Ncardia
Stem cell experts

Assessment of pro-arrhythmic effects in Pluricyte® Cardiomyocytes

using the Axion BioSystems Maestro™ MEA system

Application note
Version 2.0

Contents

1. Introduction

Pluricyte® Cardiomyocytes are highly suitable for Axion Maestro MEA assays

Pluricyte® Cardiomyocytes are fully functional human induced pluripotent stem cell (hiPSC) derived ventricular cardiomyocytes that are particularly suitable for electrophysiology-based multi-electrode array (MEA) assays for predictive safety pharmacology, toxicity testing and efficacy screening in early drug discovery. The combination of Pluricyte® Cardiomyocytes and the Axion Maestro MEA system enables detailed electrophysiological detection of potential cardiotoxic/proarrhythmic effects of test compounds at 48- and 96-well plate formats. Pluricyte® Cardiomyocytes' well-pronounced depolarization and repolarization peaks permits easy detection of electrophysiological parameters (e.g. depolarization/repolarization peak amplitudes, beat rate, field potential duration) and facilitate efficient data analysis and interpretation of studies performed with the Axion Maestro MEA system.

Pluricyte® Cardiomyocytes strengths and characteristics

Pluricyte® Cardiomyocytes exhibit a relatively high level of maturity, when compared to other human stem cell-derived cardiomyocytes and present the following unique characteristics:

- High purity of ventricular cardiomyocytes
- Low resting membrane potentials (~-78 mV)
- Fast upstroke velocities and action potential amplitudes
- Organized sarcomeric structures
- Monolayer field potential contains well-pronounced depolarization and repolarization peaks, enabling easy detection of field potential durations in MEA assays

This application note describes the assessment of the effects of a set of pro-arrhythmic compounds in Pluricyte® Cardiomyocytes, showing the expected pharmacological responses. Pluricyte® Cardiomyocytes, cultured in Pluricyte® Cardiomyocyte Medium, in combination with the Axion Maestro system provide a highly relevant in vitro assay platform to study the cardiac safety profile of compounds during drug development.

For more data and information on how to use Pluricyte® Cardiomyocytes in combination with the Axion Maestro MEA system, please refer to our **User Guide**.

Technical support

Our scientists are ready to help you with any questions you may have regarding this application note or our Pluricyte® Cardiomyocytes. In addition, in-lab training is available upon request. For further information please visit our website **ncardia.com**, or contact us directly by **e-mail**.

2. Assessment of pro-arrhythmic effects using Pluricyte® Cardiomyocytes on the Axion Maestro MEA System

Pluricyte® Cardiomyocytes Field Potential measured using the Axion Maestro MEA system

The Axion Maestro MEA system records the extracellular field potential of cardiomyocytes in real-time using proprietary microelectrode array (MEA) technology. The majority of cardiac side effects, like torsade de pointe (TdP) and ventricular fibrillation, are associated with drugs interfering with the function of ion channels such as the human Ether-à-go-go-Related Gene (hERG) K^+-channels, the Nav1.5 Na^+-channels or the Ca^{2+}-channels. Through the MEA measurements, the Axion Maestro MEA system captures changes in the extracellular field potential of Pluricyte® Cardiomyocytes, generated by the electrophysiological processes across the cell membrane. Drugs affecting different ion channels can consequently be studied using the MEA measurement. Figure 2.1 depicts a typical waveform of the extracellular field potential signal of Pluricyte® Cardiomyocytes obtained using the Axion Maestro MEA system. Indicated are the depolarization phase, characterized by the robust sodium spike, during which an influx of sodium occurs (I_{Na}), the plateau phase, during which an influx of Calcium ($I_{Ca\text{-}L}$) occurs, and the repolarization phase, during which an efflux of potassium occurs (I_{Kr}/I_{Ks}), characterized by the clear repolarization peak. Parameter measurements that can be analyzed using the AxIS software may include the sodium spike amplitude, the beat period (time period between two successive sodium spikes, a parameter from which the beat rate can be derived as follows: “beat rate (BPM) =” 60/(beat period (s))) and the field potential duration (the time period between the depolarization and repolarization peaks), as depicted in Figure 2.1.

