Electrophysiological properties of iCell Cardiomyocytes® obtained by automated patch clamp on the QPatch

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Introduction

Pluripotent stem cell-derived cardiomyocytes, iCells® Cardiomyocytes, were biophysically and pharmacologically characterized with planer automated patch clamp using the QPatch. iCells Cardiomyocytes are differentiated from human induced pluripotent stem cells. They are differentiated in large numbers and cryo-preserved, which make them highly suitable for automated patch clamping and facilitates their use in drug screening.

Here we present the results obtained during assay optimization of the cell culture, assay set-up on the QPatch as well as biophysical and pharmacological validation of the cardiac currents.

Implementing optimal cell culture routines, the iCells Cardiomyocytes demonstrated spontaneous rhythmical contractions indicating functional properties of adult cardiomyocytes. We tested the cells in two different QPatch recording modes; single-hole recordings and multi-hole recordings where up to ten cells are patched at the same time and the total current is measured per site. Three different types of currents were studied including sodium, calcium and potassium. By using specific buffer solutions and changing the voltage protocols it was possible to characterize calcium, and sodium currents from the same cell.

Our study showed that iCells Cardiomyocytes can successfully be applied to the QPatch. The recorded currents are similar to human cardiomyocytes and the response to known pharmacology is as expected. Using the QPatch we believe that iCells Cardiomyocytes have great potential for safety screening and other cardiovascular investigations early in the drug discovery process.

Material and Methods

Ringer solutions

Extracellular for I_{Na} (mM): 120 NaCl, 5 KCl, 3.6 $CaCl_2$, 1 $MgCl_2$, 20 TEACl, 10 HEPES. pH adjusted to 7.4 with NaOH.
Extracellular for I_{Kr} (mM): 15 NaCl, 140 KCl, 1.2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES. pH adjusted to 7.4 with NaOH
Extracelluar for I_{Ca} (mM): 125 NMG, 3.6 $CaCl_2$, 1 $MgCl_2$, 20 TEA Chloride, 10 HEPES. pH adjusted to 7.4 with HCl

Intracellular for I_{Ca} and I_{Na} (mM): 120 CsCl, 3 $MgCl_2$, 10 EGTA, 5 HEPES, 4 Na_2-ATP. pH adjusted to 7.4 with CsOH
Intracellular for I_{Kr} (mM): 5.374 $CaCl_2$, 1.75 $MgCl_2$, 3.125/10 KOH/EGTA, 120 KCl. pH adjusted to 7.2 with KOH

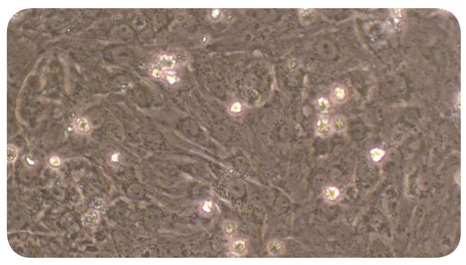
Cells

Cryopreserved iCell Cardiomyocytes were received from Cellular Dynamics International. Each vial contained approximately 4.5 mio viable cadiomyocytes with a plating efficiency of 41-53 %. The cells were thawed and plated on fibronectin coated T-25 culture flasks using 60,000 cells per cm^2 to obtain a syncytial monolayer. The culture flasks were stored in a controlled environment in an incubator (37°C, and 5 % CO_2) and the culture media was exchanged every other day. The culturing was continued for six to eight days. After culturing the cells were harvested and prepared for QPatch experiments according to the SOP developed by Sophion.

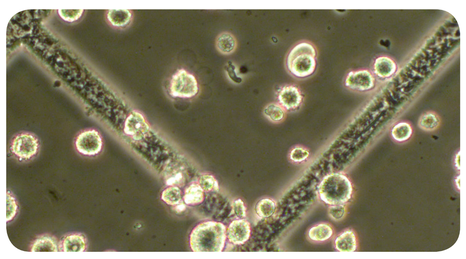
QPatch

Small volumes of 50-100 µl cell suspension were applied to the QPatch. Cell positioning, giga sealing and whole-cell formation were obtained automatically with suction according to a pre-programmed suction protocol. Currents were recorded using either the single-hole or multi-hole technology. For the multi-hole technology up to 10 cells are patched per measurement site and the total current is recorded. For the single-hole technology one cell is patched per measurement site. Different voltage protocols were applied to obtain specific biophysical characteristics of the currents. Different liquids and increasing concentrations of the compounds were added automatically through the flow channels in the QPlate. Data were analyzed using the QPatch Assay Software.

