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Comprehensive Profiling of Axiogenesis Ventricular vCor.4U iPSC-Derived Cardiomyocytes

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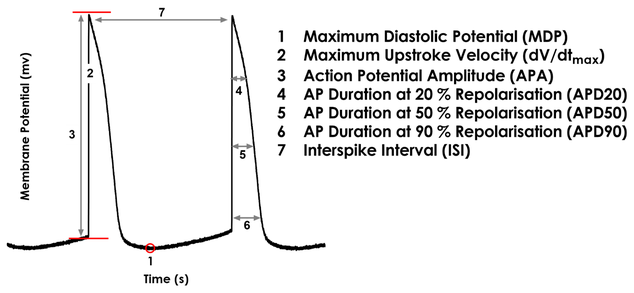
Introduction

The FDA's Comprehensive *in vitro* Proarrythmia Assay (**CiPA**) initiative aims to provide a thorough preclinical cardiac safety profile of new chemical entities that enables prediction of human clinical proarrhythmia risk. To allow the successful utilisation of commercial human iPSC-derived cardiomyocytes (iPSC-CM) as models of human CM in the CiPA safety paradigm, their biophysical and pharmacological profile needs to be fully characterised. Here we will highlight our work to assess the utility of Axiogenesis vCor.4U iPSC-CM for CiPA-relevant cardiotoxicity screening.

Voltage clamp recordings were used to record key cardiac ionic currents present in vCor.4U iPSC-CM: sodium (I_{Na}), calcium ($I_{Ca,L}$), inward (I_{Kin}) and outward (I_{Kout}) potassium currents. In current clamp, vCor.4U iPSC-CM generated spontaneous action potentials (AP) at a frequency of 0.4 Hz and were suitable for external pacing at 1 Hz using field stimulation. Cell population responses were also determined using plate-based phenotypic recordings of extracellular field potentials (EFP) on a multi-electrode array (Axion Maestro) and with dual impedance/EFP readouts of contractility and excitability (Nanion CardioExcyte96)

Materials and Methods

Axiogenesis vCor.4U were cultured on fibronectin-coated coverslips at 37°C (5 % CO_2). All data were recorded 7-10 days post-seeding at RT. Voltage clamp recordings were made from single cells under whole-cell patch clamp with protocols and solutions designed to isolate the ionic current of interest[1]. AP recordings were made using perforated patch (100 µg/ml Gramicidin). Data were acquired with EPC10 amplifiers and PatchMaster software (HEKA Elektronik, Germany). Signals were low-pass filtered at 10 kHz before digitization at 20 kHz.
Data were analysed using CAPA software (SSCE UG, Germany) and FitMaster (HEKA). The AP parameters analysed in this study are shown in Figure 1. Spontaneous APD were rate corrected using Friderica's correction. Data are reported as mean ± SEM. Statistical comparisons were performed using a Paired Student's *t*-test or One-way ANOVA with post-hoc testing.



1 Maximum Diastolic Potential (MDP)
2 Maximum Upstroke Velocity (dV/dt_{max})
3 Action Potential Amplitude (APA)
4 AP Duration at 20 % Repolarisation (APD20)
5 AP Duration at 50 % Repolarisation (APD50)
6 AP Duration at 90 % Repolarisation (APD90)
7 Interspike Interval (ISI)

Figure 1: Analysed action potential parameters.
Example action potential indicating the parameters which are quantified using FitMaster (evoked) and CAPA software (spontaneous).

For plate-based assays, vCor4U iPSC-CM were seeded on 96-well plates according to manufacturer's instructions. Plates were incubated at 37 °C (5 % CO_2) for 7-10 days and 100 % medium exchanges were performed daily.
Compounds were serially diluted in DMSO followed by a 1000 fold dilution into media. The final DMSO concentration did not exceed 0.1 %. Recordings were acquired immediately before compound treatment (baseline) and after dosing at 30 min and 1 hour for the Maestro and up to 72 hours for CardioExcyte96.

