Exploring stem cell-derived cardiomyocytes with automated patch clamp techniques

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Abstract

There is increasing interest for cardiomyocytes as models for studying cardiac cellular physiology and preclinical drug safety testing. Stem cell-derived cardiomyocytes have the potential for such a model and have the possibility for modeling human diseases. The present investigation is the first to describe current properties from stem cell-derived cardiomyocytes using multi-hole recordings with planar automated patch clamp technology.

In our study pluripotent stem cell-derived cardiomyocytes were biophysically and pharmacologically characterized. The cells are differentiated in large numbers and cryo-preserved, which make them suitable for automated patch clamping and facilitate their use in drug screening. We tested the cells in two different recording modes; single-hole and multi-hole, respectively. For multi-hole recordings up to ten cells are patched at the same time and the total current is measured per site. This recording mode can be useful for small currents (e.g. endogenous) and typically increases the success rate for useful data. For all experiments the whole-cell configuration was used and three different types of currents were studied; Na^+, Ca^{2+} and K^+. Using specific voltage protocols biophysical characteristics of each current was described and compared from single-hole and multi-hole experiments. We showed that currents recorded from these pluripotent stem cell-derived cardiomyocytes are similar to human cardiomyocytes and the response to known pharmacology is as expected. The $V_{0.5}$ values, I-V relationships, current kinetics and IC_{50} values determined for known blockers (TTX, nifedipine and cisapride) were comparable for the two recording modes. Clearly the success rate for usable data per measurement plate was significantly increased with the multi-hole technology. This is the first time current properties of stem cell-derived cardiomyocytes have been described from multi-hole recordings with planar automated patch clamp. Our study has shown that automated patch clamp is ready for stem cell-derived exploration.

Material and Methods

Ringer solutions voltage clamp experiments
EC I_{Na} (mM): 120 NaCl, 5 KCl, 3.6 $CaCl_2$, 1 $MgCl_2$, 20 TEACl, 10 HEPES. pH 7.4 with NaOH
EC I_K (mM): 15 NaCl, 140 KCl, 3.2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES. pH 7.4 with NaOH
EC I_{Ca} (mM): 125 NMG, 3.6 $CaCl_2$, 1 $MgCl_2$, 20 TEA Chloride, 10 HEPES. pH 7.4 with HCl

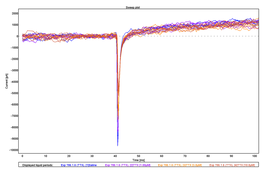
IC I_{Ca} and I_{Na} (mM): 120 CsCl, 3 $MgCl_2$, 10 EGTA, 5 HEPES, 4 Na_2-ATP. pH 7.4 with CsOH
IC for for I_K (mM): 5.374 $CaCl_2$, 1.75 $MgCl_2$, 3.125/10 KOH/EGTA, 120 KCl. pH 7.2 with KOH

Ringer solutions current clamp experiments
EC (mM): 145 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 20 TEACl, 10 HEPES, 10 glucose. pH 7.4 with NaOH
IC (mM): 5.374 $CaCl_2$, 1.75 $MgCl_2$, 3.125/10 KOH/EGTA, 10 HEPES, 120 KCl, 4 Na_2-ATP. pH 7.2 with KOH

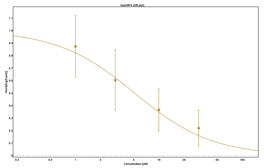
Cells
Cryopreserved human induced pluripotent stem (iPS) cell-derived cardiomyocytes, iCells®, were generously received from Cellular Dynamics. Each vial contained approximately 4.5 mio cells with a plating efficiency of 41-53%. The cells were thawed and plated on fibronectin coated T-25 culture flasks 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.
For action potential recordings human embryonic stem (hES) cell-derived Cytiva™ Cardiomyocytes were generously received from GE Healthcare. Cells were received in frozen vials. Each vial contained approximately 2 mio cells with a purity of 64%. 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. Cells were cultured according to the SOP received from GE Healthcare. 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 pulses 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 patch per measurement site and the total current is recorded. For the single-hole technology one cell is patched per measurement site. Voltage protocols were applied to obtain specific biophysical characteristics of the currents. One or more concentrations of the compounds were added sequentially and automatically through the flow channels in the QPlate. Action potential recordings were obtained after giga seal and whole cell formation. Data were analyzed using the QPatch Assay Software.