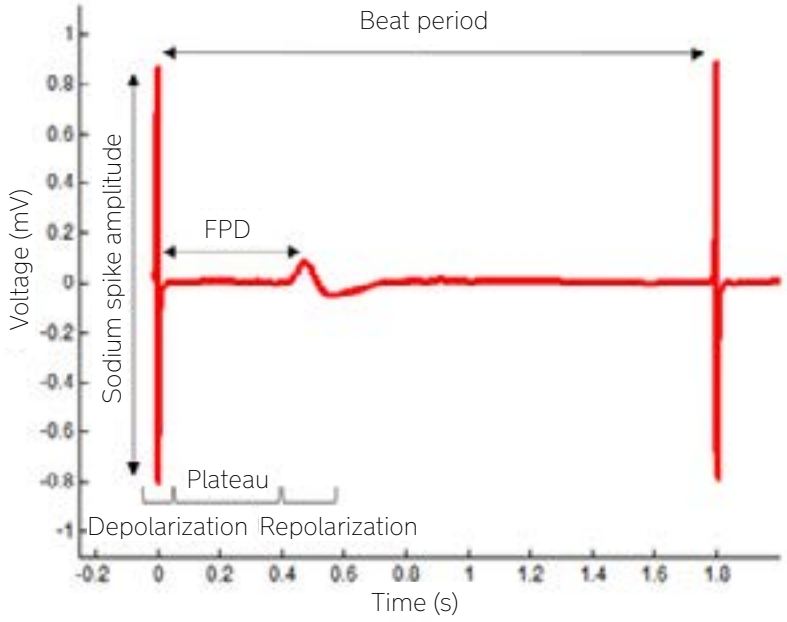

Figure 2.1. Typical waveform of the extracellular field potential signal of Pluricyte® Cardiomyocytes obtained using the Axion Maestro MEA system. The sodium spike amplitude, field potential duration (FPD), the beat period (time period between two successive sodium spikes), the depolarization and repolarization phases are indicated. [Image was generated using the Axion Cardiac Data Plotting Tool, version 1.2.1].

2.1 Experimental design to study acute drug effects

To assess the effects of a set of pro-arrhythmic compounds, Pluricyte® Cardiomyocytes were cultured on Maestro Classic MEA 48 plate in Pluricyte® Cardiomyocyte Medium for 9 days. The set of pro-arrhythmic drugs (Table 2.1) was dissolved in DMSO at a concentration of 10mM and then diluted in Pluricyte® Cardiomyocyte Medium in 10-fold serial dilutions. The Pluricyte® Cardiomyocytes were then treated with this set of pre-diluted pro-arrhythmic drugs in a cumulative dose response experiment (Figure 2.2). Acute-drug effects were directly measured using the Axion Maestro MEA system. Compound concentrations were increased by 3-fold (Figure 2.2.1) for each separate recording step (Figure 2.2.2). The data were analyzed using Axion Integrated Studio (version 2.1.1.16) to determine the compound effects on beat period, field potential duration, and spike amplitude.

Drug class	Drug	Expected effects on hiPSC-cardiomyocytes electrophysiology
hERG channel blocker (I_{Kr})	E4031	Delays repolarization phase by blocking the hERG channel, resulting in prolonged FPD and ultimately arrhythmias
hERG channel blocker (I_{Kr})	Dofetilide	Delays repolarization phase by blocking the hERG channel resulting in prolonged FPD and ultimately arrhythmias
Sodium channel blocker (I_{Na})	Mexiletine	Reduces sodium spike amplitude by blocking sodium channels. Higher concentrations also block potassium channels resulting in an increased FPD
Sodium channel blocker (I_{Na})	Flecainide	Reduces sodium spike amplitude by blocking sodium channels. Higher concentrations also block potassium channels resulting in an increased FPD
Calcium channel blocker ($I_{Ca,L}$)	Nifedipine	Decreases FPD by blocking Ca^{2+} channels
Calcium channel blocker ($I_{Ca,L}$)	Diltiazem	Decreases FPD by blocking Ca^{2+} channels
β-adrenergic receptor agonist	Isoproterenol	Increases beat rate by activating β-adrenergic receptors resulting in decreased FPD

Table 2.1. List of pro-arrhythmic drugs and their expected effects on hiPSC-derived cardiomyocytes.

Figure 2.2. Cumulative Dose-Response Experiment.

