SOPHION BIOSCIENCE

Application Report

hiPSC-derived cardiomyocyte recordings using physiological solutions on QPatch II

Electrophysiological characterization of human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), including recordings of voltage-gated ion channels (Na_V, Ca_V, hERG) and paced action potentials

Summary

- It is possible to record voltage-gated ion channels (Na_V, Ca_V and hERG) from hiPSC-CMs in physiological solutions with up to 50% success rates using QPatch II
- It is possible to record paced action potentials from hiPSC-CMs in physiological solutions with up to 20% success rates using QPatch II
- Within the hiPSC-CM population that pass quality criteria we record Na_V in ~ 90%, Ca_V in ~ 80%, hERG in ~ 60% and paced action potentials in ~ 50% of the CMs

Introduction

Primary cardiomyocytes are difficult to obtain and maintain in culture, and cardiac diseases have therefore been investigated in differentiated stem cells. Traditionally, these studies were conducted using embryonic stem cells[1]; however, this approach is challenging in many countries due to ethical issues and might fail to capture late-onset diseases.

The human-induced pluripotent stem cell (hiPSC) technology was developed in 2007[2,3], and hiPSC-derived cardiomyocytes (hiPSC-CMs) have since then been evaluated as a promising model system for cardiac drug screening and disease modelling[4,5].

Automated patch clamp (APC) studies of hiPSC-CMs have been challenging due to:

1. Cell quality (batch-batch variation, differentiation efficiency)[6]
2. Cell harvest (purity/quality of the single-cell suspension)
3. Cardiac maturity (presence of pacemaking current I_f and reduced densities of hyperpolarising current I_{K1}[7,8].

Consequently, APC recordings of hiPSC-CMs in general run with low success rates and poor reproducibility. However, as cell biologists improve the quality and maturity of hiPSC-CMs, and electrophysiologists improve the quality and purity of the cell suspension, these parameters are improving. Here we demonstrate that we can run hiPSC-CM experiments in physiological solutions with up to 50% success rates depending on the assay (quality criteria: membrane resistance (R_{mem}) > 200 MΩ, cell capacitance (C_{slow}) > 4 pF, see Table 1).

Table 1: hiPSC-CM assay success rates (R_{mem} > 200 MΩ and C_{slow} > 4 pF) are listed for four different assays. Data are avg ± sd of N_{plates}

Assay	Assay success rate [%] of 48 measurement sites	N_{plates}
Na_V assay	40 ± 10	3
Ca_V assay	21 ± 8	3
hERG assay	14 ± 1	2
Paced action potentials assay	19 ± 9	3

We record Na_V currents in ~ 90%, Ca_V currents in ~ 80%, hERG currents in ~ 60% and paced action potentials in ~50% of the hiPSC-CMs that pass the quality criteria (see Table 2). This indicates, not surprisingly, that expression of all ion channels is required to obtain physiologically relevant action potentials[8] and supports further investigation into hiPSC-CM maturation protocols[9,10].

Table 2: Ion channel and action potential recordings in hiPSC-CMs. The phenotype rate was quantified as the percentage of successful experiments that passed the current/voltage filters listed on the right (see later section for further details). Data are avg ± sd of N_{plates}.

	Phenotype rate [%] of successfull experiments	N_{plates}	Current/voltage filters
Na_V assay	87 ± 8	3	$\lvert I_{Nav} \rvert$ > 200 pA
Ca_V assay	80 ± 20	3	$\lvert I_{Cav} \rvert$ > 100 pA
hERG assay	62 ± 8	2	$\lvert I_{hERG,tail} \rvert$ > 100 pA
Paced action potentials assay	50 ± 10	3	V_p > 0 mV, RMP < -40 mV

Results and discussion

Basic evaluation

First, we performed a basic evaluation of the performance of the hiPSC-CMs on QPatch II in physiological solutions. The cell suspension was prepared according to Sophion in-house protocols.

We calculated the success rate as the percentage of sites, out of the full experiment plate (48 sites) that passed the following quality criteria:

Membrane resistance: R_{mem} > 200 MΩ

Cell capacitance: C_{slow} > 4 pF

These criteria yielded up to 50% success rates (Fig. 1A). In ~ 90% of the successful experiments we recorded Na_v currents ($|I_{Nav}|$ > 200 pA, Fig. 1B-D).