1. Biophysics of key cardiac currents

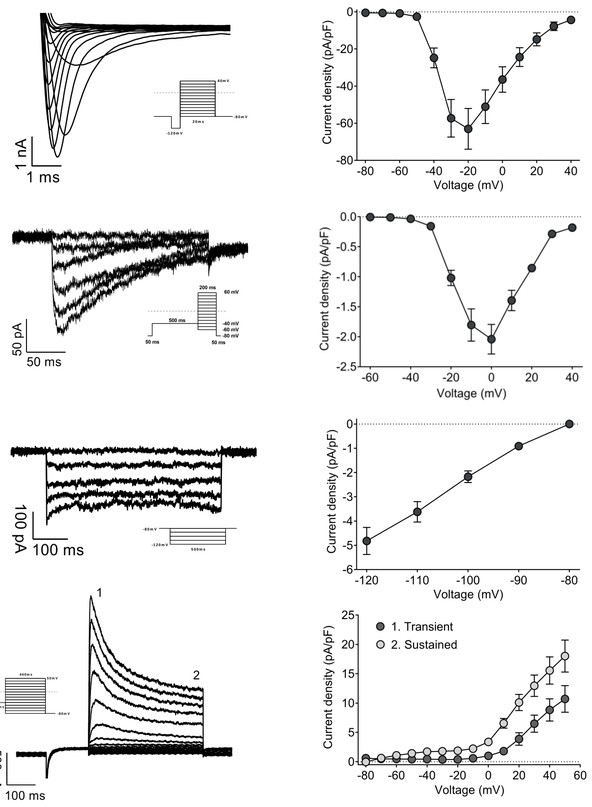
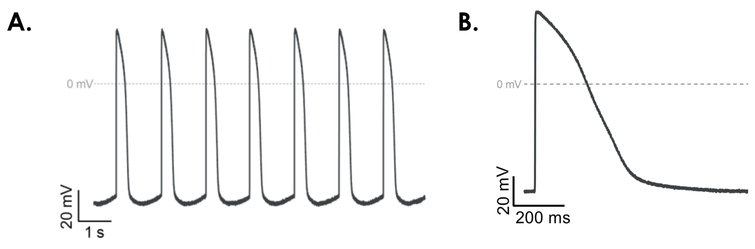


Figure 2: Quantification of key cardiac ionic currents.
Left; Example traces of sodium (I_{Na}; **A**), L-type calcium ($I_{Ca,L}$; **B**), inward (I_{Kin}; **C**) and outward potassium currents (I_{Kout}; **D**) elicited by the protocols shown. *Right*; I-V relationships shown for I_{Na} (peak; **A**), $I_{Ca,L}$ (peak; **B**), I_{Kin} (pulse end; **C**), transient (peak) and sustained I_{Kout} (pulse end; **D**). N ≥ 13.

2. Action Potential Properties



C.

AP parameters	Spontaneous (N=89)	Evoked 1 Hz (N=75)
MDP (mV)	-72.0 ± 0.3	-73.3 ± 0.6
dV/dt_{max} ($V.s^{-1}$)	56.8 ± 3.7	50.1 ± 4.8
APA (mV)	106.5 ± 0.8	119.9 ± 1.7
APD20 (ms)	133.1 ± 3.8	99.3 ± 3.0
APD50 (ms)	249.4 ± 4.9	211.6 ± 3.8
APD90 (ms)	502 ± 16.1	426.8 ± 10.9
Frequency (Hz)	0.43 ± 0.01	1

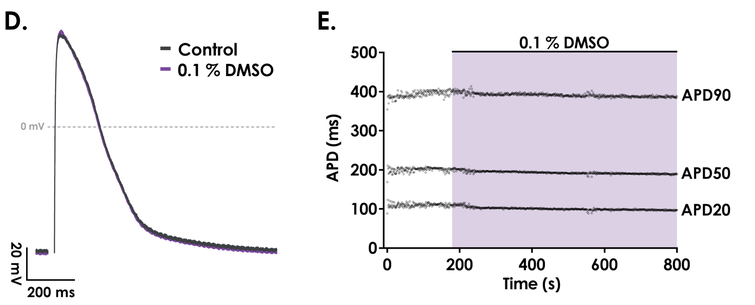


Figure 3: Characteristics of spontaneous and evoked action potentials.
Representative spontaneous (**A**) and evoked (1 Hz; **B**) AP traces under control conditions. ***C***; Average AP parameters in control conditions. ***D***; Representative trace showing evoked AP stability following 0.1 % DMSO application (10 min). ***E***; Stability of evoked AP over time (APD *vs* time) during the control period and following 0.1 % DMSO application (10 min).

