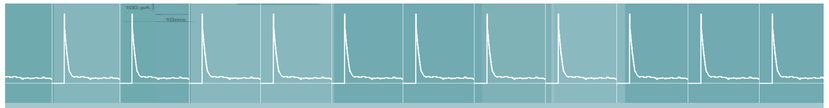
DATA QUALITY IS NOT AFFECTED BY CELL SUSPENSION DENSITY ON QPATCH 16

CHRIS MATHES | HERVØR L. OLSEN | MICHAEL STOLTENBORG | MORTEN SUNESEN | NIELS J. WILLUMSEN

SOPHION BIOSCIENCE A/S
Baltorpvej 154
DK-2750 Ballerup
DENMARK
info@sophion.dk
www.sophion.com

SOPHION BIOSCIENCE, INC.
675 US Highway One
North Brunswick, NJ 08902
USA
Phone: +1 732 745 0221
www.sophion.com



When determining the potencies of drugs in single-cell assays it is a major concern that the employed assay is affected by factors such as the density of the applied cell suspension. It is envisaged that a high density of cells may lead to titration of test compound molecules (by receptor-binding and adsorption) by cells in the vicinity of the targeted cell. This would cause a rightward shift in the concentration-response relationship, and consequently, incorrectly increased IC_{50} values.

We have tested whether IC_{50} values determined with the QPatch 16 automated patch-clamp system are dependent on the density of the applied cell suspension. We assume that cell density determines the number of cells present in the extracellular flow channel.

It is predicted that increasing the cell suspension density may reduce the time it takes to position a cell on the patch-clamp site. The cell positioning step is accomplished by suction. We tested the effect of the cell suspension density using a wide range of densities (from 0.5 to 16 million cells per ml) on the cell positioning time.

For the tests we used two expression systems: (1) CHO cells expressing hERG potassium channels, and (2) HEK cells expressing $Na_v1.2a$ sodium channels. We used test compounds with very different oil-water partition coefficient (log(P)): astemizole and verapamil with log(P) values of 6.43 and 3.45, respectively, (source: www.syrres.com/esc/physdemo) for hERG assays, and tetrodotoxin (TTX) with log(P) of -6.21 for the $Na_v1.2a$ assay.

MATERIALS AND METHODS

Cells. Cultured HEK293 cells stably expressing $Na_v1.2a$ and CHO cells stably expressing hERG potassium channels were used.

Ringer's solutions.
For CHO-hERG the following solutions were used:
Extracellular (mM): 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 145 NaCl, 10 Glucose, pH=7.4 (NaOH), ~305 mOsm.
Intracellular (mM): 5.4 $CaCl_2$, 1.75 $MgCl_2$, KOH/EGTA 31.3/10, 10 HEPES, 120 KCl, 4 Na_2-ATP, pH=7.2 (KOH), ~290 mOsm.
For HEK293-$Na_v1.2a$ the following solutions were used:
Extracellular (mM): 1 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, 5 MES, 3 KCl, 140 NaCl, 0.1 $CdCl_2$, 20 TEA-Cl, pH=7.3 (NaOH), ~320 mOsm.
Intracellular (mM): 135 CsF, 1/5 EGTA/CsOH, 10 HEPES, 10 NaCl, pH=7.3 (CsOH), ~320 mOsm.

Compounds. Astemizole (Sigma, Buchs, Switzerland) was employed in four concentrations from 1.9 to 50 nM in three-fold increments. Verapamil (Sigma) was employed in four concentrations from 0.03 to 30 µM in ten-fold increments. Tetrodotoxin (Alomone Labs., Jerusalem, Israel) was employed in concentrations from 0.5 to 500 nM in ten-fold increments.

Data analysis. Recorded ion channel whole-cell currents were stored in an integrated database (Oracle). Drug effects (IC_{50}) were analysed as a function of concentration with the QPatch assay software.

