.p., a control current, plus four drug concentration tested currents), MPC is not an efficient method and thus hamstrings ion channel research and drug development.

Since 1999, automated patch clamp (APC) technologies were developed to make ion channel recordings easier and more efficient by lowering the technical requirements whilst increasing throughput (for reviews on the early developments of APC technology see [4–7]). The main breakthrough in making ion channel recordings easier and faster was planar patch clamp: whilst MPC used a microscope and micromanipulator to locate and place a recording glass electrode pipette on an adherent cell, APC used robots to apply suspensions of ion channel expressing cells onto planar arrays of recording sites (or planar recording chips; see [8]) – see Fig. 2.1. In APC, a combination of gravity and negative pressure enables high electrical resistance seals (100 s MΩ – several GΩ) to form between the cell membrane and the recording site hole.

This chapter will discuss a number of APC platforms, describing key developments that have deepened our understanding of ion channels and advanced our ability to discover novel ion channel therapeutics.

2.2 APC Platforms: Key Developments Over Two Decades

Developments of APC technologies have grown apace since the first Apatchi-1 (Sophion Bioscience) and AutoPatch (CeNes/Xention) were made in 1999 – see Fig. 2.2. In brief, the initial technologies in APC developments used three main types of recording:

1. A robotic visualisation and movement of recording electrode to an adherent cell, essentially replicating what a researcher would do in MPC;
2. Inverted pipettes that were 'backfilled with cell suspensions', allowing trapping and sealing of a single cell in the pipette tip; and
3. Planar patch clamp where robotics applies a cell suspension to a planar array of recording sites.

After these initial format developments, the planar patch clamp quickly evolved to be the clear winner. Planar arrays of recording sites, or planar recording chips, gave the simplest and most consistent method of robotic cell application and sealing and vastly increased the recording capacity of APC by as much as >100-fold over MPC. In gaining the highest throughput capability of thousands of d.p./day, compromises were made; these compromises, it is argued, may have reduced the early adoption of the technology. One such compromise was that the early APC platforms used a 'loose-seal' (typically ~100 MΩ) configuration, which differed from the more accurate and giga-ohm (GΩ) seals routinely attained in MPC recordings [1]. The PatchXpress compromised on having lower throughput (limited to 16 simultaneous recordings) but was the first APC with the ability to achieve the favoured, accurate GΩ seal formation. Another compromise that allowed higher throughput platforms to achieve GΩ seals was the use of 'seal enhancer' in recording solutions (e.g. PatchLiner [10] and the SyncroPatch96 [11], Nanion). The high Ca^{2+} content of this 'seal enchancer' (e.g. 40 mM) has an impact on recordings that are sensitive and/or modulated by Ca^{2+}. The need to use such high divalent ion 'seal enhancer' supplements has been reduced over time, with developments in cell lines, culturing and understanding of the chemistry of glass/lipid interactions. Finally, the fixed well format used on a number of platforms (see Table 2.1) can also cause problems: the lack of wash through of cells or test compounds can have knock-on effects to later elements of the recording (e.g. desensitisation of ligand-gated ion channels

