Human iPSC-derived cardiomyocytes for high throughput *in vitro* cardiac pharmacology and cardiotoxicity studies

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Abstract

Cardiovascular disease remains the leading global cause of death. Evaluating potential cardiotoxicity continues to be a major challenge and expense in early drug development. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have emerged as a physiologically relevant and accessible model for *in vitro* cardiac pharmacology and cardiotoxicity testing.

Cardiac ion channels are key therapeutic targets and central contributors to drug-induced cardiotoxicity, highlighting the importance of electrophysiological studies using hiPSC-CMs. However, conventional manual patch-clamp techniques are labor-intensive and low-throughput, limiting their utility for large-scale screening.

Automated patch clamp (APC) systems enable high-throughput, precise electrophysiological measurements across large hiPSC-CM populations, accelerating screening workflows and lowering costs associated with cardiac pharmacology and safety assessment.

This study evaluates axoCells™ Ventricular Cardiomyocytes using Sophion's QPatch and Qube 384 APC platforms to characterize electrophysiological properties and assess their suitability for evaluating cardiac action potentials and preclinical cardiotoxicity screening.

Cardiac voltage-gated currents (Na_v and Ca_v) and action potentials were recorded on QPatch with 40% whole-cell success rates in physiological solution. Pharmacological and biophysical evaluation identified the presence of TTX-resistant $Na_v1.5$ channels and L-type $Ca_v1.2$ channels. Qube 384 was used to perform a concentration-response experiment with a known Na_v - channel blocker, tetracaine. The assay ran with more than 80% whole-cell success rate and yielded IC50 values in good agreement with reported values for $Na_v1.5$ – channels†.

APC recordings on the QPatch platform assessed key metrics including whole-cell success rates, sodium (Na_v) and calcium (Ca_v) currents, and action potentials. Pharmacological profiling confirmed the presence of TTX-resistant $Na_v1.5$ currents, inhibition by nifedipine, and potentiation by bay K8644. These findings were reproducible on the Qube 384 platform, supporting the utility of axoCells for scalable cardiac electrophysiology studies. By leveraging the scalability and precision of this APC technology we wish to enable higher-throughput cardiotoxicity testing with greater physiological relevance than the equivalent animal models and heterologous cells, and with easier access and more consistent performance than human primary tissue.

Methods

Cells and Culture: Recordings were performed using axoCells human iPSC-derived ventricular cardiomyocytes (ax2508). Cell culture was performed using Axol's reagents and protocols.

Cell Suspension: Cell suspension preparation was performed according to internal Sophion methods.

Electrophysiology Solutions: Contact Sophion for information regarding solutions.

Electrophysiological Analysis: All analysis was performed with Sophion Analyzer.

Immunocytochemistry of axoCells Ventricular Cardiomyocytes

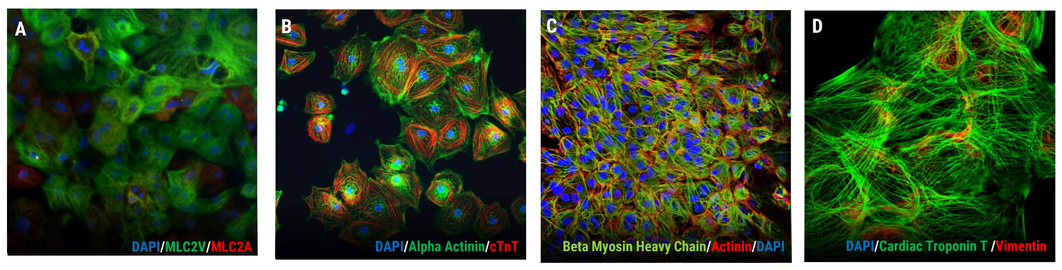

Figure 1. Representative Immunocytochemistry for ventricular cardiac-specific markers. axoCells ventricular cardiomyocytes fixed and stained 7 days post-thaw.

