Patch clamp technique: review of the current state of the art and potential contributions from nanoengineering

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Abstract: The patch clamp technique permits high-resolution recording of the ionic currents flowing through a cell's plasma membrane. In different configurations, this technique has allowed experimenters to record and manipulate the currents that flow either through single ion channels or those that flow across the whole plasma membrane. Unfortunately, the conventional patch clamp method is laborious, requiring the careful fabrication of electrodes, skillful manipulation of the patch pipette towards a cell, and the clever design of electronics and apparatus to allow low-noise recordings. Advances in microfabrication offer promising technologies for high-throughput patch clamp recordings, particularly suitable for drug screening. This paper provides a review of the advances that have been made in the patch clamp technique over the years and considers where application of nanotechnology might provide significant contributions in the future.

Keywords: patch clamp technique, automation, microfabrication, electrophysiology, nanoactuation

1 INTRODUCTION

Cells are packed with nanoscale structures: the components of most cellular organelles have dimensions in the nanoscale range. The endoplasmic reticulum, nuclear and mitochondrial membranes, the Golgi apparatus, microtubules, and actin and myosin filaments are all examples of cellular structures that are only rendered visible through electron microscopy or other techniques that can resolve detail in the 100 nm or lower range. The cell's plasma membrane is another example. This less-than-10 nm-thick skin separates the cell's cytoplasm from the extracellular medium in which it resides. Although this membrane is formed mostly of a bilayer of lipid molecules, buried within the lipid bilayer are protein molecules that serve the functions of cell recognition, transport of chemicals into and out of the cytoplasm and ion channels that underlie changes in cellular function. These nanoscale-sized channels show ion species selectivity and, for different varieties, are gated by intra- or extracellular ligands, by mechanical stress, by changes in extracellular pH, or by changes in the electrical potential across the membrane. Currently, the most widely used method for studying the electrophysiological properties of biological membranes and the currents that flow through their ion channels is the patch clamp technique. This technique permits low noise measurement of the currents passing through the low conductance (pS) ion channels, by isolating a small patch of the membrane, which sometimes can contain just a single channel.

By regulating the flow of specific ions across the cell membrane, ion channels are involved critically in

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a variety of physiological processes, from maintenance of the heartbeat to muscle contraction to the discharge of neural action potentials. The adoption of the patch clamp technique by numerous laboratories has led, therefore, to significant advances in the life sciences, notably the characterization of the properties of ion channels in a variety of cells and the pharmacological treatment of diseases implicated with ion channels such as diabetes and heart disease [**1**]. Although it was pioneered in a specialized field [**2**], the potential applications of the patch clamp technique are vast, especially in combination with other techniques in imaging (e.g. microfluorimetry), electrochemistry (e.g. amperometry), and molecular biology. This review discusses the development of the patch clamp technique where nanoengineering has played a part in this development, and areas where nanoengineering may have a significant future impact.

2 HISTORICAL DEVELOPMENT

The patch clamp technique stems from the development of increasingly refined electrodes for intracellular recordings of bioelectrical signals from ever smaller structures. Luigi Galvani provided the first experimental evidence of electrical activity in animals, evoking muscular contractions in frog nerve-muscle preparations by electrical stimulation with metal wires [**3**]. For the first intracellular measurement of the action potential, Hodgkin and Huxley inserted long glass capillaries filled with saline or metal into the giant squid axon, whose diameter measures up to 1 mm [**4**]. Impaling micropipettes developed by Graham *et al.* [**5**, **6**] allowed the study of finer structures such as skeletal muscle fibres, where pipettes with smaller tips (outer diameter less than 5 μm), and hence higher resistances, produced successful and consistent recordings. Around the same time, Cole [**7**] and Marmont [**8**] developed the voltage clamp technique, which, combined with micropipettes, permitted intracellular recordings of both the membrane voltage and current of individual cells in single [**9**] and two-electrode [**10**] modes. Glass micropipettes also facilitated intracellular perfusion [**11**, **12**]; however, the impalement of the cell membrane generated large leakage currents.

The desire for low-noise recordings of current flowing through ion channels of the cell membrane and the ability to analyse their regulatory mechanisms by internal perfusion prompted Sakmann and Neher to develop the patch clamp technique, for which they received the Nobel Prize in Physiology and Medicine in 1991. Their initial methodology involved pressing the blunt tip (diameter 0.5–2 μm) of a heat-polished pipette onto the surface of the cell membrane to establish a MΩ seal [**2**, **13**], so that the ionic current will only flow into the pipette and not through the seal. As the seal resistance increases with decreased surface area, electrical isolation of a small patch of the membrane reduces the current leakage through the seal sufficiently so that even the small currents flowing through single ion channels could be detected extracellularly. The cornerstone discovery that gentle suction could establish a GΩ seal (gigaseal) between the pipette and the patched membrane [**14**] led to significant improvements in the quality of patch clamp recordings and the expansion of the technique through several additional configurations.

