

Chapter 2

Development of Patch Clamping

Patch clamping was first introduced into biophysical studies by Neher and Sakmann in [1] 1976 and was soon expanded to many other fields such as biology and medicine. The technique not only allowed the detection of single-channel currents in biological membranes for the first time but also enabled higher current resolution, direct membrane patch potential control, and physical isolation of membrane patches [2]. The development of the patch clamp method was honoured with a Nobel Prize in 1991. The conventional technique of patch clamping uses a glass micropipette to study every single cell individually. The nature of the technique makes it laborious, time-consuming and very low in throughput yet providing high-quality recordings. To achieve higher throughputs mainly two approaches have been adopted over the past decade: automation of conventional patch clamping, and using planar/lateral patch clamp systems. Planar and lateral patch clamping systems take advantage of microfabrication techniques, microfluidics and nanotechnology to overcome many of the difficulties of conventional patch clamping. However the low seal resistances of planar and lateral patch clamping systems have prevented them from becoming a complete alternative to the conventional technique. Because of its superior data quality confirmation of functional activities is often based on the data obtained by conventional patch clamp recordings. However no matter which approach is used, gigaseal formation is the bottleneck in developing high throughput systems capable of producing high-quality recordings.

2.1 Conventional Patch Clamping Technique

In patch clamping a patch of membrane is isolated from the external solution to record the currents flowing into the patch. To achieve this, small glass capillaries are heated and pulled to fabricate glass micropipettes with an aperture size of 1–2 μm . The pipettes are then backfilled with a conductive solution and pressed against the surface of a cell. To improve the sealing condition a gentle suction is

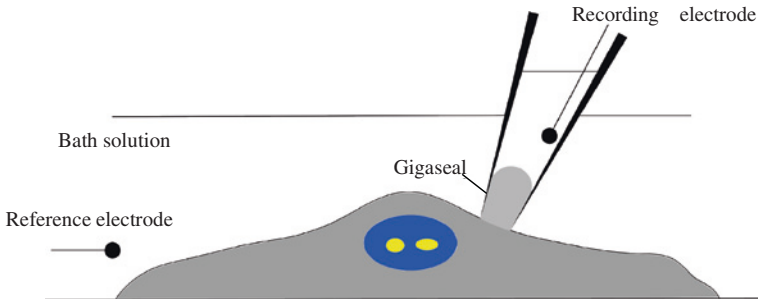


Fig. 2.1 Schematic of the patch clamping experiment. The patch is drawn to have the classical Ω shape; however, recent studies show that the patch can take various shapes [5]

applied to the backend of the pipette. As is shown in Fig. 2.1 there are two electrodes in the patch clamp set-up: a recording electrode inside the pipette and a reference electrode in the bath solution. A high-resistance seal between the glass and the patch of membrane reduces the leakage of current between the two electrodes and completes the electrical isolation of the membrane patch. It also reduces the current noise of the recording, permitting good time resolution of single-channel currents, in the order of 1 pA [3]. Since the electrical resistance of the seal is in the order of giga Ohms, it is called a gigaseal.

2.1.1 Conventional Patch Clamp Configurations

There are several configurations of the patch clamping technique (Fig. 2.2). These configurations enable the technique to study ion channels at different levels, either whole-cell or single ion channels, and to manipulate the fluid on both the extracellular and the intracellular sides of the membrane during experiments [3, 4]. The configurations are:

- Cell-attached mode
- Whole-cell mode
- Inside-out mode
- Outside-out mode
- Perforated patch clamp mode.

2.1.1.1 Cell-Attached Mode

The micropipette is positioned against the cell membrane and suction is applied to the backside of the pipette. A gigaseal forms between glass and cell. This enables the experimenter to study the activities of the ion channels in the patch of