3. Core Cardiac Channel Pharmacology

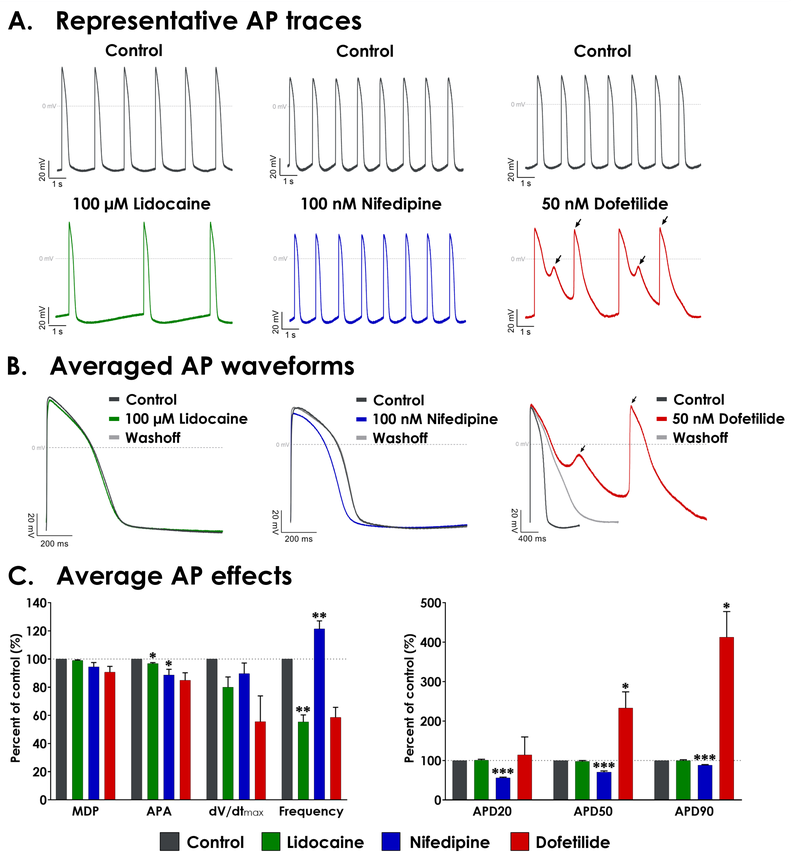


Figure 4: Effects of core cardiac channel modulation.
A; Typical spontaneous AP recorded under control conditions (grey) and in the presence of 100 µM Lidocaine (green), 100 nM Nifedipine (blue) or 50 nM Dofetilide (red). Early after depolarisations (EADs) were detected following Dofetilide addition (arrows). ***B***; Average AP waveform for each condition, mean of N > 3 individual AP. **C**; Average effect (% of control) for each compound. N ≥ 4. * p<0.05, ** p<0.01, *** p<0.001.