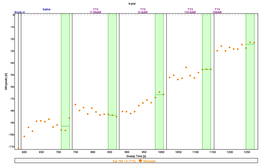
I_{Nav} multi-hole



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)

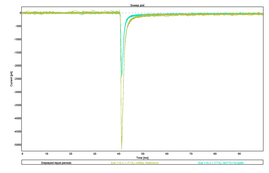


Dose-response plot. Data were fitted to the Hill equation and the IC_{50} value for TTX was 6.3 µM (± 4.5, n=12)

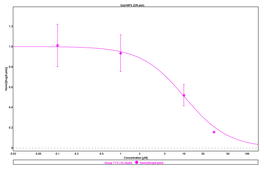


I-T plot; I_{Na} current blocked by increasing concentrations of TTX (1, 3, 10, 30 µM)

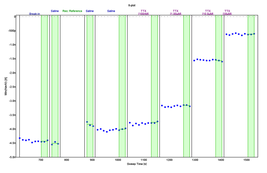
I_{Nav} single-hole



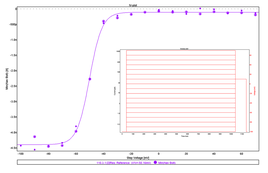
I_{Na} current sweeps obtained at 0 mV in the absence (large peak) and presence of 10 µM TTX (small peak)



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)

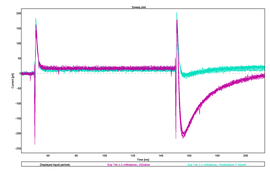


I-T plot; I_{Na} current blocked by increasing concentrations of TTX (0.1, 1, 10 µM)

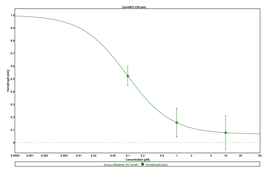


Boltzmann fit of I_{Na} tail current obtained at +10 mV from currents pre-stimulated with voltage steps from -100 mV to +70 mV. The $V_{0.5}$ was -49.4 mV (± 2.9, n=5). ($V_{0.5}$ lit. value -62 to -72 mV, Schneider et al. 1994)

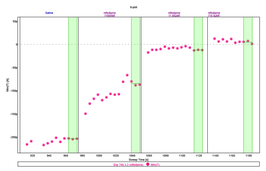
I_{Ca} single-hole



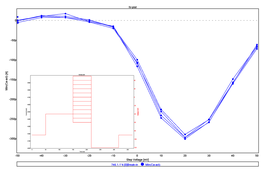
I_{Ca} currents sweeps obtained at +10 mV in the absence (large peak) and presence of nifedipine (1 µM) (small peak). t-type I_{Ca} current was inhibited by a 150 ms pre-pulse at -40 mV



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)

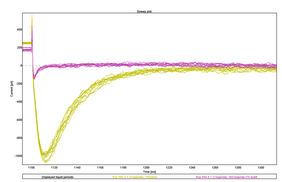


I-T plot; I_{Ca} current blocked by increasing concentrations of nifedipine (0.1, 1, 10 µM)

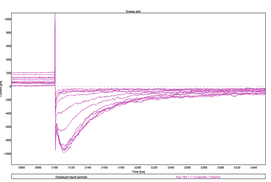


I-V plot of the I_{Ca} current. I_{Ca} was activated by voltage steps from -60 mV to +50 mV. The current began to activate close to -40 mV and peaked at voltages between 0 and +10 mV. (Similar I-V relations, Pelzmann et al. 1998)

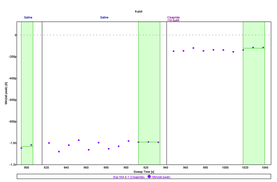
I_K single-hole



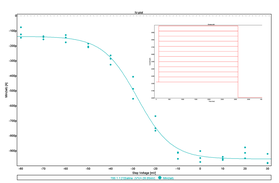
I_K inward tail current sweeps recorded at -100 mV from a pre-pulse at +20 mV (1000 ms). The tail current was blocked by 10 µM cisapride



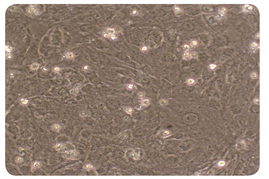
I-V plot of the I_K current. I_K was activated by voltage steps from -60 mV to +50 mV. The current began to activate close to -40 mV and peaked at voltages between 0 and +10 mV. (Similar I-V relations, Pelzmann et al. 1998)