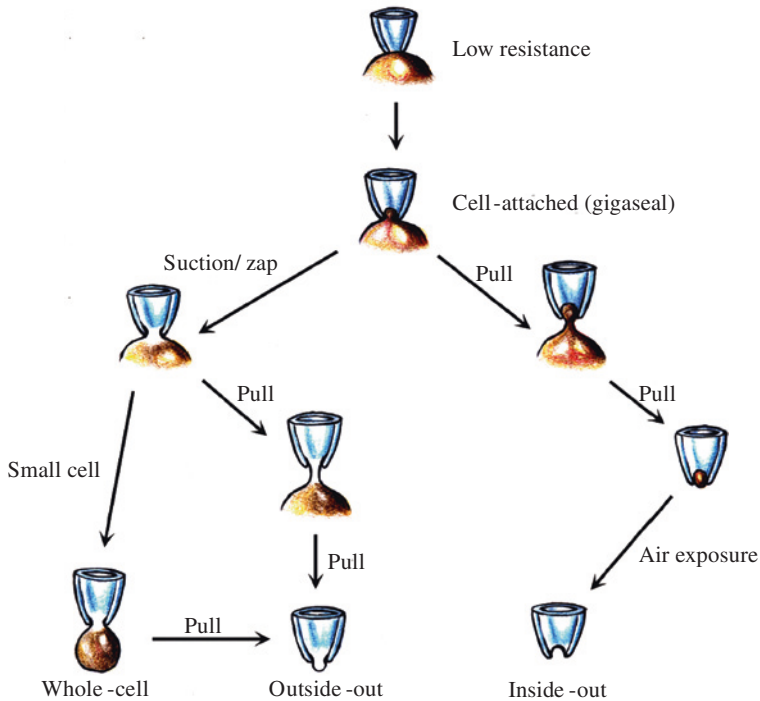


Fig. 2.2 Patch clamp configurations [4]

membrane. The cell-attached patch mode is a single-channel configuration and is the simplest to obtain. Every patch clamp experiment starts with this situation.

2.1.1.2 Whole-Cell Mode

If a higher suction is applied the patch of membrane under the pipette tip in cell-attached mode will rupture and the pipette solution will make direct contact with the cytoplasm. The response of all ion channels within the cell membrane can then be studied.

2.1.1.3 Inside-Out Mode

If the pipette is quickly withdrawn from the cell after the cell-attached mode is obtained, the patch of membrane within the tip of the electrode will tear from the cell while maintaining the gigaseal. This configuration enables study of the effects of intracellular factors on channels. This mode is also a single ion channel configuration.

2.1.1.4 Outside-Out Mode

If the pipette is slowly withdrawn from the cell after whole-cell configuration is obtained, a bleb of cell separates from the cell and forms a patch on the tip of the pipette. This is a single ion channel configuration and because the bath composition can be altered easily during recordings, it enables study of the effects of extracellular factors on the channels.

2.1.1.5 Perforated Patch Clamp Mode

As the volume of the cell is negligible compared with the volume of the patch pipette, the intracellular fluid will be replaced by that of the pipette in the whole-cell configuration. This is a disadvantage when intracellular factors are being studied. To avoid this problem in the cell-attached mode a membrane-perforating agent is added to the pipette solution, which perforates the membrane and allows only small molecules to pass through, so that the cytoplasm's organic composition remains largely intact. This is referred to as perforated patch clamp mode and allows the study of all ion channels in the cell membrane.

There is an equivalent electrical circuit associated with each of these patch clamp configurations. Figure 2.3 shows the electrical components involved in a patch clamping experiment and an equivalent electrical circuit for cell-attached mode.

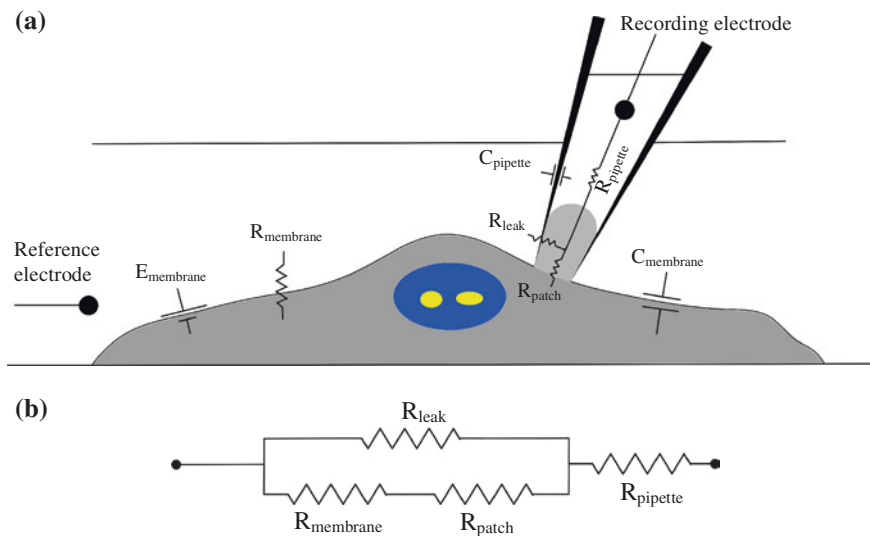


Fig. 2.3 A schematic of patch clamping. **a** electrical components involved in patch clamping, **b** an equivalent circuit for the cell-attached patch configuration

