Application Report:

Cor.4U on QPatch

Cells provided by AxioGenesis

This report shows how cardiomyocytes from Axiogenesis can be used on the QPatch. Action potentials can be induced in a controlled and reproducible manner using a mix of voltage- and current clamp and pharmacological effects can easily be detected.

AR_PUBLIC19236-4

Sophion Bioscience A/S, Baltorpvej 154, DK-2750 Ballerup, Danmark
Phone: + 45 44 60 88 00 Fax: +45 44 60 88 99 E-mail: info@sophion.dk www.sophion.com

Cor.4U from on QPatch

Cells provided by AxioGenesis

QPatch

Introduction

Fully differentiated, cell lineages formed from human induced pluripotent stem cells (iPSC) are powerful cellular models for action potential based screening of compounds. Parallel recordings from up to 48 iPS derived cardiomyocytes are made on the QPatchHT with full analysis of action potential features including action potential duration, upstroke velocity and membrane firing potential.

In this report we describe the validation of pharmacology and biophysics of iPSC from Axiogenesis on the QPatch in current clamp mode.

Materials & Methods

Cardiomyocytes derived from human iPSC, Cor.4U®, were shipped in T25 culture flasks directly from Axiogenesis. This approach was found to be very solid. The cells were kept at 37 C° until they were used. One flask was found to be sufficient for 2 QPlate 48's.

The cells were prepared on the day of experiment following the protocol provided by Axiogenesis.

The harvesting steps are summarized in the appendix.

Typical cell count was 0.5 mill/mL in the final volume with a viability of around 90%.

The cells were patched using the following saline solutions (mM):

Intracellular saline: KF 120, KCl 20, HEPES 10, EGTA 10

Extracellular saline: NaCl 145, HEPES 10, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1

The cells were positioned and patched with -100 mbar pressure and whole cell configuration was obtained using either a perforated protocol (5 µM β-Escin in the intracellular solution) or a conventional whole cell formation with pressure pulses.

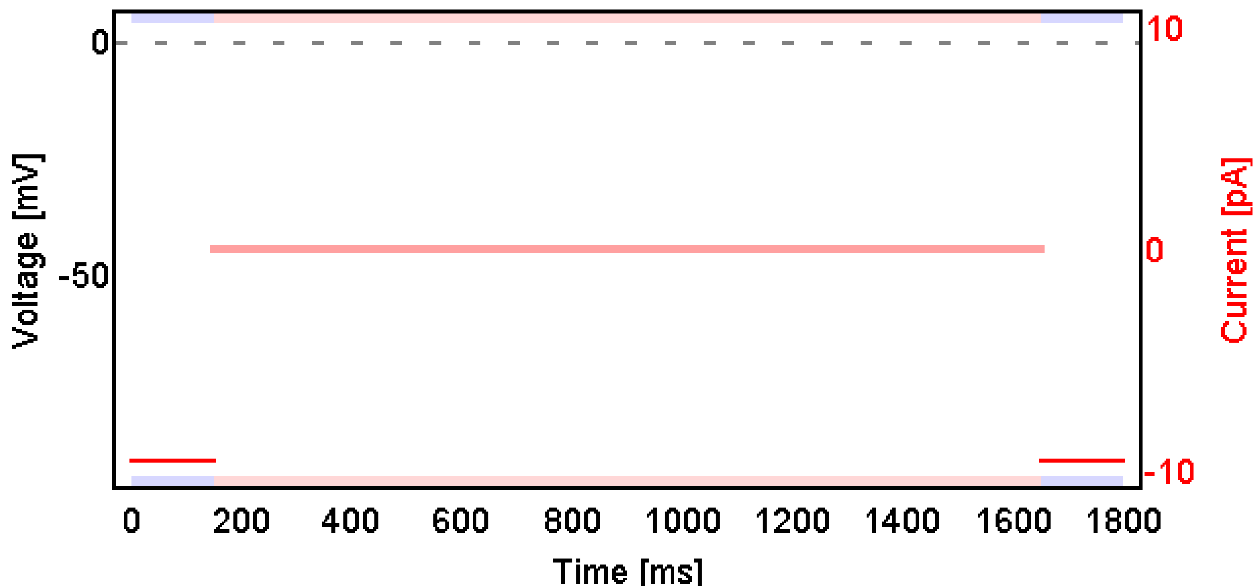


Figure 1: Typical voltage/current clamp mix protocol used for eliciting action potentials. Top blue line shows VC, top red line CC.

