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Application Report

Assessing cardiac safety - from single ion channels to engineered heart tissue

Envisioning how multi-platform approaches will enhance the predictive value of preclinical cardiac safety screening

Introduction

Cardiac safety remains a major cause of attrition in drug discovery. Compounds that are promising in early-stage screening may nonetheless fail in later development due to proarrhythmic liability, conduction abnormalities, or impaired myocardial contractility. Such failures are costly and often reflect limitations in the predictive power of early-stage assays. There is, therefore, a clear need for screening strategies that can more reliably identify cardiac risk earlier and support more informed compound selection.

Single ion-channel electrophysiology is the gold standard of cardiac safety assessment and provides detailed insight into direct target effects. The development of automated patch clamp (APC) has enabled high-throughput, reproducible ion-channel measurements thus facilitating efficient profiling of large compound libraries while retaining mechanistic detail.

Integration of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) into cardiac safety evaluations aligns with initiatives like the Comprehensive *in Vitro* Proarrhythmia Assay (CiPA) initiative, aiming to improve predictions of arrhythmogenic risk beyond single ion channel testing. APC recordings of hiPSC-CMs allow recordings of 1) endogenously expressed ion channels, in a more physiologically relevant environment, and 2) single-cell action potentials (APs), reflecting the combined activity of multiple ion channels expressed in the human cardiomyocyte.

Engineered heart tissues (EHTs) incorporate multicellular organization that allow the assessment of wholistic contractile function (force, beating rate and arrhythmogenesis) in a human pseudo-organ 3D environment. The excitation-contraction coupling, in which action potentials on the cellular level triggers tissue contraction through an increase in intracellular Ca^{2+}, relies on an intricate balance of several ion channels. Thus, EHTs allow us to evaluate inotropic effects of ion-channel drugs by measuring the force-frequency relationship at the tissue level.

Each of these approaches (single channels, APs and EHTs) interrogates a distinct level of biological organization, and their combined application provides a more comprehensive assessment than any individual assay alone. In this application note, we exemplify how different generic pharmacological compounds with known impact on cardiac ion channels can be evaluated at different scales using Sophion platforms (Figure 1). The APC platforms QPatch and Qube 384 are used for evaluating drug effects on single ion-channel currents and APs in hiPSC-CMs, while the EHT platform, Ethica M, evaluates their impact on tissue contractility. We envision that multi-platform approaches combining functional assays at different scales will provide more comprehensive assessment of drug safety.

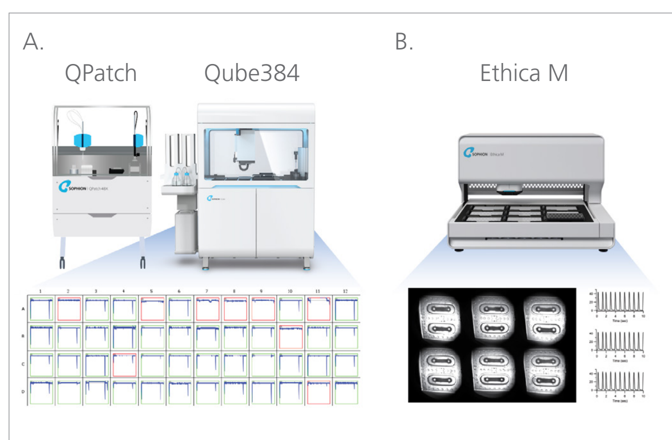

Fig. 1: Sophion platforms employed to evaluate cardiac safety targets. **A)** Sophion APC platforms, QPatch and Qube 384 including the Qube plate view (48 out of 384 experiment sites) displaying Na_V currents recorded in hiPSC-CMs. **B)** Sophion EHT platform, Ethica M including a micrograph of 6 wells each harboring two cardiac tissues (bottom left) and the resulting contraction force curves (right).

