SOPHION BIOSCIENCE

Application Report

Human iPS cell-derived cardiomyocytes (Cor.4U®) on Sophion's Qube 384: Voltage and current clamp recordings

Cor.4U® cells were kindly provided by Ncardia

Summary

- High fidelity voltage and current clamp recordings of human iPS cell-derived cardiomyocytes (Cor.4U®) on Qube
- Demonstration of the presence of I_{Na}, I_K and I_{Ca}
- High throughput current clamp recordings
- Pharmacological characterization of action potentials and isolated currents
- Using Sophion Analyzer software it is possible to easily extract key parameters relevant for studying compound effects on action potentials

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 (Ma et al., 2011). 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).

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-based, second generation automated patch clamp system that fulfills all these requirements. Stem cell technology in combination with Qube's high throughput capability holds great potential to accelerate cardiac safety studies. In the present study, we developed and assay for hiPSC-CM (Cor.4U) using Qube both in voltage and in current clamp mode.

Results and discussion

Sodium current (I_{Na})

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 I_{Na}. Figure 1 shows a typical current response to such a protocol. In a next step, the pharmacological tool agent, tetracaine, was used to further investigate this current. Tetracaine is an inhibitor of voltage-gated sodium channels and application of the compound resulted in a concentration-dependent inhibition of I_{Na} (bottom panel of figure 1). A plate view of all sodium IV curves recorded during one experiment is represented in Figure 2. It was possible to record IV curves from 72% of all tested wells.

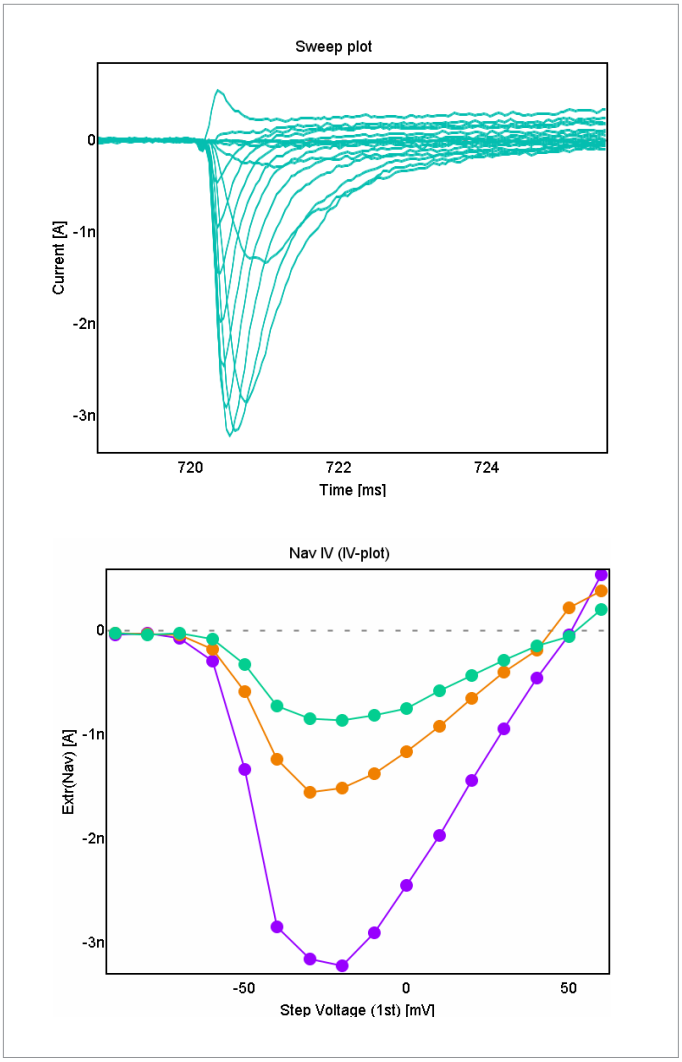

Fig. 1: Typical sodium currents (I_{Na}) in a Cor.4U cell. Top panel: Current traces were elicited using a series of voltage steps from -90 mV to +60 mV in 10 mV increments from a holding potential of -120 mV. Bottom panel: Current - voltage relationship of the peak current in control condition (blue), 1 µM (orange) and 10 µM (green) tetracaine.