3 PATCH CLAMP CONFIGURATIONS

3.1 Extensions of the cell-attached configuration

Figure 1 illustrates the various configurations of the patch clamp technique. As a non-invasive technique, the initial 'cell-attached' configuration (Fig. 1(a)), so named because the patched membrane adheres

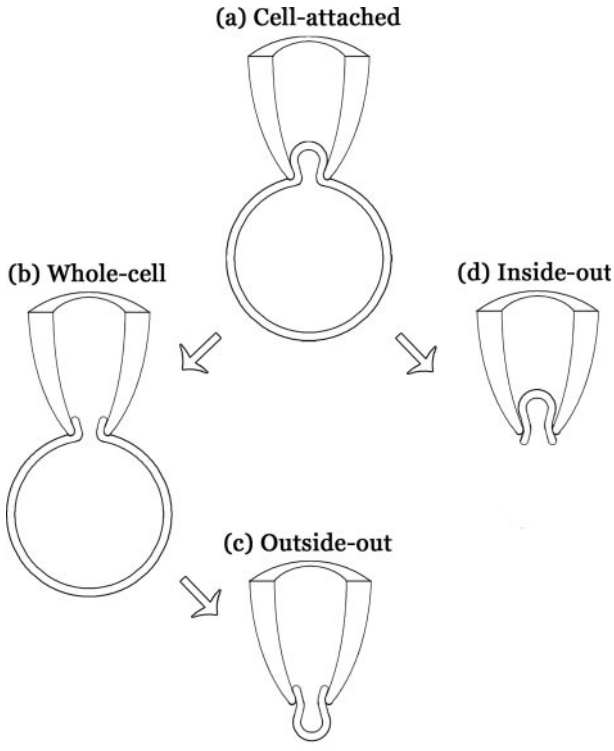

Fig. 1 Configurations of the patch clamp technique: (a) the cell-attached configuration; (b) whole-cell configuration obtained by the patched membrane being ruptured; (c) outside-out configuration, due to pulling the pipette from whole-cell mode; (d) inside-out configuration, resulting, alternatively, from pulling the pipette away from the cell in the cell-attached configuration

tightly to the pipette, controls neither the cell membrane potential nor the intracellular environment of the patch. In the most cited paper on the patch clamp technique, Hamill *et al.* detailed several variants of this technique to create complete electrical and/or mechanical isolation of the patched membrane for a variety of cells, establishing many of the methodologies still employed today [**15**].

The 'whole-cell' configuration (Fig. 1(b)), the method of choice for most cell-culture and tissue preparations, ruptures the patch created in the cell-attached configuration with a pulse of suction or voltage, thus establishing low-resistance electrical and physical continuity between the cell and the pipette lumen. Access to the cell interior allows the voltage of the whole cell to be controlled via a voltage clamp, the ensemble currents from all the ion channels in the cell membrane to be observed, and individual current types to be separated by controlling the chemical composition on both sides of the cell membrane. Furthermore, the whole-cell configuration permits the monitoring of the exocytotic activities of secretory cells through cell capacitance measurements [**16**].

Due to the stability and tightness of the gigaseal, cell-excised configurations of membrane (Fig. 1(c)–(d)) can be achieved by withdrawing the pipette from the rest of the cell, thus allowing the experimenter to control the intracellular and extracellular environments of the patched membrane. Like the cell-attached configuration, cell-excised recordings are used to study individual ion channels from a section of the cell membrane. Pulling the pipette from the cell-attached configuration establishes an 'inside-out configuration' (Fig. 1(d)) that exposes the cytosolic side of the patch to the bath solution, which can be changed through perfusion. Thus, the inside-out configuration is particularly desirable for studying ion channels that are regulated by intracellular ligands. On the other hand, withdrawing the pipette from whole-cell configuration establishes the 'outside-out' configuration (Fig. 1(c)) which generally results in a resealing of the cell membrane so that the outside of the membrane faces the bath solution. The outside-out configuration is desirable for studying extracellularly ligand gated ion channels, but is harder to obtain, given the number of steps involved. The use of pipettes with submicrometer tips may help to establish gigaseals, isolate single ion channels, and promote membrane resealing in the outside-out configuration.

