Application Report:

CHO-hERG DUO

In multi-hole mode

AR_PUBLIC15938-2 CHO hERG DUO in multihole mode

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Introduction

CHO-hERG DUO is a QCell, a cell line that can be provided by Sophion. The performance of this particular cell line was evaluated on multi-hole QPlates on QPatch HTX.

We have evaluated several features – success rates, stability, pharmacology and biophysical properties of the assay.

The report also contains introduction to some of the methodology that is used when running multi-hole experiments on the QPatch.

Introduction to timed whole cell protocols and leak subtraction

Formation of a tight seal between cell and chip is crucial in patch clamping. It is therefore important to optimize the whole cell protocol for each cell line of interest.

We have found that the use of so-called timed protocols where suction is changed at specific times is a very efficient way to form seals with multi-hole plates.

In multi-hole mode there are ten cells, which have to be patched in parallel and there is therefore ten times more cell membrane to rupture when going into whole cell configuration. We have found that the suction used for whole-cell break in in multi-hole mode must be harder than in single-hole mode, but still soft enough so that the seal is kept. Below in Figure 1, two timeline plots are shown and by combining the two plots it can be seen that the total capacitance (Cchip+Ccell) increases 35 pF when performing the whole cell suction.

Figure 1. Timelines from a representative well. By combining the two graphs, it can be seen that the total capacitance increases (red curve bottom plot) when whole cell suction is applied (red curve top plot).

Even though the seals are high, one has to take the increased leak into account when using the multi-hole plates. This originates from the fact that ten cells in parallel appear leakier than a single cell because all leak conductances from the ten holes are summarized.

The QPatch software has several methods for subtracting the ohmic linear leak current. The classic P/n leak subtraction is a method where the cells are exposed to miniature versions of the voltage protocol of use. These miniature sweeps are then used to determinate the leak component, which can then be subtracted from the current. Since the time used for each miniature sweep is the same for each voltage protocol, the P/n leak correction is not so practical for assays with long voltage protocols, such as the ones used for hERG measurements, as the total experimental time will potentially affect overall success rates. Other methods must be taken into use.

Online P/n leak subtraction can be seen in Figure 2. The benefit from using this feature is that the leak current is determined between each voltage protocol and this method is therefore less sensitive to changes in cell parameters during experiments.

The down side of using this feature with long hERG voltage protocols is the sensitivity for bad sweeps. If the parameters "jump" during one leak sweep then the whole measured sweep is affected.

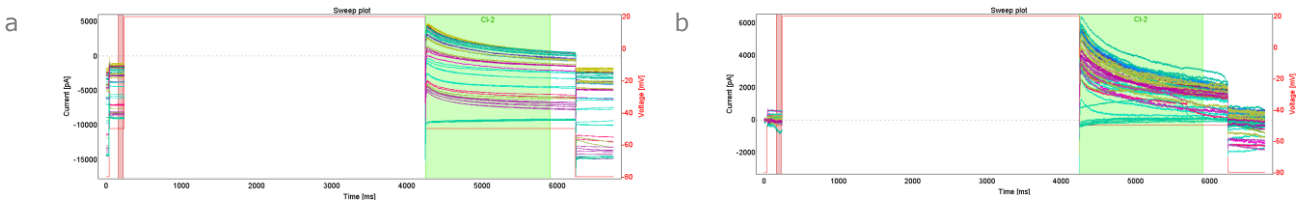

Figure 2. Example of sweeps from a multi-hole experiment without (a) and with (b) online P/n leak subtraction.

Sweep subtraction is a faster leak method where a specific control antagonist is applied to the cell at the end of the experiments and the current which is left is then subtracted from all other sweeps. The disadvantages of this method are that the seal/leak has to stay constant for the whole experiment duration and that the control antagonist must be specific for the current of interest.