Compounds	t = 0	t = 30 min	t = 60 min	t = 90 min	t = 120 min
E4031	3nM	10nM	30nM	100nM	--
Dofetilide	3nM	10nM	30nM	100nM	--
Mexiletine	300nM	1µM	3µM	10µM	30µM
Flecainide	300nM	1µM	3µM	10µM	30µM
Nifedipine	3nM	10nM	30nM	100nM	300nM
Diltiazem	30nM	100nM	300nM	1µM	3µM
Isoproterenol	300pM	1nM	3nM	10nM	30nM

Figure 2.2.1. Compound concentrations tested

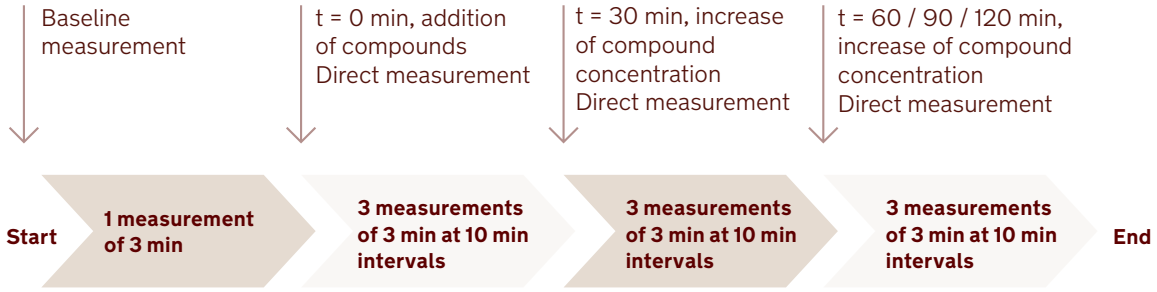

Figure 2.2.2. Measurement Scheme

2.2 Results

Calcium channel blockers affect the plateau phase between the depolarization and repolarization phase, resulting in a shortening of the field potential duration.[1] As shown in Figure 2.3, L-type calcium channel blockers nifedipine and diltiazem shorten the field potential duration of Pluricyte® Cardiomyocytes in a concentration-dependent manner.

hERG potassium channel blockers block the rapid component of the delayed rectifier outward potassium current (I_{Kr}), thereby delaying the repolarization phase. This results in an increase in field potential duration and flattening of the repolarization peak. At higher concentrations, blocking of the hERG channel may lead to TdP-like arrhythmias.[2] Figure 2.4 shows this prolongation of the field potential duration (FPD) of Pluricyte® Cardiomyocytes induced by hERG potassium channel blockers E4031 and dofetilide. Furthermore, TdP-like arrhythmias were frequently observed at high concentrations of E4031 and dofetilide.

Sodium channel blockers affect the depolarization phase of the field potential by blocking sodium channels (INa), resulting in a decrease in sodium spike amplitude.[6] Figure 2.5 shows that sodium channel blockers mexiletine and flecainide indeed decrease the sodium spike amplitude in Pluricyte® Cardiomyocytes in a concentration-dependent manner. Both mexiletine and flecainide also block hERG (I_{Kr}) potassium channels,[3] shown here by an increase in field potential duration.

Isoproterenol is a **β-adrenergic receptor agonist**; activation of this receptor results in an increased beat rate (decreased beat period) and consequently a reduction in field potential duration.[4] Figure 2.6 shows that isoproterenol has a concentration-dependent effect on the beat rate of Pluricyte® Cardiomyocytes, as well as on the absolute field potential duration.

Figure 2.7 provides an overview of the different cardioactive compounds and their effects on beat period, field potential duration and sodium spike amplitude.