3.2 Perforated patch recording

During whole-cell recordings, irreversible 'washout' of diffusible intracellular constituents (e.g. adenosine triphosphate (ATP), phosphorylating molecules, and intracellular Ca^{2+}) into the relatively larger volume of the pipette through dialysis [**17**] can significantly impair the properties and functions of ion channels, hence decreasing the ionic currents through them over time [**18–21**] and complicating data analysis. The perforated patch clamp technique seeks to overcome this problem by accessing the intracellular space not by seal rupture but by forming pores in the cell membrane in the cell-attached mode. Loading ATP [**22**] into the recording pipette permeabilizes the membrane to monovalent ions but blocks larger ions and molecules, thus reducing current rundown. However, using ATP as a pore-forming agent creates pipette access resistances that are much higher than those in the standard whole-cell configuration. Consequently, the bandwidth of the measurable signals, inversely proportional to the product of the access resistance and membrane capacitance, decreases, earning the technique the name 'slow whole-cell'. On the other hand, the use of the polyene antibiotics nystatin [**17**] and amphotericin B [**23**] generates access resistances comparable to those in the standard whole-cell technique. However, these hydrophobic ionophores interfere with gigaseal formation (overcome by filling the pipette tip with polyene-free solution), are unstable in electrolyte, being light and heat sensitive and losing potency over time, and cause Cl^- redistribution between the cell and the pipette, generating a Donnan potential difference [**24**] across the semiporous membrane. Since the concentration gradient of Cl^- affects several signalling mechanisms and the Donnan voltage errors can be quite high (10 mV), gramicidin D, a mixture of antibiotics which forms channels selectively permeable to monovalent cations and uncharged molecules but not Cl^-, has subsequently been used to perforate the cell membrane [**25**, **26**]. At low concentrations, gramicidin D solutions do not interfere with seal formation, but prefilling the pipette tip with gramicidin-free solution is necessary for higher concentrations, which frequently spontaneously rupture the cell membrane. The access resistances achieved are slightly higher than when using antibiotic-free pipette-filling solutions and the potency of the pore-forming agent degrades over 1–2 h [**26**]. Due to their interference with gigaseal formation, quick loss of potency, and difficulties in preparation, polyene and gramicidin antibiotics were found to lead to low success rates, prompting the use of the hydrophilic saponin β-Escin in perforated recordings from myocytes [**27**] and neurons [**28**]. Pipette solutions with this ionophore can be stored for a long time and interfere less with gigaseal formation.

Electroporation, using high voltages to cause dielectric breakdown of the cell membrane, can also create pores that allow compounds to cross the lipid bilayer and is routinely used for inserting plasmid

DNA into bacteria. This method can be extended to the patch-clamp technique to transfect single cells [**29**, **30**]. Whereas early work on electroporation used large voltages, the modified technique exploiting the patch clamp electrode applies potentials of just a few volts to the membrane, creating localized pores through which genes and other molecules may pass. Since only a small portion of the membrane is disturbed, the cell remains healthy after electroporation. The use of nanoscale pipettes might further prevent washout of diffusible intracellular constituents.

4 PATCH CLAMP TECHNOLOGY

4.1 Conventional patch clamp technology

4.1.1 Patch pipettes

While the patch pipette acts simply as a fluid bridge between a cell and the transducing Ag/AgCl electrode, its properties significantly determine how easily gigaseals are formed, how stable they remain, and the quality of the recordings. The wide variety of glass capillaries available for fabricating the patch clamp electrode can be classified according to their thermomechanical and electrical properties. Thermal properties determine how easily glass capillaries can be shaped into the desired pipette profile (e.g. orifice diameter, the taper length of the conical tip, and wall thickness) during pulling and fire polishing, which smoothens the pipette edges to avoid damaging the cell membrane. Electrical properties, on the other hand, affect the response of the patch electrode. The series resistance of the electrode, determined in part by the tip geometry, and the distributed pipette capacitance constitute a low-pass filter that slows the response of the current measuring circuit. The capacitance of the patch pipette, a function of its material composition, wall thickness, and immersion depth, also affects the noise produced by the glass and the size of the capacitive transients in response to changes in the stimulating voltage signal. Coating the pipette tip with an insulating material such as Sylgard 184 (Dow Corning, Midland, MI) decreases the capacitance between the pipette lumen and the bath meniscus that creeps up the electrode, and hence the capacitive noise [**31**]. Glass material composition also influences the sealing properties of the pipette with different cells and ion channel behaviour if leachable components enter the pipette filling solution. In general, the ideal patch pipette has a blunt tip that will not damage the cell membrane, and low-access resistance and capacitive transients that can be easily compensated for by the patch clamp amplifier.

Soft glasses (e.g. soda lime) that soften at a lower temperature than hard glasses (e.g. borosilicate and quartz) produce pipettes with smoother tips and are easier to fire-polish [**32**]. By pressurizing the pipette lumen during fire-polishing, the pipette cone angle of even hard glasses can also be widened [**31**]. Flyion GmbH (Tübingen, Germany) offers a fully automatic Feedback Microforge, based on the pressure-polishing technique, to reshape the pipette tip to any desired geometry. In particular, larger cone angles were shown to have lower access resistances and are thought to achieve whole-cell configurations more easily, possibly by providing a larger surface area for glass–lipid interaction inside the pipette. Lower access resistances reduce errors in the command current needed to maintain the membrane voltage clamp, increase the signal bandwidth, and decrease the RC noise associated with the access resistance and the cell membrane capacitance.