Yet another leak subtraction method is available in the QPatch software packages. This is a prepulse leak subtraction method where a small pre-pulse or ramp is use for a AC measurement of the leak current at two given potentials thereby allowing for a more accurate leak subtraction at other potentials. This method is therefore mainly useful for IV protocols (see Figure 3).

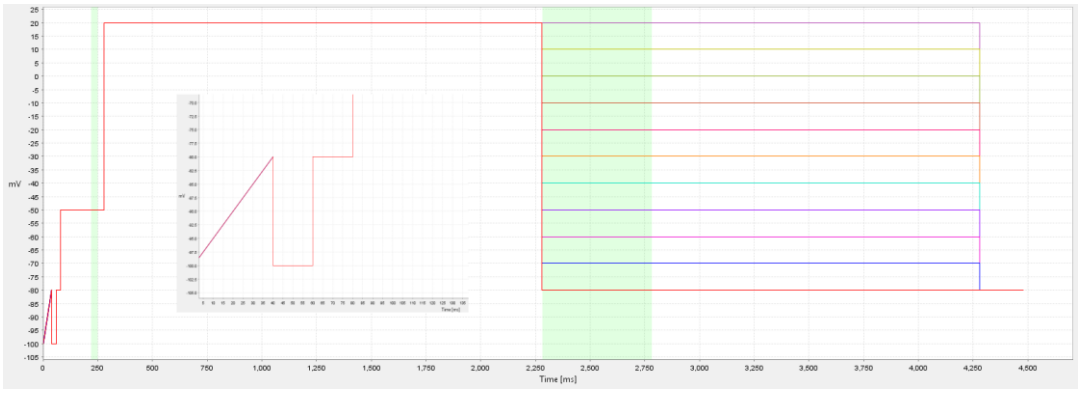

Figure 3. Voltage protocol with ramp and pre pulse for reversal potential investigations with leak corrections.

All three methods available in the software have been tried in this study and they all have their strengths.

In Figure 4 an example of a single experiment/measurement site can be seen, with sweep subtraction and without sweep subtraction. Note that the base line current is shifted to 0 pA after sweep subtraction.

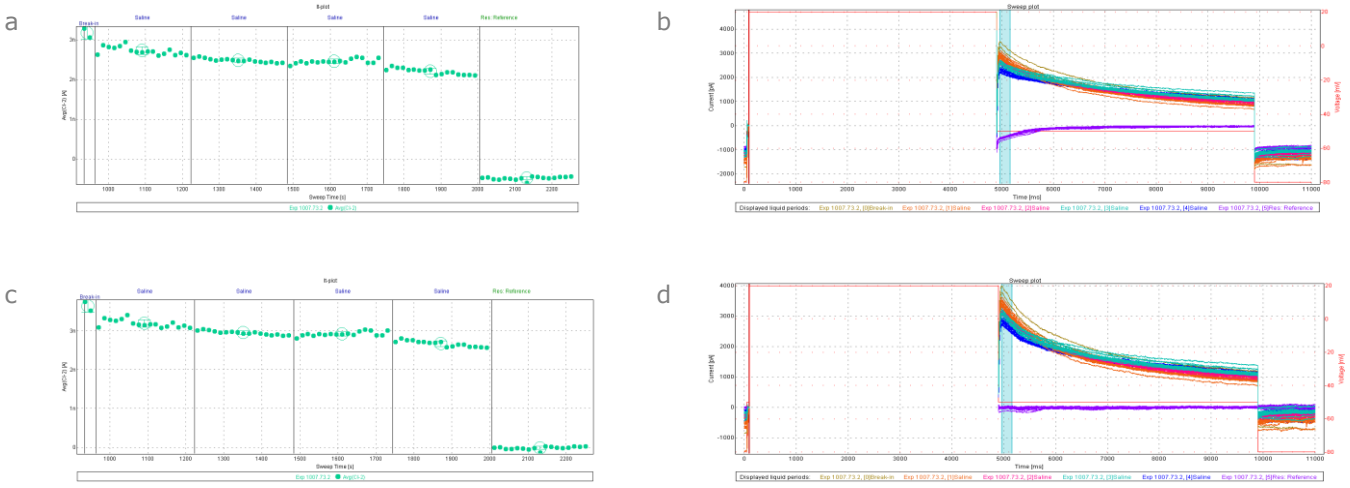

Figure 4. Example of use of sweeps subtraction, all four figures originate from the same raw data. a and c) current versus time plot (I/t-plot) with and without sweep subtraction respectively b and d) hERG responses with and without sweep subtraction.