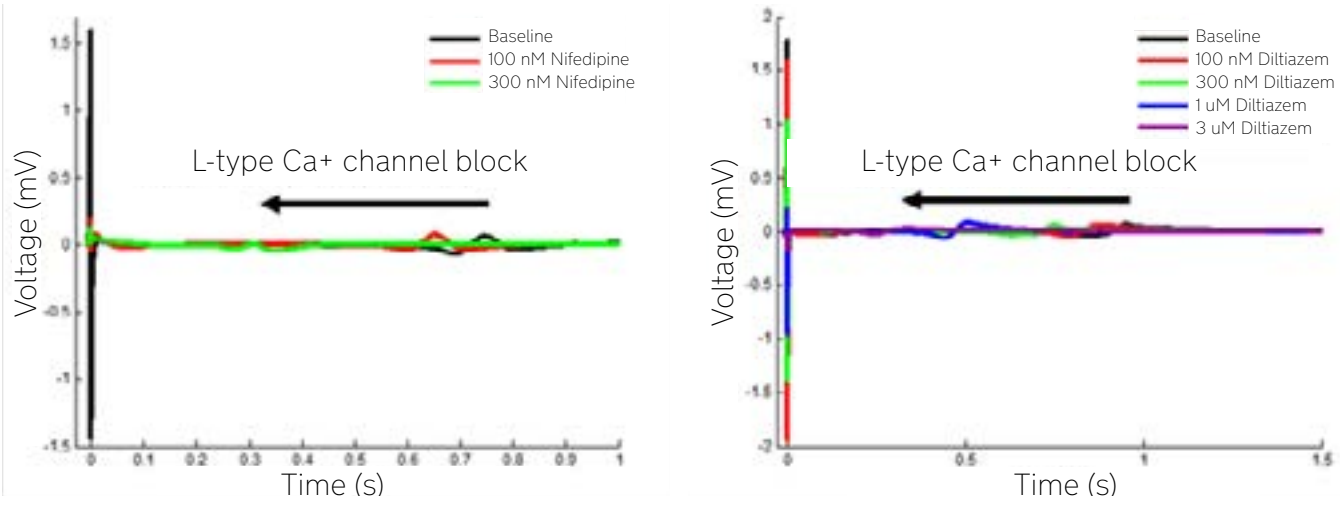

Figure 2.3. Ca^{2+} channel blockers. Calcium channel blockers nifedipine (left panel) and diltiazem (right panel) reduce the field potential duration of Pluricyte® Cardiomyocytes, as shown here in an overlay of averaged waveforms.
[Images were generated using the Axion Cardiac Data Plotting Tool, version 1.2.1]