Glasses with low dielectric loss factors, the imaginary component of the relative dielectric constant, generate lower noise levels and smaller capacitance transients [**33**]. Unfortunately, quartz glass [**34**], which has the lowest dielectric loss factor, has a high melting point and is capable of being pulled only by laser-based pipette pullers (e.g. Sutter P-2000). Alternatively, in addition to coating soft glasses with Sylgard to decrease pipette capacitance, high-lead borosilicate glass that has a low softening temperature and good electrical properties can be used. However, lead glass contains leachable components that can block ionic currents [**35**, **36**].

4.1.2 Ag/AgCl electrodes

A metal electrode inside the pipette accomplishes transduction of the ionic current into the electrical current while another one in the bath solution serves as ground. The low impedance of metal electrodes at a high frequency makes them amenable for recording rapid signals. Pt and Ag/AgCl electrodes are especially attractive for their low junction potentials and weak polarization; however, Pt electrodes are expensive. Ag/AgCl electrodes, which are commonly used in patch clamp electrodes, must, on the other hand, maintain a layer of AgCl, responsible for the reversible redox reaction at the electrode surface and stable half-cell potential in electrolytes containing Cl^- as the main anion. As this layer scrapes off during pipette changes, the electrode must be recoated periodically, either by dipping into bleach mainly consisting of sodium hypochlorite or through electrophoresis in a chloride solution, which affords more control over the coating thickness. A thin coat of AgCl stabilizes the potential difference between the electrode and electrolyte while excessive coating tends to increase the resistance of the electrode, since AgCl has a higher specific resistivity than Ag. Moderate coating may minimize the electrode–electrolyte interface impedance by increasing the effective

surface area of the electrode [**37**]. An intermediate coating thickness can also be obtained by first heavily coating an Ag wire and then removing a portion of the initial deposit by reversing the voltage bias, resulting in electrodes with a lower and more stable electrode impedance [**38**].

It is also desirable that the electrode–electrolyte junction potential be stable during an experiment to avoid offset artefacts. Because changing the superfusate could cause large shifts in the ionic composition of the bath, the ground electrode is placed in a separate compartment from the working electrode and connected to the bath via an agar salt bridge with KCl. Similarly, soft agar bridges have been implemented in patch clamp holders to minimize the junction potential between the Ag/AgCl electrode and the pipette solution with large changes in the Cl^- concentration, leading to large drifting offset potentials [**39**–**41**].

4.1.3 Pipette perfusion

In many studies of ion channel functions, electrophysiologists analyse the effects of various drugs on the channel currents, most simply accomplished by probing different cells or different locations on the cell membrane with pipettes loaded with different solutions. Due to cell-to-cell variation and variations in channel density along the cell membrane, a better approach is to perfuse the pipette by altering the patch clamp holder. Cull-Candy *et al.* inserted a multibarrel pipette inside a patch electrode within 100 μm of the tip and the desired solution was released by applying air pressure to a hypodermic needle connected to the appropriate barrel [**42**]. Alternatively, a short inflow polyethylene tubing [**41**] or polymer-coated quartz capillary [**43**] near the tip connected to the perfusion reservoir can also provide the exchange electrolyte while an outflow tube further up from the tip serves to collect the unwanted solution. Finally, Alpert *et al.* used a double-barrel pipette, with the septum partially broken, to add new solution to one barrel and remove old solution from the other [**44**].

4.1.4 Measurement of exocytosis

Using basic impedance analysis, cell exocytosis can also be quantified by the patch clamp technique by measuring the membrane capacitance, membrane conductance, and access resistance in the whole-cell configuration. The 'time domain' technique derives these parameters from analysis of the capacitive transients in response to square wave stimulation whereas the 'frequency domain' technique applies a sine wave stimulus to the cell, resolves the amplitude and phase of the resulting complex current signal using a lock-in amplifier, and determines the parameters from basic circuit analysis [**16**, **45**, **46**].

Artefacts and errors in membrane capacitance measurements using these methods are avoided by using amperometry, which detects the actual secreted products. Dernick *et al.* combined these two complementary methods in patch amperometry, in which a carbon fibre electrode that oxidizes the released molecules and the reference electrode are placed inside the patch pipette while the Ag/AgCl electrode is placed instead in the bath [**47**]. A nanomotor inside the electrode holder controls the distance between the carbon fibre electrode and the pipette tip. Using this technique, exocytosis of single vesicles are detected in the cell-attached configuration by changes in the cell membrane capacitance measurements, indicative of vesicular fusion with the cell membrane and by amperometric detection of the released particles.