Definitions of the elements in Fig. 2.3 are as below [3]:

R_{pipette} is the pipette resistance.

C_{pipette} is the pipette capacitance.

R_{leak} is the leak resistance and represents the quality of the seal between the glass micropipette and membrane.

R_{patch} is the resistance of the patch of membrane inside the pipette.

R_m is the whole-cell membrane resistance.

C_m is the whole-cell membrane capacitance. A membrane and the intracellular and extracellular media form a capacitor.

E_m is the potential difference across the membrane.

2.2 Other Methods for Measuring Ion Channel Activities

A major constraint in developing new ion channel-based drugs has been the difficulty of screening ion channels at the throughput required of the modern industry in a cost-effective way. The throughput of conventional patch clamping is around 100 data point/week which does not satisfy the needs of pharmaceutical industry [6]. Therefore a good deal of effort has been expended in developing alternative methods of monitoring ion channels activities that can be integrated into industry-standard compound screening formats with corresponding high throughput. The principal methods of high-throughput ion channel screening are: receptor binding assays, flux measurements and fluorescence detection techniques. The advantage of these approaches is their medium to high throughput (15-60 K dp/wk) [7]; however, these techniques measure ion channel activities indirectly and adequate amount of patch clamping experiments are required to validate the results.

2.3 Attempts to Improve Patch Clamping

As was discussed earlier, patch clamping suffers from major drawbacks such as being very laborious and time-consuming, and requiring a lot of experience to get satisfactory results. These drawbacks have made conventional patch clamping a very low-throughput technique. To overcome these problems automated patch clamp systems have been introduced. Automation can reduce the level of complexity and increase the throughput of conventional patch clamping. However, automation of patch clamping presents three major obstacles [8]:

1. An ultraclean surface on a proper substrate is required to be able to obtain gigaseals.
2. A single cell should be positioned on a micron-sized hole without using a microscope and micromanipulator.
3. To automatically perform the complicated steps involved in a patch clamping, complex fluidic and electronic procedures are required.

Efforts to obtain high-throughput patch clamping systems fall into two categories:

- Automation of conventional patch clamping and
- Developing automated planar and lateral patch clamp systems.

In the next two sections, some examples of these attempts are presented. During the past 10 years, many patch clamp systems have been designed. It is not the purpose of the book to review them. A comprehensive review of these efforts can be found in [9].

2.3.1 Automation of Conventional Patch Clamping

A novel idea has been introduced by Lepple-Wienhues et al. [8] for making seals inside a micropipette (Fig. 2.4). The configuration has the potential to be automated and inverts the cell-electrode interface. The pipette is backfilled with a solution containing cells and suction is applied to the tip. The cell forms a seal with the inner side of the pipette. This approach enabled them to develop a fully automated patch clamp robot. Using conventional glass micropipettes for seal formation has some advantages: first, glass is proven to be a good material for seal formation; second, pulling micropipettes from glass tubes is easy and cheap; third, it is a reliable process for making holes at micrometre scale.

Another attempt to improve conventional patch clamping was introduced by D. Vasilyev et al. and is called RoboPatch (Fig. 2.5) [11]. The pipette is inserted into the cell suspension under a positive pressure. A gigaseal is obtained by switching the internal patch pipette pressure from positive to negative (suction). This suction near the vicinity of the patch pipette tip attracts cells to the opening

