IN VITRO VALIDATION OF HUMAN IPSC-DERIVED ATRIAL CARDIOMYOCYTES FOR CELL-BASED ASSAYS AND DRUG DISCOVERY

Broadbent, S[1]., Clare, N[1]., Dark, N[1]., Paonessa, M[1]., El-Haou, S[2]., Harper[1]., S, Shi, Y[1].

[1]Axol Bioscience Ltd., Cambridge, UK, support@axolbio.com; [2] Metrion Biosciences., Cambridge, UK

Discovery Stems From Here

INTRODUCTION

The development of atrial cardiomyocytes from iPSCs offers the potential for disease models of atrial fibrillation to be established from patients which may provide information on therapeutics that modify the phenotypic markers of cardiovascular disease and atrial fibrillation.

Atrial fibrillation is one of the most common arrhythmias to affect the heart, as such there is a need to develop drugs to target atrial arrhythmia. However, current mouse models fail to translate *in vitro* due to fundamental differences in the electrophysiology of cardiac action potentials[1].

Here we present data on the molecular and electrophysiological characterization of Axol's Human iPSC-derived Atrial Cardiomyocytes. We determined the protein and gene expression, beat rate and action potential parameters, along with identifying the functionality of the core cardiac and atrial-specific ion channels.

Molecular characterisation of Axol's Human iPSC-derived Atrial Cardiomyocytes reveals the expression of cardiac and atrial-specific markers troponin T, atrial myosin light chain 2 (MLC2a) and atrial natriuretic peptide (ANP) and key ion channels, $K_V1.5$ and $K_{ir3.1/3.4}$.

Functionally, Axol's Human iPSC-derived Atrial Cardiomyocytes elicit spontaneous action potentials, express functional core cardiac ion channels, INa, ICa,L and IKr and exhibit a steady beat rate.

Axol's Human iPSC-derived Atrial Cardiomyocytes are shown to be a highly validated, physiologically relevant model that offers the opportunity to study atrial-specific disorders, such as atrial fibrillation, and develop cell-based assays to identify disease modifying treatments.

MATERIALS AND METHODS

Culture of hiPSC-derived atrial cardiomyocytes: Human iPSC-derived atrial cardiomyocytes (hiPSC-ACMs) (Axol Bioscience ltd., UK) were cultured at $8.0x10^5$ cells/cm^2 on a 384-channel 24-well multi-electrode array (MEA) chip (Alpha Med Scientific Inc., Japan) and a 24-well plate both coated with Axol SureBond Coating Solution (Axol Bioscience ltd., UK) at 37°C in a 5% CO_2/95% air atmosphere.

Immunofluorescent imaging: Immunostains (Troponin T, ANP, MLC2a and MLC2v along with DAPI counterstains) were applied to mature hiPSC-ACMs on 8 DIV. Immunofluorescent imaging using an EVOS FI Auto (Life Technologies Corporation, UK) was used to obtain images of the cardiomyocytes to characterize their morphology and receptor expression.

Multi-electrode Arrays (MEA): Spontaneous extracellular field potentials were acquired at 37°C using the high-throughput MEA system, here we simultaneously recorded extracellular potentials for 16 channels per well across 24-well plates (MED64 Presto, Alpha Med Scientific Inc., Japan) at a sampling rate of 20 kHz/channel and stored on a personal computer. Synchronised, spontaneous beats were first observed and recorded after four DIV on most electrodes across all wells. The hiPSC-ACMs continued to be viable until 32 DIV when the experiment was terminated.

Manual Patch Clamp (MPC): Action potentials (AP) were recorded from hiPSC-ACMs 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/dtmax), 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.

