Differentiation and Validation of Human iPSC-Derived Atrial Cardiomyocytes

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Introduction

There is a growing trend for utilisation of native human cells in drug discovery to overcome common translational disconnects between *in vitro* screening data, preclinical animal models, and clinical trials in man. Translational assays using cardiomyocytes derived from human induced pluripotent stem cells (hiPSC) are increasingly appreciated as an accessible cell source for cardiac disease modelling, drug screening, and safety pharmacology.

Previously, we have shown that human embryonic stem cell derived atrial cardiomyocytes (ACMs) can be used for assessing atrial-selective pharmacology (Devalla *et al.*, 2015). Here, we show improved, robust monolayer-based production of functional hiPSC-ACMs for atrial drug screening.

Materials and Methods

Differentiation of hiPSC-ACMs: LDN
hiPSCs seeded as a monolayer were differentiated to ACMs using a method described previously (Devalla *et al.*, 2015). Differentiated ACMs were enriched by flow cytometry for the cardiac surface marker, Signal-regulatory protein alpha (SIRPA).

mRNA/protein expression: LDN
Gene and protein expression was assessed in hiPSC-ACMs at day 21 using standard techniques. Primer sequences are available on request.

Manual patch clamp: MET
hiPSC-ACMs were cultured onto Matrigel-coated coverslips at 37 °C (5 % CO_2). All data were recorded 7-10 days post-seeding at RT using perforated patch clamp (100 µg/ml Gramicidin) with physiological solutions (Ma *et al.*, 2011). Data were acquired with EPC10 amplifiers and PatchMaster software (HEKA Elektronik, Germany). Analog 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. All 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.

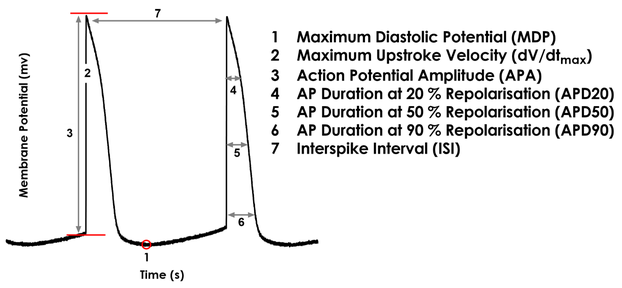


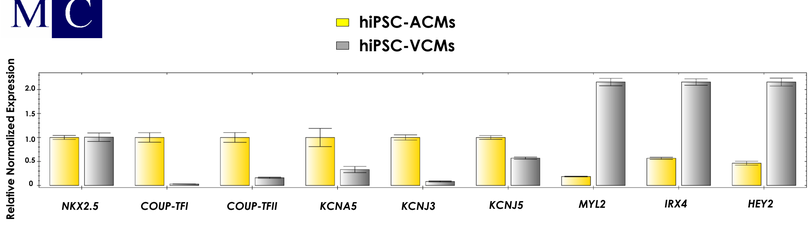
Fig. 1: Action potential parameters analysed.
Example action potential (AP) indicating the parameters quantified in this study.

Automated patch clamp (APC): NAN
The APC experiments presented on this poster were performed on the Patchliner (PL) or SyncroPatch (SP) 384/768PE as indicated. Experiments were all performed in regular electrophysiological salt solutions. Both systems have the option of temperature control and their amplifiers the option of current clamp (Patchliner: HEKA EPC10, SP 384PE: Tecella).

CardioExcyte 96: NAN
The CardioExcyte 96 is a hybrid system combining impedance and EFP recordings from the same cell monolayer. It provides complementary data to other assays such as patch clamp.

