SOPHION BIOSCIENCE

Application Report

QPlate, a unique design enabling high performance automated patch clamp

An introduction to the biochip that enables experiments on QPatch

Introduction

The QPlate is the biochip applied for electrophysiological measurements using the QPatch automated patch clamp (APC) instrument. The unique QPlate design ensures high and consistent experiment quality and secures several vital properties for high-throughput patch clamp, including:

- Giga-ohm seal formation in physiological solutions
- Minimal compound adsorption
- Fast and efficient (100%) liquid exchange
- Maintenance-free, single-use electrodes
- Several QPlate configurations to accommodate different experiment- and throughput requirements
- A non-compromised testing environment

QPlate key design features

The QPlate is the biochip consumable used in Sophion's QPatch APC system for high-throughput recordings of ion channel currents in living cells. The QPlate is assembled from several components (Fig. 1A) and designed to ensure a trusted testing environment with a range of properties that are advantageous for APC measurements (Fig. 1B-E).

The four components that make up the QPlate are (Fig. 1A): (i) A glass-coated plastic cover, which shields the testing environment and provides inlets for liquid access to the experiment site. (ii) A silicon/glass chip, which is embedded in the plastic cover and contains the patch hole where the cell is positioned during measurement. (iii) A flexible gasket used to form the liquid- and electrical seal between the top and bottom sections. (iv) A ceramic substrate containing the embedded Ag/AgCl electrodes. Once assembled, the components form two microfluidic channels placed above and below the patch site which contain the extra- and intracellular solutions respectively (Fig. 1D). The liquid inlets and outlets of the channels are placed away from the patch site to protect it from contamination.

Giga-ohm seal formation in physiological solutions

The high purity of the glass surface at the experiment site enables the formation of a tight, giga-ohm (GΩ) seal between the cell membrane and patch hole using physiological solutions (Fig. 1B). This allows the user to avoid "seal-enhancing" ions, such as fluoride, which are often required in APC experiments and have been reported to modulate ion channel function (1,2).

Minimized compound adsorption

The microfluidic channels connecting the extracellular liquid inlet to the patch site are covered with a layer of pure, hydrophilic glass, which minimizes compound adsorption (Fig. 1C). This ensures reliable compound concentrations in concentration-response experiments.

Fast and efficient liquid exchange

Efficient liquid exchange is vital, especially when working on ligand-gated ion channels. The microfluidic channels on the plate ensure rapid and precise delivery of liquid (Fig. 1D). The intracellular solution is not exchanged during the experiment. The microfluidic channel on the extracellular side allows rapid liquid exchange with multiple compound additions to the same cell during the experiment. The compound consumption is significantly reduced as solution exchange can be achieved with less than 10 µL of solution. The laminar flow in the microfluidic channel prevents dilution of the compound during addition and subsequently allows complete compound washout (see Fig. 2 and Table 1). This eliminates the need for concentration compensation which might cause overshoot responses.

Maintenance-free, single-use electrodes ensure minimal voltage drift

The embedded, single-use, Ag/AgCl electrodes eliminate the need for manual electrode maintenance (i.e. chlorination) and ensure consistent, high-quality measurement performance with minimal drift in offset voltage (V_{off}) and site resistance (R_{site}) (Fig. 3).