Results

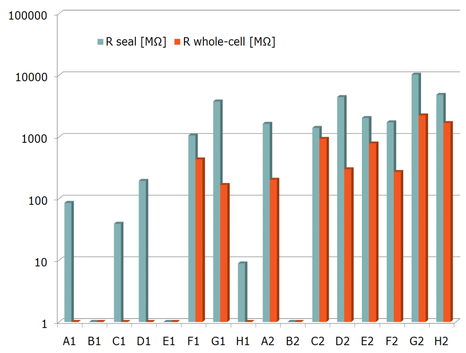


iCell cardiomyocytes cultured for 7 days in a fibronectin coated culture flask



iCell cardiomyocytes harvested by trypsination procedure. The cell density is approximately 1.5 mio cells per ml.

QPlate sealing overview



QPlate sealing overview from a **single-hole** experiment. The success rate for sealing for single-hole experiments could reach up to 70 % for sealing and 60 % for whole-cells

	Single-hole
Seal	53 % (±12, n=9)
Whole-cell	22 % (±13, n=9)

Table 1. Success rates for obtained seals and whole-cells with iCells®. n refers to the number of QPlates 16's used for single-hole experiments

	Single-hole	Multi-hole
Cell size	29 pF (±15, n=41)	N.D
Cells expressing I_{Na}	91 % (±16, n=40)*	N.D
Cells expressing I_{Ca}	41 % (±39, n=58)*	N.D
Cells expressing I_K	80 % (±35, n=17)*	N.D
Usable I_{Na} data/QPlate	16 % (±10, n=224)	58 % (±22, n=80)
I_{Nav} amplitude	-3.5 nA (±2.2, n=24)	-6.4 nA (±42., n=46)
IC_{50} TTX for I_{Na}	10.3 µM (±0.5, n=6)	6.3 µM (±4.5, n=12)
Tau for I_{Na} inactivation* **	0.85 ms (±0.29, n=22)	0.99 ms (±0.16, n=20)

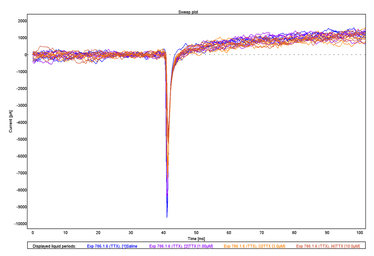
Table 2. Comparing different results from I_{Na}, I_{Ca} and I_K obtained from single-hole and multi-hole recordings. *Specific currents from established whole-cells. **Recorded at 0 mV. ***Data fitted to a mono-exponential curve

References:
Schneider M, Proebstle T, Hombach A, Rüdel R. Plügers Arch, 1994;84-90
Drolet B, Khalifa M, Daleau P, Hamelin B, Turgeon J. Circulation, 2011;204-210
Sanguinetti MC, Jurkiewicz NK. J Gen Physiol, 1990;195-215
Pelzmann B, Schaffer P, Bernhart E, Lang P, Mächler H, Rigler B, Kiodl B. Cardiovascular Research, 1998;424-432

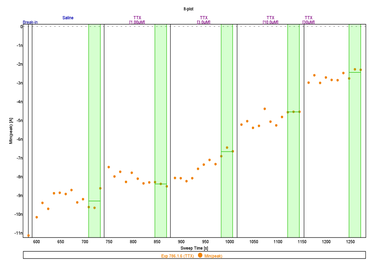
In cooperation with:

Cellular Dynamics international

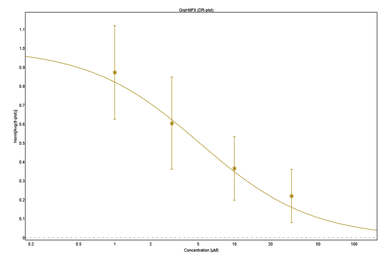
I_{Nav} multi-hole



I_{Na} current sweeps obtained at 0 mV in the absence (large peak) and presence of increasing concentrations of TTX (1, 3, 10, 30 µM)

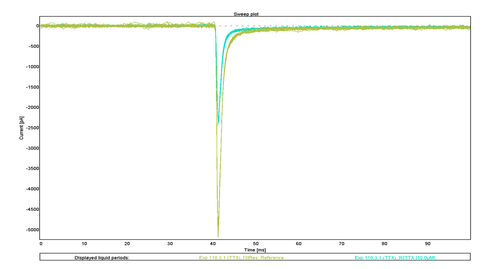


I-T plot; I_{Nav} current blocked by increasing concentrations of TTX (1, 3, 10, 30 µM)