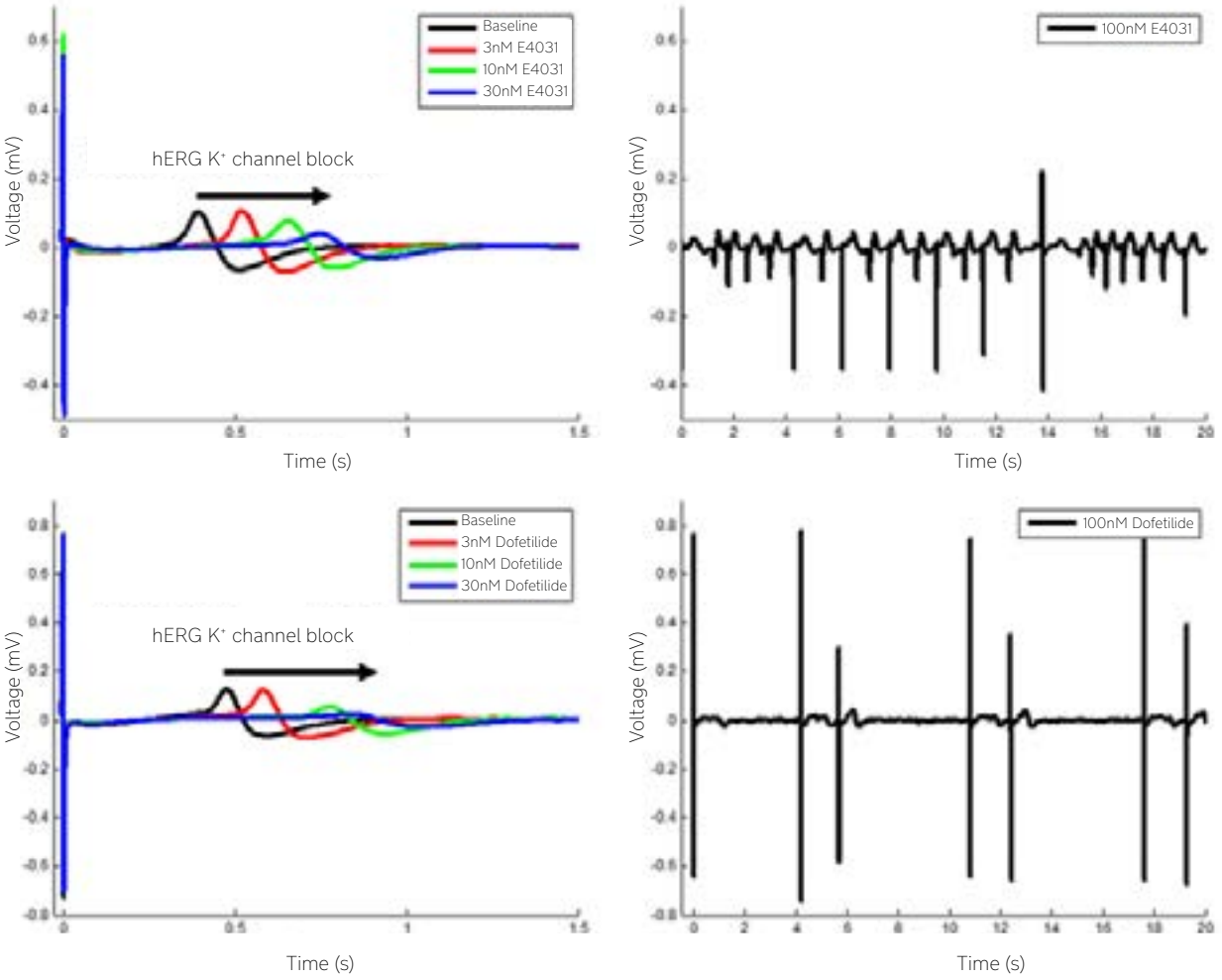

Figure 2.4. hERG channel blockers.hERG potassium channel blockers E4031 (top panel) and dofetilide (bottom panel) increase the field potential duration and cause flattening of the repolarization peak of Pluricyte® Cardiomyocytes, as shown here in an overlay of averaged waveforms. Despite flattening of the peak, Axis software could accurately detect the repolarization of Pluricyte® Cardiomyocytes even in the presence of high concentration hERG channel blockers. TdP-like arrhythmias were observed at high concentrations for both E4031 and dofetilide. [Images were generated using the Axion Cardiac Data Plotting Tool, version 1.2.1]