1. hiPSC-ACM Molecular characterisation

A. Quantitative expression levels of key cardiac markers



B. hiPSC-ACMs express PITX2

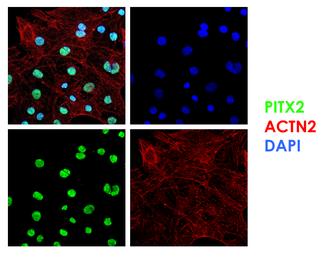


Fig. 2A: hiPSC-ACMs preferentially expressed atrial markers such as transcription factors, *COUP-TFI*, *COUP-TFII* and ion channel genes *KCNA5*, *KCNJ3*, *KCNJ5*, compared to hiPSC-derived ventricular cardiomyocytes (hiPSC-VCMs). As expected, hiPSC-VCMs preferentially expressed ventricular markers such as *MYL2*, *IRX4* and *HEY2*. **B:** hiPSC-ACMs express left-atrial marker PITX2.

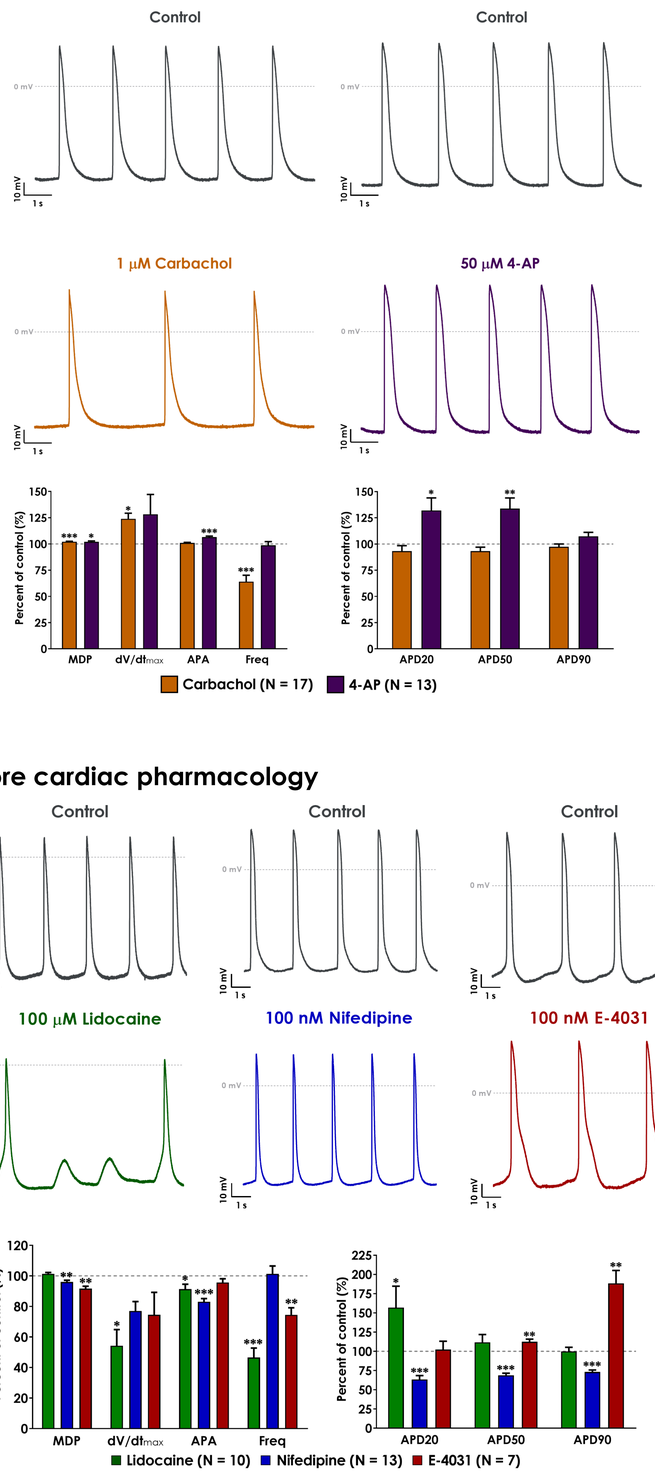
2. Electrophysiological validation of hiPSC-ACMs

A. Control conditions

AP Parameter	Spontaneous (n = 56)
MDP (mV)	-66.7 ± 0.9
dV/dt_{max} (V/s)	25.5 ± 2.6
Amp (mV)	88.6 ±1.6
APD20 (ms)	139.9 ± 7.4
APD50 (ms)	204.1 ± 7.9
APD90 (ms)	485.8 ± 10.3
Frequency (Hz)	0.4 ± 0.0

Table 1: Spontaneous action potential parameters.