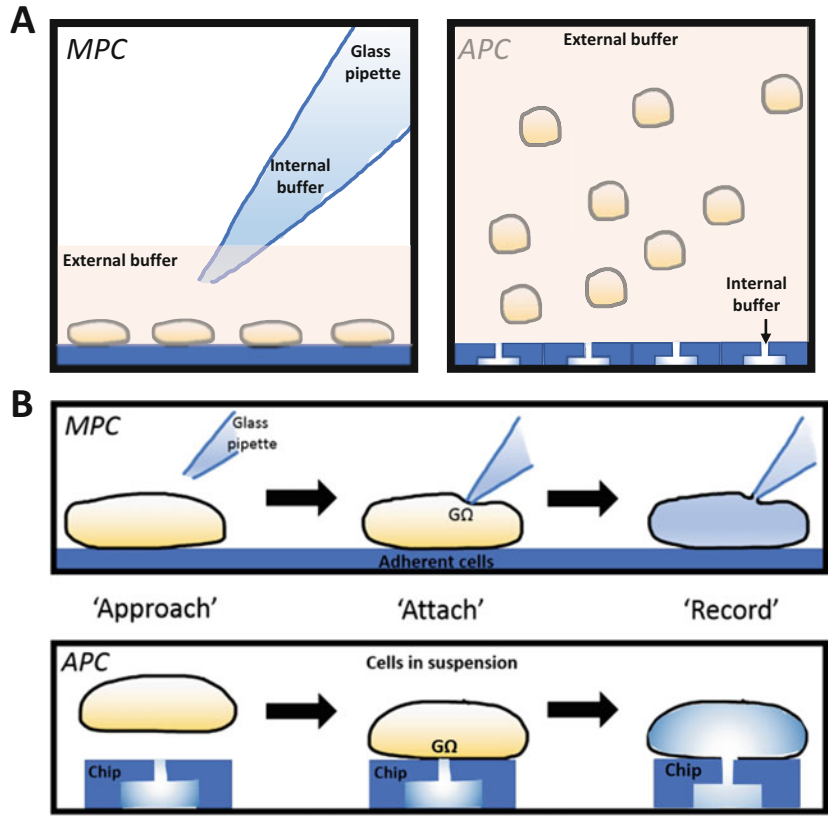

Fig. 2.1 Manual and automated patch clamp methodologies. (**a**) (*Left panel*) Manual patch clamp (MPC) involves the use of a glass pipette, filled with an internal recording buffer, containing a silver-chloride electrode in circuit with a patch clamp amplifier. Adherent cells on coverslips are maintained in an external recording buffer. (*Right panel*) Automated patch clamp (APC; also referred to as planar patch clamp) involves the use of recording chips with planar arrays of recording sites (four recording sites are shown); each site contains an internal buffer and an electrode in circuit with an amplifier. A suspension of cells in an external buffer is applied to the recording sites via an automated pipettor. (**b**) (*Top panel*) To obtain recordings in MPC the glass pipette is manoeuvred using a micro-manipulator onto an adherent cell ('Approach'). The glass pipette then makes contact with the cell and then through the application of negative pressure forms an electrical seal, in the order of GΩ magnitude ('Attach'). Via the application of negative pressure a hole is made in the patch of membrane patch under the glass pipette: this allows the electrode, the recording solution (shaded blue) in the pipette and cell to become one compartment and electrically contiguous ('Record'). (*Bottom panel*) APC, uses cells in suspension that are applied to planar arrays of recording sites on the recording chip (for clarity only a single recording site is shown; 'Approach'). Under gravity and negative pressure a GΩ electrical seal forms between cell membrane and the recording site ('Attach'). Further negative pressure is applied to form a hole in the cell membrane to give the whole cell configuration, whereby the electrode and internal recording solution (shaded blue) situated beneath the recording site are made electrically contiguous with the cytosol ('Record'). Adapted from Perkel et al. [8] and Bell and Dallas [9]

due to incomplete or inefficient washout of applied ligand). Another documented example was that when cell suspension applications were made to a fixed recording well: Surplus cells (i.e. all the cells added but not sealed on the recording site or sites of a recording well) acted as 'lipid sinks', whereby lipophilic compounds (e.g. terfenadine) were absorbed into the surplus cell lipid membranes. This lipid absorption lowered the free concentration of the compounds

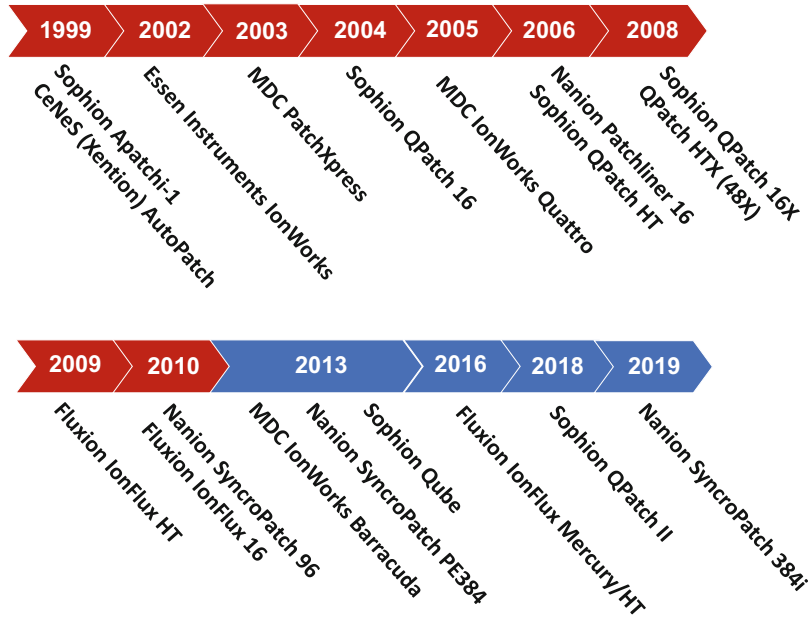

Fig. 2.2 Two decades of automated patch clamp platform development. A timeline showing the main automated patch clamp (APC) platforms. First-generation development years are shaded red and second generation are shaded blue

and resulted in lower potencies being measured for these lipophilic compounds [12].

A number of reviews describe the key developments between the first and the second generation of APC platforms [9, 13–15]. Table 2.1 summarises the main capabilities of the second generation of APC platforms. For example, IonWorks, the first 384-recording capable APC platform, only allowed two compound additions, whilst its improved, later sibling, the IonWorks Barracuda, had multiple additions by using an add-dilute-remove solution addition cycle [16, 17]. Further improvements in fluidics followed in other platforms, Nanion's

Table 2.1 A comparison of key features of the second generation of APC platforms (2013 onwards)

Feature		IonWorks Barracuda (MDC, USA)	Qube (Sophion, Denmark)	IonFlux HT/Mercury/Ultra (Fluxion, USA)	QPatch II (Sophion, Denmark)	SyncroPatch 384PE/384i (Nanion, Germany)
Recording sites		384	384	16/64/256	48	384 (768) (+2nd module)
Throughput (d.p./day)		~8 k	~16 k	~1.5 k/5 k/18 k	~3–5 k	~18 k (36 k)
Substrate		Planar, plastic	Planar, polymer	Lateral, PDMS	Planar, silicon/glass	Planar, glass
Seal resistances		~100–300 MΩ	>1 GΩ	>1 GΩ	> 1 GΩ	>1 GΩ
Amplifier compensation		No	Yes	Yes	Yes	Yes
Internal perfusion		No	Yes (offline)	No	No	Yes (online)
External solution exchange	Format	Fixed well	Microfluidics	Microfluidics	Microfluidics	Fixed well
	Cycle	Add-dilute-remove	Add-displace-replace	Continuous	Add-displace-replace	Add-dilute-remove
Current clamp		No	Yes	Yes	Yes	Yes
Temperature control		No	Yes – BoN	Yes – ambient	Yes - BoN	Yes - ambient

d.p./day data points per day, *PDMS* poly-dimethyl-siloxane, *BoN* bed-of-nails. Copied with permission from Bell & Fermini, J. Pharm. Tox. Methods, 2021

SyncroPatch 384 PatchEngine (384PE) and 384i [15, 18], use low-volume pipettor additions and recording site wells, whilst Sophion's QPatch II [19], Qube [20, 21] and Fluxion's HT, Mercury and Ultra platforms all have planar patch clamp recording plates with built-in micro-fluidics channels allowing low-volume, efficient and rapid exchange of solutions into and through the cell recording sites. The second-generation APC platforms maintain high throughput whilst achieving high-quality GΩ seals. With the exception of the IonWorks Barracuda, all of the second-generation APC platforms have current-clamp and temperature control modes, adding further functionality. Finally, developments made from first to second generation have seen the estimated cost per d.p. fall some 3- to 10-fold [9].

