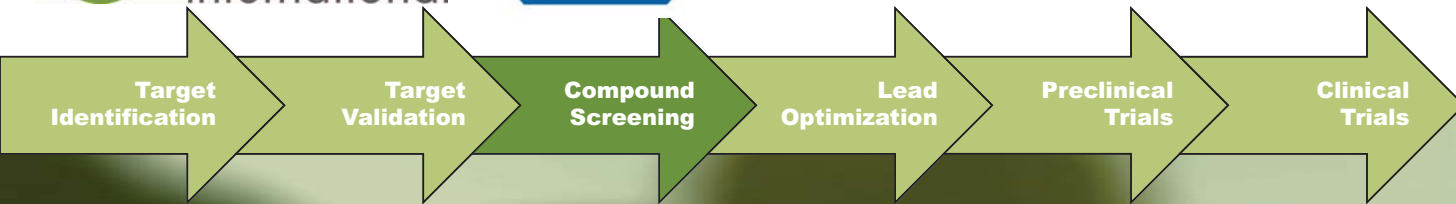
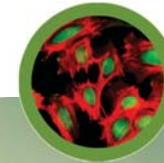


Assessment of drug-induced QT prolongation in human induced pluripotent stem cell-derived cardiomyocytes using Microelectrode Arrays

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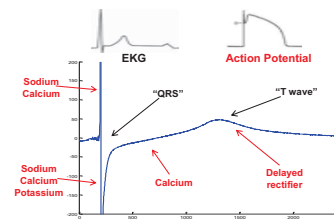


Abstract

Assessing the potential for drug-induced cardiac arrhythmias is a vital component of the toxicological and safety pharmacological profile of new chemical entities (NCEs). Traditional methods for electrophysiological assessment of NCE arrhythmogenic propensity employ human ion channels heterologously expressed in non-cardiac cell lines and/or animal cardiac models. Neither system completely reflects the human condition; heterologous expression systems do not typically express ancillary channel subunits and may miss the functional impact of NCEs that interact with multiple channels, while animal models do not express human proteins and may miss species-specific effects. iCell Cardiomyocytes are a human-based induced pluripotent stem (iPS) cell-derived cardiomyocyte test system where ion channels are expressed in their native environment and thus surmount many of the vagaries associated with traditional models. The sensitivity and robustness of iCell Cardiomyocytes and microelectrode array (MEA) recording technology as a cardiac arrhythmogenicity assessment platform was tested by measuring changes, or lack thereof, in MEA-recorded field potential waveforms from spontaneously electrically active syncytia of iCell Cardiomyocytes before and during cardioactive compound exposure. Beta-adrenergic activation by 300nM Isoproterenol produced an approximate 2-fold increase in beating frequency. Sodium channel block by 3µM tetrodotoxin ceased spontaneous beating frequency. Calcium channel block by 30 – 3000nM nifedipine shortened the spontaneous field potential duration. hERG channel block by 3 to 100nM E-4031 or KCNQ channel block by 3 – 100µM chromanol 293B prolonged the spontaneous field potential duration. These results demonstrate that the iCell Cardiomyocytes / MEA platform is suitable for assessing drug-induced changes in electrical activity of a relevant human-based cardiac model.

Introduction

iCell Cardiomyocytes are derived from human induced pluripotent stem (iPS), express all major cardiac ion channels, and beat spontaneously in culture. MEA technology uses external electrodes to measure changes in the local field potential, and thus the activity of the underlying ion channels. A prototypical field potential recorded over a single beat and the EKG and intracellular action potential correlates are shown illustrated on the right.



The MEA was used to assess the effects of known cardioactive agents on iCell Cardiomyocytes beating rate and field potential duration (FPD).

Methods

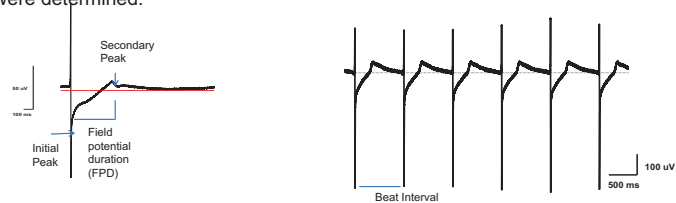
Cell Preparation:

iPS cell-derived cardiomyocytes were cultured onto 6-well multi-electrode arrays at a density of ~30x10³ cells per well and allowed to form spontaneously beating syncytial monolayers.



Electrophysiology:

Spontaneous field action potentials (sfAP) were recorded and digitized at 10 kHz. Experiments were conducted in an environmentally controlled chamber (Plas-Lab 850-LCS) with a 5% air:CO₂ mix at ~36 °C. Following an equilibration period of 15 mins, the test substances were added directly to the bath in a cumulative manner and FPD and beating rates were determined.