Materials and Methods

CHO-hERG DUO cells cultured and harvested according to Sophion SOP.

Ringer's solutions:

Extra cellular Ringers solution: In mM: 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 145 NaCl, 10 Glucose. pH=7,4 (w. NaOH), 305 mOsm (w. sucrose)

Intra cellular Ringers solution: In mM: 5.374 $CaCl_2$, 1.75 $MgCl_2$, 10 EGTA, 10 HEPES, 120 KCl, 4 Na_2-ATP. pH=7.2 (w. KOH), 295 mOsm (w. sucrose)

Results

Throughput

The throughput was evaluated in a series of 6 pt. dose response experiments. Throughput was calculated as the number of experiments that was found useful after analysis ie. the rightmost column in the histogram below. See Figure 5.

IC_{50} values were determined by use of group Hill fit and each useful liquid period was included in the analysis in the multi-hole experiments. See Figure 5.

Figure 5. Top: Success rate in single hole mode (n=64). Bottom: Success rate in multi-hole mode (n=240).

Stability of recordings

Rundown can be a problem when running hERG. The effect on rundown and current stability was evaluated by measuring coefficient of variance (CV) and rundown of the last saline period before compound was added.

CV was measured as SD/average current derived with the assay software from 500 sites on both single-hole and multi-hole QPlates.

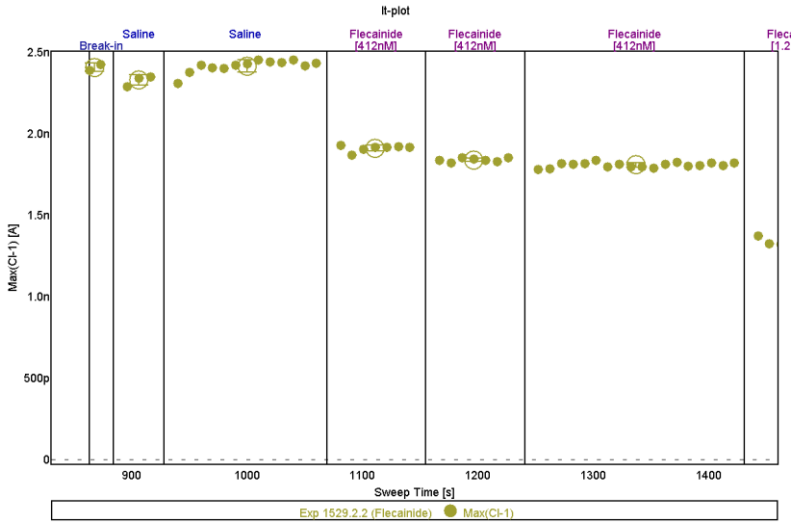

Figure 6. hERG statistical analysis It-plot. SD and average of current in the last saline period is used for CV determination.

The distribution of the found CV's is shown in Figure 7. CV was found to be slightly but not significantly lower with multi-hole compared to single-hole mode.