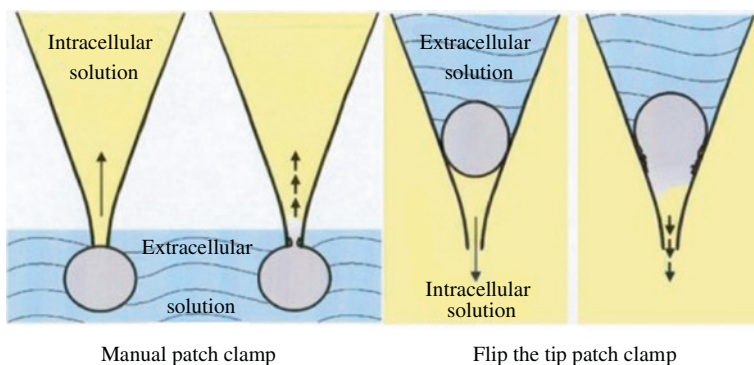


Fig. 2.4 Comparison of conventional and flip the tip patch clamping. Cells are added into glass micropipette and suction is applied to the tip to automatically achieve a gigaseal. Microscope and micromanipulator are no longer required, in contrast to conventional patch clamping [8, 18]

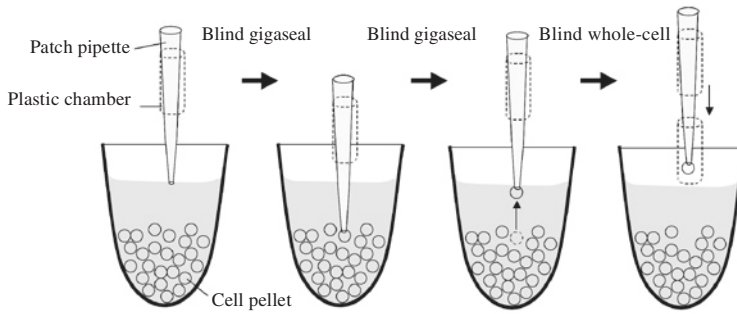


Fig. 2.5 RoboPatch automated system. It utilizes a blind patch voltage-clamp recording method for ion channel drug screening [11]

and eventually results in a physical contact between cellular membrane and patch pipette, allowing the formation of a gigaseal.

Although these approaches overcome some of the drawbacks of conventional patch clamping they still have some limitations. For example, there is no optical access to the cell and the throughput, although higher than conventional patch clamping, does not meet pharmaceutical industry needs. Using a glass micropipette to study an individual cell limits the highest throughput that can be achieved. Applicability of these systems at research level is also limited since they do not have the flexibility often required in research projects.

2.3.2 Planar and Lateral Patch Clamping

In a planar patch clamping process, the pipette is replaced with a micron-sized pore in a flat chip. In lateral patch clamping the pore is in the side wall of the channel. Figure 2.6 compares the three types of patch clamping designs. In both lateral and planar patch clamping, the cell is positioned on the pore and suction is applied to facilitate gigaseal formation. In comparison with conventional techniques, planar and lateral patch clamping configurations do need costly equipment, such as a precise manipulator, a high-magnification microscope and an anti-vibration table. However if the material of the chip is transparent then lateral patch clamping can provide optical access to the patching site.

Fertig et al. [12] conducted one of the earliest attempts in planar patch clamping. Their design is shown in Fig. 2.7. The figure also shows the difference between conventional and planar patch clamp configurations.

The idea of bringing cells to the patching site rather than bringing the pipette to the cells has drawn the attention of many researchers and hundreds of different designs have been developed, each with some advantages over the others. In planar patch clamping the same hole is used for both cell positioning and gigaseal



Fig. 2.6 Three types of patch clamping configuration: (a) conventional, (b) planar and (c) lateral