2.3 Early APC Adoption: Ion Channels in Drug Discovery

With such automation and vastly increased throughput, a clear application was in drug discovery safety pharmacology. To this end, cardiac ion channel safety testing researchers were early adopters and advanced the assay capabilities of APC platforms. For example, in some of the earliest APC screening campaigns, drug libraries were tested against the hERG (Kv11.1) ion channel using IonWorks HT [22, 23]. More recently, a wide range of industry and academic cardiac safety researchers have worked together to define a standardized hERG liability definition [24]. Although much of the focus of cardiac safety APC assays has been on the chemotype promiscuous hERG channel, other critical cardiac sodium, potassium, and calcium ion channel currents have seen increasing attention using these platforms [25–28]. This work has culminated in a broader scope of cardiac safety pharmacology: The comprehensive in vitro pro-arrhythmia assay (CiPA) is a strategy aimed at standardising assays on key cardiac ionic currents (I_{Kr}, I_{Ks}, I_{K1}, I_{NaFast}, I_{NaLate}, I_{CaL}), across different labs and various APC platform assays [29].

Alongside the early adoption in cardiac safety pharmacology, an early application of APC technology was the secondary screening of high-throughput primary screening, checking that 'hits' arising in the primary screen were actually modulating the ion channel target and not an artefactual response observed in the primary screen (e.g. fluorescent screens can give false positive hits due to compound auto-fluorescence). After using a large, first (primary) fluorescence-based HTS, drug discovery researchers might follow-up with a secondary, smaller medium-throughput screen (MTS) to validate the primary HTS compound 'hits' (e.g. [30]); [7], provide a good summary of such a screening cascade). A number of drug discovery ion channel programmes have followed this screening cascade format, with APC routinely used to determine and verify compound activity in a 'hit' focused, limited secondary screen: for example, HCN channels [31]; Nav channels [32–35], Cav3 (T-type) calcium channels [36] and Cav2 (N-type) calcium channels [37].

However, more recently, the high-throughput and high-quality recordings afforded by the second generation of APC (see Table 2.1) have given drug discovery researchers the tools to make APC-driven HTS possible earlier in the cascade. A screening model being adopted is that the HTS primary and hit-validation secondary screens are being combined into a single mid-to-high throughput screen with the rationale that the improved, data-rich read-out of APC gives stronger, more robust data earlier. One such single APC 158 k compound screen was made by Chambers et al. [20] in a drug discovery programme versus the Nav1.7 ion channel, a chronic pain target, using the Qube. Along these lines, Danahay et al. [38] adopted a mixed fluorescence-based assay and mid-throughput QPatch APC screening model, running the two assay formats in parallel to find compounds that potentiated TMEM16A chloride currents for the development to treat cystic fibrosis [38]. In these APC screens. the traditional primary and secondary screens are driven by a single APC HTS screen, making ion channel drug discovery faster, more efficient and consequently cheaper.

2.4 APC: Advancing Ion Channel Research

Apart from the higher throughput and lowered user technical hurdles, the most obvious advantage that APC brings is in the name: automation. Unlike MPC, APC allows full walk-away recording capability: Most APC platforms have fully automated cell preparation (onboard centrifugation of cell suspensions and resuspension in external recording solution); membrane seal and whole cell formation; and voltage (or current) clamp and test/drug solution exchange protocols. Consequently, as long as sufficient cell suspension volume, solutions, recording and compound plates (both often queued in automated elevator stacks) are provided, several hours (e.g. overnight) of unattended recordings are readily achievable. Add to this the capability that the second-generation APC platforms (see Table 2.1) all have remote access and control which allows monitoring of experimental progress, writing and execution of voltage and test solution application protocols and analysis of completed experimental data. With the growing trend of working remotely – accelerated by the Covid-19 pandemic – such remote access and control of APC platforms will become increasingly advantageous and routinely adopted in ion channel labs.

Earlier, when describing the development of the first generation of APC platforms, the sacrifices that APC R&D adopted were defined that allowed a broader user base by lowering the technical ability needed to make ion channel recordings whilst vastly increasing throughput. However, the most efficient and widely adopted solution, that of planar patch clamp (i.e. arrays of recording sites in a planar recording plate), in the design and development of APC technology provided advantages, giving researchers additional capabilities as standard, not readily achievable in MPC.

Table 2.1 summarises these additional capabilities that APC technology allows, further described here:

2.4.1 Control of Internal Cell Solution Dialysis or Exchange

SyncroPatch 96/384PE/384i APC platforms allow the exchange of the internal cell solution: On these platforms, internal solution exchange can be performed ‘online’ during ongoing recordings, with the experimental protocol set to exchange the internal solution, i.e. allowing continuous recording throughout and following exchange. Using the Patchliner and Qube, the internal solution can be changed ‘off-line’: recordings can be paused and internal solution changed manually before continuing with recordings. A number of labs have used APC platforms to study internal solution exchange: TREK-1 channel modulation via intracellular pH was demonstrated using the 384PE [39]; direct PIP2 modulation of human EAG channels was shown on the Patchliner [40]; activation of TRPC5 or TMEM16A channels was shown on the SyncroPatch 384PE via Ca^{2+} containing internal solutions [41].