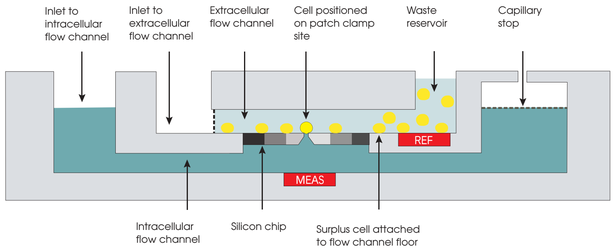


Figure 1.
Schematic transection of the QPlate™The targeted cell is positioned on top of the patch clamp hole in the silicon chip. Throughout the extracellular (upper) flow channel cells are shown sticking to the floor of the flow channel. Electrodes are indicated in red.

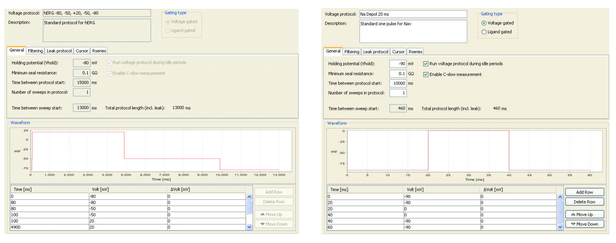


Figure 2.
Graphic representation of voltage protocols used for hERG (left) and $Na_v1.2a$ (right) as viewed with the QPatch assay software.

WHOLE-CELL CURRENT TRACES

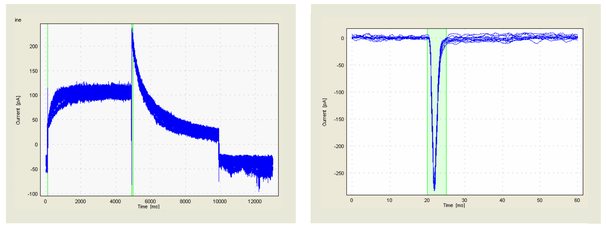


Figure 3.
Characteristic hERG (left) and $Na_v1.2a$ (right) whole-cell currents as viewed with the QPatch assay software. Twelve individual current traces are superimposed in each panel.

CONCENTRATION-RESPONSE RELATIONSHIPS

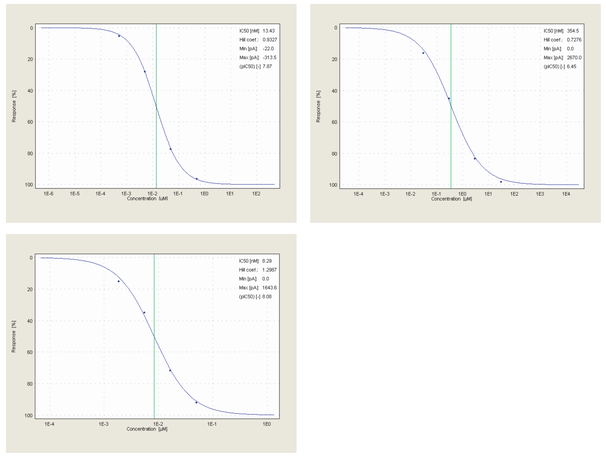


Figure 4.
Concentration-response relationships for TTX (top left), verapamil (top right) and astemizole (bottom) as viewed with the QPatch assay software. Green vertical lines indicate IC_{50}.

CELL POSITIONING TIMES

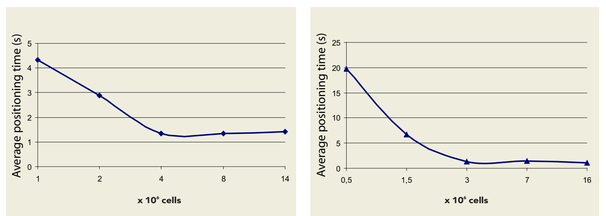


Figure 5.
The cell positioning time was reduced similarly at higher cell suspension densities when using CHO (left) and HEK (right) cells in suspension.