B. Atrial selective pharmacology



C. Core cardiac pharmacology

Fig. 3: Characterisation of hiPSC-ACM action potentials.
A: Average spontaneous AP parameters in control conditions.
B: Pharmacological confirmation of atrial phenotype using Carbachol (I_{KACh}) and 4-AP (I_{Kur}). *Top*: Representative spontaneous AP under control conditions (grey) and in the presence of 1 µM Carbachol (orange) and 50 µM 4-AP (purple). *Bottom*: Average effect (% of control) for each compound.
C: The presence of key cardiac currents, I_{Na}, $I_{Ca,L}$, and I_{Kr}, in hiPSC-ACM were confirmed using Lidocaine, Nifedipine, and E-4031, respectively. *Top*: Representative spontaneous AP under control conditions (grey) and in the presence of 100 µM Lidocaine (green), 100 nM Nifedipine (blue), and 100 nM E-4031 (red). *Bottom*: Average effect (% of control) for each compound.
Data are presented as mean ± SEM, N ≥ 7. * p<0.05, ** p<0.01, *** p<0.001.

3. Suitability of hiPSC-ACM for automated patch clamp

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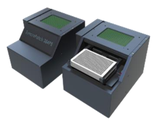
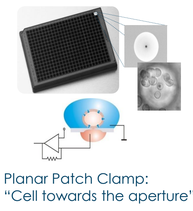


Patchliner & SyncroPatch 384/768PE

hiPSC-ACM were recorded in automated patch clamp systems Patchliner and SyncroPatch 384 PE. Na_v, Ca_v and K_v currents could be identified in the voltage clamp mode. Furthermore, in current clamp, APs could be elicited successfully.



Planar Patch Clamp:
"Cell towards the aperture"

	Parameter (n)
Success rate cell catch/seal	65 %
$Na_v1.5$ peak (pA)	772 ± 1889 (11)
$Ca_v1.2$ peak (pA)	162 (1)
$K_v1.5$ peak (pA)	264 ± 140 (4)
Cells with AP	10 %

Table 2: Success rate and recorded values. Only cells reaching a threshold of Na_v current showed APs in the current clamp mode.

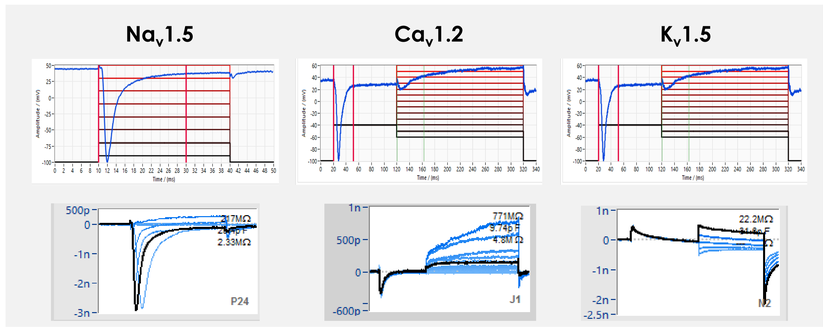
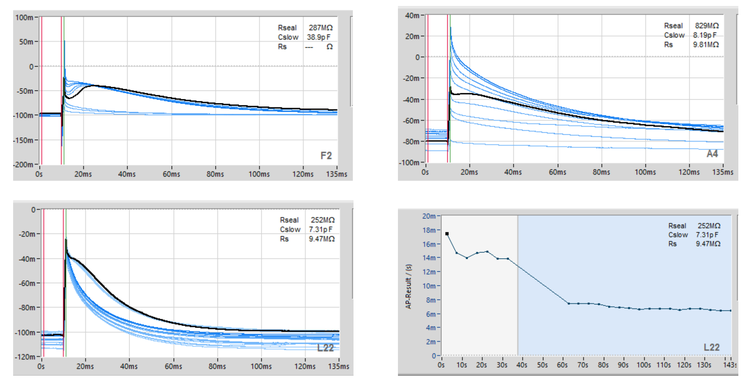