In MPC recordings, the internal solution exchange is limited to the internal pipette solution dialyzing the intracellular environment: Consequently, the timing of cell dialysis is diffusion dependent and a one-time event after achieving whole-cell access to the recording cell.

2.4.2 Control of Experimental Temperature

APC platforms use two formats to control experimental temperature (see Table 2.1):

(a) control by the ambient environment, whereby the platform cabinet temperature and/or recording solutions can be heated or cooled [e.g. TRPV1 and TPRV3 studies on the Patchliner platform [11, 42].
(b) control by bed-of-nails (BoN; an array of amplifier electrode connectors), whereby temperature measured at the BoN controls

the recording site temperature by heating/cooling fluid that flows via tubes that snake in and out of the BoN. This format takes into account the significant heat generated by the BoN, which can alter the recording site temperature by over +3 °C [43].

Of particular importance in ion channel, safety testing is the biophysical and pharmacological effects of temperature [44]. The cardiac safety implications of temperature on compound modulation of hERG (Kv11.1) ion channels have been studied using IonFlux [45, 46]. An examination of the effects of temperature on kinetics was performed across 40 different Kv channels using the Patchliner [47].

Temperature-controlled experiments though possible on MPC would require specialised add-on temperature control equipment.

2.4.3 Recording Site Fluid Applications: Microfluidics and Fixed-Well

The use of microfluidic channels (e.g. IonFlux HT, Mercury and Ultra; QPatch, QPatch II and Qube) or fixed-well formats (IonWorks Barracuda, Patchliner, SyncroPatch PE384 and 384i; see Table 2.1) allows expensive, limited quantity molecules (e.g. crude venom fraction purifications, synthesized peptides or antibodies) to be tested in low-volume (2–20 µl) applications [48–56].

Microfluidic channels or pipettor robot 'stacking' (where a liquid column 'stacks' different test solutions in the pipettor to be applied sequentially) also provide rapid external solution exchange rates (with complete solution exchange in 10–50 ms), fast enough to allow fast desensitising ligand-gated ion channel recordings [57–63].

Such perfusion is possible in MPC; however, it requires specific add-on fast perfusion microinjection units.

2.4.4 Planar Patch Clamp Recording Plates

In MPC, using a long, glass micropipette as the recording electrode introduces mechanical noise to the recording. Although this mechanical noise is abrogated by the use of vibration-dampening air tables, mechanical noise is still picked up by the glass electrode acting as a mechanical 'antenna'. Planar patch clamp does not have this problem, so anti-vibrational air tables are not needed on APC platforms. Furthermore, this reduced mechanical noise in APC is best seen in the greater success rates for longer recordings (>30 min) that are possible on APC platforms [5].

2.4.5 Multi-Hole Patch Clamp

APC recording plates can employ more than one recording site per recording well. With multiple recording sites per well, multiple cells can seal and be recorded from in a single recording well. This multi-hole patch clamp (also known as population or ensemble) allows multiple whole-cell currents to be averaged in a single recording well. Consequently, by recording currents across multiple cells, multi-hole patch clamp gives improved recording success rates and averages variable ion channel expression across several cells [64, 65].

2.4.6 Pressure Control

To position, seal and attain whole-cell electrical access on cells landing on recording sites in planar arrays, APC platforms have the ability to control pressure at each recording site. This pressure control has been extended beyond its original role in seal and whole-cell formation to apply different pressures to cells during recordings, allowing pressure protocols to be applied to mechanosensitive channels. For instance, QPatch

was used for cell volume and membrane stretch pressure studies on cells expressing the mechanosensitive BK and KCNQ channels [66].

2.4.7 Optogenetics and Optical Stimulation

Both Sophion and Nanion have adapted their Qube and SyncroPatch 384PE APC platforms to have a fully integrated LED-based optogenetics unit allowing simultaneous optical and voltage stimulation. Using these specially modified APC platforms, they have shown that optogenetically modified cell lines (i.e. cell lines expressing optically activated channelrhodopsins – for an optogenetics review see [67]) can be simultaneously optically stimulated and voltage-clamped with the resulting currents recorded [68, 69]. Additional functionality is possible with these LED-capable APC, such as releasing caged compounds and in light-based actuation studies of intracellular events [68] and investigating photoswitchable compounds in the modulation of ion channels [69]. These LED-modified APC platforms to date are in-house R&D proof-of-concept platforms and as such are currently not on the market. However, researchers interested in using the optogenetically capable Qube 384-Opto or SyncroPatch 384 PE should contact the manufacturers (*personal communications* Dr. Sandra Wilson, Sophion or Dr. Alison Obergrussberger, Nanion).

Optopatch spiking HEK cells, an optogenetics assay-ready cell line has been developed by researchers at Harvard University [70, 71], allowing an optogenetics approach to high-throughput screening of Nav1.7 ion channel modulators. Recently, this optogenetics screening cell line was used to generate data in a pilot screen of Nav1.7 blockers: these optogenetics screen data were compared to data generated on the IonWorks Barracuda APC (MDC) with the two sets of data showing good correlation [72]. Another example where optogentically modified ion channel cell lines have been used to good effect in combination with APC are in human-induced pluripotent stem cell-derived cardiomyocytes (Cor.4U cells, NCardia) expressing the light-activated channel channelrhodopsin-2 (ChR2). Using the LED capable SyncroPatch 384PE action potentials in Cor.4U-ChR2 cells can be elicited via 1 ms pulses of blue light [69]. These examples of optogenetically modified ion channel cell lines provide further evidence that combining APC with optogenetic screens and experimentation is likely to become increasingly viable assay formats in the future.

