Human stem cell-derived cardiomyocytes: *in vitro* assays and screening platforms for exploring ventricular and atrial phenotypes

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Introduction

Metrion offers high quality cardiac ion channel services to predict human arrhythmia risk, as required by the FDA's Comprehensive *in vitro* Proarrhythmia Assay (**CiPA**) initiative.

- High quality *in vitro* human cardiac ion channel patch clamp assays,
- Comprehensive *in silico* human action potential (AP) models
- Predictive assays utilising induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs).

For CiPA, iPSC-CMs expressing a mature ventricular phenotype are required. At Metrion Biosciences we have focused on electrophysiological profiling of Axol Human iPSC-Derived Ventricular Cardiomyocytes (hiPSC-vCMs) by evaluating their biophysical and pharmacological characteristics using:

- Whole-cell voltage clamp recordings to quantify inward Na^+ and Ca^{2+} currents (I_{Na} and I_{Ca}), as well as outward and inward K^+ currents (I_K).
- Current clamp measurements of AP parameters and pharmacology, utilising:
  - Compounds to discriminate between atrial and ventricular phenotypes
  - Core cardiac channel modulators from the CiPA validation toolbox

These data confirmed a ventricular phenotype, the functional expression and pharmacology of typical cardiac currents, including I_{Na}, I_{Ca}, and I_{Kr}.

Materials and methods

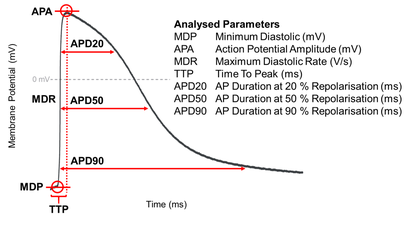
Cell Culture: Human iPSC-Derived Ventricular Cardiomyocytes (ax2505, Axol Bioscience Ltd.) cultured in Cardiomyocyte Maintenance Medium (ax2530-500) at 37 ˚C (5 % CO_2). First 24 h with 10 % FBS, Pen/Strep, thereafter, serum-free.
Immunocytochemistry: Cells were fixed in 3 % PFA, permeabilized with 0.2 % Triton X-100 and blocked with BSA. Primary antibody was incubated overnight 4 ˚C, and secondary antibody coupled to Alexa Fluor® dyes (Invitrogen) applied for 2 h.
Western Blot: 30 µg protein run on 10 % SDS-PAGE gel for 70 min at 130 V and transferred to PVDF membrane. Membranes incubated with primary antibody overnight at 4 ˚C, washed and incubated with secondary antibody for 1 h. Chemiluminescent imaging.
Manual Patch Clamp: Axol hiPSC-vCMs were seeded onto fibronectin-coated flasks and cultured at 37 °C (5 % CO_2) for 4-5 days before re-seeding onto fibronectin-coated coverslips for MP recordings. AP were recorded 7-10 days after cell seeding at RT in current clamp mode using perforated patch (100 µg/ml gramicidin). For evoked AP, cells were typically paced at 1 Hz with a field stimulator. Voltage clamp recordings were obtained from single cells using the conventional whole-cell patch clamp configuration with protocols and solutions designed to isolate the ionic current of interest.

	External				Internal			
	I_{Na}	I_{Ca}	I_K	CC	I_{Na}	I_{Ca}	I_K	CC
NaCl	50	-	140	140	5	5	5	5
TEA-Cl	1	140	-	-	-	-	-	-
KCl	5.4	5.4	5.4	5.4	-	-	20	125
K-Asp	-	-	-	-	-	-	110	-
$CaCl_2$	1.8	1.8	1.8	1.8	-	-	-	-
$MgCl_2$	1	1	1	1	-	-	1	5
CsCl	90	-	-	-	130	130	-	-
Glucose	10	10	10	10	-	-	-	-
HEPES	10	10	10	10	10	10	10	10
MgATP	-	-	-	-	5	5	5	-
$CdCl_2$	0.3	-	0.3	-	-	-	-	-
EGTA	-	-	-	-	10	10	10	5
4-AP	-	2	-	-	-	-	-	-
pH	7.4 CsOH	7.4 CsOH	7.4 NaOH	7.4 NaOH	7.2 CsOH	7.2 CsOH	7.2 KOH	7.2 KOH

Table 1: Manual patch clamp solutions. Composition (in mM) of external and internal solutions used for voltage and current (CC) manual patch clamp experiments.

Data were acquired with EPC-10 amplifiers and PatchMaster software (HEKA Elektronik, Germany). Analog signals were low-pass filtered at 10 kHz before digitization at 20 kHz. Spontaneous AP were analysed with CAPA software (SSCE UG, Germany). The AP parameters analysed are shown in **Figure 1**. In this dataset Maximum Depolarisation Rate (MDR) could not be calculated accurately for evoked AP, therefore Time To Peak (TTP) was used by measuring the time (ms) from the end of the stimulation artefact to the AP peak. Data are reported as mean ± SEM.