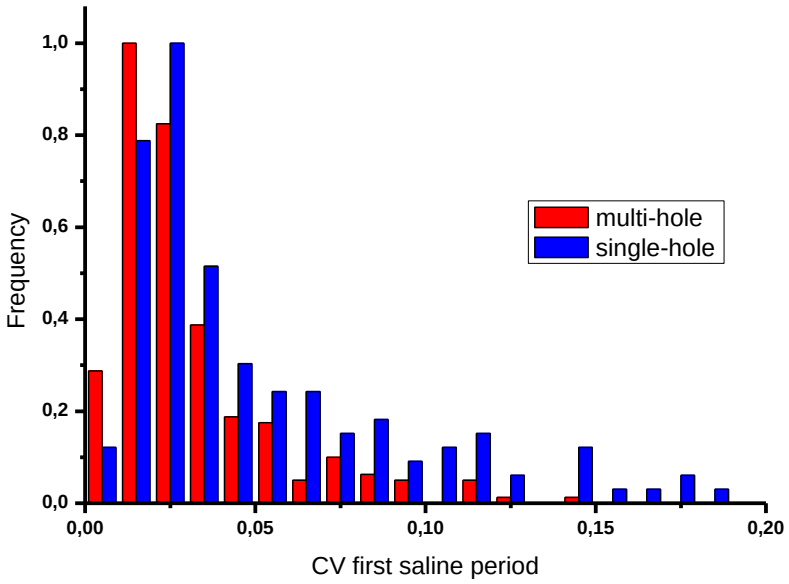

Figure 7. Distribution of saline CV in single-hole and multi-hole.

The rundown in these hERG experiments could be described with a linear function. The assay software was therefore used to make a linear fit to the last saline period and the rundown rate (% rundown/min) was derived from this result. The distributions of rundown rates are shown below in Figure 8. It was found that the rundown was reduced slightly in multi-hole mode, however it was also found that there were more run-up in multi-hole mode than seen in single-hole mode.

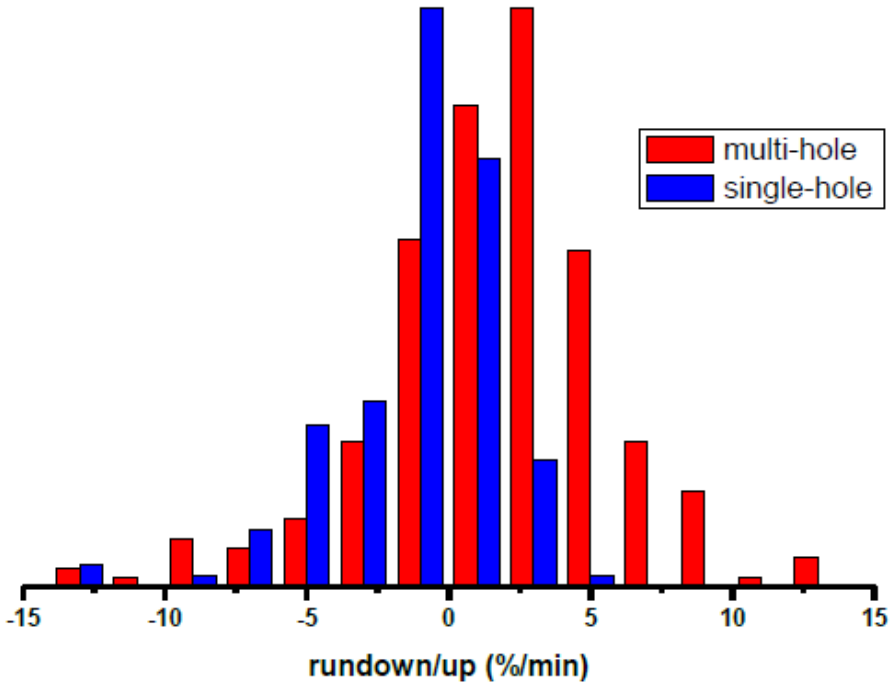

Figure 8. Distribution of rundown rates in multi-hole and single-hole mode.

Biophysics of hERG in multi-hole mode

When performing current vs. potential experiments (IV-experiments) on reversal potential, an off-line leak protocol is needed. By using a pre-pulse or a pre-ramp, it is possible in the QPatch assay software to subtract a leak at any potential. See Figure 3. IV curves by using this method can be seen in Figure 9, when reversal potential is measured to -80 mV.

