Assessment of pharmacological responses to cardioactive compounds in human induced pluripotent stem cell-derived (hiPSC) cardiomyocytes using MEA and calcium transient analysis

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Abstract

Introduction

hiPSC-derived cardiomyocytes hold great potential for safety pharmacology testing. Therefore, they are currently evaluated within the CiPA (Comprehensive *in vitro* Proarrythmia Assay) consortium as novel models for cardiac safety assessment.

Objective

Here, we assessed the effects of a set of cardioactive compounds, including CiPA compounds that hold a low (mexiletine, nifedipine, and diltiazem) and high (dofetilide) risk for manifesting human TdP, on the electrophysiology and calcium handling of hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes). Pluricyte® Cardiomyocytes were cultured in serum-free Pluricyte® Cardiomyocyte Medium and compound-effects were assessed using the Axion Maestro multiwell 768-channel platform and the Hamamatsu FDSS®/µCell platform, to evaluate them.

Results

Both MEA and calcium transient analysis showed predictive and reproducible responses of Pluricyte® Cardiomyocytes to cardioactive compounds. As expected, dofetilide appeared to cause more dramatic effects (prolonged field potential durations and TdP-like arrhythmias) compared to the low risk CiPA compounds.

Conclusion

Our data support the use of Pluricyte® Cardiomyocytes as a relevant *in vitro* model for cardiac safety assessment at an early stage of drug development.

Electrophysiological characteristics of Pluricyte® Cardiomyocytes

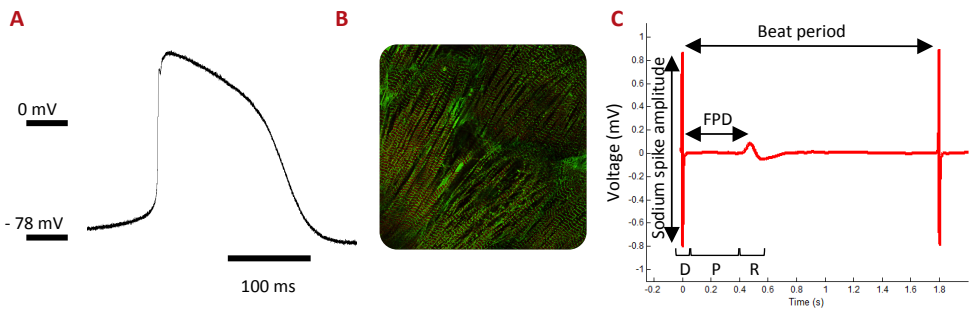

Figure 1.
A: Typical action potential of Pluricyte® Cardiomyocytes generated using the perforated patch-clamp technique, showing a low resting membrane potential, fast upstroke velocity and robust action potential amplitude.
B: immunofluorescence analysis (green: alpha actinin, red: MHCβ) showing a high degree of ultra-structural organization of the cells. **C:** Typical waveform of a Pluricyte® Cardiomyocyte monolayer acquired through MEA analysis using the Maestro multiwell 768-channel platform. The field potential shows a robust sodium spike amplitude and a well-pronounced repolarization peak.

Pharmacological responses of Pluricyte® Cardiomyocytes to CiPA reference compounds using the Maestro multiwell 768-channel platform

Example of a Na^+ channel blocker, **mexiletine** (a low-risk CiPA compound)

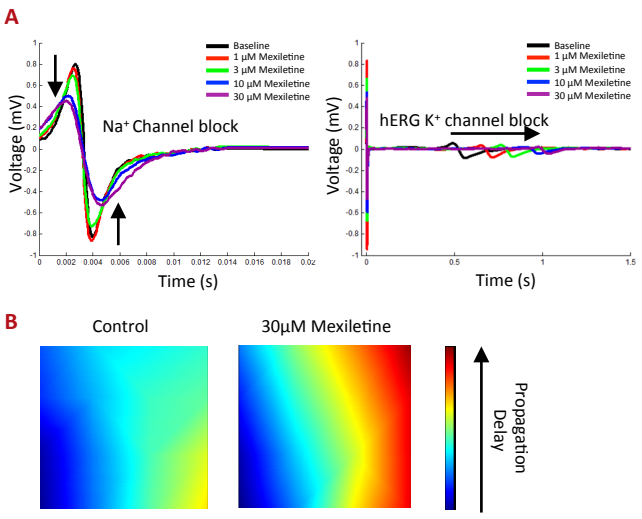

Figure 2.
A: Mexiletine blocks the rapid inward sodium current as well as the hERG K^+ channels, leading to a reduction in the sodium spike amplitude (**left panel**) and a prolongation of the field potential duration (**right panel**), respectively, as shown here in averaged waveforms.
B: Conduction velocity of a Pluricyte® Cardiomyocyte monolayer field potential. Blue shading marks the beat origin and red shading indicates a long beat propagation delay. Mexiletine reduced the maximum rate of depolarization and decreased the conduction velocity.
[Images were generated using the Axion Cardiac Data Plotting Tool, version 1.2.1 and Axion integrated studio, version 2.1.1.16]

