Are stem-cell cardiomyocytes a viable cellular reagent for automated patch-clamp?

APCONIX
INTEGRATED TOXICOLOGY AND ION CHANNEL EXPERTISE

Lauren EADES, Michael MORTON
ApconiX Ltd., Biohub at Alderley Park, Cheshire, SK10 4TG, UK.
michael.morton@apconix.com

Introduction

ICH guidelines state that compounds in drug discovery must be tested for inhibition of hERG cardiac ion channel. It is often prudent to test compounds against a wider array of cardiac ion channels, e.g. hNaV1.5 and hCaV1.2 (Kramer et al., 2013). The CiPA initiative will demand testing and additional ion channel targets as well; namely hNaV1.5 late current, hKir2.1, hKvLQT1 and Kv4.3 (Gintant *et al*., 2016). These ion channel assays are all amenable to automated patch-clamp and have typically been run using recombinant cell lines over-expressing an individual ion channel. The aim of this research was to investigate whether human induced pluripotent stem cell-derived cardiomyocytes are a useful, affordable and predictive cellular reagent for use on the QPatch automated patch-clamp system.

Methods

iPS-SC cardiomyocytes were purchased from two vendors, AXOL and Supplier B. Cells were cultured according to the manufacturers recommended instructions with the recommended proprietary medium. Briefly, cells were thawed and cultured for 7-9d in fibronectin-coated T25 flasks. On the day of assay, cells were dissociated with Accumax for 20mins before addition of culture medium to stop the reaction. Cold EC buffer was added and cells were incubated at 4°C for 15mins prior to assay on QPatch HTX (Sophion). A variety of extracellular and intracellular buffers were used (in mM):

EC1: NaCl 140, KCl, 4.5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, glucose 10, 5μM blebbistatin, pH 7.45

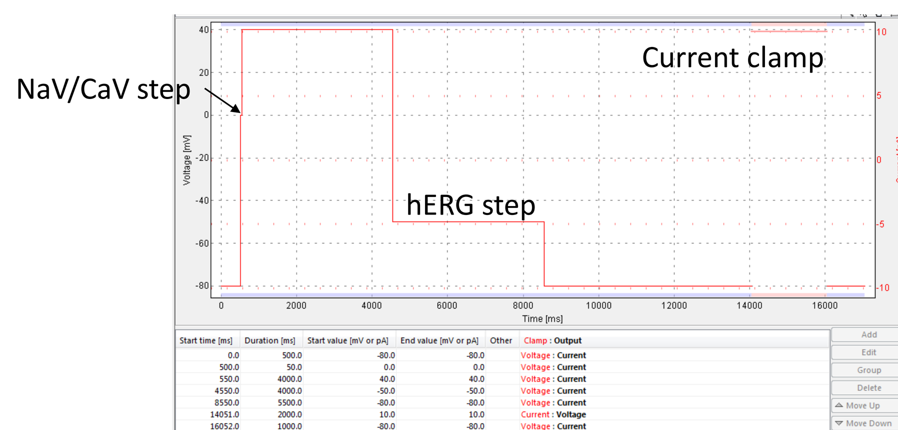
EC2: NaCl 135, KCl 4.5, $BaCl_2$ 10, $MgCl_2$ 1, HEPES 10 , glucose 10, 5μM blebbistatin, pH 7.45

IC1: CsF 140, EGTA 1, HEPES 10, NaCl 10, 4mM K_2ATP, pH 7.25

IC2: KF 120, KCl 20, EGTA 10, HEPES 10, 4mM K_2ATP, pH 7.25

Standard QPatch cell positioning, sealing, and whole-cell access protocols were used. Perforated patch was also used for some experiments with the addition of 10μM escin to the IC buffer.

Voltage/current-clamp protocol:



Results

iPS-SC cardiomyocytes from two sources, AXOL and Supplier B, were tested. Both cells cultured well and after a few days in culture began to beat asynchronously. AXOL and Supplier B cells beat at frequencies of 0.1-0.2 Hz and 3-5 Hz, respectively. Cells dissociated completely after 20mins of Accumax. A T25 flask yielded sufficient cells for two sequential experiments. During this time, cells could be successfully stored at 4°C for up to one hour.