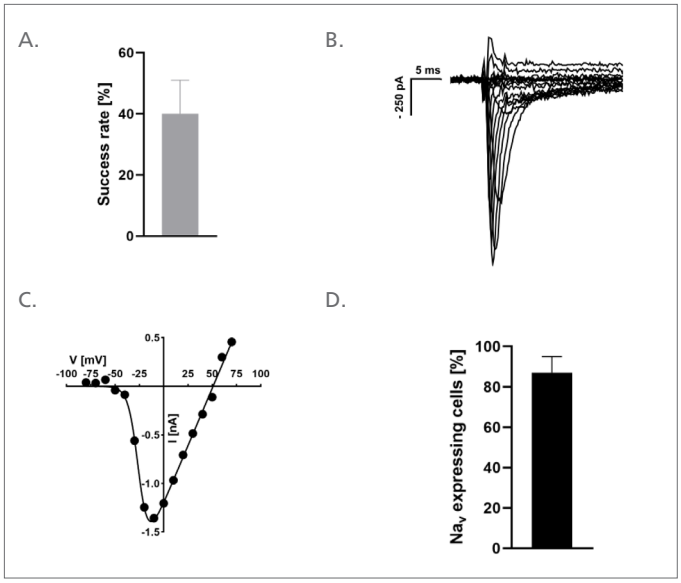

Fig. 1: Performance of hiPSC-CMs in physiological solutions on QPatch II. A) Experiment success rate in percentage of 48 experiment sites (R_{mem} > 200 MΩ and C_{slow} > 4 pF). Data is avg ± sd of three measurement plates. B) Representative voltage-gated Na_V current trace in response to a voltage step protocol from –80 mV to +70 mV (ΔV = +10 mV) following a pre-step to -110 mV (see methods). C) Na_V current (I) vs. step voltage (V) plot. D) Percentage of successful experiments with Na_V currents. Data is avg ± sd of three experiment plates.

The hiPSC-CMs that passed the quality criteria displayed a heterogeneous size distribution, as illustrated in the histogram of C_{slow} values shown in Figure 2, ranging from 5 pF to 50 pF. The sealing in physiological solutions was good with Rmem = 1.5 GΩ ± 0.3 GΩ (avg ± sem) (see Fig. 2A + B for histograms).

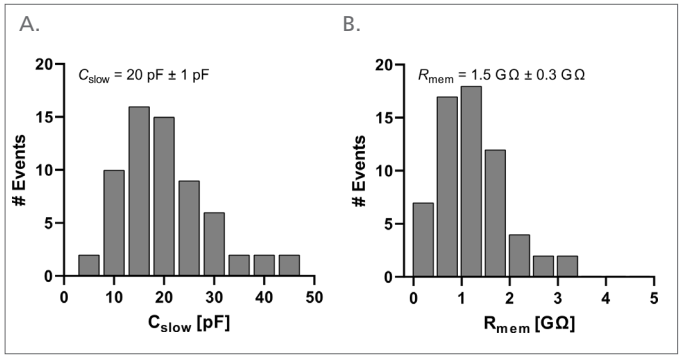

Fig. 2: Histograms of C_{slow} and R_{mem} values of the measured cell population (N_{cells} = 70) together with the avg ± sem values.

Ca_V measurements

Next, we quantified the percentage of the tested hiPSC-CMs that expressed $Ca_V1.2$ channels. The assay was conducted with up to a 30% success rate, and in ~80% of successful experiments, we recorded Ca_V currents ($|I_{Cav}$ > 100 pA, see Figure 3). We quantified the $Ca_V1.2$ current-voltage relationship in physiological solutions before and after block by 10 µM nifedipine (Fig. 3B + C).

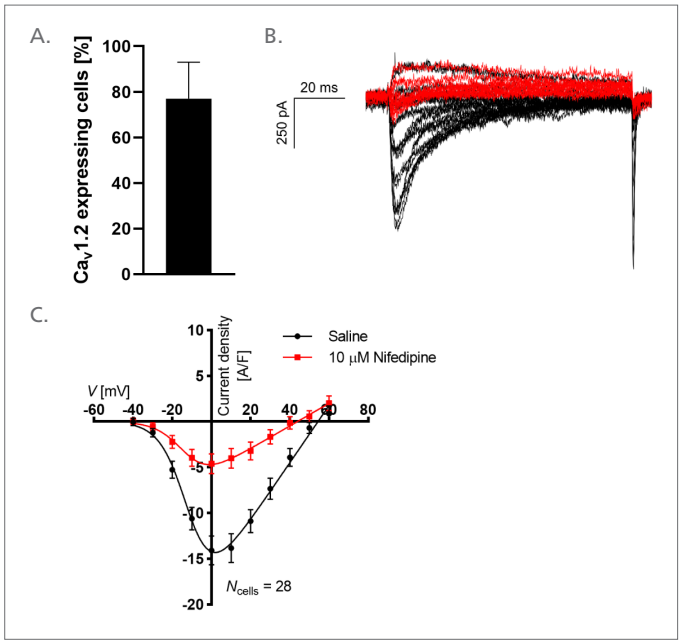

Fig. 3: QPatch II measurements of $Ca_V1.2$ in hiPSC-CMs in physiological solutions. A) The percentage of successful experiments with a Ca_V current (I_{Cav} < -100 pA), avg ± sd of 3 measurement plates. B) Representative Ca_V current in response to a voltage step protocol from –40 mV to +80 mV with 10 mV step size (see methods), before (black) and after (red) the addition of 10 µM nifedipine. C) Ca_V current density as a function of voltage before (black) and after (red) the addition of 10 µM nifedipine. Data points are avg ± sem of N_{Cells} = 28.