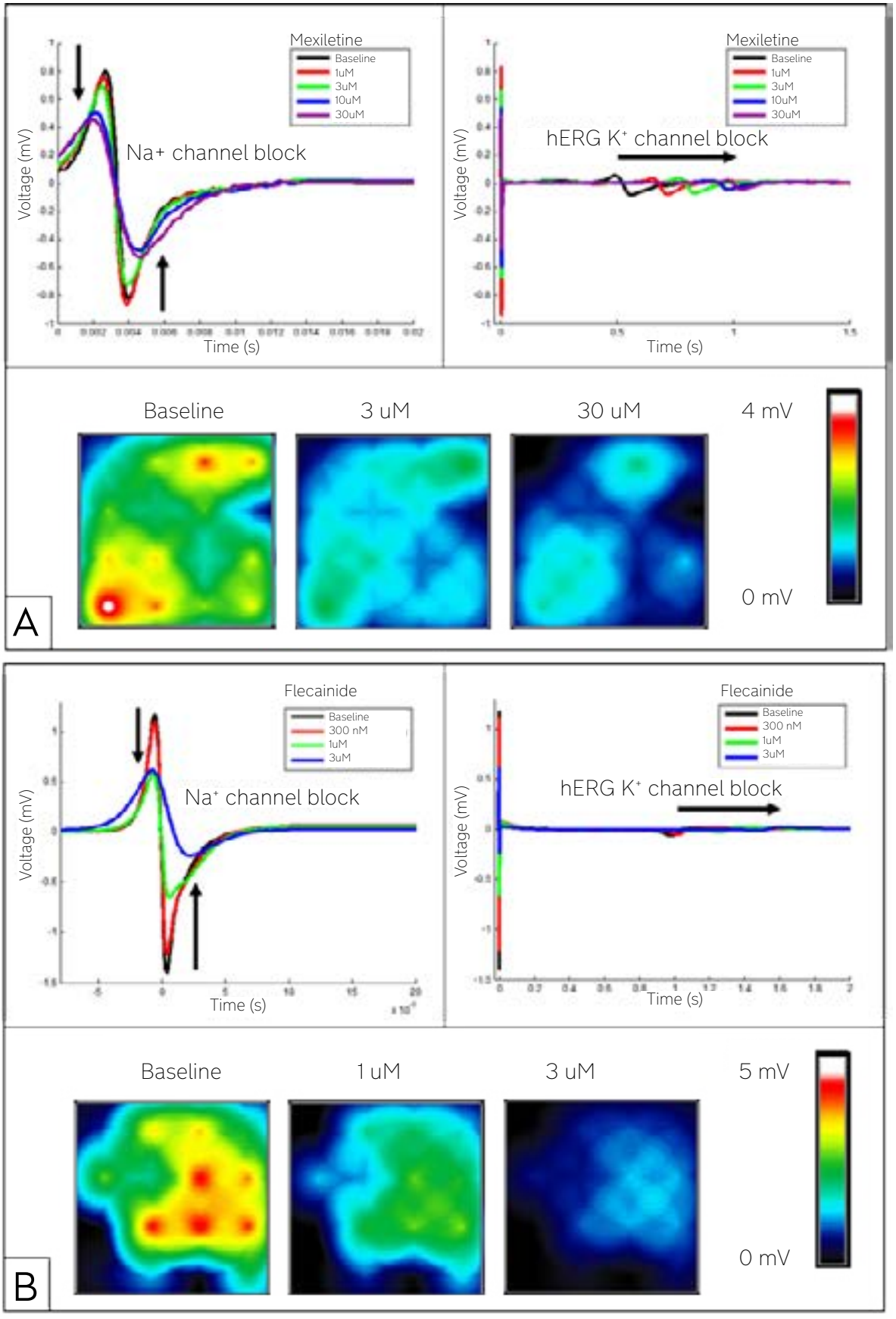

Figure 2.5. Na^+-channel blockers mexiletine and flecainide. By blocking the Nav1.5 Na^+ -channels, mexiletine (A) and flecainide (B) reduce the amplitude of the sodium spike of Pluricyte® Cardiomyocytes, shown here in an overlay of the sodium spike (top left panels), and in heat plots depicting the sodium spike amplitude (bottom panels). Both mexiletine and flecainide also block hERG K^+-channels resulting in a prolongation of the field potential duration and flattening of the repolarization peak (top right panels). Top panels show overlays of 10 averaged waveforms for each condition. [Images were generated using the Axion Cardiac Data Plotting Tool, version 1.2.1 and Axion Integrated Studio, version 2.1.1.16]