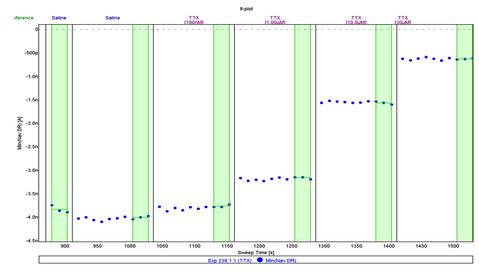


Dose-response plot. Data were fitted to the Hill equation and the IC_{50} value for TTX was 6.3 µM (± 4.5, n=12)

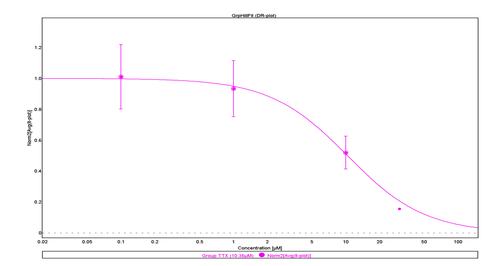
I_{Nav} single-hole



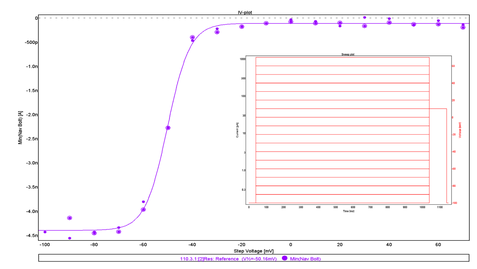
I_{Nav} current sweeps obtained at 0 mV in the absence (large peak) and presence of 10 µM TTX (small peak)



I-T plot; I_{Na} current blocked by increasing concentrations of TTX (0.1, 1, 10 µM)

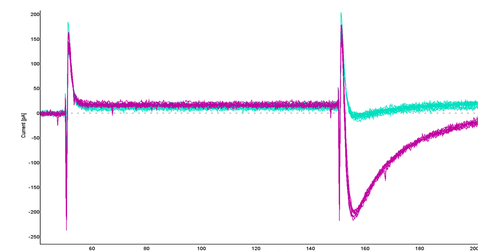


Dose-response plot. Data were fitted to the Hill equation and the IC_{50} value for TTX was 10.3 µM (± 0.5, n=6). (IC_{50} lit. value 10.6 µM, Schneider et al. 1994)

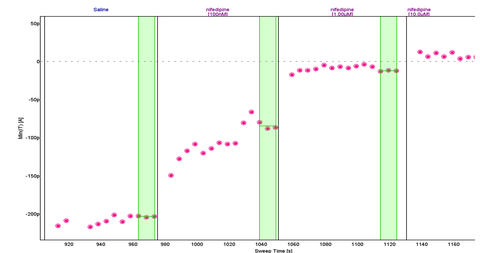


Boltzmann fit of I_{Na} tail current obtained at +10 mV from currents pre-stimulated with voltage steps from -100 mV to +70 mV. The $V_{0.5}$ was -49.4 mV (± 2.9, n=5). ($V_{0.5}$ lit. value -62 to -72 mV, Schneider et al. 1994)

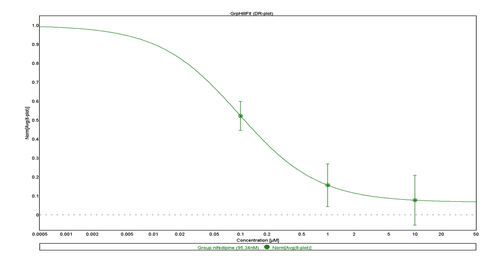
I_{Ca} single-hole



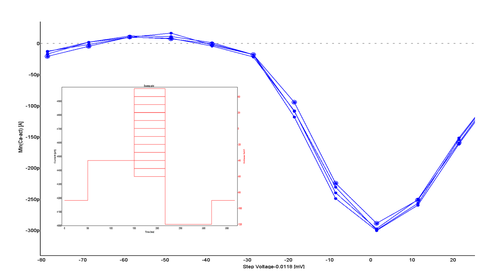
I_{Ca} currents sweeps obtained at +10 mV in the absence (large peak) and presence of nifedipine (1 µM) (small peak). t-type I_{Ca} current was inhibited by a 150 ms pre-pulse at -40 mV



I-T plot; I_{Ca} current blocked by increasing concentrations of nifedipine (0.1, 1, 10 µM)