Cell positioning, sealing, and whole-cell access

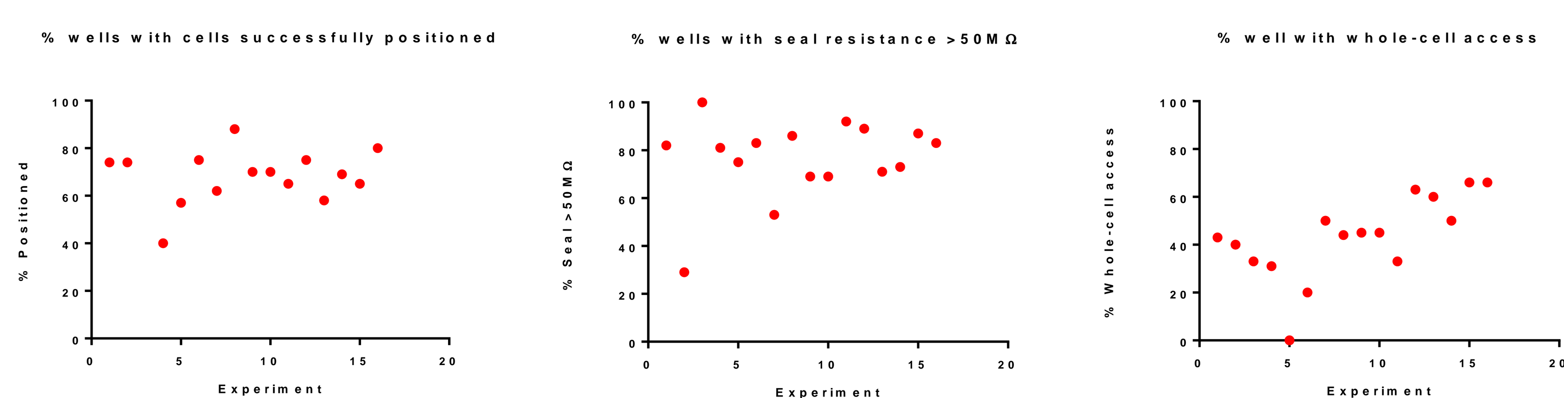


Figure 1. Success rates for positioning, sealing, and whole-cell access of SC-CMs. Each experiment is a different cell preparation. 1-7 are Supplier B. 8-16 are AXOL.

A limiting factor with SC-CMs was clearly gaining whole-cell access. Attempts were made to optimise QPatch whole-cell protocol parameters, but were unsuccessful. As an alternative to suction/zap-based whole cell access, escin at 10μM was used as a perforating agent. This too proved no benefit.

Kramer *et al*. (2013) MICE Models: Superior to the HERG Model in Predicting Torsade de Pointes. Scientific Reports 3:2100

Gintant *et al*. (2016) Evolution of strategies to improve preclinical cardiac safety testing. Nat Rev Drug Disc 15:457-471

Mannikko *et al*. (2015) Pharmacological and electrophysiological characterization of AZSMO-23, an activator of the hERG K(+) channel. *BJP* 172: 3112-25

AXOL – Ion channel profile

A number of ionic currents were identified in SC-CMs from AXOL (Figure 2):

A. A verapamil-sensitive peak sodium current, ranging from 100 to 2000pA was present in 53% recordings (32/60);
B. When recordings were made in Ba-containing EC2, a verapamil-sensitive barium current ranging from 100 to 2500pA was present in 84% of recordings;
C. A ranolazine-sensitive late sodium current up to 200pA was present in several recordings;

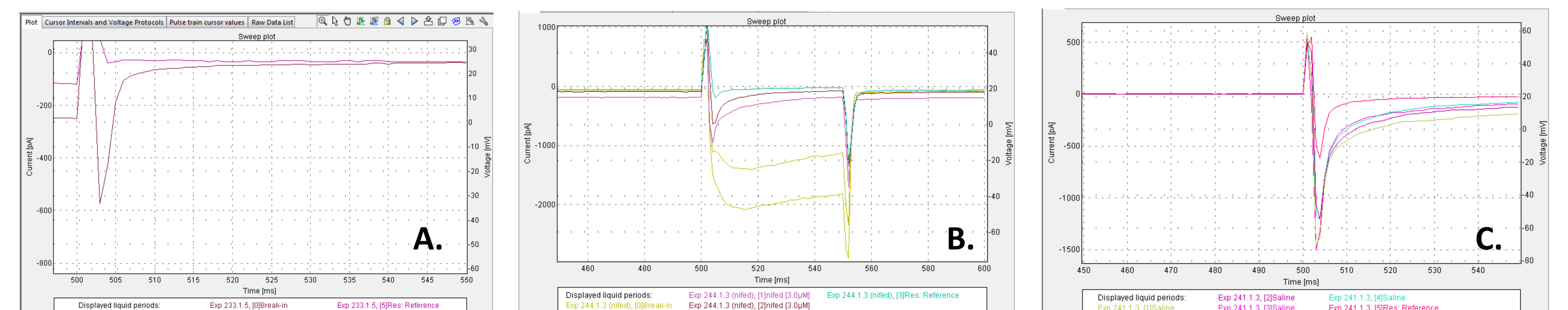


Figure 2. Ionic currents recorded in cells from AXOL. A, peak sodium; B, barium; C, late sodium.