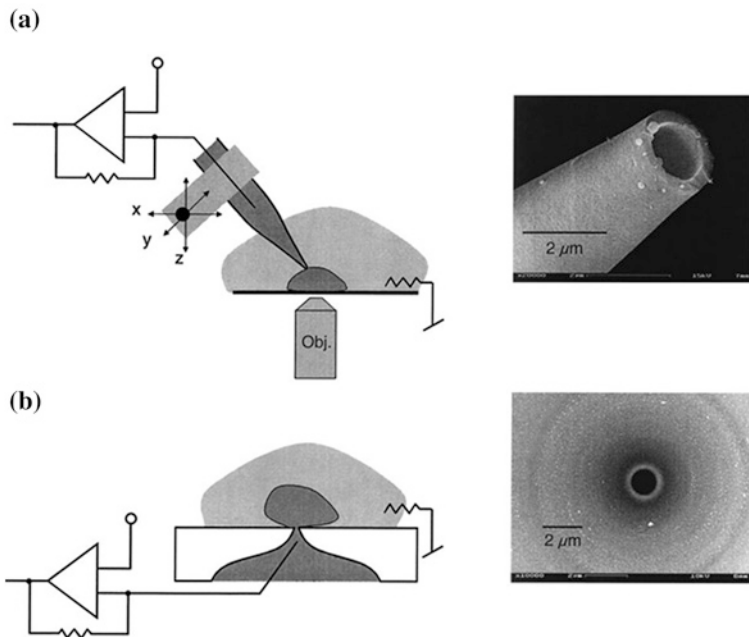


Fig. 2.7 Replacing the patch clamp pipette with a microstructured chip. **a** Whole-cell configuration of the conventional patch clamp technique. The tip of a glass pipette is positioned onto a cell using a micromanipulator and an inverted microscope. **b** Whole-cell recording using a planar chip device having an aperture of micrometre dimensions. Cells in suspension are positioned and sealed onto the aperture by brief suction [12]

formation. This greatly affects the gigaseal quality since debris in the solution may block or contaminate the pore. To overcome this problem, Stett et al. [13] developed a concentric double pipette-like structure (Fig. 2.8). The outer channel is used for cell positioning and the inner channel for current measurements. Positive pressure is initially applied in the inner channel to prevent debris from approaching its surface. Suction in the outer channel directs a cell to the top of the measurement site, which looks exactly like the tip of a pipette. When a cell is placed at the measurement site, suction is used in the inner channel to encourage seal formation.

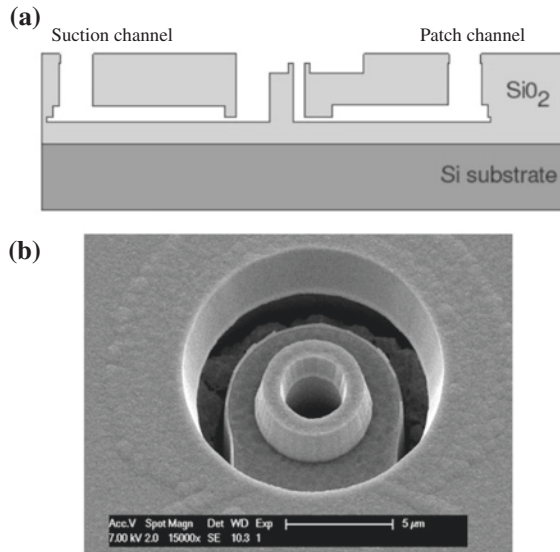


Fig. 2.8 The Cytocentering technology. **a** schematic drawing of the cytocentering site with suction and patch channels; **b** SEM image of two concentric opening formed with focused ion beam milling in a 10 μm thick quartz layer [19]

Planar patch clamping systems increase throughput mainly by taking advantage of their potential to be parallelised and integrated with microfluidic systems. Microfluidic systems facilitate cell manipulation and provide good control on intracellular and extracellular solution exchange. Matthews et al. [14] developed a microfabricated planar patch-clamping system with polydimethylsiloxane (PDMS) microfluidic components (Fig. 2.9).

The design shown in Fig. 2.9 uses the advantages of PDMS as a transparent, easy way to process material. The use of PDMS components has made the exchanging of planar patch clamp chips easier and facilitated quick microfluidic connections. The integration of microfluidics into planar patch clamp systems can enhance the solution control by providing laminar-flow conditions, rapid fluid exchange, and micro-scale control elements such as valves and a pump.

Another advantage of planar patch clamping systems is their potential to be parallelised. Nagarah et al. [15] reported the fabrication of quartz films with high-aspect ratio pores in order to be used as a planar-patch electrophysiology device (Fig. 2.10). The smooth surfaces of pores, the material type and the high depth of pores (which will increase the length of the membrane-glass opposition) resulted in formation of high-resistance seals comparable with those in conventional patch clamping.