Results and discussion

In Figure 2 we present the cardiac action potential along with its 5 phases (0 – 4) and the associated ion channels. To exemplify how ion-channel current measurement, action potential and EHT approaches might serve as complementary cardiac safety assays we focus on generic drugs for three cardiac ion channels:

1. $Na_V1.5$ responsible for I_{Na} during the rapid depolarization phase (phase 0)
2. $Ca_V1.2$ responsible for I_{CaL} during the plateau phase (phase 2)
3. hERG responsible for I_{Kr} during the repolarization phase (phase 3)

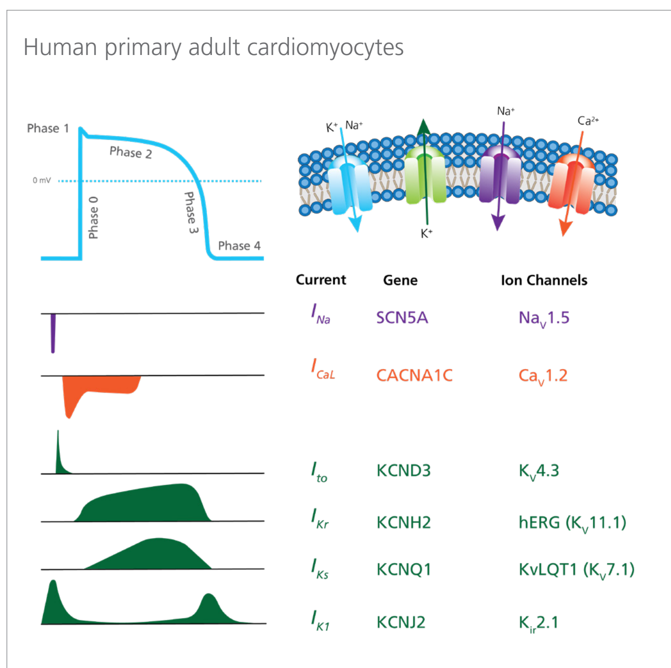

Fig. 2: The cardiac action potential including the five phases (0-4) and the associated genes and ion channels. The phases are characterized by a rapid depolarization (phase 0), initial repolarization (phase 1), plateau phase (phase 2), rapid repolarization (phase 3) and resting membrane potential (phase 4). From the top the currents are: I_{Na}, sodium current; I_{CaL}, L-type calcium channel current; I_{to}, transient outward potassium current; I_{Kr}, rapid delayed- rectifier potassium current; I_{Ks}, slow delayed-rectifier potassium current; and I_{K1}, inward-rectifier potassium current. Adapted from (Karbassi *et al.*, 2020).

Sodium currents - I_{Na}

Voltage-gated sodium channels, primarily $Na_V1.5$, drive the rapid depolarization phase (phase 0) of the cardiac action potential and thereby determine cardiac excitability which, through its interplay with potassium and calcium channels, translates into tissue contraction.

A compound-dependent block of the $Na_V1.5$ current can cause cardiac conduction delay and an increased risk of proarrhythmic events. In Figure 3 we show a proof-of-concept APC compound screen on $Na_V1.5$ endogenously expressed in hiPSC-CMs, applying the state-dependent blocker tetracaine. This type of safety screen will typically reveal IC_{50} values and biophysical details regarding compound mode-of-action (MoA).

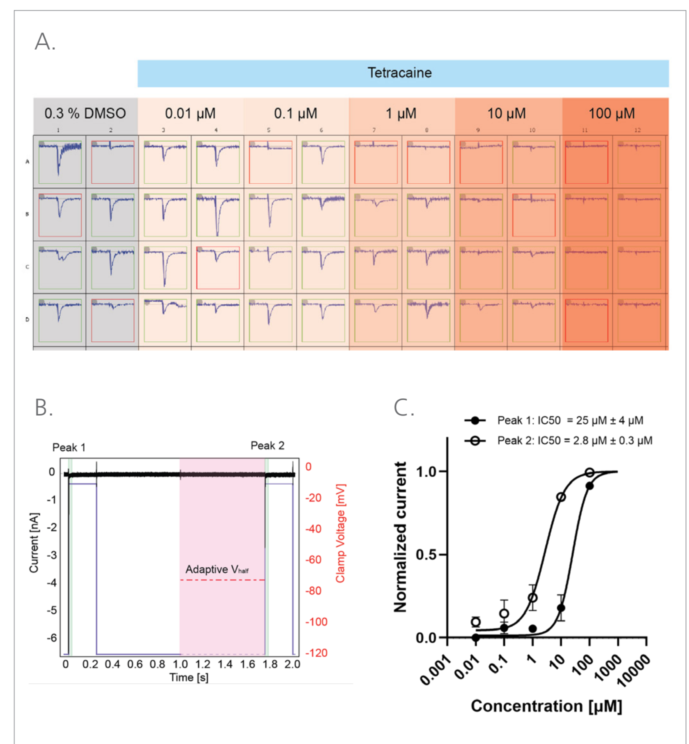

Fig. 3: $Na_V1.5$ blocker concentration-response experiment using hiPSC-CMs on Qube 384. **A)** Plate view (48 out of 384 experiment sites) displaying Na_V peak currents recorded in hiPSC-CMs upon the addition of 0.3 % DMSO (control, grey) or increasing concentrations of the state-dependent Na_V blocker 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 line with previous studies.

