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Characterization of Human iPSC-derived cardiomyocytes (Cor.4U®) on an automated planar patch clamp set up (Qube)

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Introduction

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC–CM) are providing new, highly predictive strategies to assess cardiotoxicity in vitro and can thus reduce costs for cardiac safety assessment in drug development[1].

Different technologies are available to assess compound effects on cardiomyocytes, out of all, the patch clamp technique remains the gold standard as it allows to study compound effects both on individual currents but also on the entire ion channel ensemble in form of an action potential (AP). However, such studies have traditionally been limited by the labour-intensive and low-throughput nature of patch clamp electrophysiology[2].

Here we present the electrophysiological characterization of hiPSC–CM (Cor.4U) cells using our automated patch clamp (APC) platform Qube 384. Electrophysiological investigation of hiPSC-CM requires a high quality set up that offers the possibility to both record APs in current clamp mode and to isolate individual ion channel currents using voltage clamp. Qube is a 384-channel automated patch clamp system that fulfills all these requirements.

Our data illustrate that stem cell technology in combination with Qube's high throughput capability holds great potential to accelerate cardiac safety studies.

Conclusion

We developed an assay for iPSC-derived cardiomyocytes on Qube. With this assay it is possible to isolate I_{Na}, I_K and I_{Ca} using voltage clamp. Moreover, using the current clamp mode it is possible to evoke APs and study compound effects on different AP characteristics including the action potential duration.

Materials and methods

Cell culture: Cor.4U human iPS cell-derived cardiomyocytes were cultured according to Ncardia's guidelines. Cell density was between 1 and 2 million/mL and 2.7 mL of cell suspension were sufficient for one 384-well QPlate.

Cell preparation: Different harvesting protocols were tested. Briefly, the cell detachment solution Detachin did not properly separate the cells. A two-step protocol with 5 min Accutase and 12-18 h Collagenase type II incubation resulted in a well separated preparation with good seal resistances, but results were variable. The best results were obtained by dissociating the cells using Accutase for 10 min.

References

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2. Dunlop, J., Bowlby, M., Peri, R., Vasilyev, D. & Arias, R. High-throughput electrophysiology: an emerging paradigm for ion-channel screening and physiology. Nat. Rev. Drug Discov. 7, 358–368 (2008).
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Section 1: High fidelity voltage clamp recordings of Cor.4U hiPSC - CMs demonstrating the presence of Na_V, K_V and Ca_V channels

The voltage clamp mode was used to isolate individual currents in Cor.4U cells. A family of voltage steps from – 90 mV to + 60 mV in 10 mV increments (holding potential = -120 mV) was applied to the cells to elicit sodium current (I_{Na}) and potassium current (I_K) (Fig. 1 and 2). To study calcium current (I_{Ca}) in Cor.4U cells, a double pulse protocol was employed. First, a voltage pre-step to -40 mV was applied that deactivated voltage – gated Na^+ and T-type Ca^{2+} channels. Second, the membrane was clamped to a series of voltages from -40 to 70 mV in 10 mV increments (Fig. 3).