These advancements in APC were initially based on what was capable in MPC recordings. However, APC R&D has made significant improvements in ion channel recordings, and in many respects surpasses existing MPC capabilities (e.g. microfluidics channels, internal perfusion and planar patch clamp). In other capabilities where MPC has traditionally held a clear advantage, in recent years, APC developments are making good progress to catch up, allowing far higher throughput in these capabilities typically dominated by MPC. For instance, due to low numbers and potentially mixed, heterogeneous populations of native cells in acutely isolated cell preparations, ion channel recordings on primary cells have often been challenging on APC platforms. However, there are now several labs that have successfully employed APC to make recordings on primary cells [5], and specific examples include pancreatic cells [73], T-cells [74, 75], red blood cells [76] and cortical neurons [77]. An area of research that better lends itself to APC is induced pluripotent stem cells (iPSC), which allow greater numbers and homogeneity of these pseudo-native cells [11, 73, 78]. A potential improvement in human iPSC cardiomyocyte APC recordings is ‘dynamic clamp’: In Patchliner recordings, an electronic adjustment of the resting membrane potential is made by adding an I_{K1}-modelled current, resulting in a more negative resting membrane potential, redolent of native and mature cardiomyocytes [25, 26, 79].

2.5 Concluding Remarks

APC technology has made ion channel recordings easier, faster and more efficient, widening the potential user base for a previously technically challenging technique. The 'democratisation' of ion channel recordings by APC is arguably the biggest advancement in our ability to study ion channels, since Neher and Sakmann's [80] seminal publication ushered a golden era in patch clamp techniques [1, 2, 80]. Understandably, biotechs, pharmas and CROs were early adopters of APC technology, but more academic labs are accessing and adopting the technology via collaborations, consortia and shared, core facilities. For example, a recent publication highlights the democratizing advantages of APC (fostering collaborations, higher throughput, lower technical hurdles and rapid advancement of the field): in a collaboration between Washington University and Genentech, Wandi Zhu, a summer internship student, completed an impressively thorough biophysical and pharmacological analysis of 39 compounds against the bacterial NaChBac and human Nav1.7 ion channels [81].

With APC technology becoming commonplace, the future of ion channel research looks to be in fine fettle. Indeed, this APC-enabled explosion in ion channel research is likely to lead to significant breakthroughs in our understanding of ion channel physiology and pathophysiology. For instance, the improved capabilities provide the potential for greater definition of selected patient cohorts, channelopathies and rare diseases, giving more scope to treat individuals with increasingly personalised (or stratified) medicinal regimes. For instance, the application of APC allowed researchers to test gain or loss of function mutations in Nav and Cav channels – these mutant channel data will inform potential future precision medicine treatments in patients carrying these channelopathies [82]. Consequently, on the back of this accelerated research, ion channel treatments will be found helping patients across a wide range of diseases, and the first blockbuster drug is imminent.

Acknowledgements We would like to thank Dr. Alison Obergrussberger (Nanion), Drs. Sandra Wilson and Göran Mattsson (Sophion) and Dr. Ali Yehia (Fluxion) for their help with publications and clarifications. MD acknowledges the support received to carry out ion channel research in both MPC and APC formats from various sources.

Competing Interests The authors declare no competing interests. Since writing this chapter DCB has joined Sophion Biosciences A/S; however, before he joined the manuscript was written and completed as an objective review of the APC field.

References

1. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch: Eur J Physiol 391:85–100
2. Sakmann B, Neher E (1984) Patch clamp techniques for studying ionic channels in excitable membranes. Annu Rev Physiol 46:455–472
3. Nurse, P. (2015). Ensuring a successful UK research endeavour: a review of the UK Research Councils
4. Dunlop J, Bowlby M, Peri R, Vasilyev D, Arias R (2008) High-throughput electrophysiology: an emerging paradigm for ion-channel screening and physiology. Nat Rev Drug Discov 7:358–368
5. Milligan CJCJ, Li J, Sukumar P, Majeed Y, Dallas MLML, English A et al (2009) Robotic multiwell planar patch-clamp for native and primary mammalian cells. Nat Protoc 4:244–255
6. Priest, B.T., Cerne, R., Krambis, M.J., Schmalhofer, W.A., Wakulchik, M., Wilenkin, B., et al. (2004). Automated electrophysiology assays (Eli Lilly & Company and the National Center for Advancing Translational Sciences)
7. Terstappen GC, Roncarati R, Dunlop J, Peri R (2010) Screening technologies for ion channel drug discovery. Future Med Chem 2:715–730
8. Perkel JM (2010) High-throughput ion channel screening: a 'patch'-work solution. BioTechniques 48:25–29
9. Bell DC, Dallas ML (2018) Using automated patch clamp electrophysiology platforms in pain-related ion channel research: insights from industry and academia. Br J Pharmacol 175:2312–2321
10. Brüggemann A, Stoelzle S, George M, Behrends JC, Fertig N (2006) Microchip technology for automated and parallel patch-clamp recording. Small 2:840–846
11. Stoelzle S, Obergrussberger A, Brüggemann A, Haarmann C, George M, Kettenhofen R et al (2011) State-of-the-art automated patch clamp devices: heat activation, action potentials, and high throughput in ion channel screening. Front Pharmacol 2:76
12. Bridgland-Taylor MH, Hargreaves AC, Easter A, Orme A, Henthorn DC, Ding M et al (2006)