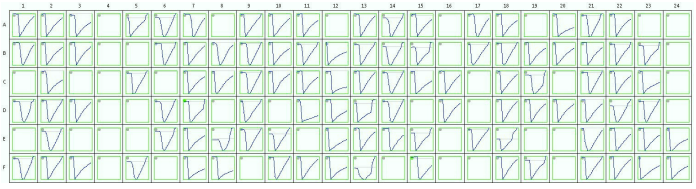

Fig. 2: Plate view of sodium IV curves in Cor.4U cells. Using the cell clone cell transfer plate (ccCTP) it is possible to only add cells to a certain number of rows, here only the top six rows of the QChip were loaded with Cor.4U cells. It was possible to record sodium IV curves from 72% of all tested wells.

Potassium current (I_K)

I_K were characterized using the same protocol as described in figure 1. A time and voltage-dependent outward current was recorded that exhibited pronounced outward rectification when analyzed between 190-200 ms after the voltage step (figure 3). Figure 4 shows a plate view of all voltage-gated K^+ IV curves.

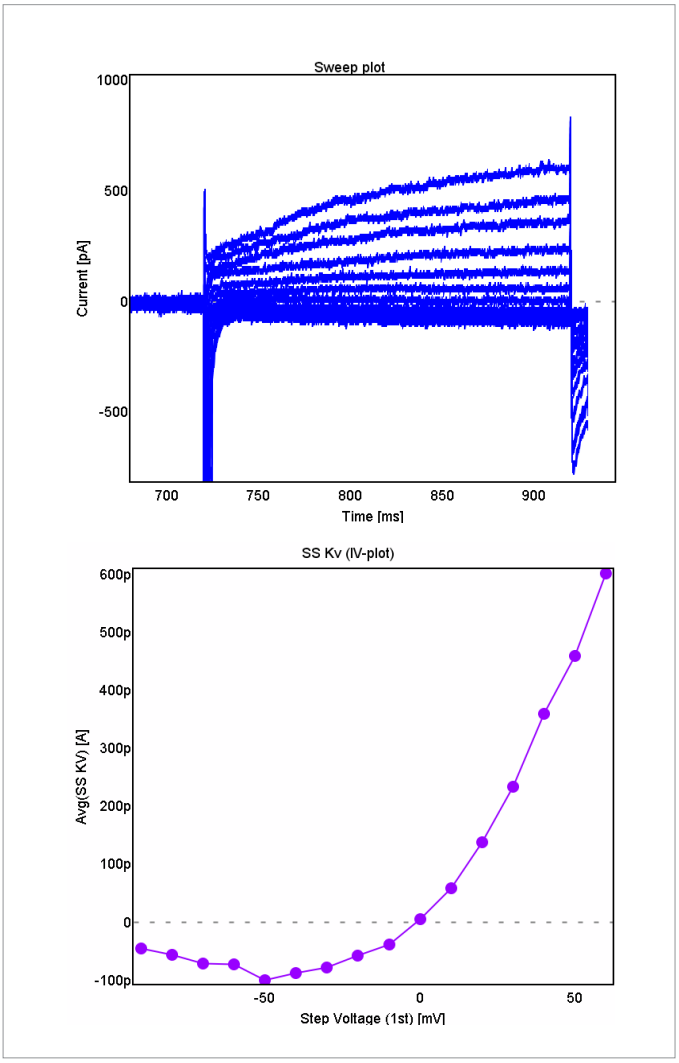

Fig. 3: Typical potassium currents (I_K) in a Cor.4U cell. Top panel: Current traces were elicited using the same voltage protocol as described in Figure 1. Bottom panel: Current-voltage relationship of the steady state current (measured 190-200 ms after the voltage step).

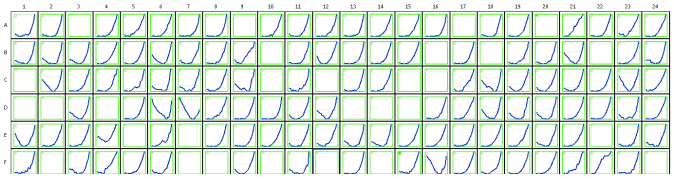

Fig. 4: Plate view illustrating potassium IV curves in Cor.4U cells. Only the top six rows of the QChip were loaded with Cor.4U cells.