Electrophysiological analysis: Raw data was acquired using MEA Symphony (Alpha Med Scientific Inc.). Trace plotting, beat extraction, beat count, waveform creation, interspike intervals (ISIs) and raster plots were produced by Clampfit (Molecular Devices, LLC, US). Graphs created by GraphPad Prism (GraphPad Software Inc., US). Beats were extracted automatically by Clampfit using a positive detection threshold of at least 300µV compared to average baseline noise of ±6µV. Waveforms were created by Clampfit from 25ms pre-trigger to 400ms post-trigger and averaged across at least a minute's section of recording. FPAs, beat counts, beat timings, ISIs and their standard deviation (SD) were determined automatically by Clampfit.

Arrhythmia monitoring and identification: Any arrhythmias within the cells were to be identified by daily monitoring of the beat rate under a microscope (Nikon Diaphot, Nikon, Japan), the appearance of after-depolarisations on the whole trace plot and within the waveform, unstable raster plots and large standard deviations for the ISIs.

1. ELECTROPHYSIOLOGICAL CHARACTERIZATION OF hiPSC-ATRIAL CARDIOMYOCYTES

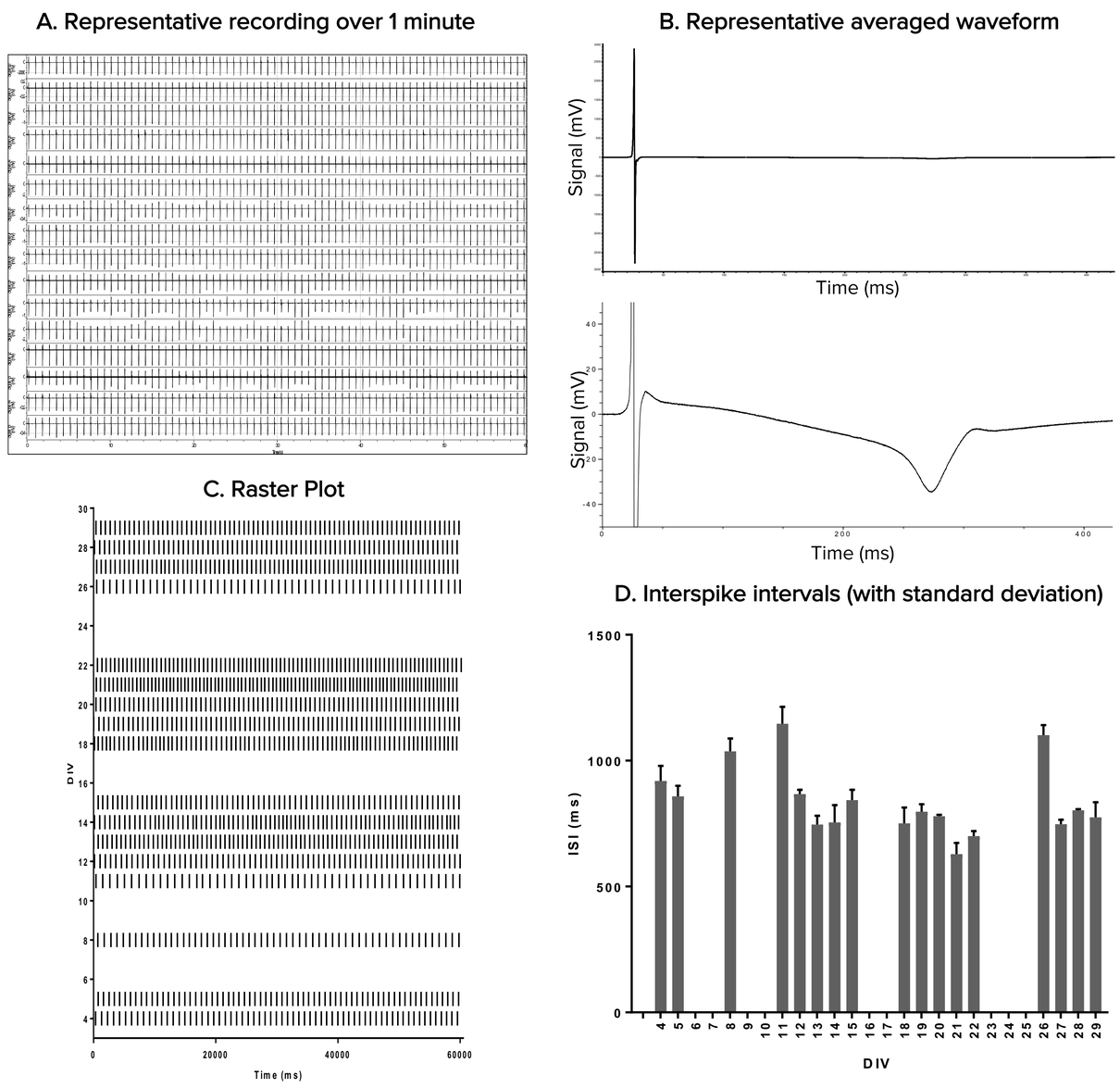
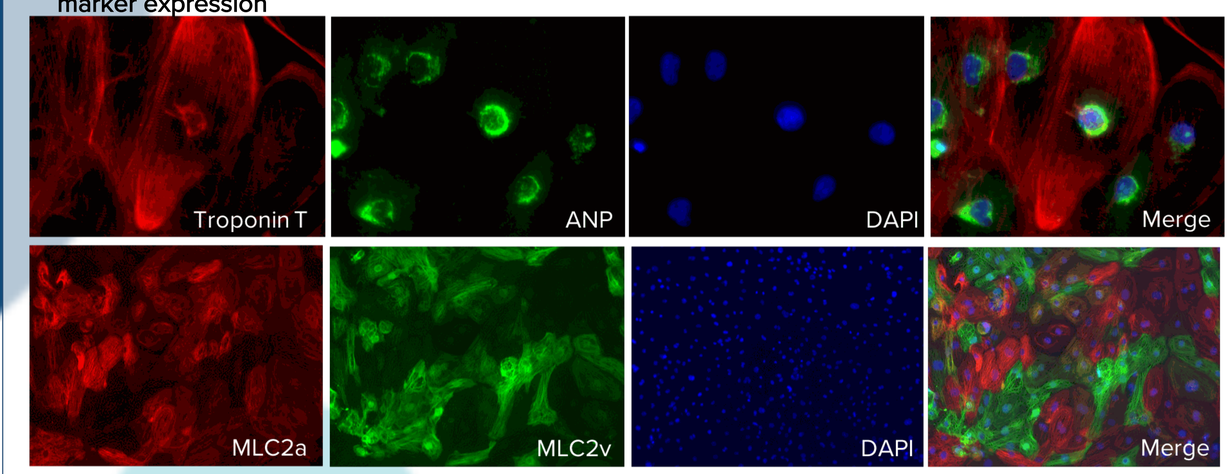