Optimisation and validation of a medium-throughput electrophysiology-based hERG assay using IonWorks™ HT. J Pharmacol Toxicol Methods 54:189–199
13. Liu C, Li T, Chen J (2019) Role of high-throughput electrophysiology in drug discovery. Curr Protoc Pharmacol 87:e69
14. McGivern JG, Ding M (2020) Ion channels and relevant drug screening approaches. SLAS Discov 25:413–419
15. Obergrussberger A, Friis S, Brüggemann A, Fertig N (2020) Automated patch clamp in drug discovery: major breakthroughs and innovation in the last decade. Expert Opin Drug Discov:1–5
16. Gillie DJ, Novick SJ, Donovan BT, Payne LA, Townsend C (2013) Development of a high-throughput electrophysiological assay for the human ether-à-go-go related potassium channel hERG. J Pharmacol Toxicol Methods 67:33–44
17. Kuryshev YA, Brown AM, Duzic E, Kirsch GE (2014) Evaluating state dependence and subtype selectivity of calcium channel modulators in automated electrophysiology assays. Assay Drug Dev Technol 12:110–119
18. Obergrussberger A, Brüggemann A, Goetze TA, Rapedius M, Haarmann C, Rinke I et al (2016) Automated patch clamp meets high-throughput screening: 384 cells recorded in parallel on a planar patch clamp module. J Lab Autom 21:779–793
19. Schupp M, Park SH, Qian B, Yu W (2020) Electrophysiological studies of GABAA receptors using QPatch II, the next generation of automated patch-clamp instruments. Curr Protoc Pharmacol 89:e75
20. Chambers C, Witton I, Adams C, Marrington L, Kammonen J (2016) High-throughput screening of NaV1.7 modulators using a giga-seal automated patch clamp instrument. Assay Drug Dev Technol 14:93–108
21. Qian B, Park SH, Yu W (2020) Screening assay protocols targeting the Nav1.7 channel using qube high-throughput automated patch-clamp system. Curr Protoc Pharmacol 89(1):e74
22. Danker T, Möller C (2014) Early identification of hERG liability in drug discovery programs by automated patch clamp. Front Pharmacol 5:203
23. Sorota S, Zhang XS, Margulis M, Tucker K, Priestley T (2005) Characterization of a hERG screen using the IonWorks HT: comparison to a hERG rubidium efflux screen. Assay Drug Dev Technol 3:47–57
24. Windley MJ, Abi-Gerges N, Fermini B, Hancox JC, Vandenberg JI, Hill AP (2016) Measuring kinetics and potency of hERG block for CiPA. J Pharmacol Toxicol Methods
25. Becker N, Horváth A, De Boer T, Fabbri A, Grad C, Fertig N et al (2020) Automated dynamic clamp for simulation of IK1 in human induced pluripotent stem cell–derived cardiomyocytes in real time using patchliner dynamite8. Curr Protoc Pharmacol 88:e70
26. Goversen B, Becker N, Stoelzle-Feix S, Obergrussberger A, Vos MA, van Veen TAB et al (2018) A hybrid model for safety pharmacology on an automated patch clamp platform: using dynamic clamp to join iPSC-derived cardiomyocytes and simulations of Ik1 ion channels in real-time. Front Physiol 8
27. Guo D, Jenkinson S (2019) Simultaneous assessment of compound activity on cardiac Nav1.5 peak and late currents in an automated patch clamp platform. J Pharmacol Toxicol Methods 99
28. Sanson C, Schombert B, Filoche-Rommé B, Partiseti M, Bohme GA (2019) Electrophysiological and pharmacological characterization of human inwardly rectifying Kir 2.1 channels on an automated patch-clamp platform. Assay Drug Dev Technol 17:89–99
29. Brinkwirth N, Takasuna K, Doi M, Becker N, Obergrussberger A, Friis S et al (2020) Reliable identification of cardiac liability in drug discovery using automated patch clamp: benchmarking best practices and calibration standards for improved proarrhythmic assessment. J Pharmacol Toxicol Methods
30. Gilbert DF, Islam R, Lynagh T, Lynch JW, Webb TI (2009) High throughput techniques for discovering new glycine receptor modulators and their binding sites. Front Mol Neurosci 2:17
31. Vasilyev DV, Shan QJ, Lee YT, Soloveva V, Nawoschik SP, Kaftan EJ et al (2009) A novel high-throughput screening assay for HCN channel blocker using membrane potential-sensitive dye and FLIPR. J Biomol Screen 14:1119–1128
32. Bagal SK, Chapman ML, Marron BE, Prime R, Storer RI, Swain NA (2014) Recent progress in sodium channel modulators for pain. Bioorg Med Chem Lett 24:3690–3699
33. Klement G, Babich O, Larsson O, Lund P-E, Malmberg A, Sandberg L et al (2012) Identification of novel NaV1.7 antagonists using high throughput screening platforms. Comb. Chem High Throughput Screen 15:713–720
34. Kornecook TJ, Yin R, Altmann S, Be X, Berry V, Ilch CP et al (2017) Pharmacologic characterization of AMG8379, a potent and selective small molecule sulfonamide antagonist of the voltage-gated sodium channel NaV1.7. J Pharmacol Exp Ther
35. Trivedi S, Dekermendjian K, Julien R, Huang J, Lund PE, Krupp J et al (2008) Cellular HTS assays for pharmacological characterization of Na V1.7 modulators. Assay Drug Dev Technol 6:167–179
36. Xie XS, Van Deusen AL, Vitko I, Babu DA, Davies LA, Huynh N et al (2007) Validation of high throughput screening assays against three subtypes of Cav3 T-type channels using molecular and pharmacologic approaches. Assay Drug Dev Technol 5:191–203
37. Swensen AM, Herrington J, Bugianesi RM, Dai G, Haedo RJ, Ratliff KS et al (2012) Characterization of the substituted N-triazole oxindole TROX-1, a small-molecule, state-dependent inhibitor of Ca(V)2 calcium channels. Mol Pharmacol 81:488–497

