Functional characterisation of human iPSC-derived atrial cardiomyocytes

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Introduction

Atrial fibrillation (AF) is the most common arrhythmia observed in the clinic, considerable effort has been made to identify the cellular mechanisms of AF and develop new safe and effective antiarrhythmic drugs(1). However, preclinical studies using non-cardiac cells and non-human animal models may not replicate the physiology of human atrial cardiomyocytes or predict patient efficacy and safety(2).

Here we outline our results from studies to validate human induced pluripotent stem cell-derived atrial cardiomyocytes (hiPSC-ACMs) generated by Axol Bioscience. The atrial phenotype of Axol hiPSC-ACMs was first characterised at the molecular level using immunocytochemistry with atrial specific markers before functional validation using manual patch clamp recordings of action potential (AP) parameters.

The atrial phenotype was further confirmed using modulators of the atrial specific acetylcholine-activated inward-rectifying potassium current (I_{KACh}) and the ultrarapid delayed rectifier potassium current (I_{KUr}) which are also targets in AF drug discovery(1).

Materials and Methods

Cell Culture: Human iPSC-derived atrial cardiomyocytes (Axol Bioscience Ltd.) were generated based on a variation (retinoid acid) of the Burridge *et al* method(3) seeded and cultured according to the manufacturer's instructions.
Immunocytochemistry: Cells were fixed in 4 % PFA, permeabilized with 0.3 % Triton X-100 and blocked with 5 % donkey serum. Primary antibody was incubated overnight 4 ˚C, and secondary antibody coupled to Alexa Fluor® dyes (Invitrogen) applied for 2 h.
Manual Patch Clamp (MPC): AP were recorded from Axol hiPSC-ACM 7-10 days after cell seeding. Recordings were made at room temperature in current clamp mode using perforated patch (100 µg/ml gramicidin). Data were acquired with EPC10 amplifiers and PatchMaster software (HEKA Elektronik, Germany). Analog signals were low-pass filtered at 10 kHz before digitisation at 20 kHz. Spontaneous AP were analysed with CAPA software (SSCE UG, Germany). AP parameters analysed: maximum diastolic potential (MDP), upstroke velocity (dV/dt_{max}), AP amplitude (APA), AP duration at 20, 50 and 90 % repolarisation (APD20, APD50, APD90), and frequency (Freq). Data are presented as mean ± SEM. Significance was determined by paired student's *t*-test comparing control values to the effect of compound application. * P<0.05, ** P<0.01, *** P<0.001.

1. Expression of cardiac markers in Axol hiPSC-ACM

Axol hiPSC-ACM express protein makers characteristic of atrial cardiomyocytes

A) MLC2v/MLC2a/DAPI B) ANP/MLC2a/DAPI C) ANP/TropT/DAPI

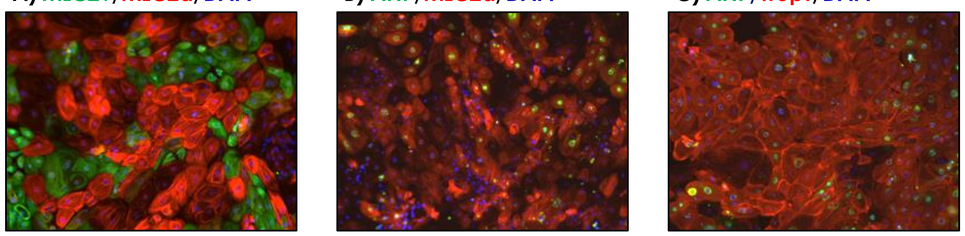
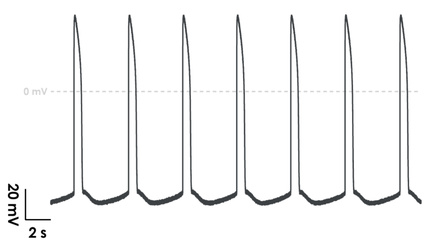


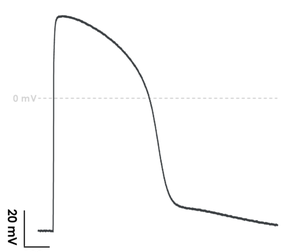
Figure 1: Cardiac protein marker expression was detected in Axol hiPSC-ACM by immunocytochemistry
The expression of two atrial-specific markers (atrial myosin light chain ***MLC2a*** and atrial natriuretic peptide ***ANP***), one ventricular marker (ventricular myosin light chain; ***MLC2v***), and one general cardiac marker (Troponin T; ***TropT***) were evaluated in hiPSC-ACM. (**A**) Higher expression levels of MLC2a (red) were detected compared to MLC2v (green). (**B**) Cells positive for MLC2a (red) also expressed ANP (green). (**C**) Expression of Troponin T (red) was also confirmed in ANP (green) positive cells.

2. Action potential characteristics

Axol hiPSC-ACM elicit spontaneous action potentials and are suitable for pacing experiments

A) Spontaneous AP B) Evoked AP (1 Hz) C) AP parameters in control conditions





AP parameter	Spontaneous (N = 31)	Evoked 1 Hz (N = 28)
MDP (mV)	-73.6 ± 0.8	-73.0 ± 0.9
dV/dt_{max} (V/s)	44.6 ± 3.4	30.9 ± 3.8
APA (mV)	120.2 ± 1.0	126.3 ± 1.5
APD20 (ms)	252.4 ± 6.3	235.2 ± 5.6
APD50 (ms)	338.0 ± 6.7	352.1 ± 4.8
APD90 (ms)	419.0 ± 7.0	468.7 ± 11.4
Frequency (Hz)	0.28 ± 0.01	1

Figure 2: Characteristics of spontaneous and evoked action potentials from Axol hiPSC-ACM
Representative traces of spontaneous (**A**) and evoked (1 Hz; **B**) AP recorded under control conditions. (**C**) Average AP parameters.