4.1.5 Push pen electrode

Our laboratory has designed a patch clamp electrode assembly for low-series resistance whole-cell recordings using a 'push pen' approach to actuate an etched Ag/AgCl electrode through the patch pipette (Fig. 2). The technical details of the device will be described elsewhere. The movable Ag/AgCl electrode, as opposed to the typically stationary Ag/AgCl electrode which is placed far from the pipette tip, can clear debris from the pipette orifice to sustain longer recording sessions and decrease the access resistance

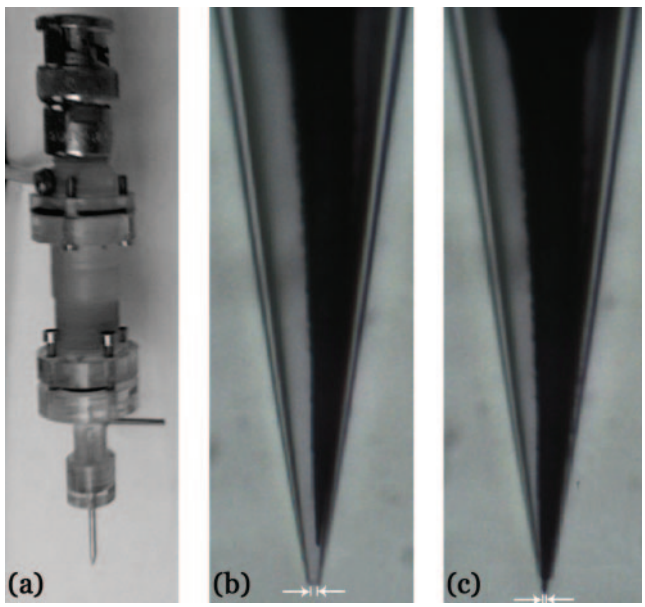

Fig. 2 Push pen patch clamp electrode: (a) electrode holder, (b) pipette (1.1 μm tip radius) and Ag/AgCl wire (0.5 μm tip radius) inside the glass pipette, and (c) protruding from the pipette tip

between the interior of the pipette and the cytoplasm, thereby increasing the measurable signal bandwidth. Impedance measurements have shown that positioning the metal electrode closer to the pipette orifice provides improvement in the electrode impedance, particularly in the 1–100 kHz frequency range, owing to displacement of the less conductive electrolyte. Positioning the Ag/AgCl electrode closer to the pipette tip can also limit the diffusion of intracellular regulatory molecules from the cytoplasm into the pipette, much like the perforated patch technique.

4.1.6 Patch clamp electronics

While traditional microelectrode amplifiers employ voltage followers, the patch clamp headstage is a sensitive current-to-voltage (IV) converter. The initial amplifier design of Hamill *et al.* monitors the pipette current (I_p) through the voltage drop (V_{R_f}) across the feedback resistor (R_f) of an inverting amplifier (Fig. 3) [**15**]. Since the operational amplifier has high gain, the pipette voltage (V_p) also follows the command voltage (V_{cmd}). Using large values of R_f minimizes the Johnson thermal current noise, important for resolving the minute currents during single channel recordings, but limits the current resolution of whole-cell recordings, which require lower feedback resistances to allow the passage of large currents. Moreover, the stray capacitances C_{R_f} across R_f limits the time resolution of the amplifier to $\tau_f = R_f C_{R_f}$ (about 1 ms). To increase the bandwidth of the IV converter, resistive headstages are coupled to compensation circuits that correct the limited high-frequency response through pole cancellation.

Alternatively, capacitive headstages [**48**, **49**] use a feedback capacitor to integrate the pipette current, which is subsequently differentiated to yield I_p (Fig. 4). Unlike GΩ resistors that possess intrinsic noise in excess of thermal noise, commercial capacitors have more ideal properties, thus generating lower noise levels, responding faster, and having a wider dynamic range than resistive headstages. However, the charge across C_f must be periodically discharged when its voltage approaches the supply voltage to accommodate steady current input that would otherwise saturate the operational amplifier. Meanwhile, V_O is held by a sample and hold circuit during the short reset time. The frequency of discharge depends on the magnitude of the current.

Commercial headstages such as the Axopatch 200B (MDS Analytical Technologies, Toronto, Canada, formerly Axon Instruments) contain both resistive and capacitive feedback circuits for whole-cell and single channel recordings respectively. In whole-cell recordings, the amplifier must compensate the series resistance between the cytosol and the pipette, thus