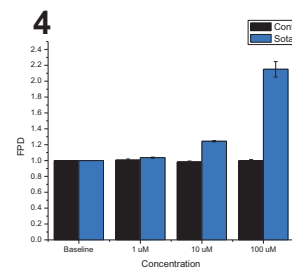
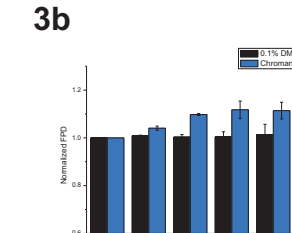
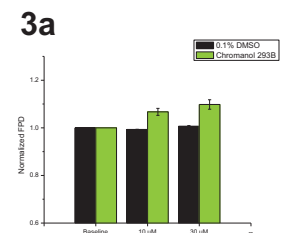
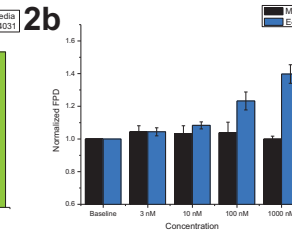
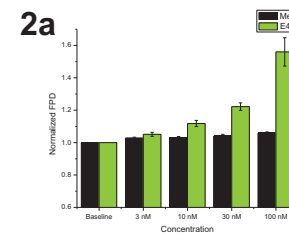
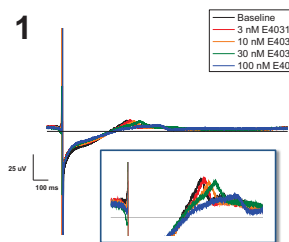


Signal Analysis:

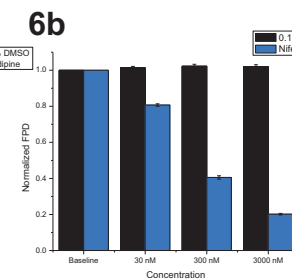
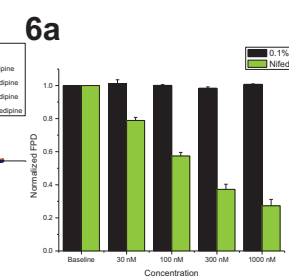
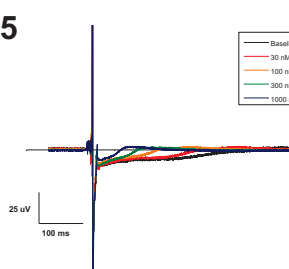
All data were analysed off-line using Spike2 (CED). Measurements were taken from a field potential trace averaged over the last minute of recording. Values were normalized to the last minute of the equilibration period.

Results

1. Effects on Field Potential



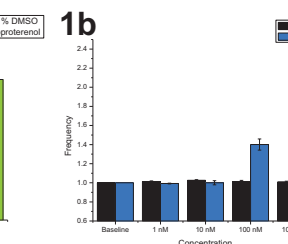
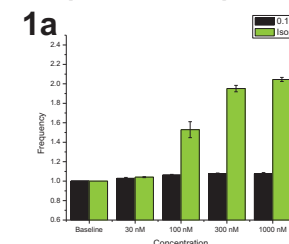
Potassium Channel Block: Potassium channels are responsible for the repolarization phase of an action potential and their block prolongs the FPD. 1) MEA field potential recorded Before and during application of the I_{Kr} blocker E4031. 2) Effects of E4031 on FPD as performed at CDI (2a) and Roche (2b). 3) Effects of I_{Ks} blocker Chromanol 293B on FPD as performed at CDI (3a) and Roche (3b). 4) Effects of I_{Kr} blocker Sotalolol. All three compounds produced significant prolongation of the field potential.



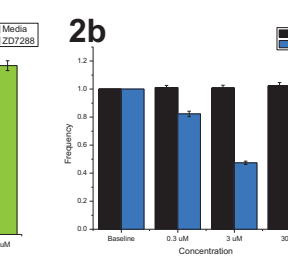
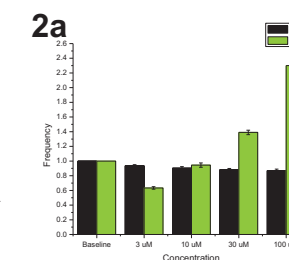
Field potential shortening via calcium block: Calcium channels responsible for the plateau phase of an action potential shorten FPD when blocked. 5) MEA measurements of FPD at varying concentrations of the L-type Calcium channel blocker, Nifedipine. 6) Measurements performed at CDI (6a) and Roche (6b).

1. Effects of selected compounds on spontaneous beat rate

Changes in frequency: 1) Rate increases changes via beta-adrenergic stimulation. Effect of Isoproterenol on spontaneous beating as performed at CDI (1a) and Roche (1b).



Changes in frequency: 2) Rate changes via I_f block. Effect of ZD7288 on spontaneous beating as performed at CDI (2a) and Roche (2b). Lower concentrations cause a slowing of beat rate. Non-specific channel block of Ca⁺⁺ can cause an increased beat rate at higher concentrations



Changes in frequency: Rate changes via Na⁺ block TTX at both sites completely blocked spontaneous field potentials at 3µM TTX.

Summary

Choosing an appropriate test system and analytical technique is crucial for accurate and meaningful assessments.

iCell Cardiomyocytes are human iPS cell-derived cardiomyocytes that demonstrate robust, physiologically meaningful, and appropriate electrophysiological responses across experiments and across test sites.

iCell Cardiomyocytes show;

- Significant prolongation in field potential duration.
 - I_{Kr} blocking effects at 10 to 30 nM E-4031 and 100 nM Sotalolol.
 - I_{Ks} blocking effects at 10 – 30 µM Chromanol 293B.
- Significant shortening of field potential duration.
 - I_{Ca} blocking effects at 30 to 100 nM Nifedipine.
- Significant changes in beat interval / frequency.
 - β-adrenergic stimulation with 100 nM isoproterenol increased beat rate.
 - I_f biphasic effects at 3 and 30 µM block by ZD7288.
 - Cessation of beating with 3 µM tetrodotoxin.

These results were repeated across two independent sites, thus verifying the sensitivity and robustness of the iCell Cardiomyocyte system.