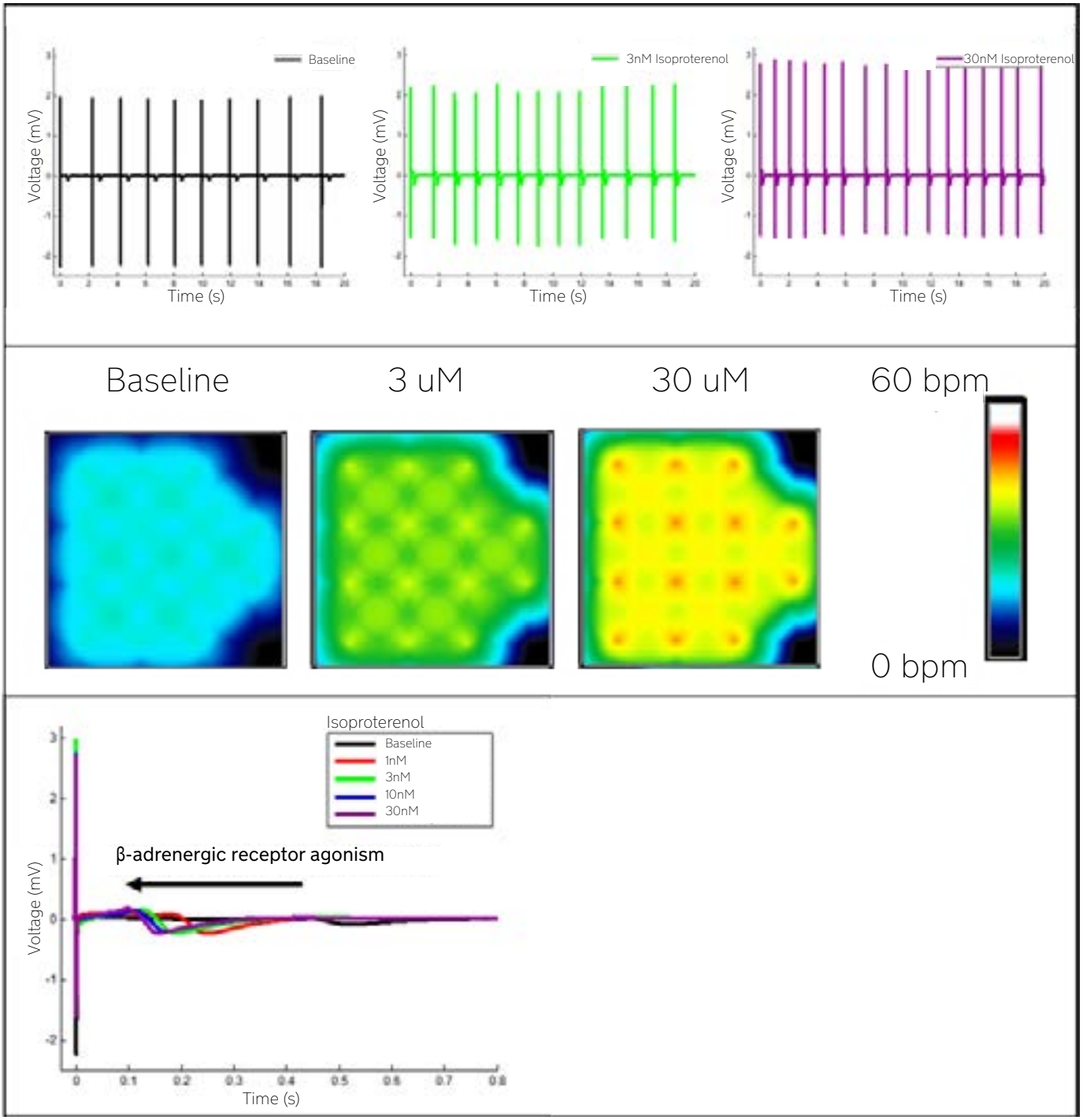

Figure 2.6. β-adrenergic receptor agonist.Isoproterenol activates the β-adrenergic receptor, resulting in an increase in beat rate in Pluricyte® Cardiomyocytes, shown here by an increase in spike frequency in a 20 seconds measurement (top panel), and in heat plots depicting beat rate in beats per minute (bpm) (the reciprocal of the beat period, middle panel). In addition, isoproterenol shortens the field potential duration of Pluricyte® Cardiomyocytes, as shown in an overlay of averaged waveforms (bottom panel). [Images were generated using the Axion Cardiac Data Plotting Tool, version 1.2.1 and Axion integrated studio, version 2.1.1.16]