axoCells hiPSC-CMs contain the TTX-resistant cardiac $Na_v1.5$ channel

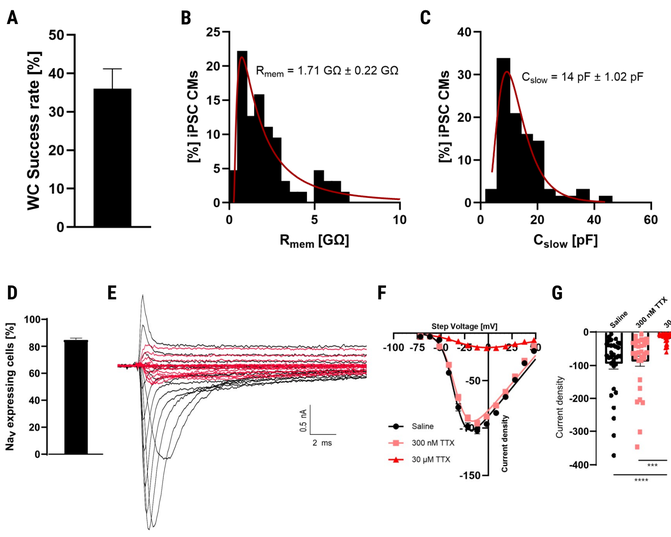

Figure 2. Performance of axoCells hiPSC-CMs in physiological solutions on QPatch. (A) Experiment whole-cell (WC) success rate in percentage of 48 experiment sites (R_{mem} > 200 MΩ and C_{slow} > 4 pF). Data is mean ± SEM of three measurement plates. **(B)** and **(C)** Histograms of R_{mem} and C_{slow} values of the measured cell population (N_{cells} = 65) together with the mean ± SEM values and best fit of the distribution (red line). **(D)** Percentage of successful experiments with Na_v current. Data is mean ± SEM of three experiment plates. **(E)** Representative Na_v current trace in response to a voltage step protocol before (**BLACK**) and after (**RED**) addition of 30 µM Tetrodotoxin TTX. **(F)** Na_v current density vs. step voltage plot recorded in saline (**BLACK**), 300 nM TTX (PINK) and 30 µM TTX (**RED**). Data points are mean ± SEM of 32 cells. **(G)** Bar diagram of maximum Na_v current density recorded in saline (**BLACK**), 300 nM TTX (PINK) and 30 µM TTX (**RED**) showing significant block by 30 µM, but not 300 nM TTX. Statistical comparison was performed with a one-way ANOVA with Tukey's Multiple Comparison Test, p < 0.001 (***) and p < 0.0001 (****).