Producing planar patch clamp chips is costly and as with patch pipettes, the planar chip cannot be reused once a recording is made. Therefore low-cost and easy fabrication processes are desirable. There is no optical access to the patching site in planar patch clamping. Lateral patch clamping was introduced to address

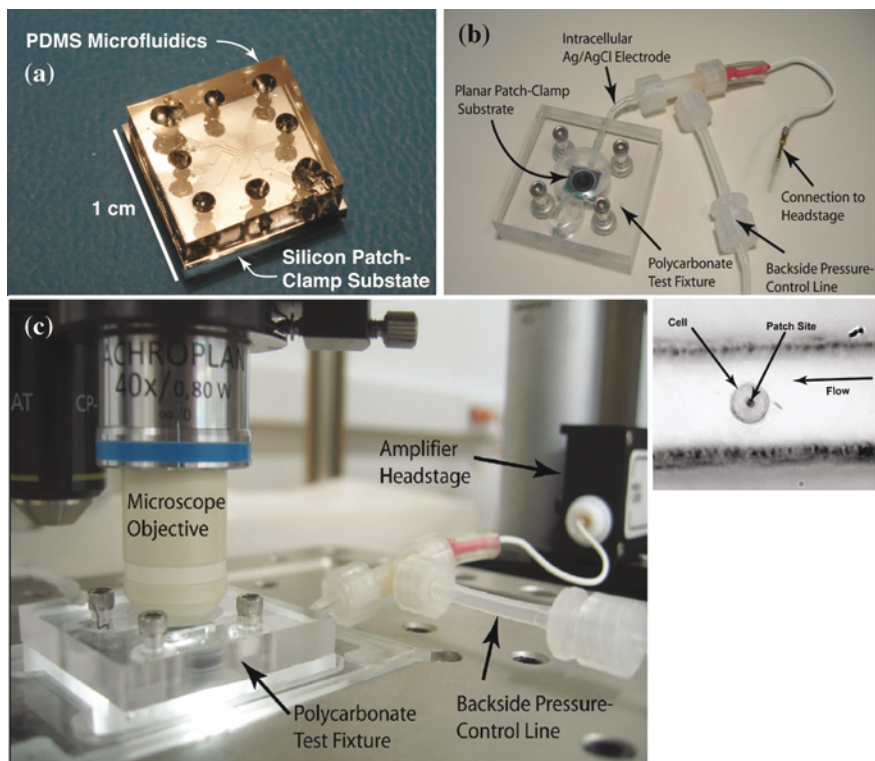


Fig. 2.9 A micro-fabricated planar patch clamp substrate and PDMS microfluidic components. **a** overview of the eight-port microfluidic system, **b** electrodes and capillaries can be simply connected to the micro-machined planar patch clamp system, **c** use of PDMS has enabled optical access, the inset shows a cell, positioned on the pore. Images were kindly provided by Dr Brian Matthews, Electrical Engineering Department, UCLA

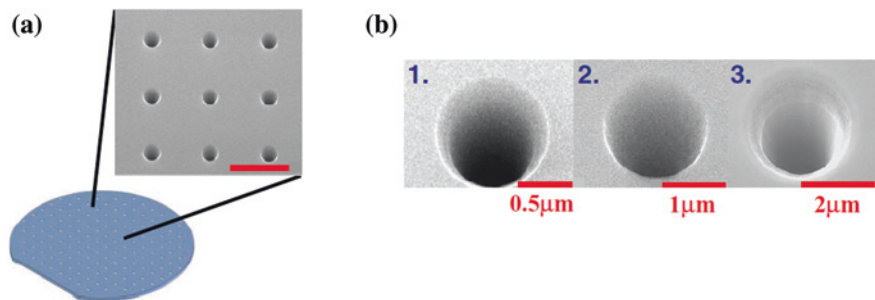


Fig. 2.10 Fabrication of high-aspect ratio pores in quartz films. The pores are polished electrochemically to have smooth surfaces. **a** An SEM image of an array of pores etched 5 μm deep in silicon dioxide. Scale bar = 5 μm. **b** SEM images of high-aspect ratio pores in silicon dioxide with very smooth sidewalls. Pores #1-2 are etched in LTO films while pore #3 is etched in fused quartz. The greater depth of pores increases the length of the membrane-glass interaction in seal formation [15]