Calcium current (ICa)

To study 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. A typical current response to such a protocol is shown in figure 5. Nifedipine, an inhibitor of voltage-gated Ca^{2+} channels, was used to characterize the current. The right panel of figure 5 shows IV curves in the presence of various concentrations of the compound. In line with reports in the literature, 1 µM nifedipine completely abolished I_{Ca} (Harraz & Welsh, 2013).

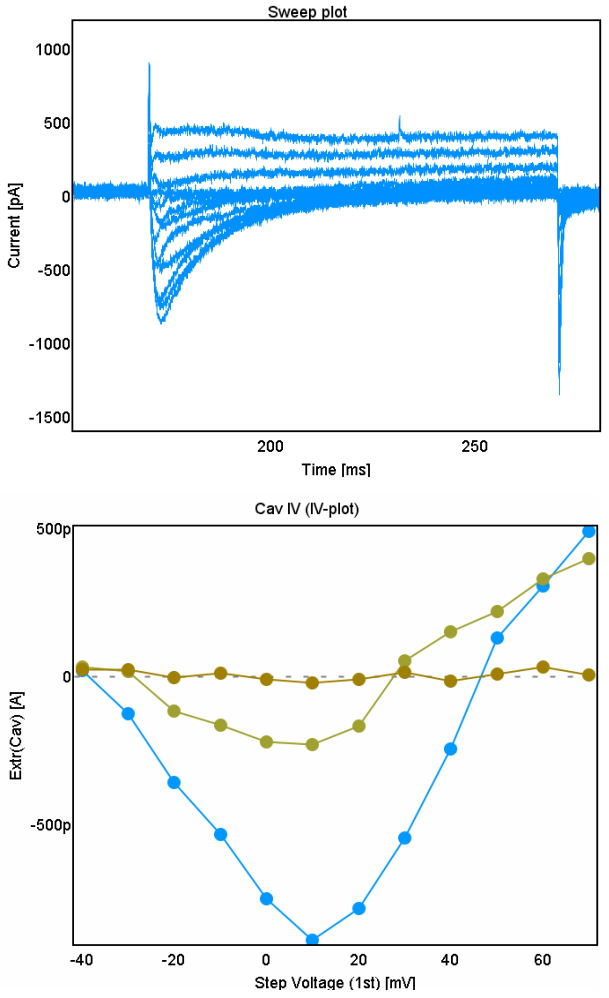

Fig. 5: Typical Ca_v and K_v current in a Cor.4U cell. Top panel: Current traces were evoked using a double pulse protocol with a pre-pulse to -40 mV followed by a set of voltage steps from –40 mV to +70 mV in 10 mV increments. Bottom panel: Current voltage relationship in control (blue) condition, 0.1 µM (orange) and 1 µM (red) nifedipine. The peak current measured between 2-10 ms after the voltage step was used to construct the IV curve.

Action potentials (APs)

Finally, 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 mode during a sweep. Changing the clamp from V = -90 mV to I = 0 pA elicited an AP in many of the cells (Figure 6).

Another possibility to evoke an AP is a brief current injection that depolarizes the membrane. An example of this is shown in figure 7, where an AP was generated by injecting 1 nA current for 1 ms. 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 7). 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, $APD90_{1µM\ Nif.}$ = 240 ± 90 ms and $APD90_{1µM\ Nif.}$ = 140 ± 20 ms (SD; n=9).

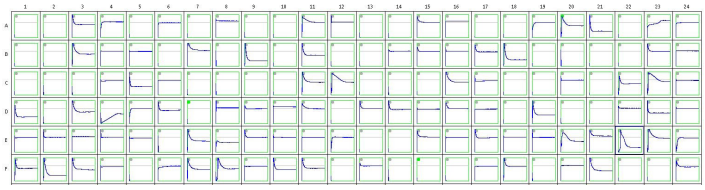

Fig. 6: Plate view of APs in Cor.4U cells. APs were elicited by changing from voltage clamp (V=-90 mV) to current clamp (I = 0 pA). Only the top 6 rows of QChips were loaded with Cor.4U cells in this experiment.