axoCells hiPSC-CMs contain the cardiac L-type $Ca_v1.2$ channel

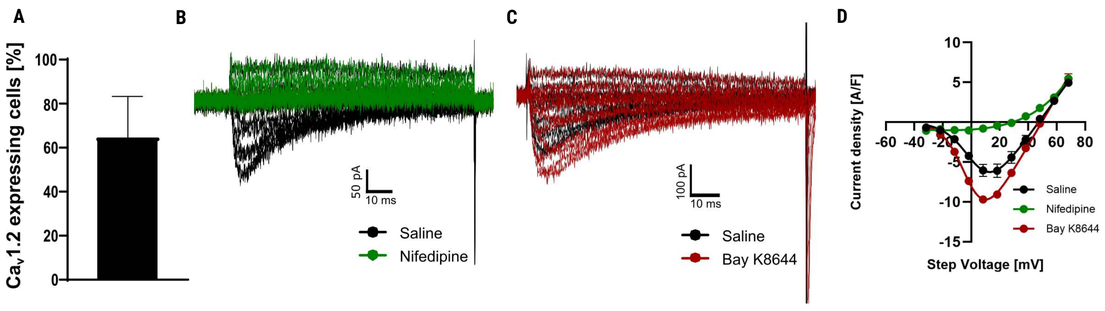

Figure 3: Measurements of Ca_v current in axoCells hiPSC-CMs in physiological solutions. (A) Percentage of successful experiments with Ca_v current. Data is mean ± SEM of three experiment plates. **(B)** Representative Ca_v current trace in response to a voltage step protocol before (**BLACK**) and after (**GREEN**) addition of an L-type Cav channel blocker (10 µM nifedipine). **(C)** Representative Ca_v current trace in response to a voltage step protocol before (**BLACK**) and after (**RED**) addition of an L-type Ca_v channel agonist (0.3 µM bay K8644). **(D)** Ca_v current density vs. step voltage plot recorded in saline (**BLACK**), 10 µM nifedipine (**GREEN**) and 0.3 µM bay K8644 (**RED**). Data points are mean ± SEM of 7 – 10 cells.