38. Danahay HL, Lilley S, Fox R, Charlton H, Sabater J, Button B et al (2020) TMEM16A potentiation: a novel therapeutic approach for the treatment of cystic fibrosis. Am J Respir Crit Care Med 201:946–954
39. Sauter DRP, Sørensen CE, Rapedius M, Brüggemann A, Novak I (2016) pH-sensitive K+ channel TREK-1 is a novel target in pancreatic cancer. Biochim Biophys Acta Mol Basis Dis 1862:1994–2003
40. Han B, He K, Cai C, Tang Y, Yang L, Heinemann SH et al (2016) Human EAG channels are directly modulated by PIP 2 as revealed by electrophysiological and optical interference investigations. Sci Rep 6:1–13
41. Brinkwirth N, Friis S, Goetze T, Rapedius M, Costantin J, Brüggemann A et al (2017) Investigation of the ion channels TMEM16A and TRPC5 and their modulation by intracellular calcium. Biophys J 112:413a
42. Papakosta M, Dalle C, Haythornthwaite A, Cao L, Stevens EB, Burgess G et al (2011) The chimeric approach reveals that differences in the TRPV1 pore domain determine species-specific sensitivity to block of heat activation. J Biol Chem 286:39663–39762
43. Lei CL, Clerx M, Beattie KA, Melgari D, Hancox JC, Gavaghan DJ et al (2019) Rapid characterization of hERG channel kinetics II: temperature dependence. Biophys J 117:2455–2470
44. Guo J, Zhan S, Lees-Miller JP, Teng GQ, Duff HJ (2005) Exaggerated block of hERG (KCNH2) and prolongation of action potential duration by erythromycin at temperatures between 37 °C and 42 °C. Hear Rhythm 2:860–866
45. Golden AP, Li N, Chen Q, Lee T, Nevill T, Cao X et al (2011) IonFlux: a microfluidic patch clamp system evaluated with human ether-à-go-go related gene channel physiology and pharmacology. Assay Drug Dev Technol 9:608–619
46. Kauthale RR, Dadarkar SS, Husain R, Karande VV, Gatne MM (2015) Assessment of temperature-induced hERG channel blockade variation by drugs. J Appl Toxicol 35:799–805
47. Ranjan R, Logette E, Marani M, Herzog M, Tâche V, Scantamburlo E et al (2019) A kinetic map of the homomeric voltage-gated potassium channel (Kv) family. Front Cell Neurosci 13:358
48. Chow CY, Chin YK-Y, Walker AA, Guo S, Blomster LV, Ward MJ et al (2020) Venom peptides with dual modulatory activity on the voltage-gated sodium channel Na V 1.1 provide novel leads for development of antiepileptic drugs. ACS Pharmacol Transl Sci 3:119–134
49. Gonçalves TC, Benoit E, Kurz M, Lucarain L, Fouconnier S, Combemale S et al (2019) From identification to functional characterization of cyriotoxin-1a, an antinociceptive toxin from the spider *Cyriopagopus schioedtei*. Br J Pharmacol 176:1298–1314
50. Israel MR, Dash TS, Bothe SN, Robinson SD, Deuis JR, Craik DJ et al (2020) Characterization of synthetic Tf2 as a NaV1.3 selective pharmacological probe. Biomedicines 8:155
51. Nicolas S, Zoukimian C, Belgium FB, Montnach J, Diochot S, Cuypers E et al (2019) Chemical synthesis, proper folding, nav channel selectivity profile and analgesic properties of the spider peptide phlotoxin 1. Toxins (Basel) 11:367
52. Sharma G, Deuis JR, Jia X, Mueller A (2020) Recombinant production, bioconjugation and membrane binding studies of Pn3a, a selective Na V 1. 7 inhibitor. Biochem Pharmacol:114148
53. Tzakoniati F, Xu H, Li T, Garcia N, Kugel C, Payandeh J et al (2020) Development of photocrosslinking probes based on Huwentoxin-IV to map the site of interaction on Nav1.7. Cell Chem Biol 27:306–313. e4
54. Williams WA, Linley JE, Jones CA, Shibata Y, Snijder A, Button J et al (2019) Antibodies binding the head domain of P2X4 inhibit channel function and reverse neuropathic pain. Pain 160:1989–2003
55. Xu H, Li T, Rohou A, Arthur CP, Tzakoniati F, Wong E et al (2019) Structural basis of Nav1.7 inhibition by a gating-modifier spider toxin. Cell 176:702–715. e14
56. Zoukimian C, Meudal H, De Waard S, Ouares KA, Nicolas S, Canepari M et al (2019) Synthesis by native chemical ligation and characterization of the scorpion toxin AmmTx3. Bioorganic Med Chem 27:247–253
57. Alijevic O, McHugh D, Rufener L, Mazurov A, Hoeng J, Peitsch M (2020) An electrophysiological characterization of naturally occurring tobacco alkaloids and their action on human α4β2 and α7 nicotinic acetylcholine receptors. Phytochemistry 170:112187
58. Gilbert DF, Islam R, Lynagh T, Lynch JW, Webb TI (2013) High throughput techniques for discovering new glycine receptor modulators and their binding sites. Front Mol Neurosci 2
59. Harvey AJ, Avery TD, Schaeffer L, Joseph C, Huff BC, Singh R et al (2019) Discovery of BNC375, a potent, selective, and orally available Type i positive allosteric modulator of α7 nAChRs. ACS Med Chem Lett 10:754–760
60. Skarratt KK, Gu BJ, Lovelace MD, Milligan CJ, Stokes L, Glover R et al (2020) A P2RX7 single nucleotide polymorphism haplotype promotes exon 7 and 8 skipping and disrupts receptor function. FASEB J 34:3884–3901
61. Sophocleous RA, Berg T, Finol-Urdaneta RK, Sluyter V, Keshiya S, Bell L et al (2020) Pharmacological and genetic characterisation of the canine P2X4 receptor. Br J Pharmacol 177:2812–2829
62. Stead C, Brown A, Adams C, Nickolls SJ, Young G, Kammonen J et al (2016) Identification of positive allosteric modulators of glycine receptors from a high-throughput screen using a fluorescent membrane potential assay. J Biomol Screen 21:1042–1053
63. Yehia A, Wei H (2020) Studying nicotinic acetylcholine receptors using the IonFlux™ microfluidic-based automated patch-clamp system with continuous