2. Functional expression of core cardiac ion channel pharmacology

Pharmacological modulation of spontaneous AP confirmed functional expression of core cardiac currents

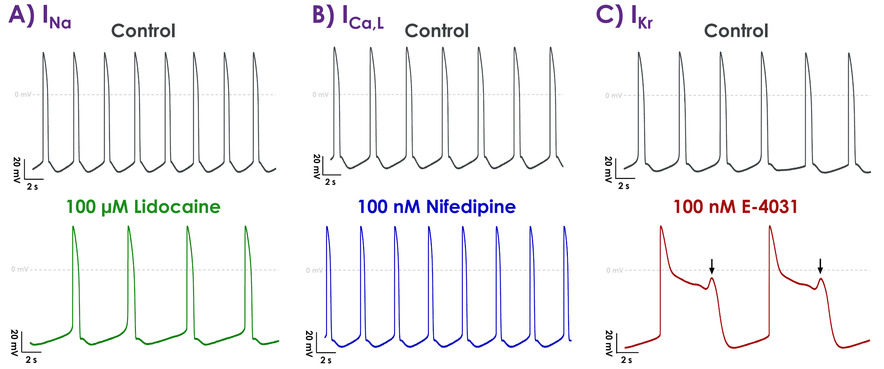


Figure 3: Confirmed functional expression of core cardiac currents in Axol hiPSC-ACM
The presence of three core cardiac currents in Axol hiPSC-ACM action potentials were confirmed using Lidocaine (I_{Na}), Nifedipine ($I_{Ca,L}$), and E-4031 (I_{Kr}). Representative spontaneous AP under control conditions (grey) and in the presence of 100 µM Lidocaine (**A**, green), 100 nM Nifedipine (**B**, blue), and 100 nM E-4031 (**C**, red). Early after depolarisations (EADs), indicative of arrhythmic events, were observed following E-4031 application (**C**, arrow). **D**: Average effect (% of control) on AP parameters, N ≥ 4.

3. Atrial-specific ion channel pharmacology

Pharmacological modulation of spontaneous AP confirmed expression of functional atrial phenotype

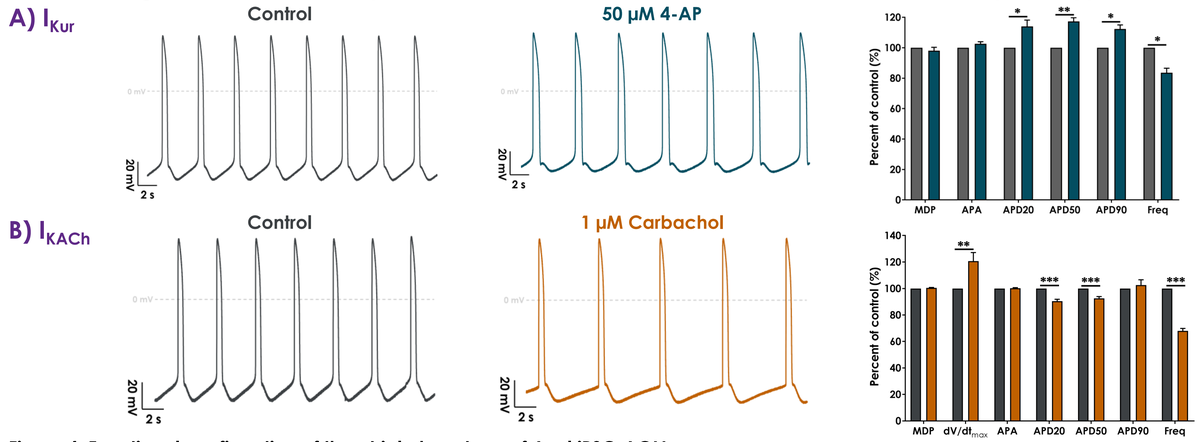


Figure 4: Functional confirmation of the atrial phenotype of Axol iPSC-ACM
The atrial phenotype of Axol iPSC-ACM was confirmed using compounds (4-AP for I_{Kur} and Carbachol for I_{KACh}) which modulate atrial specific currents that are also targets for AF. Representative spontaneous AP in control conditions (grey) and the presence of 50 µM 4-AP (**A**, blue) and 1 µM Carbachol (**B**, orange). Concordant bar graphs show the average effect (% of control) on AP parameters, N ≥ 5.

Conclusions

- **The molecular and pharmacological data presented here confirm that Axol hiPSC-ACMs express an atrial-like phenotype suitable for use on electrophysiological platforms**
- **Axol hiPSC-ACMs represent a promising tool to develop improved translational models of AF as:**
  - Axol iPSC-ACMs express atrial-specific markers
  - Spontaneous APs showed sensitivity to a core panel of cardiac ion channel inhibitors
  - An atrial phenotype was confirmed using selective modulators of atrial-specific currents such as I_{KACh} and I_{KUr} which are validated targets of AF drug discovery

References

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(3) Burridge *et al.* (2014). Nat Methods. **11**(8): 855-60

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