Fig. 4: Currents in voltage clamp. Na_v, Ca_v and $K_v1.5$ currents recorded in physiological conditions.

A.



B.

Fig. 5A: AP elicitation in current clamp mode. APs elicited by increasing current stimuli (from 0 pA, in 50 pA increments) **B: APD50.** Duration change by 250 µM Tetracaine

4. hiPSC-ACMs in label-free contractility and extracellular field potential assays

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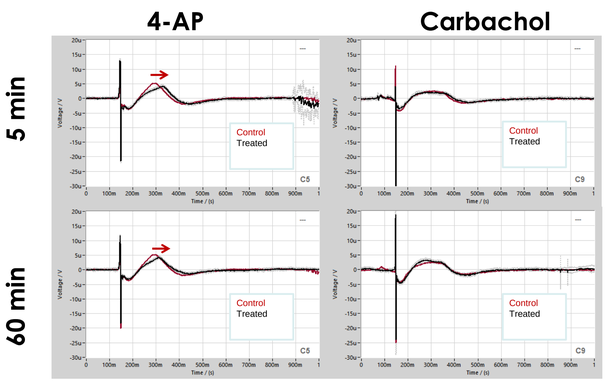
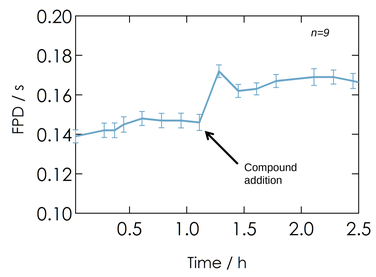
CardioExcyte 96

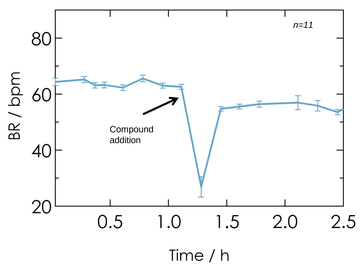
A. EFP readings (Field Potential Duration, FPD)

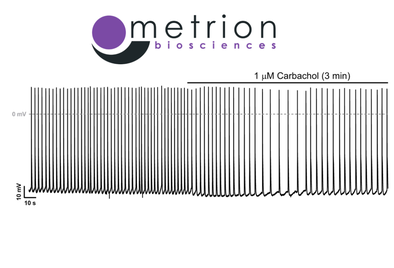




- **4-AP** increased the FPD (no effect with **Carbachol**)

B. Impedance (beat rate)





- **Carbachol** caused a temporary decrease in beat rate on the CE96 (mimicking the transient nature of the electrophysiological pharmacology response, right)

Fig. 6: EFP and Impedance recordings. hiPSC-ACMs were successuflly grown on sensor plates and revealed atrial-specific pharmacological reactions to compounds 4-AP (50 µM) and Carbachol (1 µM).

Conclusions

- hiPSC-ACMs derived using a monolayer differentiation protocol express an atrial-like phenotype and are amenable for screening on both electrophysiological and phenotypic platforms.
- hiPSC-ACMs are a promising assay reagent for studying the role of ion channels implicated in atrial fibrillation.

Acknowledgements

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References

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Ma *et al.* (2011) Am J Physiol Heart Cir Physiol. **301**; H2006-H2017.