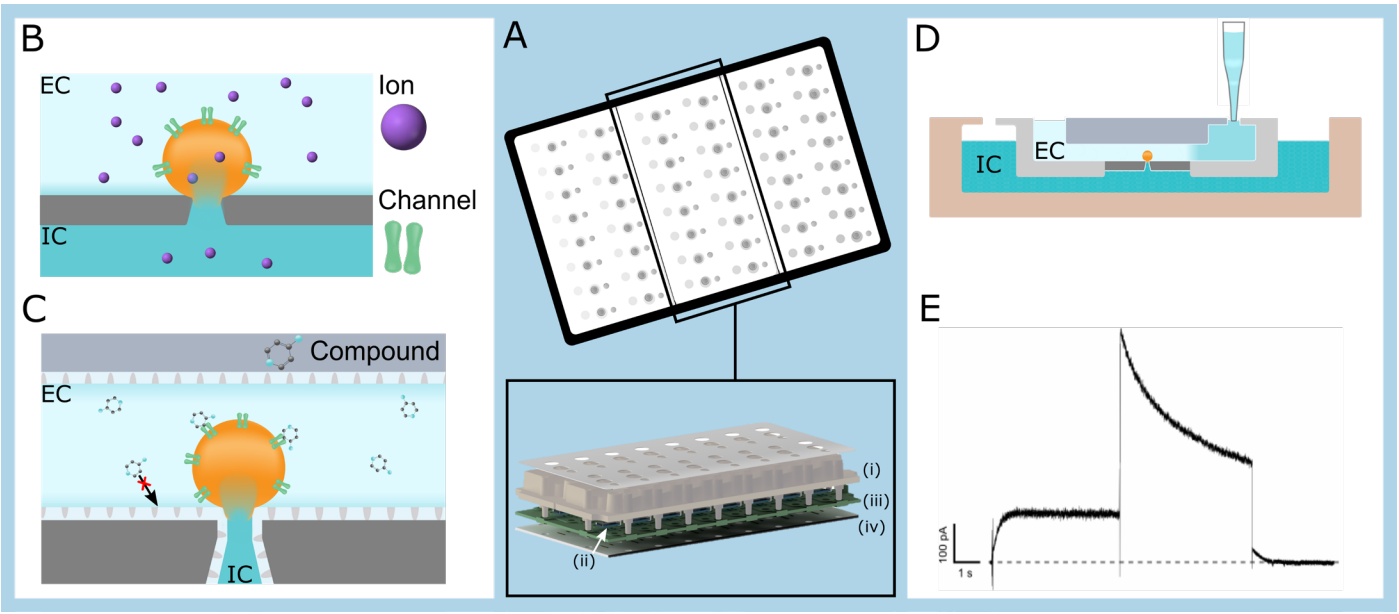

Fig. 1: The QPlate is designed to obtain high quality APC recordings. (A) The QPlate is constructed as a sandwich structure comprised of four components: (i) Glass coated plastic cover, (ii) silicon/glass chip containing the experiment site, (iii) a flexible gasket for sealing, and (iv) a ceramic substrate containing the embedded electrodes. Surface coating in high-purity glass enables the formation of giga-ohm seals in physiological extracellular (EC) and intracellular (IC) solutions (B) and limits adsorption of compound (C). The microfluidic channels ensure rapid and precise delivery of solutions to the experiment site (D) and the integrated, disposable Ag/AgCl electrodes alleviates the need for electrode maintenance while supporting stable experiments (E). The graph shows a representative hERG current measurement.

Different QPlate configurations accommodate experiment and throughput requirements

The QPlate contains 16 (suitable for QPatch 8/16) or 48 (suitable for QPatch 48) measurement sites, depending on the required throughput. Partial (rolling) QPlate allows usage of down to 8 measurement sites per experiment if greater flexibility is required. Furthermore, the user can choose from a single patch hole (single-hole QPlate) or ten patch holes (multi-hole QPlate) per measurement site. The multi-hole QPlate can be used for cells with low endogenous current levels, such as primary and iPSC cells, to improve the signal by simultaneously measuring and adding the signals of ten cells together. Additionally, plates can also be custom made with different sizes or numbers of patch holes to meet specific user needs.

A non-compromised testing environment

The QPlate storage and packaging conditions are optimized to ensure a non-compromised testing environment which consistently delivers high-quality results. The QPlates are vacuum packed in protective plastic bags (5 pcs per bag) to avoid contamination and shipped at room temperature. Upon arrival, the QPlate packages must be stored unopened at 5 °C until usage. Before opening a new package, it must equilibrate at room temperature for one hour to prevent condensation. Open packages are ideally stored in an exicator for up to one week to prevent moisture from entering the plate, as this can affect the QPlate performance.