Action potentials were also recorded in 52% of cells (31/60). These were typically 100-300ms in duration and sensitive to verapamil (Figure 3). In Ba^{2+}-containing EC, the action potentials were prolonged to >2000ms.

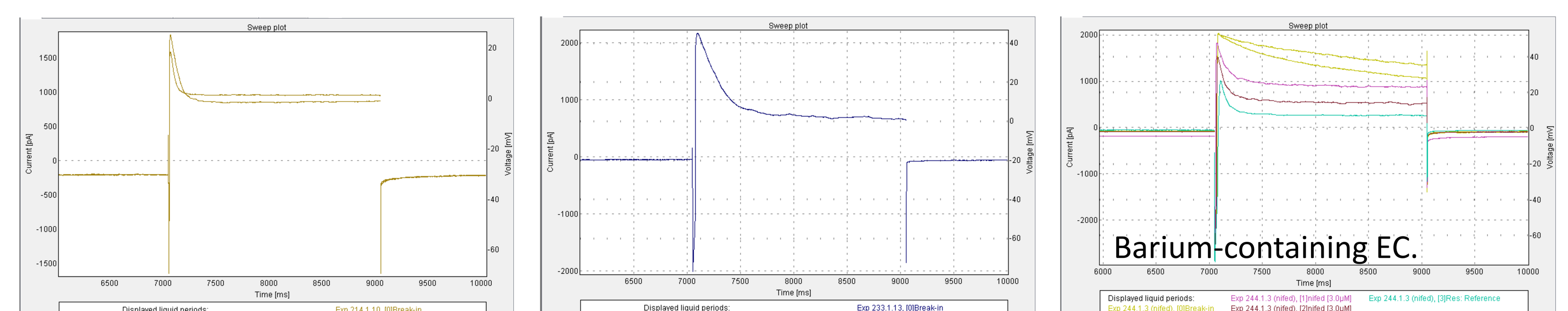


Figure 3. Action potentials recorded in cells from AXOL.

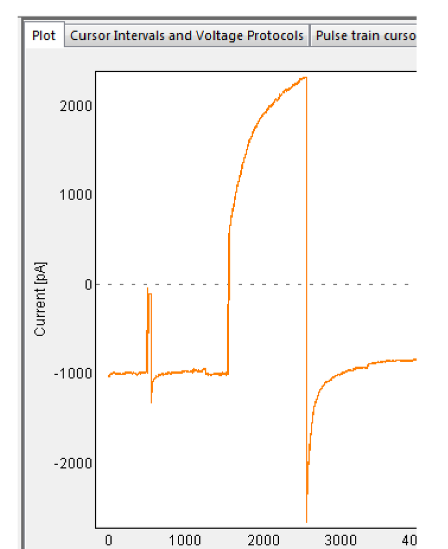
No hERG currents were identified, even in the presence of the hERG activator, AZSMO-23 (Mannikko *et al*., 2015).

Supplier B – Ion channel profile

The ionic currents recorded from these cells were predominantly peak sodium currents expressed in 14% (3/21) cells, ranging from 50 to 400pA. Action potentials were recorded in 29% (6/21) cells.

What is the outward potassium current?

When recording with potassium-containing IC2 buffer, at +40mV, a slowly activating, outward potassium current could be identified, as large as 2000 pA (inset). The time course of activation was inconsistent with cardiac IKs current.



Conclusions

- SC-CMs from different suppliers vary in their beating frequency and ion channel profile.
- Our data implies SC-CMs from AXOL express cardiac sodium and calcium channels, hNaV1.5 and hCaV1.2, respectively.
- SC-CMs are also capable of firing action potentials.
- The percentage of experiments resulting in successful whole cell recordings on the QPatch, averages approximately 40%. Attempts at optimisation have failed to significantly increase this success rate.
- The QPatch combines voltage and current-clamp in the same sweep making it a useful and novel platform for working with SC-CMs.
- SC-CMs are a challenging and expensive reagent to use on automated patch-clamp. They are heterogenous in nature but express significant and relevant cardiac ion channels.

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