EXPERIMENTAL SUCCESS RATES

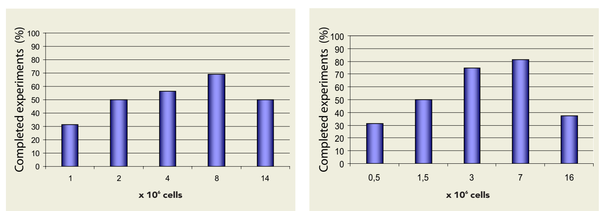


Figure 6.
Variations in the cell suspension density affected the number of successfully completed experiments similarly for CHO-hERG (left) as well as for HEK-$Na_v1.2a$ (right). The figures demonstrate that a cell density of 3-8 million cells per ml is optimal.

IC_{50} VALUE DETERMINATIONS

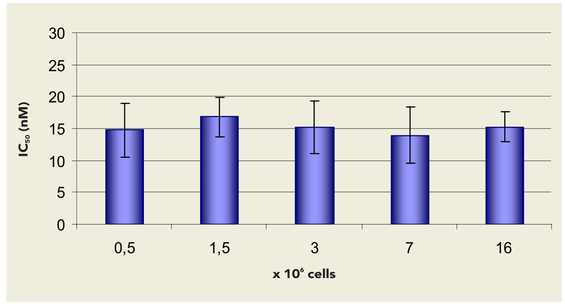
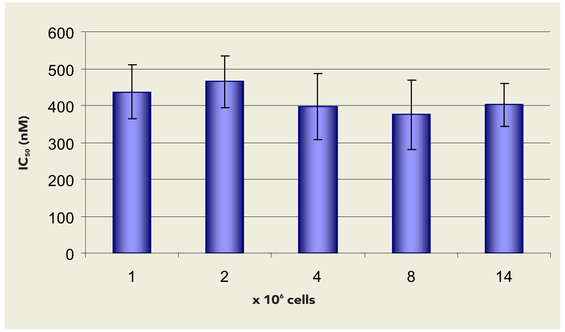
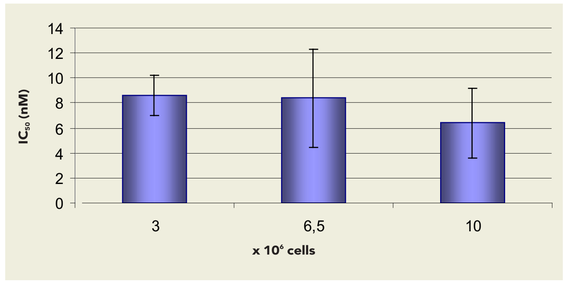


Figure 7.
No effect of cell suspension density was observed for any of the three compounds tested. The figure depicts the results for the compounds arranged after increasing degree of hydrophobicity.

TTX log(P) = -6.21.
N = 4-13 (average: 8.4)



Verapamil log(P) = 3.45.
N = 4-10 (average: 7.0)



Astemizole log(P)=6.43.
N = 6-16 (average: 9.7)

CONCLUSION

The QPatch experiments presented here demonstrate that cell suspension density has a clear effect on cell positioning time which is reduced with increasing density. The experimental success rate is also affected, and it appears that the optimal density is 3-8 million cells per ml. It can be estimated that at such densities, sedimented cells do not cover the flow channel floor area completely. This likely explains why the experimental success rate is optimal in this density range. Finally, and most importantly, we observed no effect of cell suspension density on the obtained IC_{50} values neither for very hydrophilic (TTX) nor for very hydrophobic compounds (astemizole). Thus, no right-shift of the concentration-response relationship is introduced by using high density cell suspensions (10-16 million cells per ml) in the QPatch. This is likely a consequence of the small volume of the extracellular flow channel which leads to sedimentation of few cells in the vicinity of the patch clamp site as compared to open-well systems