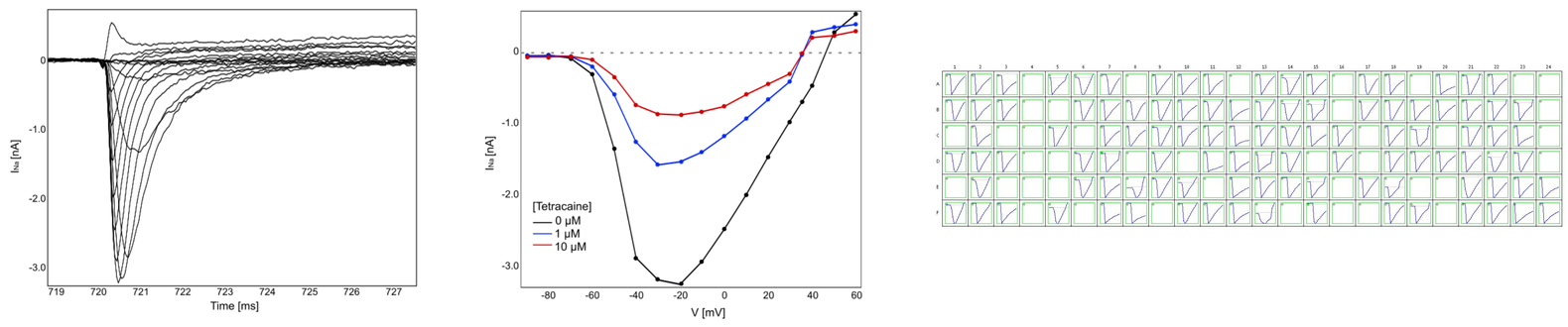

Fig. 1: **Typical sodium currents (I_{Na}) in a Cor.4U cell**
Top left panel: Sodium current traces (I_{Na}) were elicited using a series of voltage steps from – 90 mV to + 60 mV in 10 mV increments from a holding potential of -120 mV. Top right panel: Current-voltage relationship of the peak current in control condition (black), 1 µM (blue) and 10 µM (red) tetracaine. Application of tetracaine, which is an inhibitor of voltage-gated sodium channels, resulted in a concentration-dependent inhibition of I_{Na}. Bottom panel: Plate view of sodium IV curves in Cor.4U cells. Using the cell clone cell transfer plate (ccCTP) it is possible to add Cor.4U cells to the top 6 rows of the QChip, only. It was possible to record sodium IV curves from 72% of all tested wells.

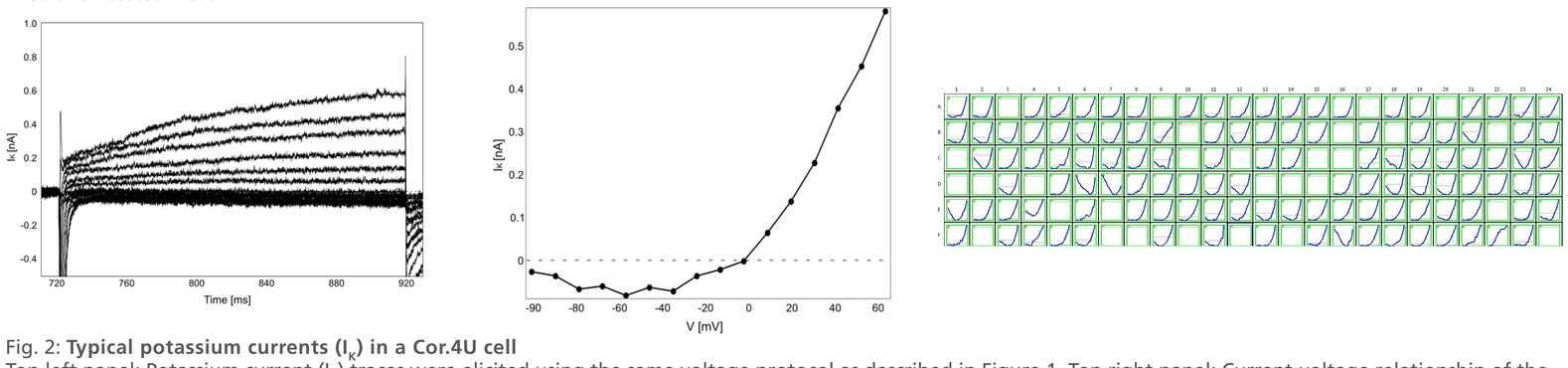

Fig. 2: **Typical potassium currents (I_K) in a Cor.4U cell**
Top left panel: Potassium current (I_K) traces were elicited using the same voltage protocol as described in Figure 1. Top right panel: Current-voltage relationship of the steady state current (measured 190-200 ms after the voltage step). The current recorded exhibited pronounced outward rectification. Bottom panel: Plate view illustrating potassium IV curves in Cor.4U cells. Only the top 6 rows of the QChip were loaded with Cor.4U cells.