In the EHTs, reduction of the peak $Na_V1.5$ current impacts the excitation-contraction coupling leading to decreased cytosolic Ca^{2+} transients and a reduced contractile force. This negative inotropic effect is visualized in Figure 4 where the EHT contraction amplitude decreases with increasing concentrations of tetracaine.

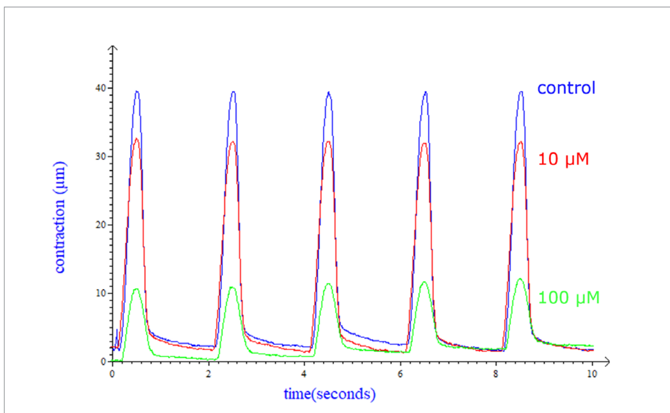

Fig. 4: Representative contraction peaks before application (blue) or after application of 10 µM (red) and 100 µM (green) tetracaine on EHTs.

Calcium currents – I_{CaL}

The L-type calcium channel $Ca_V1.2$ is the primary ion channel responsible for calcium influx and for maintaining the plateau phase (phase 2) of the cardiac action potential. In Figure 5 we show how modulation of the $Ca_V1.2$ channel, endogenously expressed in hiPSC-CMs, by either a blocker (nifedipine) or potentiator (bay K8644) affects its biophysical properties (current-voltage relationship).

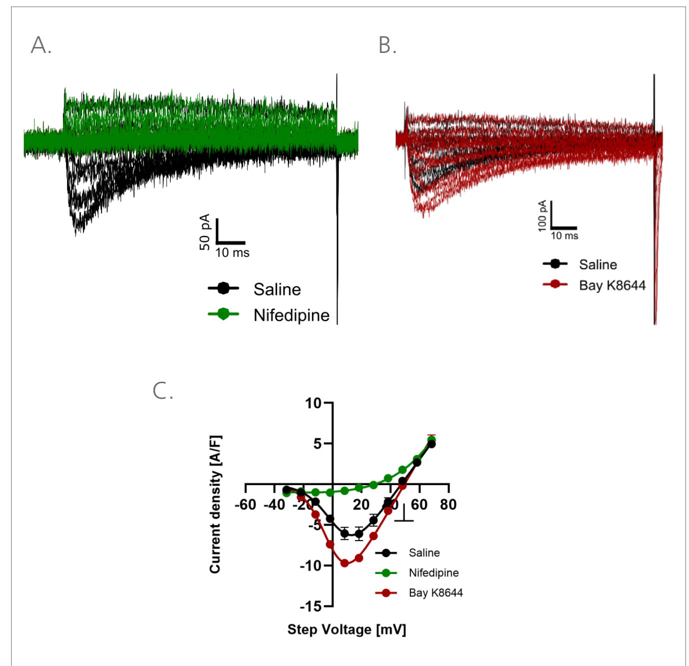

Fig. 5: Recordings of $Ca_V1.2$ currents in hiPSC-CMs on QPatch. **A)** Representative Ca_V current trace in response to a voltage-step protocol before (black) and after (green) addition an L-type Ca_V channel blocker (10 µM nifedipine). **B)** Representative Ca_V current trace in response to a voltage-step protocol before (black) and after (red) addition of an L-type Ca_V channel agonist (0.3 µM bay K8644). **C)** 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.

This modulation of the $Ca_V1.2$ ion channel causes a shortening or prolonging of the cardiac action potential, which can be assessed in single hiPSC-CMs using the current-clamp technology on QPatch (Figure 6). Plotting action potential duration at 90% (APD90) as a function of compound concentration yielded IC_{50} and EC_{50} values, in good agreement with literature values.