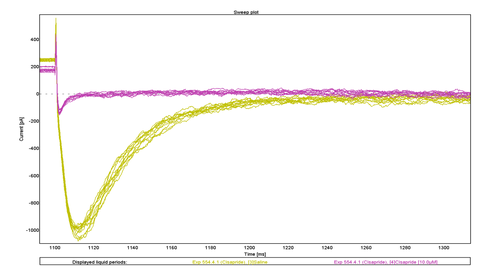


Dose-response plot. Data were fitted to the Hill equation and the IC^{50} value for nifedipine was 95.3 nM (± 42.3, n=4)

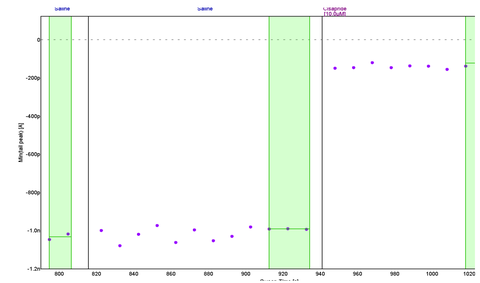


I-V plot of the I_{Ca} current. I_{Ca} was activated by voltage steps from -60 mV to +50 mV. The current began to activate close to -40 mV and peaked at voltages between 0 and +10 mV. (Similar I-V relations, Pelzmann et al. 1998)

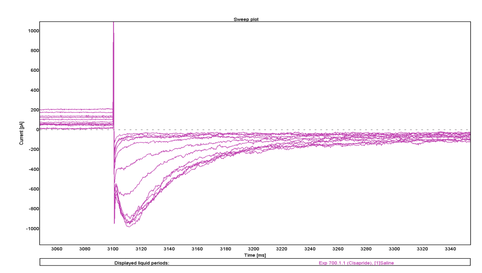
I_K single-hole



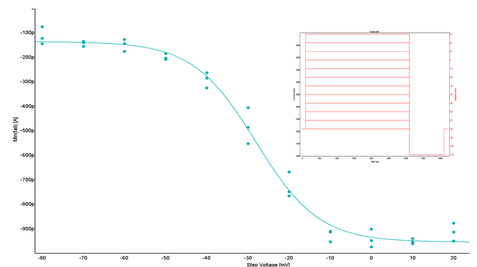
I_K inward tail current sweeps recorded at -100 mV from a pre-pulse at +20 mV (1000 ms). The tail current was blocked by 10 µM cisapride



I-T plot; I_K tail current blocked by 10 µM cisapride. (IC_{50} lit. value 15 nM, Drolet et al. 1998)



I_K tail current recorded at -110 mV after a pre-pulse stimulation with voltage steps from -80 mV to 30 mV



Boltzmann fit of the I_K tail current. The $V_{0.5}$ was -23.1 mV (± 7.5, n=5). ($V_{0.5}$ lit. value -21.5 mV, Sanguinetti and Jurkiewicz 1990)

Conclusions

The study of ion channels in their native tissue using automated patch clamp has historically been impeded by factors like inadequate cell number and non-homogenous population. With the recent efforts in deriving cardiomyocytes from human pluripotent stem cells the challenges of cell number and homogeneity have been removed for automated patch clamp. Still, the stem cell derived cardiomyocytes exhibit some greater heterogeneity compared to heterologous cell lines in expression of I_{Na}, I_{Ca}, I_K, which is limiting the data point throughput in automated patch clamp.

For the first time iCell Cardiomyocytes have been recorded using the multi-hole technology for automated patch clamping. Our results showed that biophysical and pharmacological characteristics of the I_{Na} currents were comparable using the single-hole and the multi-hole technique, respectively. The success rate for useable I_{Na} currents in the experiments increased significantly using the multi-hole technology. From our single-hole recordings we found that most cells expressed the I_{Na} and I_K currents, and less than half of the cells expressed the I_{Ca} current. The recordings from the three different currents, I_{Na} I_{Ca}, I_K, were similar to the recordings from human and other mammalian cardiomyocytes.

In conclusion, the iCell Cardiomyocytes can be cultured in sufficient numbers, can be cryo-preserved, are easy to handle regarding culturing and harvesting, show satisfactory success rates with the QPatch automated patch clamp system, and show expected biophysical and pharmacological behavior from three important currents (I_{Na}, I_{Ca}, I_K). These characteristics make the iCell Cardiomyocytes a potential candidate for *in vitro* cardiac electrophysiological studies. Due to the flow channels in the QPlate extracellular liquids can be exchanged. This provides the possibility of more data points from each experiment (e.g full dose-response or recordings of different currents from the same cell) and therefore gives rise to potential cost savings for the user. Thus, we believe that with QPatch it is possible to obtain high quality recordings, and large number of data points obtained per experiment from a human endogenous cell type with iCells Cardiomyocytes, which makes these cells an attractive application for cardiac safety research.