Solution handling in QPlate

Solutions are handled by the QPatch system and automatically added to the QPlate channels at designated inlets for intra- and extracellular solutions (Fig. 1D). The QPatch pressure system allows for individual pressure control on the intracellular side of the patch hole. This enables precise regulation of cell priming and whole-cell formation in an adaptive manner, which increases the experiment success rate. The priming protocol ensures the complete evacuation of air from the microfluidic channels before the beginning of the experiment. Each column of measurement sites shares a common pressure system on the extracellular side.

Cells are applied through the extracellular channel and positioned at the patch hole by low, negative pressure across the patch hole. When properly sealed, a brief suction pulse brings the cell into the whole-cell configuration, where it is held in place by low, negative pressure throughout the experiment. The extracellular solution can be exchanged throughout the experiment allowing precise addition and washout of compounds. The channel design allows solution exchange using volumes as low as 3 µL, thereby limiting compound consumption. The volume of the waste reservoir at the channel outlet, enables a total of 250 µL solution being added in the course of the experiment.

The microfluidic channels on the QPlate ensure fast and efficient solution exchange. Figure 2 shows quantification of solution exchange kinetics from the change in current conducted by the inward-rectifier potassium (K_{ir}) channel in response to the addition of 10 µL extracellular solution of high potassium ion concentration ($[K^+]_{out}$ = 75 mM). The solution exchange time constant (**T**) is extracted by fitting an exponential function (red) to the K_{ir} current (black) and listed for the addition of 5 µL and 10 µL in Table 1 (first row).

Subsequent washout can happen within the same measurement, with a minimum exposure time of 3 s. The percentage solution exchange upon washout was quantified from the change in potassium reversal potential upon a decrease in $[K^+]$ from 75 mM to 4 mM. If required, multiple repetitions (up to 52) of solution addition or washout is possible with a minimum of 0.05 s delay. 100% solution exchange can be obtained with two subsequent additions of 10 µL wash solution (see Table 1).

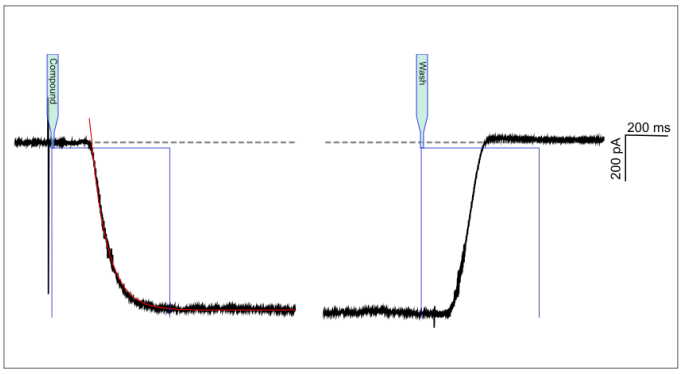

Fig. 2: Solution exchange on QPatch II. Recordings of K_{ir} channel current response (black) to the addition (left) and washout (right) of 10 µL extracellular solution containing 75 mM K^+. The time constant (T) of solution exchange was quantified by the fitting of an exponential function (red) to the current response. The percentage liquid exchange was quantified from the change in K^+ reversal potential upon washout using 4 mM K^+. All quantified parameters are listed in Table 1.

Table 1: The solution exchange time constant (top), T, and the percentage solution exchange upon one (middle) or two (bottom) additions of wash solution quantified for the addition of 5 µL or 10 µL solution. Values are given as average ± SD between three QPlates (100 cells in total).

	5 µL injection	10 µL injection
T (ms)	97 ± 10	100.3 ± 0.6
Solution exchange 1st (%)	90 ± 10	88 ± 8
Solution exchange 2nd (%)	95 ± 2	100 ± 4

Stability of embedded Ag/AgCl electrodes

The embedded, single-use Ag/AgCl electrodes ensure a high degree of measurement stability without the need for chlorination known from manual patch-clamping systems. The embedded electrodes in QPlate allow stable measurements over time with minimum drift in voltage offset (dV_{off}/dt ~ 0.005 mV/min) and site resistance (dR_{site}/dt ~ 0.003 MΩ/min), as demonstrated in Figure 3. The drift observed is primarily attributed to heating over time and can be minimized even further (dV_{off}/dt ~ 0.001 mV/min and dR_{site}/dt ~ 0.001 MΩ/min) by solution exchange or temperature control during the experiment.