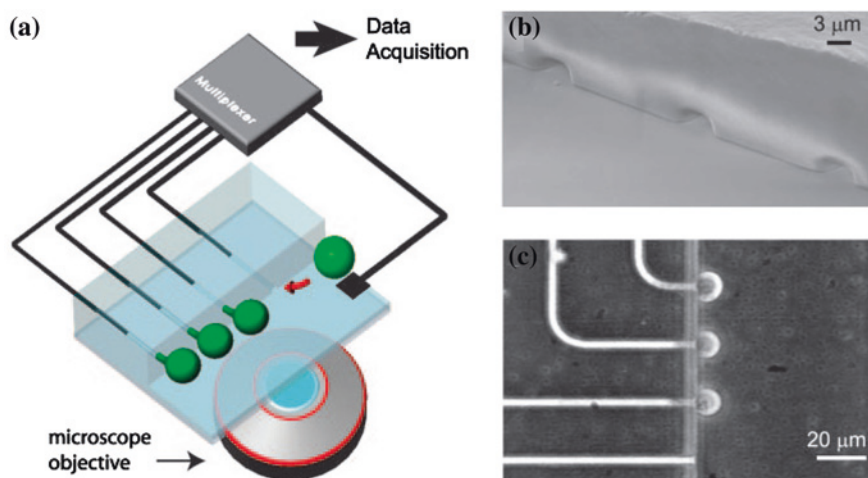


Fig. 2.11 A PDMS-based lateral patch clamping chip developed by Ionescu-Zanetti et al. [16]. **a** Cells were trapped by applying negative pressure to recording capillaries. The device is bonded to a glass coverslip for optical monitoring. **b** Scanning electron micrograph of three recording capillary orifices. The capillary dimensions are $4 \times 3 \mu\text{m}$. **c** Darkfield optical microscope image of cells trapped at three capillary orifices

some of these issues. PDMS has been widely used in lateral patch clamping systems and offers some unique advantages: firstly, the fabrication process is simple (requiring only moulding and bonding); and secondly, it is transparent, which enables the visualization of the cells during recording.

Figure 2.11 shows a PDMS-based lateral patch clamping system developed by Lee and his colleagues [16].

The device can be easily integrated with microfluidics and provides optical access. Cell-trapping sites in this design are at the bottom plane of the chip which gives the patched cell an unusual deformation. Another design was introduced, by the same group, which has an elevated trapping site [17]. In the new design a $20 \mu\text{m}$ thick PDMS layer is bonded to the main PDMS microfluidic channels. The success rate of the device is reported to be higher than 80 % but the low values of seals at $250 \text{ M}\Omega$ are just sufficient for whole-cell recording.

2.4 Comparison of Patch Clamping Methods

In this section a comparison is made between the different kinds of patch clamping. The advantages and drawbacks of each kind are presented in Table 2.1.

As can be understood from the table conventional patch clamping has better data quality and more flexibility but lower throughput, while planar and lateral patch clamping have lower data quality and flexibility and higher throughput.

Table 2.1 Comparison of three kinds of patch clamping: conventional, planar and lateral

	Conventional patch clamping	Planar patch clamping	Lateral patch clamping
Quality of recordings	Superior data quality [11, 15]	Lower data quality [11, 15]	Lower data quality
Throughput	Lower throughput [20–22]	Higher throughput [10, 14, 23, 24]	Higher throughput [16, 17]
Value of gigaseals	Higher [11, 15]	Lower	Lower [16, 17]
Optical access	Yes	No [10]	Yes ^a [16, 25]
Microfluidic integration	N/A	Yes [14, 22, 26–28]	Yes [16, 25]
Ability to control pipette and bath solution	More difficult [29]	Easier [14, 27]	Easier
Density of cell trapping sites	N/A	Lower	Higher [16, 17, 25]
Level of complexity	Higher [17, 21]	Lower [12]	Lower
Level of user expertise	Higher [17, 21]	Lower	Lower
Cost	Expensive equipment, cheap pipettes	Expensive single-use chips [10, 30]	Cheaper than planar, but more expensive than conventional patch clamping
Pipettes/chips fabrication processes	Easy	Difficult	Easier than planar, but more difficult than conventional patch clamping
Time consumption	Higher [12, 17, 21]	Lower	Lower
Ability to choose the best cell for patching	Yes	No	Yes
Potential for automation	Yes [8, 11, 32, 31]	Yes [10, 14, 13, 33, 34]	Yes [16, 17]
Patch clamp configurations	Cell-attached Whole-cell Inside-out Outside-out Perforated	Whole-cell	Whole-cell

^aIf the substrate is made of glass or other transparent materials

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