Figure 1: Electrophysiology of Axol's hiPSC-ACMs

Axol's hiPSC-ACMs produce typical atrial-like field action potential (fAPs) waveforms with no evidence of arrhythmias. **A.** Typical minute's trace of fAPs recorded from hiPSC-ACMs from a single well (16 electrodes). Note stable, synchronous beating and no evidence of arrhythmia or after-depolarisations. **B.** Averaged waveform from 10-minute recording of fAPs recorded from hiPSC-ACMs by a single electrode showing typical atrial-like fAPs at full-scale (top) and zoomed-in to show the T-wave. No evidence of after-depolarisations within waveform. **C.** Raster plot of recorded beats from a single electrode from DIV4 to DIV29, showing rhythmic and stable beat rates. **D.** Interspike Intervals (±SD) for a single electrode from DIV4 to DIV29 showing stable ISIs with small SD.

2. EXPRESSION OF CARDIAC MARKERS IN hiPSC-ACM

A. Troponin T, atrial natriuretic peptide (ANP) and atrial myosin light chain 2 (MLC2a) protein marker expression



B. Phase Contrast

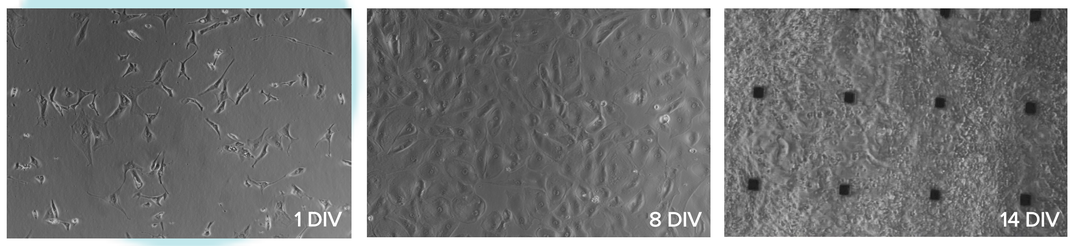


Figure 2: Morphology and Immunocytochemistry of Axol's hiPSC-ACMs

Atrial cardiomyocytes express the cardiac and atrial-specific markers troponin T, atrial myosin light chain 2 (MLC2a) and atrial natriuretic peptide (ANP). The presence of these proteins was confirmed in hiPSC-ACMs. **A.** Immunocytochemistry data showed the expression of the cardiac- and atrial-specific proteins. Troponin T staining (red) confirmed the presence of cardiac myocytes which are responsible for sarcomere contraction. ANP is specifically secreted by atrial myocytes upon atrial stretching and MLC2a facilitates cardiac contractility. DAPI counterstain. **B.** Phase contrast images of hiPSC-ACMs at DIV 1, 8 and 14.