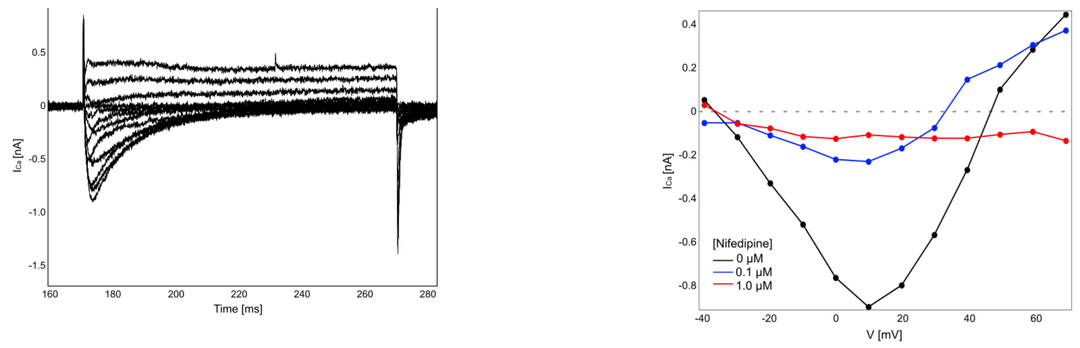

Fig. 3: **Typical calcium currents (I_{Ca}) in a Cor.4U cell**
Left panel: Calcium current (I_{Ca}) traces were evoked using a double pulse protocol with a pre-pulse to -40 mV followed by a set of voltage steps from –40 mV to +70 mV in 10 mV increments. Right panel: Current-voltage relationship in control (black) condition, 0.1 µM (blue) and 1 µM (red) nifedipine. In line with reports in the literature, 1 µM nifedipine completely abolished I_{Ca}[3]. The peak current measured between 2 - 10 ms after the voltage step was used to construct the IV curve.

Section 2: Current clamp recordings of paced and spontaneous action potentials (APs)

The current clamp mode was used to study APs in the human iPSC-derived cardiomyocytes. With Qube it is possible to change between voltage and current clamp within the same sweep. We evoked an AP by a brief current injection that depolarizes the membrane. An example of this is shown in Figure 4, where an AP was generated by injecting 1 nA current for 1 ms. The Sophion Analyzer software features several advanced analysis methods, one of which allows to easily extract the action potential duration (APD) at a given height (in percentage, Figure 4). This feature can be used to study compound effects on APs. Application of nifedipine resulted in a shortening of the AP with the following mean values $APD90_{Control}$ = 350 ± 140 ms, $APD50_{0.1µM\ Nif.}$ = 240 ± 90 ms and $APD30_{1µM\ Nif.}$ = 140 ± 20 ms (SD; n=9).

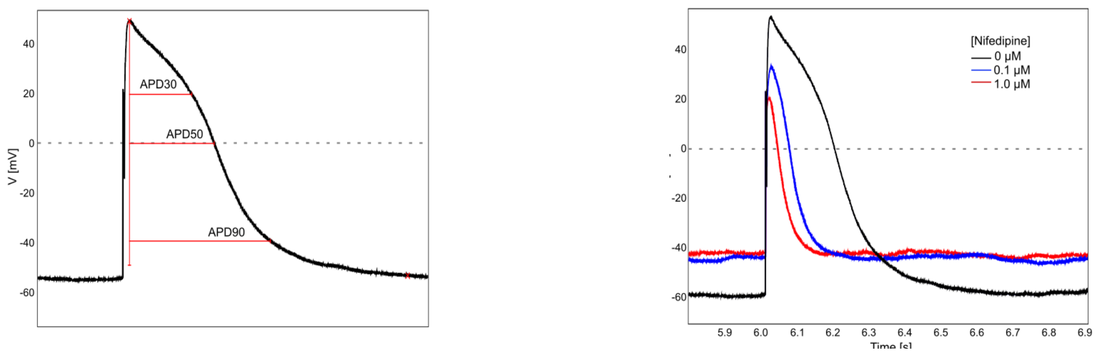

Fig. 4: **Action potential recorded in the current clamp mode in Cor.4U cells**
Left Panel: Action potential with APD30, 50 and 90 indicated with horizontal lines. The automatically detected maximum and minimum voltage are illustrated by small red crosses. These and many other characteristics of the action potential can directly be used in the further analysis. Right panel: AP recorded in control condition (black), 0.1 µM (blue) and 1 µM (red) nifedepine. The mean of n=9 ± SD was $APD90_{Control}$ = 350 ± 140 ms, $APD50_{0.1µM\ Nif.}$ = 240 ± 90 ms and $APD30_{1µM\ Nif.}$ = 140 ± 20 ms.

Cor.4U® cell were kindly provided by NCardia

Ncardia
Stem cell experts