Modulation of the L-type $Ca_v1.2$ channel influences action potential duration

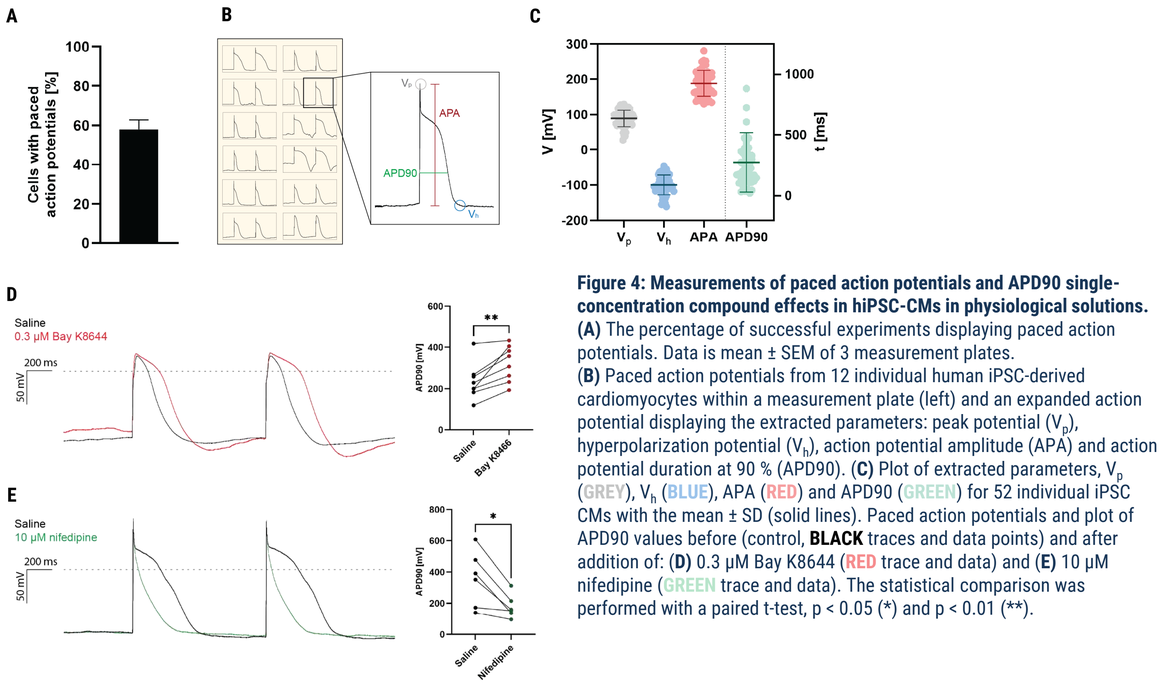

Figure 4: Measurements of paced action potentials and APD90 single-concentration compound effects in hiPSC-CMs in physiological solutions. (A) The percentage of successful experiments displaying paced action potentials. Data is mean ± SEM of 3 measurement plates. **(B)** Paced action potentials from 12 individual human iPSC-derived cardiomyocytes within a measurement plate (left) and an expanded action potential displaying the extracted parameters: peak potential (V_p), hyperpolarization potential (V_h), action potential amplitude (APA) and action potential duration at 90 % (APD90). **(C)** Plot of extracted parameters, V_p (GREY), V_h (BLUE), APA (**RED**) and APD90 (GREEN) for 52 individual iPSC CMs with the mean ± SD (solid lines). Paced action potentials and plot of APD90 values before (control, **BLACK** traces and data points) and after addition of: **(D)** 0.3 µM Bay K8644 (**RED** trace and data) and **(E)** 10 µM nifedipine (GREEN trace and data). The statistical comparison was performed with a paired t-test, p < 0.05 (*) and p < 0.01 (**).

Biophysical and pharmagological evaluation of Na_v currents on Qube 384

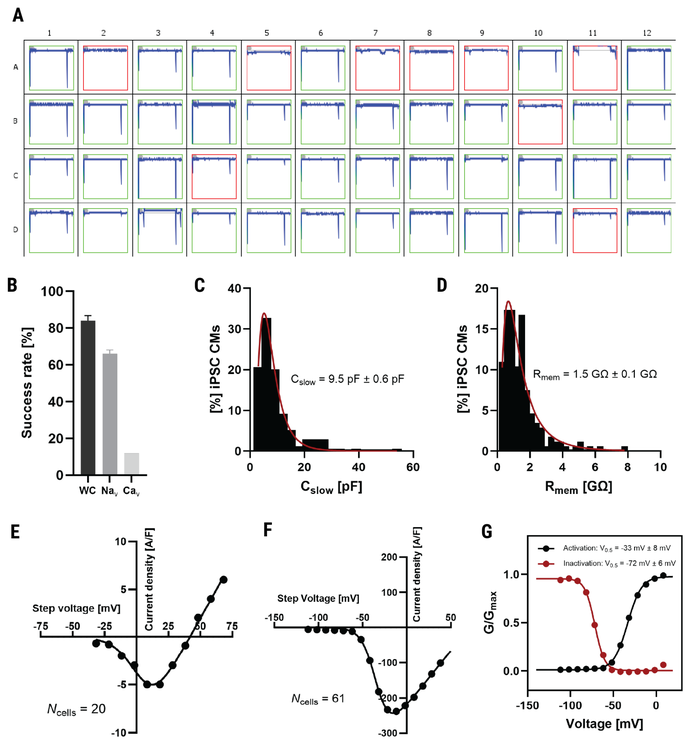

Figure 5: Performance of axoCells hiPSC-CMs on Qube 384. (A) Plate view (48 out of 384 experiment sites) displaying Na_V currents recorded in hiPSC-CMs. GREEN and **RED** are the experiments that pass and fail the quality filter, respectively. **(B)** Experiment success rates plotted for whole-cell (WC) (**DARK GREY**); Na_v current (GREY) and Ca_v current (LIGHT GREY) in percentage of experiment sites. Data is mean ± SEM of one or two measurement plates. **(C)** and **(D)** Histograms of C_{slow} and R_{mem} values of the measured cell population (N_{cells} = 177) together with the mean ± SEM values and best fit of the distribution (red line). **(E)** Ca_v current density vs. step voltage plot. Data points are mean ± SEM of 20 cells. **(F)** Na_v current density vs. step voltage plot. Data points are mean ± SEM of 59 cells. **(G)** Normalized conductance vs voltage plotted for Na_v activation (**BLACK**) and inactivation (**RED**). Fit of the Boltzmann equation resulted in a $V_{half.act}$ and $V_{half.inact}$ of approximately −33 mV and -72 mV, respectively, in good agreement with reported values for $Na_v1.5$.