4. Impedance Measurements

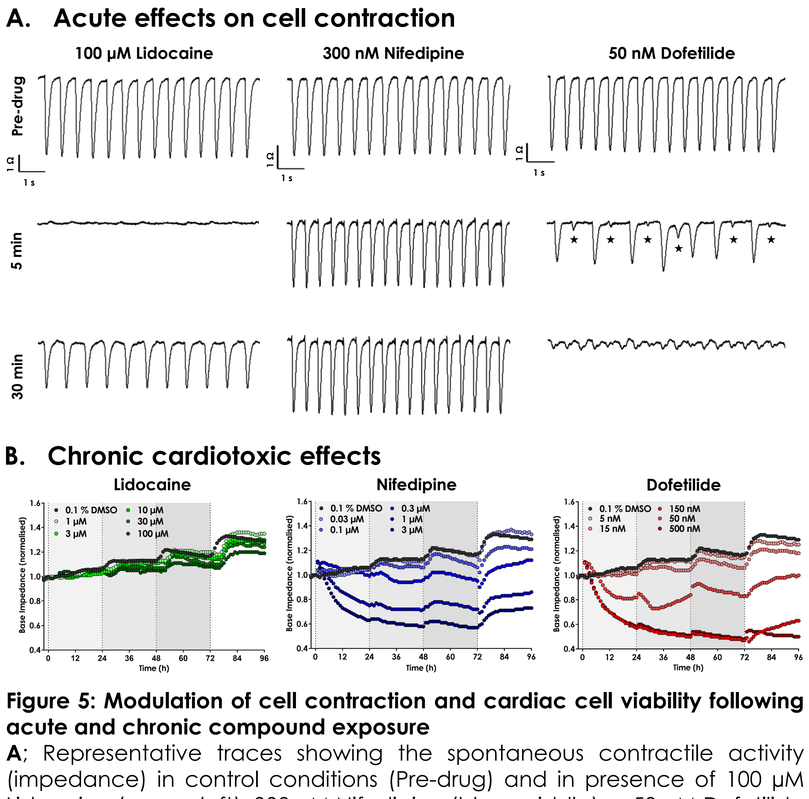
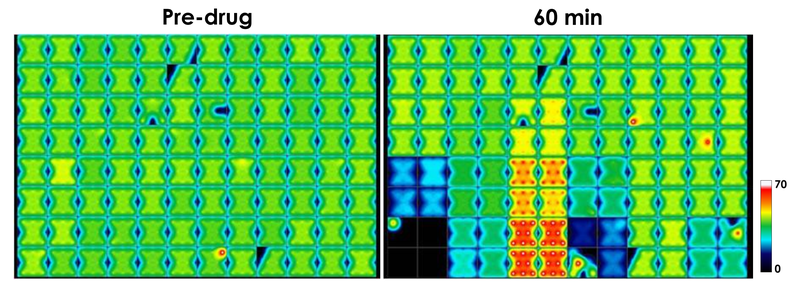


Figure 5: Modulation of cell contraction and cardiac cell viability following acute and chronic compound exposure
A; Representative traces showing the spontaneous contractile activity (impedance) in control conditions (Pre-drug) and in presence of 100 µM Lidocaine (green; left), 300 nM Nifedipine (blue; middle) or 50 nM Dofetilide (red; right) after 5 and 30 min drug application. **B**; Graphs showing chronic drug effects on base impedance over time for increasing concentrations of Lidocaine (left), Nifedipine (middle), and Dofetilide (right). Data are normalised to the last baseline value before drug application (time = 0). ★: secondary beat

5. Multi-electrode Array Measurements

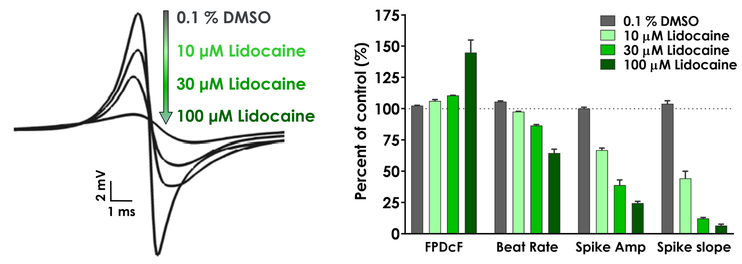
A. Beat Rate 'heat map'