Example of hERG channel blockers, **dofetilide** (a high-risk CiPA compound) and **E4031**

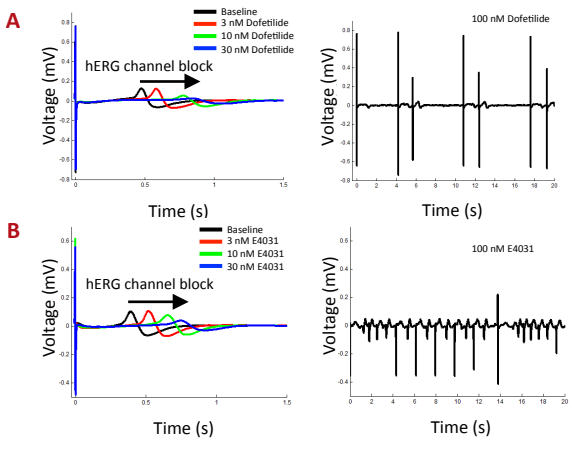

Figure 3.
hERG channel blockers dofetilide (**A**) and E4031 (**B**) induced both a concentration-dependent increase in the field potential duration and flattening of the repolarization peak of Pluricyte® Cardiomyocytes, as shown here in an overlay of averaged waveforms (**left panels**).
Despite flattening of the peak, Axis software could accurately detect the repolarization of Pluricyte® Cardiomyocytes.
TdP-like arrhythmias (**right panels**) were observed at high concentrations for both E4031 and dofetilide.
[Images were generated using the Axion Cardiac Data Plotting Tool, version 1.2.1]

Acknowledgments

We greatly acknowledge Jean Marc D'Angelo from Hamamatsu Photonics for his contribution to this study.
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 726513.

Example of L-type Ca^{2+} channel blockers, **nifedipine** and **diltiazem** (low-risk CiPA compounds)

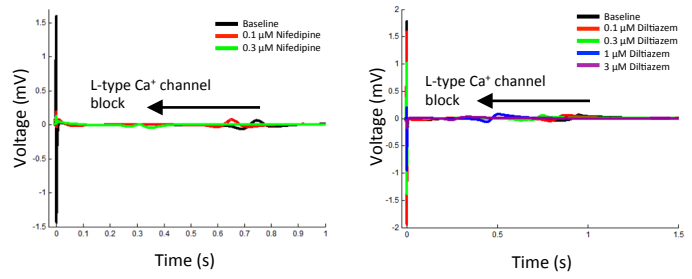

Figure 4.
Ca^{2+} channel blockers nifedipine (**left panel**) and diltiazem (**right panel**) reduce the field potential duration of the Pluricyte® Cardiomyocyte monolayer, as shown here in an overlay of averaged waveforms.
[Images were generated using the Axion Cardiac Data Plotting Tool, version 1.2.1]

Example of a β-adrenergic receptor agonist: **isoproterenol**

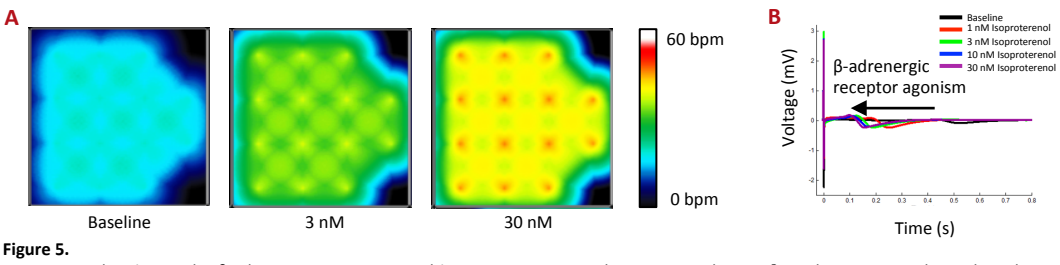

Figure 5.
Isoproterenol activates the β-adrenergic receptor, resulting in an increase in beat rate in Pluricyte® Cardiomyocytes, shown here by an increase in spike frequency in heat plots (**A**) depicting beat rate in beats per minute (bpm). In addition, isoproterenol shortens the field potential duration of Pluricyte® Cardiomyocytes, as shown in an overlay of averaged waveforms (**B**).
[Images were generated using the Axion Cardiac Data Plotting Tool, version 1.2.1 and Axion integrated studio, version 2.1.1.16]

Overview of *in vitro* effects of cardioactive compounds on Pluricyte® Cardiomyocyte electrophysiology