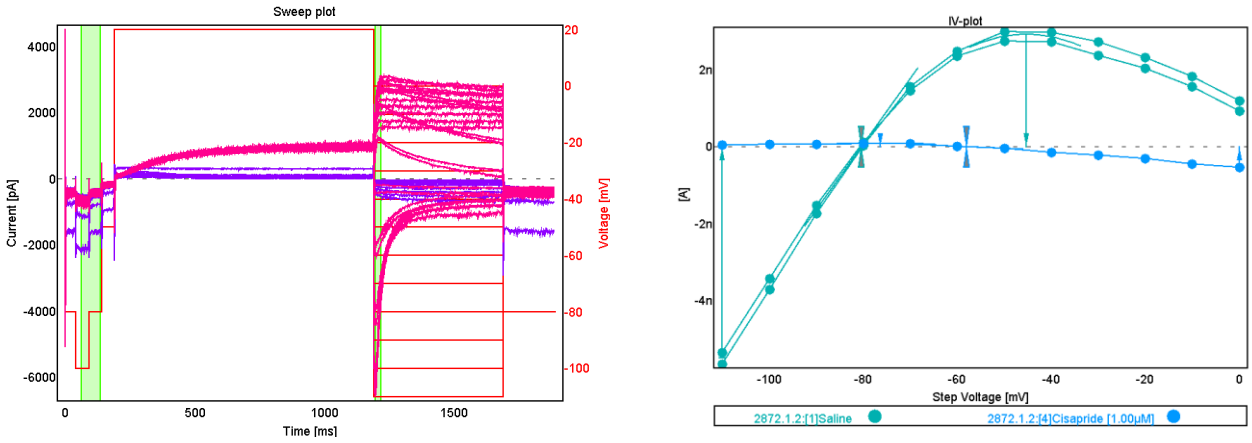

Figure 9. Current-voltage relationship for determination of the reversal potential. Left: voltage protocol and raw current traces. Right: IV relationship, green line with physiological Ringer's solutions and blue line in the presence of 1 µM Cisapride.

Pharmacology

We have tested six known blockers of the hERG current. The obtained results were used to validate the multi-hole technology against single-hole recordings and literature values.

	Literature values (nM)	Single-hole mode * (nM)	Multi-hole mode # (nM)
Astemizole	37 (2)	23 (n=8)	16 (n=153 data points)
Cisapride	20-10000 (3)	56 (n=9)	26 (148 data points)
Flecainide	3910 (4)	1360 (n=1)	1779 (97 data points)
Pimozide	2-20 (3)	41 (n=2)	13 (103 data points)
Quinidine	200-2000 (3)	1259 (n=4)	945 (108 data points)
Verapamil	100-1000 (3)	340 (n=3)	137 (107 data points)

Table 1. Half maximal inhibitory concentration (IC_{50}) values from six known blockers of the hERG current. All values are in nM. *) determined by individual Hill fits #) data normalized and IC_{50} value determined by group Hill fit.

References

1. Armstrong,CM, Bezanilla,F: Charge movement associated with the opening and closing of the activation gates of the Na channels. *J.Gen.Physiol* 63:533-552, 1974

2. Brown,AM: HERG block, QT liability and sudden cardiac death. *Novartis.Found.Symp.* 266:118-131, 2005

3. Ficker,E, Dennis,A, Kuryshev,Y, Wible,BA, Brown,AM: HERG channel trafficking. *Novartis.Found.Symp.* 266:57-69, 2005

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Conclusion

By using an appropriate whole cell protocol and a proper leak subtraction the multi-hole technology is a powerful tool in testing hERG pharmacology.

We have been able to increase the throughput due to more experiments being accepted both because more experiments completes (42 vs. 66 %, see Figure 5), but also because the stability of the recording is improved (see Figure 6).

The pharmacological data are in good agreement with both single-hole experiments and already published literature values.