Figure 1: Action potential parameters
Example action potential trace indicating the parameters which were quantified using HEKA FitMaster (evoked AP) and CAPA software (spontaneous AP) in this study.



1. Characterisation of Axol ventricular iPSC-CM

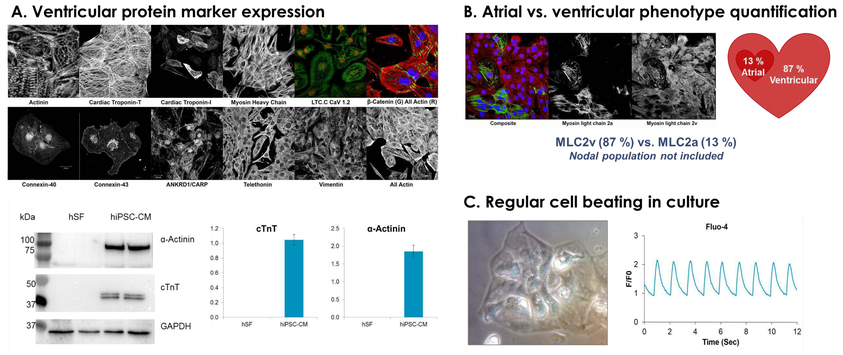


Figure 2: Molecular and Physiological Characterisation of Axol hiPSC-vCMs
A; Top Immunocytochemistry data showed the expression of ventricular cardiomyocyte markers (cardiac troponin-I; 93.5% expression). Data from Dr Christian Zuppinger, University of Bern and Prof Matt Daniels, University of Oxford. *Bottom* Western blot data confirmed that Axol hiPSC-vCMs express more cardiac troponin-T (cTnT) and α-Actinin than human skin fibroblasts (hSFs). Data from Abigail Robertson, University of Manchester. *B;* 87 % of Axol hiPSC-vCMs have a ventricular phenotype determined by MLC2v expression, compared to 13 % expressing atrial MLC2a (n=1). Data from Dr Christian Zuppinger, University of Bern. *C;* Image showing Axol hiPSC-vCMs after 10 days in 2D culture. After 7 days in culture, cells treated with Fluo-4 chemical dye show regular beating. Data from Professor Matt Daniels, University of Oxford.

2. Voltage clamp snapshot of native ionic currents

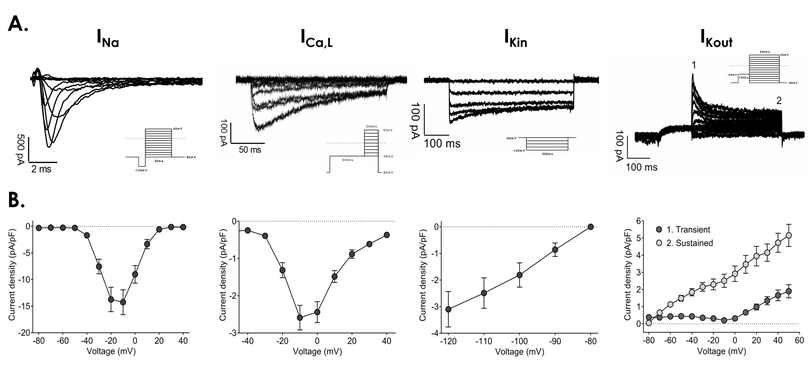
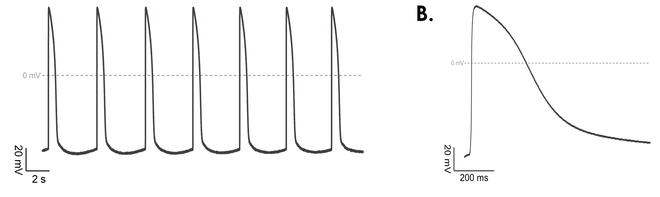


Figure 3: Voltage clamp snapshot of cardiac ionic currents in Axol hiPSC-vCMs
A; Representative traces of sodium (I_{Na}), L-type calcium ($I_{Ca,L}$), inward (I_{Kin}) and outward (I_{Kout}) potassium currents elicited by the voltage protocols shown. *B; I-V* relationships shown for I_{Na} (peak), $I_{Ca,L}$ (peak), I_{Kin} (end of the pulse), transient I_{Kout} (peak current) and sustained I_{Kout} (end of the pulse).

3. Action potential characterisation



C.