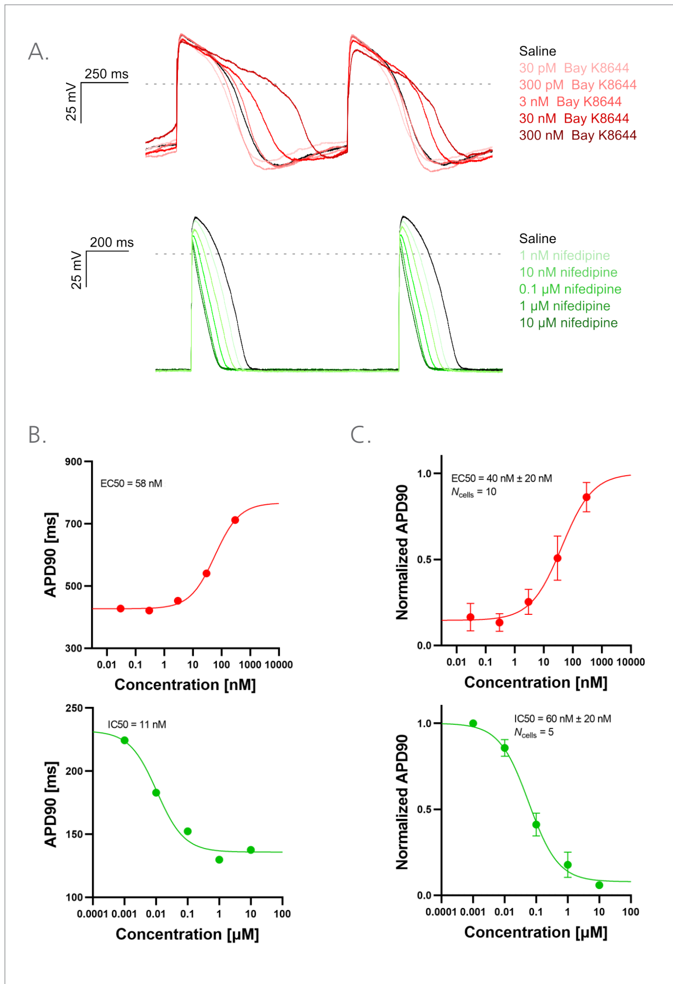

Fig. 6: Measurements of APD90 concentration-response plots in hiPSC-CMs on QPatch. **A)** Paced action potentials for saline controls (black traces) and in response to increasing concentrations of bay K8644 (top, traces in shades of red) and nifedipine (bottom, traces in shades of green). **B)** Plots of APD90 versus compound concentration with Boltzmann fits including EC_{50} or IC_{50} calculated values, recorded in a single cell. **C)** Concentration-response (average, normalized APD90) relationships with Boltzmann fits including EC_{50} or IC_{50} calculated values. Data points are mean ± SEM of 5-10 cells.

Through the maintenance of phase 2 in the action potential, $Ca_V1.2$ is responsible for initiating excitation-contraction coupling, and consequently its blockage by nifedipine in EHTs decreases contraction amplitudes in a concentration-dependent manner (Figure 7). Nifedipine reduced EHT-contraction force with an IC_{50} of 41 nM ± 3 nM in good agreement with the observed effect on single hiPSC-CM action potentials reported in figure 6 (60 nM ± 20 nM) and literature values (39 nM)[1].

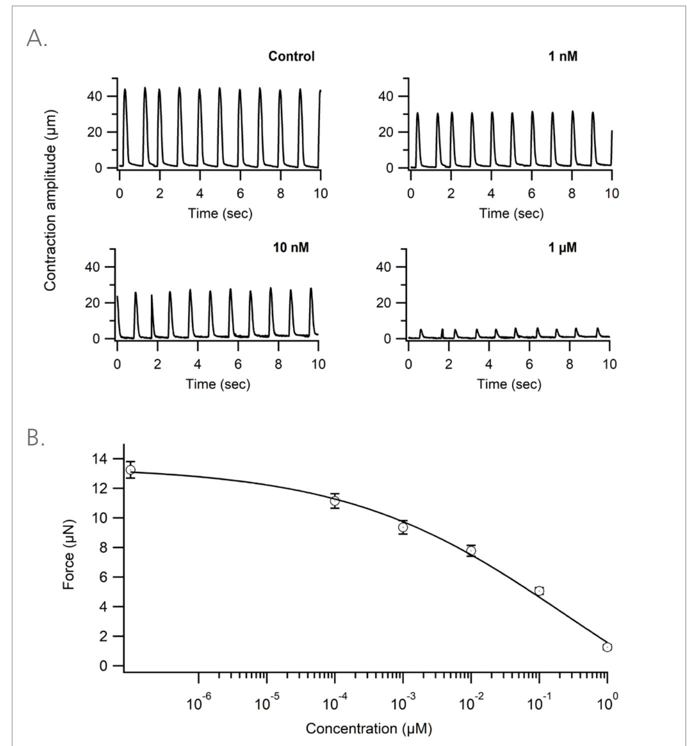

Fig. 7: A) Representative contraction peak in baseline (without compound) and when exposed to increasing concentration of nifedipine. **B)** Nifedipine concentration-response plot on EHT contraction force.