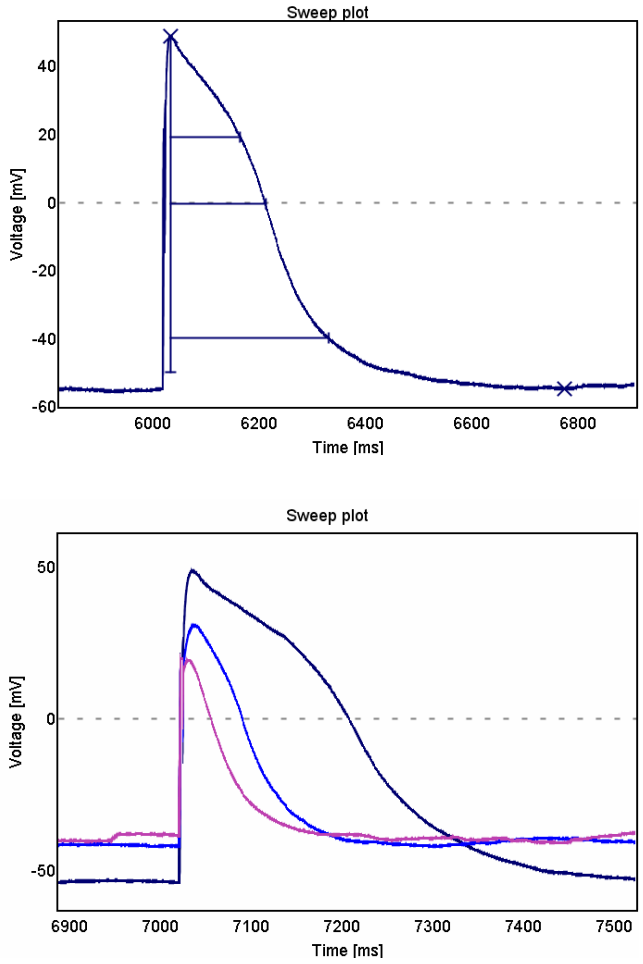

Fig. 7: Action potential recorded in the current clamp mode in Cor.4U cells. Top panel: Action potential with APD30, 50 and 90 indicated with horizontal lines. The automatically detected maximum and minimum voltage are illustrated by ×. These and many other characteristics of the action potential can directly be used in the further analysis. Bottom panel: AP recorded in control condition (dark blue), 0.1 µM (blue) and 1 µM (purple). The mean of n=9 ± SD was $APD90_{Control}$ = 350 ± 140 ms, $APD90_{1µM\ Nif.}$ = 240 ± 90 ms and $APD90_{1µM\ Nif.}$ = 140 ± 20 ms

In 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 APD.

Methods

Cell culture

- Cor.4U human iPS cell-derived cardiomyocytes were cultured and harvested according to Ncardia's guidelines. Cell density was between 1 and 2 mio/ml and 200 µl of cell suspension were sufficient for one 48-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 using the following harvesting protocol.

- Harvest protocol for Cor.4U in T25 flask
  1. Remove culture media and wash two times with PBS
  2. Add 3 mL Accumax and then remove two mL (leaving 1 mL)
  3. Place the culture flask in a 37°C incubator for 10 min
  4. Add 4 mL serum-free media and gently resuspend
  5. Transfer the suspended cells to centrifuge tube
  6. Centrifuge for three minutes in 1000 rpm
  7. Remove supernatant and add 1.5 mL extracellular saline
  8. Place in fridge (4°C) for 20-60 min prior to the experiment

References:

1. Harraz, O. F., & Welsh, D. G. (2013). Protein kinase A regulation of T-type Ca^{2+} channels in rat cerebral arterial smooth muscle. Journal of Cell Science, 126(Pt 13), 2944–54.
2. Ma, J., Guo, L., Fiene, S. J., Anson, B. D., Thomson, J. A., Kamp, T. J., … Ct, J. (2011). High purity human-induced pluripotent stem cell-derived cardiomyocytes : electrophysiological properties of action potentials and ionic currents. Am J Physiol Heart Circ Physiol, 301(5), 2006–2017.

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