hERG measurements

hERG tail currents were induced by increasing the extracellular K^+ concentration from 5 mM to 75 mM, as previously shown[11] and subsequently blocked by addition of 0.5 µM E4031 (see Fig. 4). The assay runs with up to 30% success rates, and in ~60% of successful experiments we recorded hERG tail currents ($|I_{herg,tail}| > 100$ pA). We quantified hERG tail current-voltage relationship in 75 mM K^+ before and after block by 0.5 µM E4031 (see Fig. 4B + C).

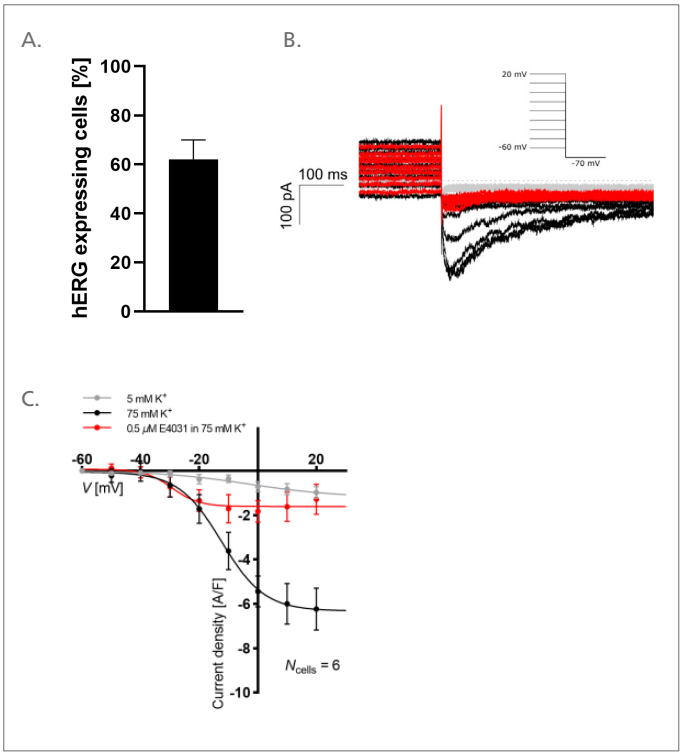

Fig. 4: QPatch II measurements of hERG in hiPSC-CMs. A) The percentage of successful experiments with a hERG tail current ($I_{hERG,tail} < -100$ pA). Data is avg ± sd of two measurement plates. B) Representative hERG tail current recorded at V = –70 mV following a voltage step protocol from –60 mV to +20 mV with 10 mV step size (see methods). The tail current was measured in 5 mM extracellular K^+ (grey) and 75 mM extracellular K^+ before (black) and after (red) addition of 0.5 µM E4031. C) hERG current density as a function of voltage in 5 mM extracellular K^+ (grey) and 75 mM extracellular K^+ before (black) and after (red) addition of 0.5 µM E4031. Data points are avg ± sem of $N_{Cells} = 6$.

Action potential measurements

Paced action potentials were recorded in response to 1 nA current injections. Analysis of the action potentials allowed us to filter according to following parameters:

Peak potential: $V_p > 0$ mV
Resting membrane potential: RMP < -40 mV

The assay yielded up to a 30% success rate, and ~50% of successful experiments passed the filtering criteria (Fig. 5A), resulting in up to 10 cells with paced action potentials per measurement plate (Fig. 5B). The threshold potential (V_t), peak potential (V_p), hyperpolarization potential (V_h) and action potential duration at 90% (APD90) were quantified. The potentials were relatively reproducible between the cells, V_t = -48 mV ± 8 mV, V_p = 35 mV ± 9 mV, V_h = -63 mV ± 6 mV, with relative standard deviation (RSD) between 10% - 26% (Fig. 5C). The APD90 was more variable with an RSD of ~ 50%.