3. hiPSC-ATRIAL CARDIOMYOCYTE ION CHANNEL PHARMACOLOGY

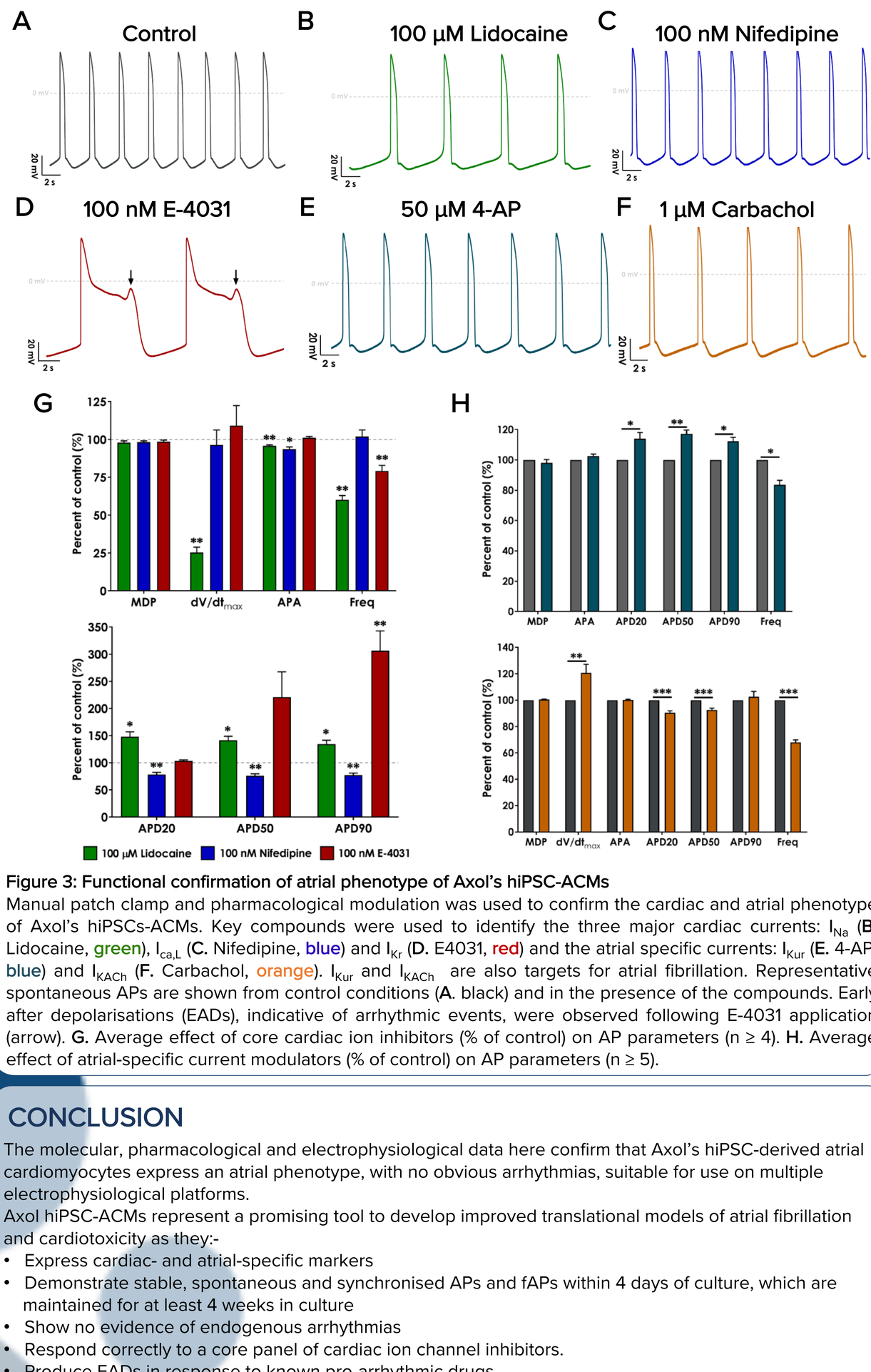


Figure 3: Functional confirmation of atrial phenotype of Axol's hiPSC-ACMs

Manual patch clamp and pharmacological modulation was used to confirm the cardiac and atrial phenotype of Axol's hiPSCs-ACMs. Key compounds were used to identify the three major cardiac currents: I_{Na} (**B.** Lidocaine, green), $I_{ca,L}$ (**C.** Nifedipine, blue) and I_{Kr} (**D.** E4031, red) and the atrial specific currents: I_{Kur} (**E.** 4-AP, blue) and I_{KACh} (**F.** Carbachol, orange). I_{Kur} and I_{KACh} are also targets for atrial fibrillation. Representative spontaneous APs are shown from control conditions (**A**. black) and in the presence of the compounds. Early after depolarisations (EADs), indicative of arrhythmic events, were observed following E-4031 application (arrow). **G.** Average effect of core cardiac ion inhibitors (% of control) on AP parameters (n ≥ 4). **H.** Average effect of atrial-specific current modulators (% of control) on AP parameters (n ≥ 5).

CONCLUSION

The molecular, pharmacological and electrophysiological data here confirm that Axol's hiPSC-derived atrial cardiomyocytes express an atrial phenotype, with no obvious arrhythmias, suitable for use on multiple electrophysiological platforms.

Axol hiPSC-ACMs represent a promising tool to develop improved translational models of atrial fibrillation and cardiotoxicity as they:-

- Express cardiac- and atrial-specific markers
- Demonstrate stable, spontaneous and synchronised APs and fAPs within 4 days of culture, which are maintained for at least 4 weeks in culture
- Show no evidence of endogenous arrhythmias
- Respond correctly to a core panel of cardiac ion channel inhibitors.
- Produce EADs in response to known pro-arrhythmic drugs
- Exhibit atrial phenotypes to known selective modulators of atrial-specific currents and known targets of atrial fibrillation

ACKNOWLEDGEMENTS

We thank our collaborators at Metrion Biosciences for providing data for Figure 3 and Alpha MED Scientific for the use of the MED64 Presto and all their technical assistance.

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