Concentration-response experiment with IC50 values in line with established values for $Na_v1.5$ – channels

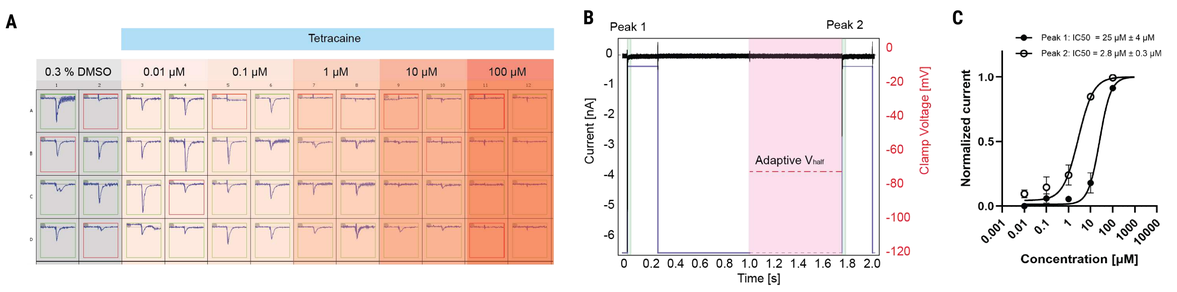

Figure 6: Na_v compound concentration-response experiment in axoCells hiPSC-CMs on Qube 384. (A) Plate view (48 out of 384 experiment sites) displaying Na_V peak currents recorded in hiPSC-CMs upon the addition of 0.3 % DMSO (control, GREY) or increasing concentrations of the state-dependent Na_V blocker tetracaine (5 point 10-fold dilution from 100 µM, ORANGE). **(B)** The two-pulse protocol used to evaluate the state-dependent blocker, consisting of two 0.2 s pulses, from -120 mV to -10 mV, separated by 1.6 s in total, 0.8 s at -120 mV and 0.8 s at the V_{half} value recorded adaptively for each single cell. **(C)** Normalized Na_v peak current as a function of tetracaine concentration plotted for Peak 1 (**SOLID BLACK**) and Peak 2 (**BLACK OUTLINE**), respectively. Fitting of the Hill equation resulted in IC_{50} values of approximately 25 µM and 2.8 µM, for Peak 1 and Peak 2 respectively, in good agreement with published values for $Na_v1.5$†.

Experiment success rate and cell quality

The performance of hiPSC-CMs was evaluated. On QPatch we obtained up to 40% success rates in physiological solutions and on Qube 384 we obtained up to 80% success rates in seal-enhancing solutions.

Of the successful experiments on QPatch, success rates for Na_V, Ca_V and paced Action Potentials were over 80%, 60% and 60% respectively (**Fig. 2D, 3A** & **4A**).

The hiPSC-CMs that passed the quality criteria displayed a heterogeneous size distribution, as illustrated in the histogram of C_{slow} values (**Fig. 2C** & **5C**), ranging from 5 pF to 45 pF.

The sealing was good both on the QPatch and Qube 384 with R_{mem} = 1.71 GΩ ± 0.22 GΩ (mean ± SEM) and 1.50 GΩ ± 0.10 GΩ (mean ± SEM) respectively (**Fig. 2B** & **5D**).

Conclusion

This study confirms that axoCells human iPSC-derived ventricular cardiomyocytes offer a robust and reliable tool for assessing the electrophysiological properties of more human cardiac cells on APC platforms, such as Sophion's QPatch and Qube 384. This will enable higher-throughput cardiotoxicity and/or drug testing with greater physiological relevance, easier access and more consistent performance than other current cardiac cell models.

The scalability and precision of this APC technology in combination with human iPSC-derived cardiomyocytes, means we can now systematically evaluate the safety and efficacy of drugs as well as toxins on a more human model earlier in the drug development process.

The integration of human-based *in vitro* models and advanced screening technologies has the potential to improve the prediction of cardiac safety liabilities, reduce animal experimentation, and accelerate the translation of promising therapeutics to the clinic.

Reference

†Karatsiompani, S and Badone, B; hiPSC-derived cardiomyocytes – $Na_v1.5$ compound screening on QPatch II; Sophion Application Report; 2022

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