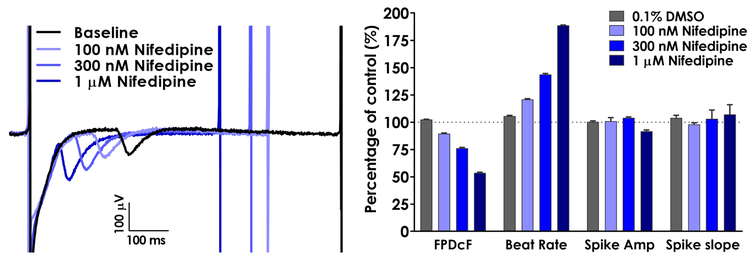
B. Field potential parameters

Mean MEA parameters	vCor.4U
Spike Amplitude (mV)	8.6 ± 1.6
Spike Slope ($V.s^{-1}$)	-13.6 ± 3
Field Potential Duration (ms)	448.5 ± 16.3
FPDcF (ms)	394.8 ± 14.9
Beat Rate (bpm)	40.9 ± 1.2
Conduction Velocity ($mm.ms^{-1}$)	0.22 ± 0.1
Beat Period Irregularity (%)	0.3 ± 0.2

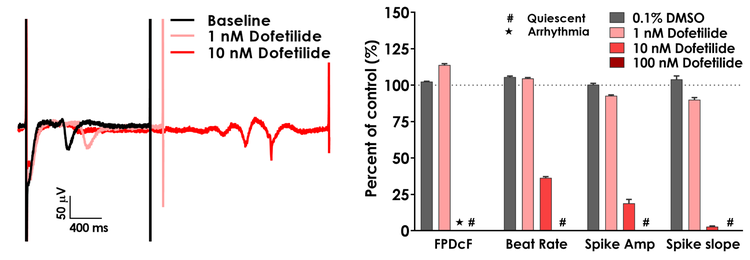
C. Lidocaine



D. Nifedipine



E. Dofetilide



F. Quinidine

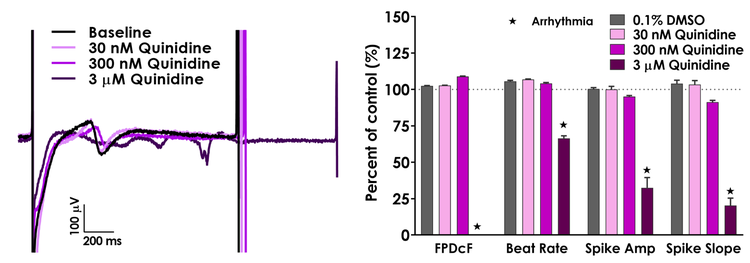


Figure 6: Pharmacological characterisation of vCor.4U MEA field potentials
A; Example of beat rate 'heat map' in control conditions and after 1 hour drug application. **B**; Average MEA parameters during baseline (pre-drug) field potential activity. **C**-**F**: *Left*; Representative traces showing the electrical activity (extracellular field potential) in control conditions (black) and the presence of increasing concentrations of Lidocaine (**C**), Nifedipine (**D**), Dofetilide (**E**), and Quinidine (**F**) which is classified as high torsade de pointe risk[2]. *Right*: Average drug effects (% of control) on MEA parameters after 60 min drug exposure: corrected field potential duration (FPDcF), beat rate, spike amplitude and spike slope. Data are normalised to the last baseline value before drug application (time = 0). FDPcF were not reported when arrhythmias occurred.★ = arrhythmias; # quiescence

Conclusions

Extensive profiling of cardiac ion channel activity with a variety of selective pharmacological tools and high fidelity recording techniques available at Metrion has enabled us to define the physiology of vCor.4U iPSC-CM:

- APs were stable over time during both spontaneous and evoked recording conditions
- The cells exhibit appropriate core cardiac channel pharmacology, including EADs and I_{Kr} inhibition
- Phenotypic readouts of contraction and excitability reflect functional core channel pharmacology

We find that both vCor.4U iPSC-CM and the Maestro MEA platform are amenable for acute and chronic drug studies. Axiogenesis vCor.4U cells represent a suitable model for *in vitro* pre-clinical cardiac safety testing of compounds according to CiPA guidelines for evaluation of potential human pro-arrhythmic cardiac risk.

Acknowledgements

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References

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2. Colatsky *et al.* (2016) J Pharmacol Toxicol. Methods. **81**; 15-20