During the experiment a step protocol of 200 ms steps ranging from -100 mV to + 80 mV were executed to identify current types in the cells. The cells were then held at -90 mV between sweeps and action potentials could be elicited by switching to current clamp mode i=0 pA as shown in figure 1 above.

Results

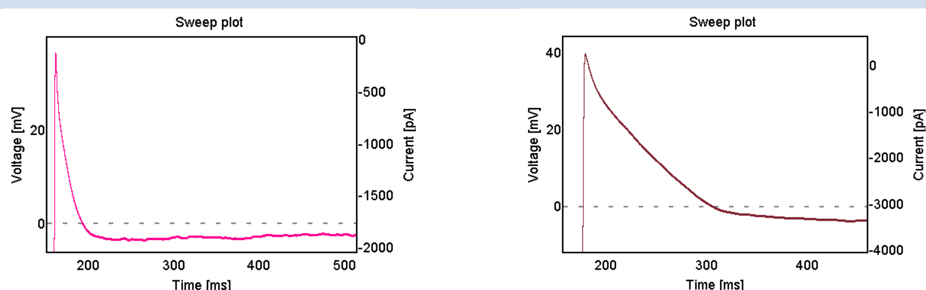
Typical seal resistances obtained with these cells are in the range 100-300 MΩ, when a cell is caught.

The rate of sites with action potentials were evaluated over 20 QPlate48's and it was found that it varied as a function of whole cell formation method from a mean around 25% with traditional pressure whole cell formation to app. 50% when using poreformer. The best runs had 75% sites with action potentials.

Action potentials could be induced by a step from V_{hold} = -90 mV to C_{hold} = 0 pA. The action potentials were seen with both atrial and ventricular characteristics – they were separated into these two groups based on their APD_{90}. Action potentials with APD_{90} below 100 ms were considered atrial and above 100 ms ventricular-like.

The characteristics measured for both groups are shown below in table 1.

Table 1:

	Atrial	Ventricular-like
Upstroke velocity (V/s)	30.9 ± 1.7	25.4 ± 3.1
Peak potential (mV)	36.8 ± 2.2	34.1 ± 3.4
APD_{90} (ms)	39.3 ± 3.7	243 ± 50
Diastolic membrane potential (mV)	-5.9 ± 1.3	-3.8 ± 1.4
n	48	19
Typical sweep		

A set of experiments were carried out in which 4 cumulative concentrations of tetracaine, a state dependent sodium channel blocker, was added to each cell. Tetracaine exhibited a clear dose dependant change to peak potential and also to the upstroke velocity, as can be seen in figure 2 below.