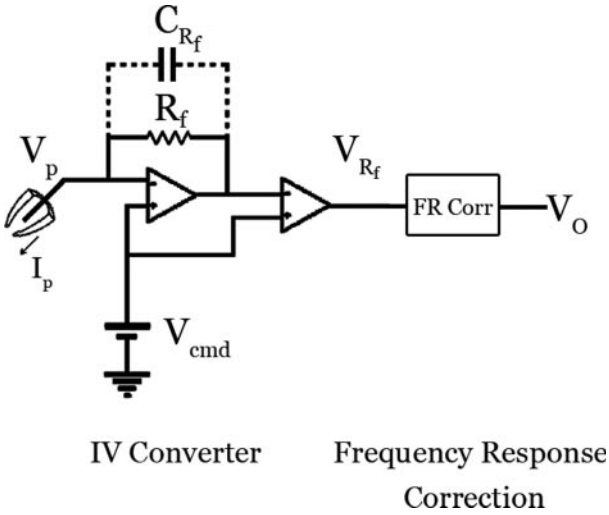

Fig. 3 Resistive headstage of the patch clamp amplifier: the recording electrode is connected to the negative input of an operational amplifier whose high gain causes the pipette voltage V_p to follow the command voltage V_{cmd} at the positive input of the operational amplifier. Due to the high input resistance of the operational amplifier, the pipette current I_p flows through the feedback resistor R_f and is thus proportional to the voltage output V_{R_f} of the differential amplifier. A subsequent frequency correction circuit boosts the limited bandwidth of the IV converter owing to the stray capacitance C_{R_f} across R_f

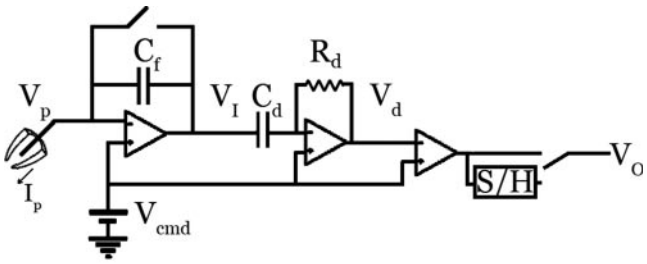

Fig. 4 Capacitive headstage of the patch clamp amplifier: as in the case of the resistive headstage, the recording electrode is connected to the negative input of an operational amplifier whose high gain causes the pipette voltage V_p to follow the command voltage V_{cmd} at the positive input of the operational amplifier. The pipette current I_p flows through the feedback capacitor C_f and is thus proportional to the derivative of the voltage output (V_I) of the negative feedback amplifier. Subtraction of the output of the differentiating amplifier ($V_d = R_d C_d \, dV_I/dt$) from V_{cmd} by a differential amplifier yields $V_O = R_d C_d \, I_p / C_f$. During the periodic discharge of C_f, the output V_O is held by a sample and hold circuit

limiting current resolution and the speed at which the cell membrane responds to the voltage clamp. Additional capacitive transient cancellation circuitry compensates for fast and slow transients that appear in the current step response owing to the charging of the pipette and the cell membrane. The use of nanotechnology to condense patch clamp amplifiers into integrated circuits may lead to superior electrical performance since minimization of parasitic capacitances reduces the noise level and increases the bandwidth, enabling parallel patch clamping operations.

4.2 High throughput patch clamp systems

4.2.1 Automated patch clamp systems

To simplify the time-consuming patching procedure and to achieve higher throughput and reproducibility, automated patch clamping systems using conventional electrodes were developed. For example, Apatchi-1 (Sophion Bioscience, Ballerup, Denmark) uses automatic cell recognition and precise pipette positioning to patch onto cells sequentially in a culture dish. Conversely, the AutoPatch (Xention Discovery Limited, Cambridge, UK) presents a 'hanging drop' of cells from a glass capillary tube (Fig. 5(a)), suspended at a liquid–air interface by virtue of surface tension, to the upright tip of a pipette. Its successor, the AP-2, performs sequential recordings, while the AP-3 operates in parallel. The Flyscreen (Flyion GmbH, Tübingen, Germany), based on the 'Flip-the-Tip' technology (Fig. 5(b)) of Lepple-Wienhues *et al.* [**50**], deviates from the conventional patch clamp methodology by dispensing cell suspensions to the back end of a standard pipette and applying suction to pull a single cell to the tip of the pipette and to form a gigaseal. An alternative to the patch clamp technique, automated two-electrode voltage clamp (TEVC) systems examine ion channels in oocytes in serial (e.g. Roboocyte from Multi Channel Systems, Reutlingen, Germany) or in parallel (e.g. OpusXpress from MDS Analytical Technologies, formerly Molecular Devices). However, none of the automated systems are capable of studying neural network dynamics, since the measurements are performed on isolated cells in separate compartments; nor can they achieve a high degree of parallelism owing to the complexity of controlling multiple electrodes.