Potassium current (delayed rectifier) - I_{Kr}

The hERG (human Ether-à-go-go-Related Gene) potassium ion channel is responsible for the rapid delayed rectifier potassium current, which is the key repolarizing current during phase 3 of the cardiac action potential[2-4]. E-4031 is a selective hERG blocker and is widely used as a reference compound in preclinical cardiac safety pharmacology to evaluate drug-induced changes in cardiac electrophysiology.

I_{Kr} currents are known to be smaller and less mature in hiPSC-CMs compared to primary CMs, and in addition, hERG channels can be sensitive to enzymatic digestion[5,6]. Therefore, APC recordings of hERG currents in hiPSC-CMs are inherently challenging. In Figure 8 we employed a previously published strategy[7] in which hERG tail currents were induced by increasing the extracellular K^+ concentration from 5 mM to 75 mM and subsequently blocked by the addition of 0.5 µM E4031.

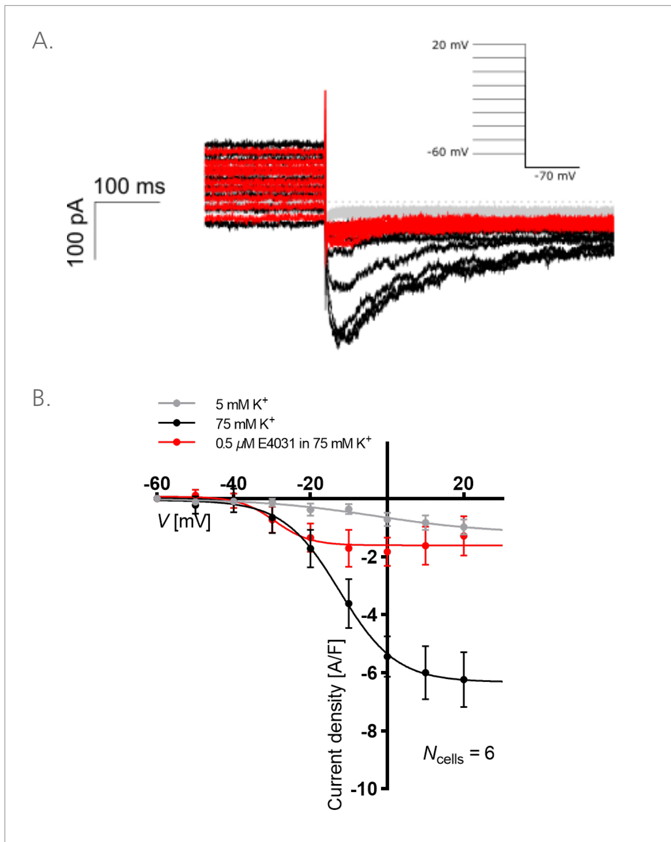

Fig. 8: Recordings of hERG currents in hiPSC-CMs on QPatch. **A)** Representative hERG tail current recorded at V = –70 mV following a voltage-step protocol from –60 mV to +20 mV with 10 mV step size. The tail current was recorded in 5 mM extracellular K^+ (grey) and 75 mM extracellular K^+ before (black) and after (red) addition of 0.5 µM E4031. **B)** hERG current density as a function of voltage in 5 mM extracellular K^+ (grey) and 75 mM extracellular K^+ before (black) and after (red) addition of 0.5 µM E4031. Data points are avg ± SEM of 6 cells.

The effect of E-4031 on hiPSC-CM APs were variable, likely due to variations in hERG expression levels. However, in Figure 9 we show an example of the expected prolonging of the action potential in response to 1 µM E-4031.