AP parameter	Spontaneous	Evoked
MDP (mV)	-65.3 ± 1.3	-73.4 ± 2.0
MDR (V/s)	27.9 ± 4.7	16.5 ± 3.0
APA (mV)	116.3 ± 2.1	128.9 ± 4.2
APD20 (ms)	425.5 ± 40.4	162.7 ± 9.5
APD50 (ms)	655.1 ± 57.2	354.8 ± 10.3
APD90 (ms)	837.5 ± 62.6	548.4 ± 12.5
Frequency (Hz)	0.33 ± 0.05	1

Figure 4: Characteristics of spontaneous and evoked action potentials
Representative traces of spontaneous (A) and evoked (1 Hz; *B*) AP recorded from Axol hiPSC-vCMs under control conditions. *C;* Average AP parameters for spontaneous (n = 18) and evoked (n = 31) AP in control conditions.

4. Atrial vs. ventricular AP phenotype

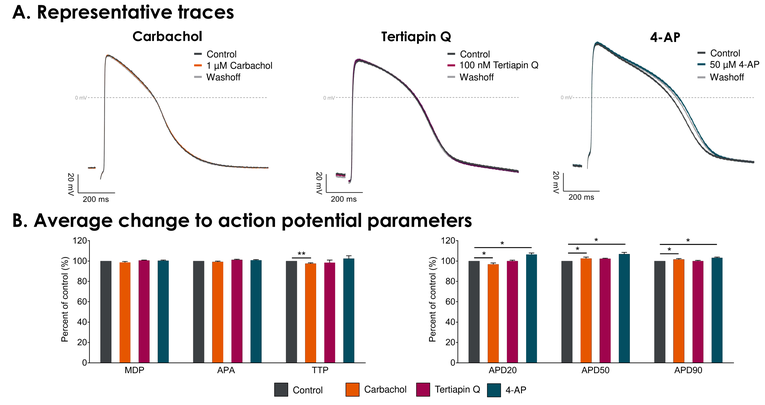


Figure 5: Pharmacological tools targeted against atrial specific currents show minimal effects
A; Representative traces of evoked AP recorded under control conditions (grey) and in the presence of 1 µM carbachol (orange), 100 nM tertiapin Q (pink) or 50 µM 4-AP (blue). *B;* Average effect of each compound on spontaneous AP parameters. Data presented as percent of control ± SEM, n ≥ 4. Significance calculated by a paired two-tailed Student's *t*-test, * p<0.05, ** p<0.01.

5. Core cardiac channel pharmacology

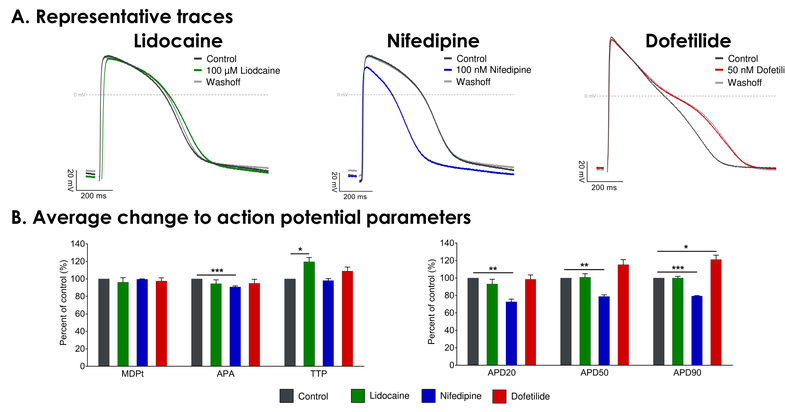


Figure 6: Core cardiac channel action potential pharmacology
A; Representative traces of evoked AP recorded under control conditions (grey) and in the presence of 100 µM lidocaine (green), 100 nM nifedipine (blue) or 50 nM dofetilide (red). *B;* Average effect of each compound on spontaneous AP parameters. Data presented as percent of control ± SEM, n ≥ 4. Significance calculated by a paired two-tailed Student's *t*-test, * p<0.05, ** p<0.01, *** p<0.001.

Conclusions

- Axol hiPSC-vCMs express a range of ventricular cardiomyocyte markers and function as a highly pure population of beating cells in culture.
- Extensive electrophysiological profiling of cardiac ion channel expression and function at Metrion determined that Axol hiPSC-vCMs:
  - Express the three major ionic currents I_{Na}, $I_{Ca,L}$, and I_K (I_{Kout} and I_{Kin})
  - Produce stable evoked AP when paced at 1 Hz
  - Express a predominately ventricular phenotype
    - Minimal effect of atrial-selective channel modulators
  - Exhibit appropriate core cardiac channel pharmacology
    - AP pharmacology confirms presence of core cardiac currents I_{Na}, $I_{Ca,L}$, and I_{Kr}

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