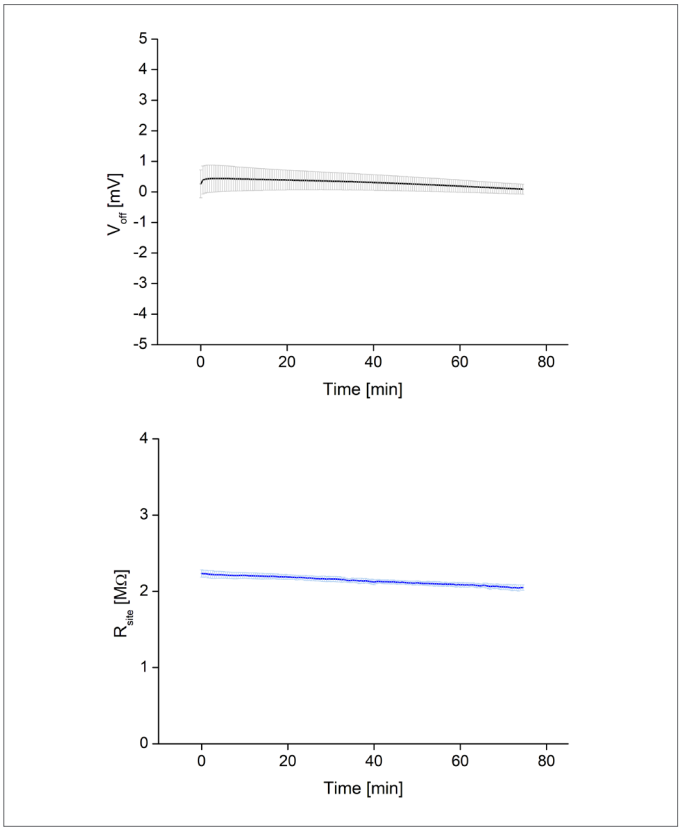

Fig. 3: Measurement stability of QPlate electrodes. The average voltage offset (V_{off}, top) and site resistance (R_{site}, bottom) were quantified in a cell-free assay and plotted as a function of time. Datapoints are average ± SD between three QPlates (16 experiment sites per QPlate).

QPlate performance and reproducibility

All individual QPlate components undergo Quality Control (QC) at multiple levels to ensure reproducible high QPlate performance. The materials are inspected for defects in an incoming QC and each batch is labelled to ensure traceability of all components. Also, critical components undergo functional and biological QC tests before being used in the assembled QPlate. The high quality of the QPlate allows for a high degree of assay reproducibility. Our internal QC assay, which is a highly standardized assay designed to detect potential production issues, runs at 89% success rate with a standard deviation of only ± 6%. However, the success rate of an APC experiment is highly dependent on cell quality as well as assay complexity.

Each finalized QPlate is uniquely labelled with a barcode that enables complete traceability of the components used and the production processes. During a QPatch experiment, the barcode is scanned, and the information saved and stored with the results. This means that every QPlate and its parts can be tracked after usage and it is therefore vital that the QPlates are kept intact.

QPlate has been used successfully on a variety of different cells and ion channels. Success rates and assay reproducibility depends to a high degree on the environment, experiment setup, cell and solutions quality, for more detailed information please visit sophion.com.

References

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2. Lei CL, Fabbri A, Whittaker DG et al. A nonlinear and time-dependent leak current in the presence of calcium fluoride patch-clamp seal enhancer. Wellcome Open Res 2020, 5:152 (https://doi.org/10.12688/wellcomeopenres.15968.1)

Author:

Kadla Røskva Rosholm, Research Scientist

Marie Louise Laub Busk, Research Scientist