4.2.2 Planar patch clamp arrays

Rapid advances in microfabrication and microfluidics led several laboratories to focus on the development of planar arrays for automated electrophysiological measurements, catering to high throughput pharmacological testing of compounds

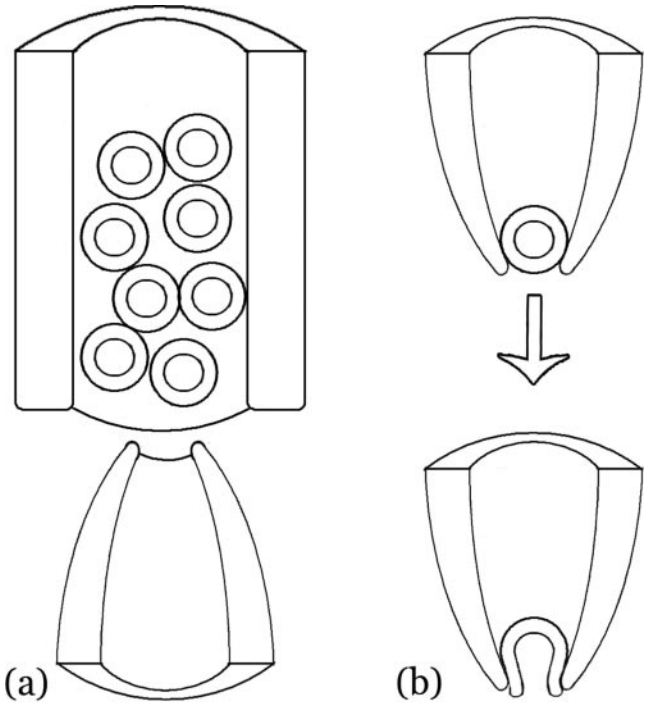

Fig. 5 (a) 'Hanging drop' interface in the autopatch: a glass capillary tube is filled with cells, suspended at the liquid–air interface by virtue of surface tension. An upright patch electrode is then inserted into the droplet; application of suction at the tip achieves gigaseal formation and whole-cell configuration. (b) 'Flip the tip' approach in the flyscreen: cell suspensions are dispensed to the back end of a patch pipette. Application of suction pulls a single cell to the tip and forms a gigaseal

on ion channel dynamics [**51**]. In general, cell cultures are suspended on a planar low-loss dielectric partition into which micrometer apertures, emulating pipette openings, are etched and connected to amplifiers via metal contacts (Fig. 6). The application of negative pressure pulls the cells onto the holes to form gigaseals and to rupture the membranes to obtain whole-cell configuration. Several planar patch clamp chip designs fabricated from different materials have been explored with varied success in forming gigaseals.

Early electrode arrays of silicon wafers insulated with silicon oxide or silicon nitride produced large capacitive noise and failed to form gigaseals with cells, possibly owing to the limited contact area between the cell membrane and the aperture, but had some success with liposomes [**52**] and artificial lipid membranes [**53**]. Nevertheless, Pantoja *et al.* incorporated microfluidic layers into each side of a silicon chip, into which a micrometer pore was etched [**54**]; however, gigaseals seldom formed, possibly due to the coarseness of the contact surface.

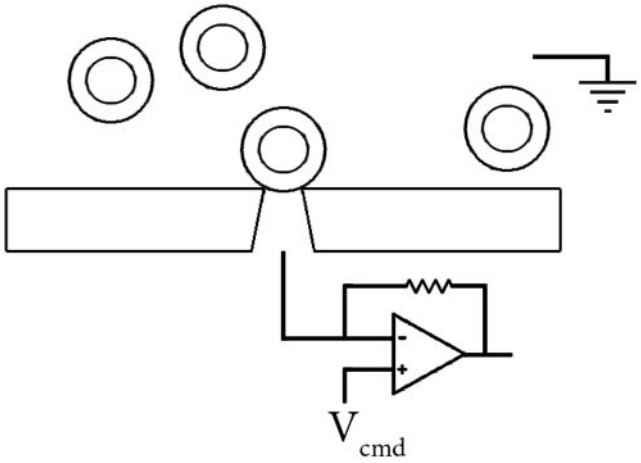

Fig. 6 Schematic of a general planar patch clamp array: cells are suspended on one side of a planar partition while metal contacts leading to current-to-voltage converters are on the other side. Application of negative pressures sucks the cells onto apertures etched into the partition, forms gigaseals, and breaks the cell membrane to achieve whole-cell configuration

Sophion Biosciences has, nevertheless, developed a silicon-based patch clamp system called the QPatch [**55**], which is the first planar system with microfluidic capabilities.

The first successful demonstration of planar patch clamp recordings from cells was made by Fertig *et al.* who used fused quartz tracked-etched to create tapering holes, a geometry similar to traditional patch pipettes. While they reported just a 30 per cent success rate of patching, the quartz planar electrodes achieved a threefold lower seal resistance than those formed with pipettes, possibly owing to surface roughness after etching [**56**]. The NPC-16 Patchliner and SyncroPatch 96 from Nanion Technologies GmbH (Munich, Germany) are based on similar borosilicate planar designs [**57**] with a miniature version available in the form of a single channel unit, the Port-A-Patch. Cytocentrics CCS GmbH is also developing a parallel planar system with disposable quartz chips, the CytoPatch Chip [**58**], in which two concentric channels are embedded: one applies suction to position the cell onto the aperture and the other to patch clamp and record from the cell.