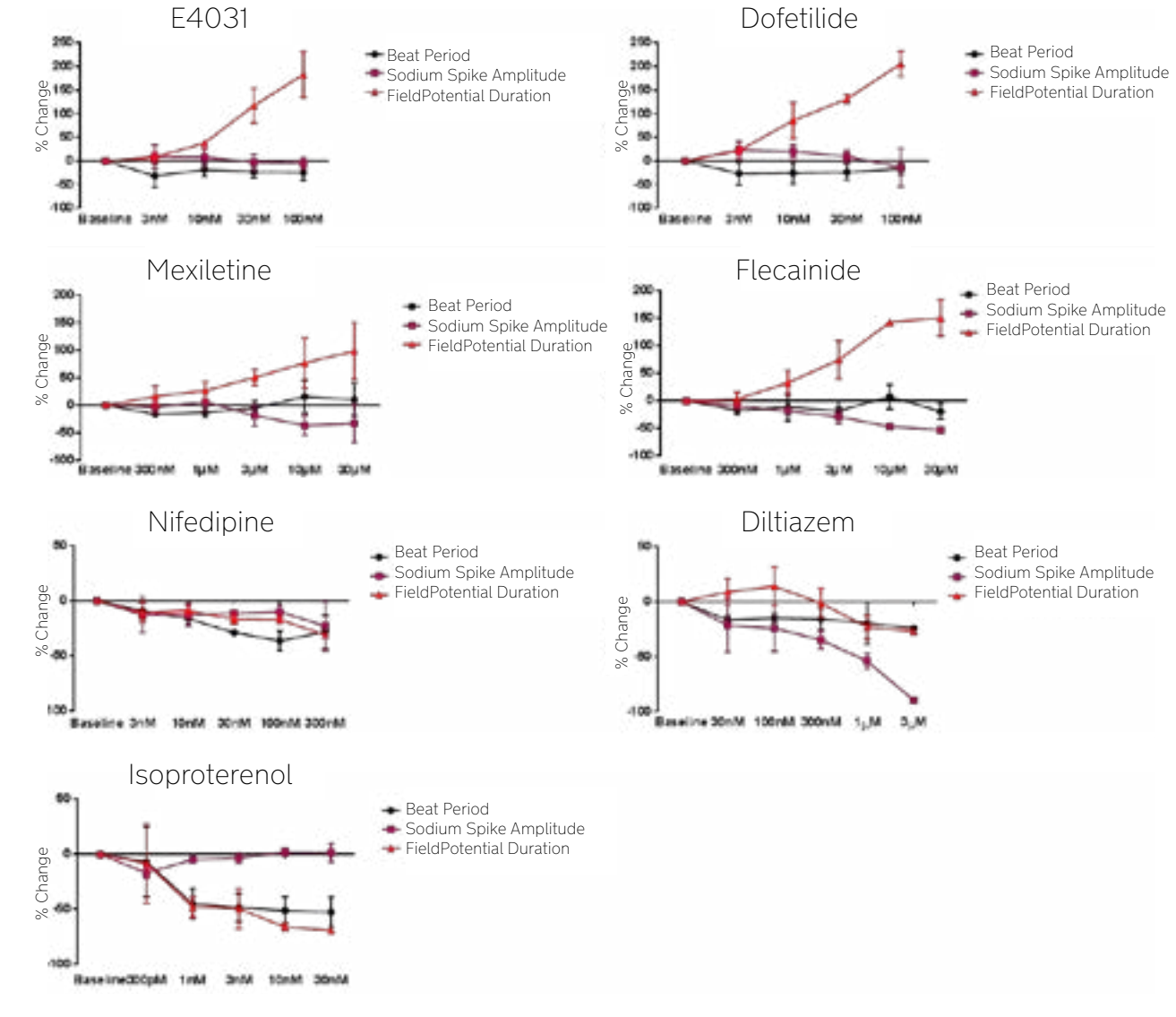

Figure 2.7. Overview of effects of cardioactive compounds on Pluricyte® Cardiomyocytes. Overview of the different cardioactive compounds and their effects on beat period (displays the time period between two successive sodium spikes), spike amplitude, and field potential duration of Pluricyte® Cardiomyocytes (time between the detected repolarization peak and the preceding sodium spike, Figure 5.1). Data are expressed as percentage of change when compared to the baseline. Mean ± SD, N= 3 wells for each condition. [Graphs were generated using GraphPad Prism version 6.07]

2.3 Concluding Remarks

In this case study, we assessed the effects of a set of pro-arrhythmic compounds on Pluricyte® Cardiomyocytes electrophysiology by MEA measurements using the Axion Maestro MEA system (Axion Biosystems, Atlanta, GA, USA). Pluricyte® Cardiomyocytes are exceptionally well-suited for implementation in safety pharmacology screening assays due to their unique strengths and characteristics (Introduction). Pluricyte® Cardiomyocytes, cultured in Pluricyte® Cardiomyocyte Medium, showed expected pharmacological responses in a reproducible manner, which could be readily detected with the Axion Maestro MEA system. The combination of Pluricyte® Cardiomyocytes with the Axion Maestro MEA platform provides a highly relevant in vitro assay to study the cardiac safety profile of compounds at an early stage of drug development.

3. References

1. Ma, J. et al. High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents. Am. J. Physiol. Heart Circ. Physiol. 301, H2006–17 (2011).
2. Sanguinetti, M. C. & Tristani-firouzi, M. hERG potassium channels and cardiac arrhythmia. Nature 440, 463–469 (2006).
3. Paul, A. A., Witchel, H. J. & Hancox, J. C. Inhibition of the current of heterologously expressed HERG potassium channels by Flecainide and comparison with quinidine, propafenone and lignocaine. Br. J. Pharmacol. 136, 717–29 (2002).
4. Matsa, E. et al. Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation. Eur. Heart J. 32, 952–62 (2011).
5. Kovács, M., Tóth, J., Hetényi, C., Málnási-Csizmadia, A. & Sellers, J. R. Mechanism of blebbistatin inhibition of myosin II. J. Biol. Chem. 279, 35557–63 (2004).
6. Clements, M. & Thomas, N. High-Throughput Multi-Parameter Profiling of Electrophysiological Drug Effects in Human Embryonic Stem Cell Derived Cardiomyocytes Using Multi-Electrode Arrays. Toxicol. Sci. 140, 445–461 (2014).

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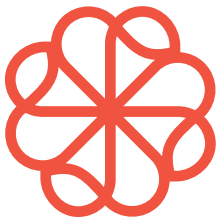

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