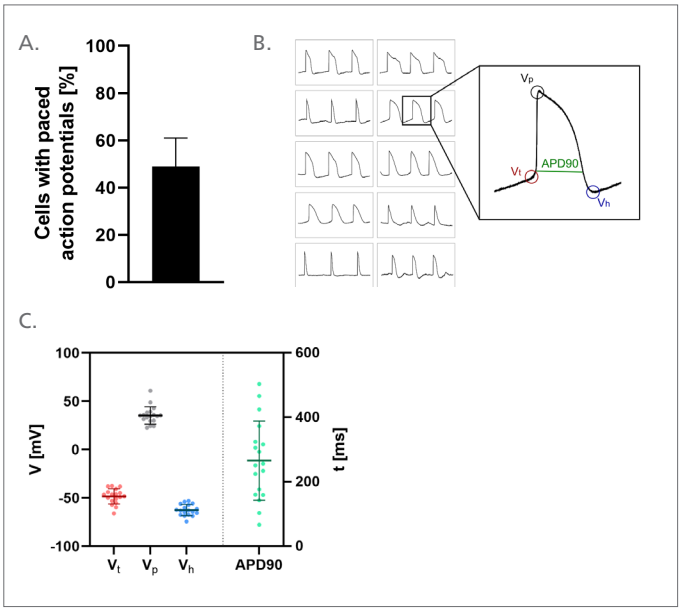

Fig. 5: QPatch II measurements of paced action potentials. A) The percentage of successful experiments displaying paced action potentials ($V_p > 0$ mV, RMP < -40 mV). Data is avg ± sd of three measurement plates. B) Paced action potentials from ten individual hiPSC-CMs within a single measurement plate (left) and a zoom on an action potential displaying the extracted parameters; threshold potential (V_t), peak potential (V_p), hyperpolarization potential (V_h) and action potential duration at 90% (APD90). C) Plot of extracted parameters, V_t (red), V_p (black), V_h (blue) and APD90 (green) for 18 individual iPSC CMs as well as the avg ± sd (solid lines).

The action potential duration at 90% (APD90) was quantified before and after adding 10 µM nifedipine and displayed on average a 50% shortening (Fig. 6) in agreement with complementary studies[12].

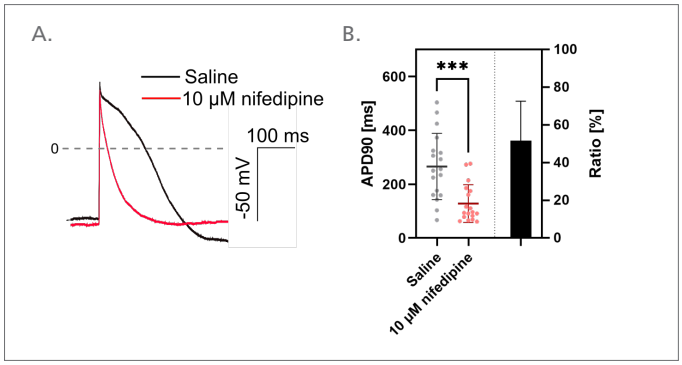

Fig. 6: Shortening of paced action potential by addition of nifedipine. A) Representative paced action potential before (black) and after (red) addition of 10 µM nifedipine. B) Plot of APD90 values before (black) and after (red) addition of 10 µM nifedipine (left) as well as the average ratio = $\frac{APD90_{comp}}{APD90_{saline}}$ (right). The action potentials were significantly shortened (~50%) by addition of nifedipine (two-tailed t-test, $p < 0.001$). Data are avg ± sd of 18 hiPSC-CMs.

Conclusion

In summary, it is possible to perform measurements on QPatch II of hiPSC-CMs in physiological ringers with voltage-gated assays yielding up to 50% success rate, depending on the ion channel target. These measurements demonstrate a heterogeneous expression of the cardiac ion channels, which consequently lowers the success rates (up to 20%) of action potential measurements. As 1) the hiPSC-CM quality and maturity increases and 2) cell suspension preparation is further optimized, we expect the throughput of these measurements to increase. Eventually, we envision that APC can be used as a characterization tool to assist the continuous development of hiPSC-CMs as well as for cardiac drug screening and disease modelling.

Methods

- **Cells were kindly provided by the laboratory of Professor Niels Voigt, Göttingen www.molecular-pharmacology.de**
- Cell culture and cell suspension preparation according to internal Sophion methods.
- Voltage protocols

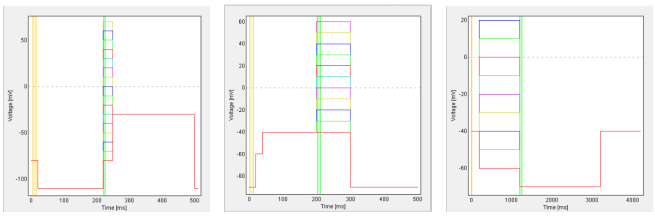

Fig. 7: Applied voltage clamp protocols. A) Na_V IV, B) Ca_V IV, C) hERG IV.

All analysis was with Sophion Analyzer

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