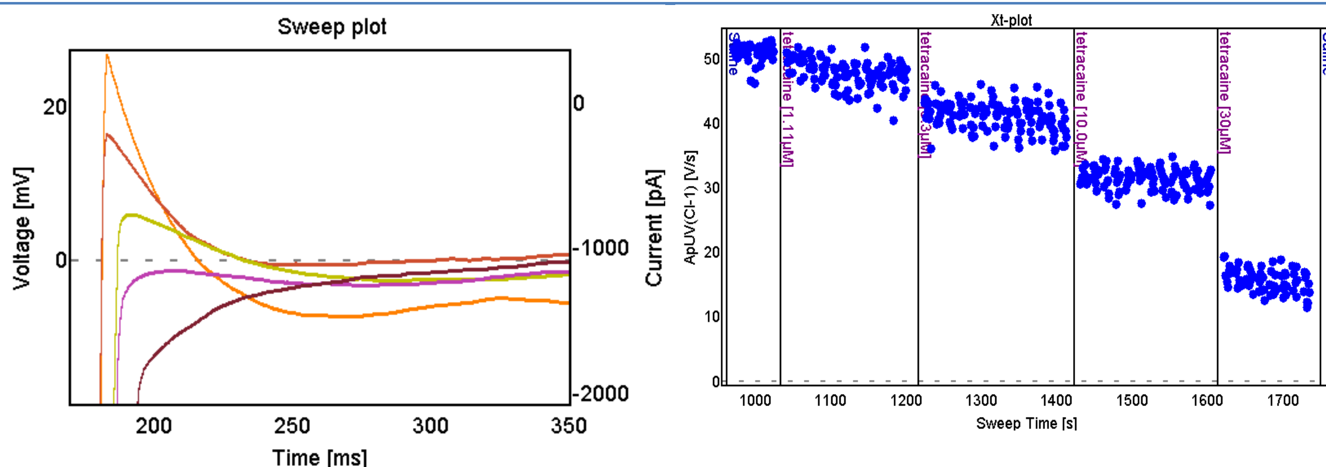


Figure 2: The effect of accumulated tetracaine concentrations ranging from 1 to 30 uM on induced atrial action potentials. The upstroke velocity was slowed in a dose dependant manner.

Appendix

Preparation of 10x EDTA stock 20 mM 100 ml:

Before starting note:

EDTA dissolves very slowly during pH adjustment. Do not heat the solution.

Do not add up to a 100 ml before after pH adjustment - leave room for NaOH.

1. Weigh out 0,585 g of EDTA.
2. Add approx. 90 ml MilliQ.
3. Adjust pH to 8,2 with NaOH.
4. When pH has reach 8,2 add MilliQ up till a 100 ml.
5. Sterile filtrate the solution and store at 5°C until use.
6. Dilute 10x with room temperature PBS to 2 mM EDTA before use.

Dissociation of Cardiomyocytes:

Before starting:

All the cell preparation is done in a flow bench.

Pre-warm TrypLE in 37°C water bath or incubator.

Prepare 2 mM EDTA as described above. *To one T25 flask you need 15 ml. room temperature*

Pre-cool Cor.4U culture medium and extracellular ringer with 10 µM Blebbistatin to 4°C. Blebbistatin will be diluted to 5 µM when mixed with Cor. 4U. *To one T25 flask you need 2 ml of each (total 4 ml).*

Make an extracellular ringer with 5 µM Blebbistatin at r*oom temperature*

1. Remove Medium.
2. Carefully wash 2x with 5 ml room temp. 2 mM EDTA.
3. Add another 5 ml of 2 mM EDTA and incubate 5 min. at room temp.
4. Remove the EDTA solution completely and add 3 ml pre-warmed (37°C) TrypLE Express – *make sure all the cells have been in contact with TrypLE by swirling the flask gently*.
5. Immediately after remove most of the TrypLE leaving only a thin layer in the culture flask.
6. Incubate at 37°C for 2-4 min.
7. Take the flask out and very gently tap on the side to see if the cells are detached. If not incubate for a little longer - *no more than 4 min. in total*.
8. Take the flask out and tap very gently again. Immediately add 2 ml of pre-cooled Cor.4U Culture medium and 2 ml extracellular ringer with 10 µM blebbistatin – *In total 4 ml solution and blebbistatin 5 µM end conc.*
9. Transfer cell suspension to a 10 ml Falcon tube with a pointy tip, and incubate the cells at 4°C for minimum 30 min. The cells will sink to the bottom and create a pellet.
10. Just before use remove as much of the medium/ringer solution as possible without touching the cell pellet.
11. Very gently resuspend the pellet in 400-500 µl extracellular ringer with 5 µM Blebbistatin – *room temp.* - by working the suspension up and down a few times - use a 1000 µl pipette - to get rid of possible cell clusters.
12. Transfer the cell suspension to the Qfuge and run the experiment with **no cell wash** on the QPatch.

Blebbistatin	Sigma Aldrich	Cat. Nr. B0560
TrypLE Express	Gibco/Life Technologies	Cat. Nr. 12605-010
DPBS	Sigma Aldrich	Cat. Nr. D8537
EDTA	Sigma Aldrich	Cat. Nr. E6758

Conclusion

Cardiomyocytes from Axiogenesis performs well in QPatch current clamp assays. Action potentials can easily be induced by using mixed protocols and the modulation of these with pharmacology can be detected and analyzed using the QPatch assay software.