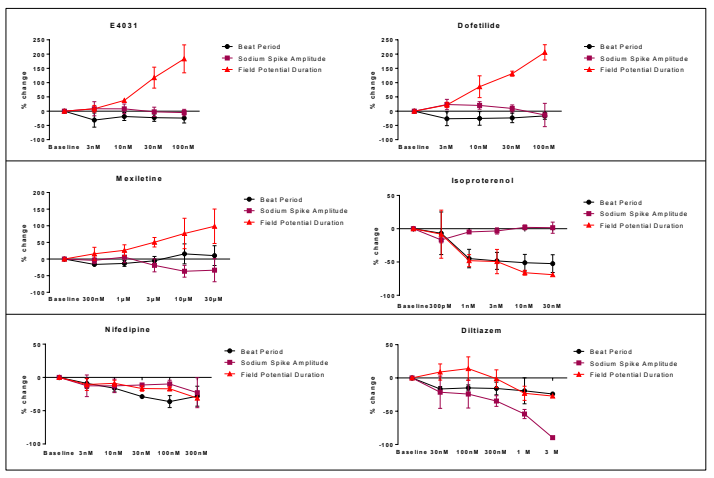

Figure 6.
Overview of the different cardioactive compounds and their effects on beat period (displays the time period between two successive sodium spikes), sodium spike amplitude, and field potential duration (time between the detected repolarization peak and the preceding sodium spike, Figure 1C) of Pluricyte® Cardiomyocytes. Data were generated using the Maestro multiwell 768-channel platform. Data are expressed as percentage change when compared to the baseline.
Mean±SD, N= 3 wells for each condition.

Screening of compound-induced alterations of calcium transients in Pluricyte® Cardiomyocytes measured using the FDSS®/µCell platform

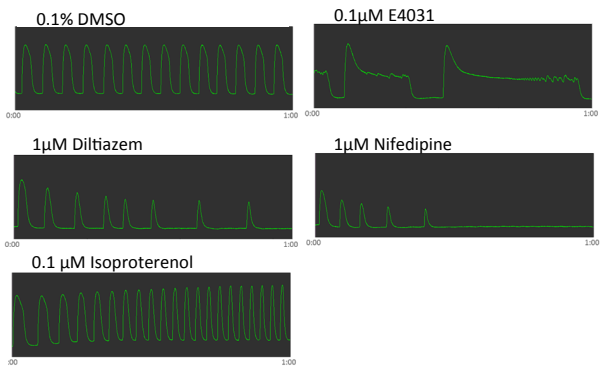

Figure 7.
Acute compound-induced alterations of calcium transients in Pluricyte® Cardiomyocytes were assessed using the FDSS®/µCell platform. Blocking of the hERG channel by 0.1 µM **E4031** resulted in TdP-like events (**top right panel**). By blocking L-type Ca^{2+} channels, CiPA reference compounds **diltiazem** and **nifedipine** reduced the calcium transient peak amplitudes, eventually leading to complete diminishing of the signal (**middle panels**). The β-adrenergic receptor agonist, **isoproterenol**, caused increased peak frequency, decreased peak width and increased peak amplitude (**lower panel**).

Concluding Remarks

Compound class	Compound	Calcium transients FDSS®/µCell platform	Field potential Maestro multiwell 768-channel platform
hERG channel block	E4031	Incidence of arrhythmias	Increased FPD, incidence of arrhythmias
	Dofetilide	Not tested	Increased FPD, incidence of arrhythmias
β-receptor agonism	Isoproterenol	Increased peak frequency, Increased peak amplitude, Decreased peak width	Increased firing rate, Decreased FPD
L-type calcium channel block	Diltiazem Nifedipine	Decreased peak amplitude	Decreased FPD

- Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium show a ventricular, relatively mature phenotype and organized sarcomere structures.
- MEA analysis of Pluricyte® Cardiomyocytes using the Maestro multiwell 768-channel platform shows monolayer field potentials with well-pronounced de- and repolarization peaks for easy detection and analysis of the field potential duration.
- Calcium transient analysis in Pluricyte® Cardiomyocytes using the FDSS®/µCell system provides a robust medium- to high-throughput screening method for cardiac safety.
- Pluricyte® Cardiomyocyte-based MEA and calcium transient assays show both predictive and reproducible responses to cardioactive compounds, including CiPA compounds holding low and high risks for manifesting human TdP.
- As expected, the high risk CiPA compound, dofetilide, appeared to cause more dramatic effects (prolonged field potential durations and even TdP-like arrhythmias) than the low risk compounds at the tested concentrations.
- Our data support the use of hiPSC-derived cardiomyocytes to predict cardiac safety of pharmaceuticals in humans at an early stage of development, in line with the new regulatory approach embodied by the CiPA initiative.