perfusion and fast solution exchange. Curr Protoc Pharmacol 88
64. Dale TJ, Townsend C, Hollands EC, Trezise DJ (2007) Population patch clamp electrophysiology: a breakthrough technology for ion channel screening. Mol BioSyst 3:714–722
65. Finkel A, Wittel A, Yang N, Handran S, Hughes J, Costantin J (2006) Population patch clamp improves data consistency and success rates in the measurement of ionic currents. J Biomol Screen 11:488–496
66. Hammami S, Willumsen NJ, Olsen HL, Morera FJ, Latorre R, Klaerke DA (2009) Cell volume and membrane stretch independently control K^+ channel activity. J Physiol 587:2225–2231
67. Fenno L, Yizhar O, Deisseroth K (2011) The development and application of optogenetics. Annu Rev Neurosci 34:389–412
68. Boddum, K., Skafte-Pedersen, P., Rolland, J.F., and Wilson, S. (2021). Optogenetics and optical tools in automated patch clamping. In: Methods in Molecular Biology.
69. Obergrussberger A, Goetze TA, Brinkwirth N, Becker N, Friis S, Rapedius M et al (2018) An update on the advancing high-throughput screening techniques for patch clamp-based ion channel screens: implications for drug discovery. Expert Opin Drug Discov 13:269–277
70. McNamara HM, Zhang H, Werley CA, Cohen AE (2016) Optically controlled oscillators in an engineered bioelectric tissue. Phys Rev X 6:031001
71. Zhang H, Reichert E, Cohen AE (2016) Optical electrophysiology for probing function and pharmacology of voltagegated ion channels. Elife:5
72. Zhang H, Moyer BD, Yu V, McGivern JG, Jarosh M, Werley CA et al (2020) Correlation of optical and automated patch clamp electrophysiology for identification of NaV1.7 inhibitors. SLAS Discov 25:434–446
73. Becker N, Stoelzle S, Göpel S, Guinot D, Mumm P, Haarmann C et al (2013) Minimized cell usage for stem cell-derived and primary cells on an automated patch clamp system. J Pharmacol Toxicol Methods 68:82–87
74. Li T, Lu G, Chiang EY, Chernov-Rogan T, Grogan JL, Chen J (2017) High-throughput electrophysiological assays for voltage gated ion channels using SyncroPatch 768PE. PLoS One 12:e0180154
75. Ong ST, Bajaj S, Tanner MR, Chang SC, Krishnarjuna B, Ng XR et al (2020) Modulation of lymphocyte potassium channel K V 1.3 by membrane-penetrating, joint-targeting immunomodulatory plant defensin. ACS Pharmacol Transl Sci
76. Rotordam MG, Fermo E, Becker N, Barcellini W, Brüggemann A, Fertig N et al (2019) A novel gain-of-function mutation of piezo1 is functionally affirmed in red blood cells by high-throughput patch clamp. Haematologica 104:e179–e183
77. Toh MF, Brooks JM, Strassmaier T, Haedo RJ, Puryear CB, Roth BL et al (2020) Application of high-throughput automated patch-clamp electrophysiology to study voltage-gated ion channel function in primary cortical cultures. SLAS Discov 25:447–457
78. Haythornthwaite A, Stoelzle S, Hasler A, Kiss A, Mosbacher J, George M et al (2012) Characterizing human ion channels in induced pluripotent stem cell-derived neurons. J Biomol Screen 17:1264–1272
79. Li W, Luo X, Ulbricht Y, Wagner M, Piorkowski C, El-Armouche A et al (2019) Establishment of an automated patch-clamp platform for electrophysiological and pharmacological evaluation of hiPSC-CMs. Stem Cell Res 41:101662
80. Neher E, Sakmann B (1976) Single-channel currents recorded from membrane of denervated frog muscle fibres. Nature 260:799–802
81. Zhu W, Li T, Silva JR, Chen J (2020) Conservation and divergence in NaChBac and NaV1.7 pharmacology reveals novel drug interaction mechanisms. Sci Rep 10:10730
82. Heyne HO, Baez-Nieto D, Iqbal S, Palmer D, Brunklaus A, Collaborative E et al (2019) Predicting functional effects of missense variants in voltage-gated sodium and calcium channels. BioRxiv:671453