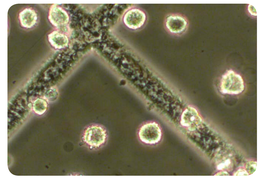
I-T plot; I_K tail current blocked by 10 µM cisapride. (IC_{50} lit. value 15 nM, Drolet et al. 1998)



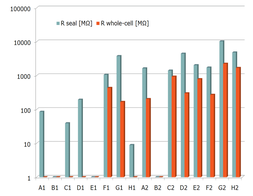
Boltzmann fit of the I_K tail current. The $V_{0.5}$ was -23.1 mV (± 7.5, n=5). ($V_{0.5}$ lit. value -21.5 mV, Sanguinetti and Jurkiewicz 1990)



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.



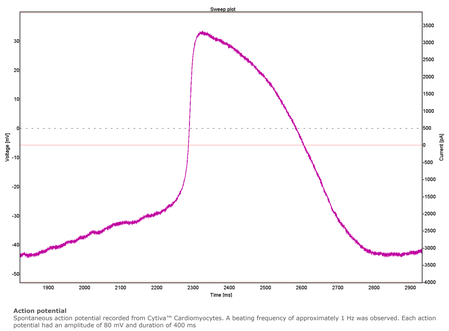
QPlate sealing overview from a **single-hole** experiment. The success rate for sealing for single-hole experiments could reach up to 70 % for sealing and 60 % for whole-cells

	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_{Navy} 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 of iCell Cardiomyocytes. *Specific currents from established whole-cells. **Recorded at 0 mV. ***Data fitted to a monoexponential curve



Action potential
Spontaneous action potential recorded from Cytiva™ Cardiomyocytes. A beating frequency of approximately 1 Hz was observed. Each action potential had an amplitude of 80 mV and duration of 400 ms

References:

Schneider M, Proebstle T, Hombach A, Rüdel R Characterization of the sodium currents in isolated human cardiocytes. Plügers Arch, 1994;84-90

Drolet B, Khalifa M, Daleau P, Hamelin B, Turgeon J Block of the rapid component of the delayed rectifier potassium current by the prokinetic agent cisapride underlies drug-related lengthening of the QT interval. Circulation, 2011;204-210

Sanguinetti MC, Jurkiewicz NK Two components of cardiac delayed rectifier K+ current. J Gen Physiol, 1990;195-215

Pelzmann B, Schaffer P, Bernhart E, Lang P, Mächler H, Rigler B, Kiodl B L-type calcium current in human ventricular myocytes at a physiological temperature from children with tetralogy of fallot. Cardiovascular Research, 1998;424-432

Conclusion

The study of ion channels in their native tissue using automated patch clamp has historically been impeded by factors like; the cells have not be available in sufficient numbers and the cell population has shown inadequate homogeneity. With the recent efforts in deriving cardiomyocytes from pluripotent-, and embryonic stem cells, cardiomyocytes are now available in adequate quantities and with acceptable purity to match a range of applications for automated patch clamp technology. Still, the stem cell-derived cardiomyocytes exhibit some variation in expression of specific current sub-types, which limits the data point throughput for automated patch clamp systems.

Here we show single-hole and multi-hole recordings of I_{Na}, I_{Ca}, I_K recorded from human iPS cardiomyocytes. 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 I_{Na} were comparable using the single-hole and the multi-hole technique, respectively. The success rate for experiments with useable I_{Na} significantly increased using the multi-hole technology. From our single-hole recordings we found that most cells expressed I_{Na} and I_K, and less than half of the cells expressed I_{Ca}. The recordings from I_{Na} I_{Ca}, I_K were similar to recordings obtained and reported from other mammalian cardiomyocytes.

We used hES Cytiva cells for current clamp recordings obtained on the QPatch system. These cells exhibited spontaneous action potentials with ectrophysiological properties expected for ventricular subtypes (similar data obtained from iPS iCell cardiomyocytes, data not shown).

In conclusion, stem cell-derived cardiomyocytes are commercially available in large quantities, are easy to handle and show satisfactory success rates especially for multi-hole recordings with the QPatch automated patch clamp system. The iPS and hES cells used in this study have shown expected biophysical and pharmacological behavior from voltage clamp and current clamp recordings. From our exploration of different stem cell-derived cardiomyocytes our data have shown that these cells are candidates for *in vitro* cardiac electrophysiological studies and can be a power full tool for cardiac safety research.