The first commercially available automated platform, the IonWorks [**59**] from MDS, formerly Molecular Devices, does not form gigaseals with cells, and therefore generates lower quality recordings, but achieves the highest throughput. Using suction to draw cells toward holes in the well plates, the IonWorks HT and Quattro form MΩ seals, sufficient only for measuring large currents, use pore-forming agents to permeabilize the cell membrane. PatchXpress from Axon Instruments, now MDS, was the first planar patch clamp system to enable gigaseal formation by using SealChips from Aviva Biosciences (San Diego, CA). The special coatings on these glass chips promote gigaseal formation, which with simple suction seals cells about 75 per cent of the time [**60**].

Alternatively, Klemic *et al.* demonstrated a 13 per cent success rate of macro patching (apertures around 4 μm) onto Xenopus oocytes with micromolded polydimethylsiloxane (PDMS) [**61**]. PDMS is better known as the silicone elastomer Sylgard (Dow Corning, Midland, MI) and must undergo plasma oxidation to render the surface hydrophilic in order to form gigaseals, a treatment whose effect wears off relatively quickly, thus limiting the shelf life of PDMS patch partitions.

Planar patch clamp arrays have the potential to increase throughput and minimize capacitive noise through the use of low-loss dielectric materials. Nevertheless, the biggest technical challenge for automated patch-clamp recording lies in guiding the cells to the recording sites, successfully forming gigaseals, and achieving low-noise recordings. Therefore, although patching isolated cells and oocyte expression systems may be automated, more technically demanding experiments such as precise patching onto multiple small structures in a preparation (e.g. axons, dendrites, and soma of neurons) continues to rely heavily on the skill of the electrophysiologist using traditional patch clamp systems. Moreover, it remains the gold standard that advances in the basic operation of the patch clamp must be tested with the traditional glass/metal patch clamp electrode before implementation in planar arrays.

5 RECORDING FROM SUBMICRON STRUCTURES

In addition to a mechanically stable manipulator to facilitate fine movement of the pipette, patch clamp recordings from multiple small structures require tools of compatible size. A major concern with nanoscale electrodes is their considerable tip resistance, inversely related to the area of the pipette opening, and the increased probability of clogging the tip, both of which significantly raise the access resistance during whole-cell recordings, slowing the charging of the cell membrane and filtering high-frequency signals. However, finite element simulations of the patch electrode indicate that the impedance increase due to downscaling is logarithmic, so that the impedance difference between a nano- and micropipette is not as large as expected (unpublished data).

6 WHERE NANOENGINEERING CAN IMPACT PATCH CLAMP TECHNOLOGY

This review has discussed the evolution of the patch clamp technique from its origins in basic science to its commercial application in drug discovery. The technique is responsible for more than 30 000 scientific papers and is the default method for studying cellular electrophysiology today. The basic limitation of this method is the art required for its use, which becomes particularly challenging as the biological structures to be studied become smaller. A few places where nanoengineering has already had an impact have been discussed and, hopefully, this review has provided a sufficiently detailed description of the state-of-the-art to prompt readers themselves to propose new nano-inspired solutions to some of the problems that remain to be overcome. Methods to reduce access resistance for small pipette orifices, technology to permit simultaneous patch clamp recordings from multisites on either one cell or from many cells in a cellular network, and the application of nanoengineering to improve the success rate of planar patch clamp arrays are obvious areas for research and development. Superior designs of integrated nanoelectronic patch clamp amplifiers may further lower noise and increase signal bandwidth. Integration of nanofluidic channels in the patch electrode or array could allow fast switching of attolitres of perfusate to study ion channel dynamics. New nanomaterials for coating or methods of functionalization of the pipette may better facilitate gigaseal formation. Novel composites for the inner electrode, perhaps with carbon nanotubes, and chemical functionalization of their surfaces are perhaps worthy of investigation in order to promote greater charge transfer and/or sense cellular biochemistry. Furthermore, advances in nanoactuation could permit inner electrodes to advance and withdraw within the patch pipette and to perform ion conductance imaging with traditional patch pipettes. The patch clamp technique has had a large impact on the life sciences temporally in parallel with the emergence of the field of nanoscience. One wonders how the application of nanoengineering to this technology will magnify its impact in the future.

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APPENDIX 1

Notation

C_d	input capacitor to the differentiating amplifier in the capacitive headstage
C_f	feedback capacitor of the IV converter in the capacitive headstage
C_{R_f}	stray capacitor associated with R_f in the resistive headstage
I_p	pipette current
R_d	feedback resistor of the differentiating amplifier in the capacitive headstage
R_f	feedback resistor of the IV converter in the resistive headstage
V_{cmd}	command voltage of the patch clamp amplifier
V_d	output voltage of the differentiating amplifier in the capacitive headstage
V_I	output voltage of the IV converter in the capacitive headstage
V_O	voltage output of the patch clamp headstage
V_p	pipette voltage
V_{R_f}	voltage across R_f in the resistive headstage
τ_f	time constant of the IV converter in the resistive headstage