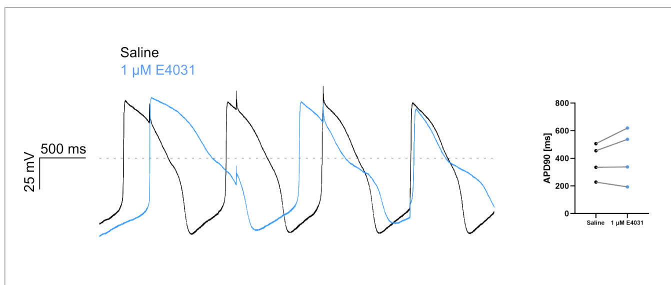

Fig. 9: QPatch recordings of hiPSC-CM action potentials before (black) and after (blue) block of the hERG channel by 1 µM E4031.

Exposing EHTs to increasing concentrations of E-4031, only resulted in a marginal change in the contraction amplitude but as expected, the pulse width of the contraction peak shows broadening in a concentration-dependent manner (Figure 10A) with an EC_{50} of ~ 5 nM.

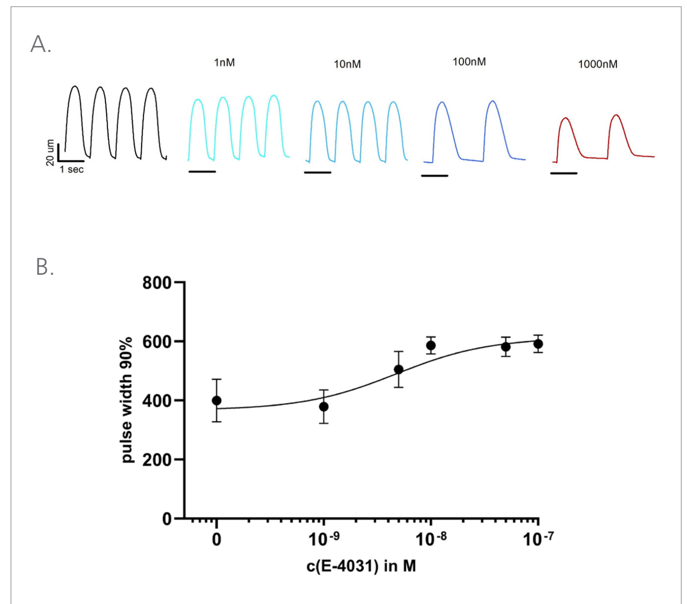

Fig. 10: A) Representative contraction trace showing concentration-dependent effect on the peak and pulse width with E4031 on the EHTs. **B)** Concentration-response plot of E4031 on pulse-width generated by the EHTs

Conclusion

Assessment of cardiac safety during drug development traditionally focuses on electrophysiological endpoints, for example testing compounds against single ion-channel activity and action potential dynamics. While electrophysiology assays provide critical information about drug effects on cardiac ion channels and action potentials, they do not fully capture downstream functional consequences on myocardial mechanical performance. Contractility assays complement electrophysiological measurements by directly evaluating the mechanical output of cardiomyocytes.
As presented in this study, combining electrophysiology and contractility measurements in hiPSC-CMs provides complementary information regarding electrical and mechanical responses to pharmacological compounds at different scales ranging from single ion channels to engineered tissues. We envision this multi-parametric approach will enable the detection of diverse cardiotoxic mechanisms, including ion channel block, calcium channel handling disruption and direct effects on cardiac-contractile function.

As a result, such integrated assays enhance the predictive value of preclinical cardiac safety screening and provide a more physiologically relevant evaluation of potential drug-induced cardiac liabilities.

Methods

APC: The APC experiments presented were conducted with hiPSC-CMs kindly provided by Axol Bioscience (Figures 3 and 5) or UM Göttingen lab (www.molecular-pharmacology.de) (Figures 6, 8 and 9). Cell culture was according to cell vendor protocols and cell suspension preparation was according to Sophion internal methods. Solutions and assay protocols are available upon request.

All analysis was performed with Sophion Analyzer and GraphPad Prism.

EHTs: This study was performed using hiPSC-derived cardiomyocytes from different sources (iBET, Portugal and FujiFilm Cellular Dynamics, USA). EHTs were produced and measured using the Ethica M solution (Sophion Bioscience) as per internal Sophion Standard Operating Procedures. EHTs formed within two days post seeding and were further cultured for at least 20 days. The EHTs were paced for two weeks to enhance functional maturity, before pharmacological evaluation studies were conducted.

All the EHT data presented is average ± SEM. Analysis and figures were prepared using the Ethica M Analyzer, Igor 6.3.7.2 (WaveMetrics, Inc. Oregon, USA) and GraphPad Prism (USA).

For information on dissociation procedures and solutions contact us at info@sophion.com.

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