Assessment of pro-arrhythmic effects using Pluricyte® Cardiomyocytes on the ACEA xCELLigence® RTCA CardioECR
Contents

1. Introduction 1

2. Assessment of pro-arrhythmic effects using Pluricyte® Cardiomyocytes on the ACEA xCELLigence® RTCA CardioECR system 2
   2.1 Experimental design to study acute-drug effects 3
   2.2 Results 4
   2.3 Concluding Remarks 5

3. References 11
1. Introduction

Pluricyte® Cardiomyocytes are highly suitable for ACEA xCELLigence® RTCA CardioECR 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 xCELLigence® RTCA CardioECR system enables detailed electrophysiological detection of potential cardiotoxic/pro-arrhythmic effects of test compounds in a 48-well plate format. The well-pronounced depolarization and repolarization peaks of Pluricyte® Cardiomyocyte monolayer field potential signals allow an 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 xCELLigence® RTCA CardioECR 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 has 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 xCELLigence® RTCA CardioECR 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 xCELLigence® RTCA CardioECR system, please refer to our User Guide at ncardia

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 www.ncardia.com, or contact us directly by e-mail (sci-support@ncardia.com).
2. Assessment of pro-arrhythmic effects using Pluricyte® Cardiomyocytes on the ACEA xCELLigence® RTCA CardioECR system

The xCELLigence® RTCA CardioECR instrument records cellular impedance (presented as Cell Index (CI)) and electrical field potential (presented as extracellular recording (ECR)) of cardiomyocytes in parallel, using proprietary microelectrode array (MEA) technology. Through the ECR measurement, the xCELLigence® RTCA CardioECR instrument captures changes in the extracellular field potential, generated by the electrophysiological processes across the cell membrane. Drugs affecting different ion channels affect different phases of the extracellular field potential which can consequently be studied using the ECR measurement. In addition, the xCELLigence® RTCA CardioECR instrument records the impedance of the cells by sending a small electrical current through the cardiomyocyte monolayer. The CI measurement thereby quantifies the relative contractile state of the cell monolayer. Drugs affecting the contractile machinery of the cardiomyocyte can therefore be studied using the CI measurement. Figure 2.1 depicts a single waveform of the Cell Index and the extracellular field potential signal (ECR) of a Pluricyte® Cardiomyocyte monolayer obtained using the xCELLigence® RTCA CardioECR instrument. Indicated are the Cell Index amplitude, a measure related to the contraction; the depolarization phase, during which an influx of sodium into the cells occurs ($I_{Na}$), characterized by the sodium spike; the plateau phase, with an influx of Calcium ($I_{Ca,L}$); the repolarization phase, during which an efflux of potassium occurs ($I_{Kr}/I_{Ks}$), characterized by the clear repolarization peak; and the field potential duration, the time period between the depolarization and repolarization phase.

![Figure 2.1](image-url)
2.1 Experimental design to study acute-drug effects

Pluricyte® Cardiomyocytes were cultured on 48-well E-plates® in Pluricyte® Cardiomyocyte Medium for 10 days. A set of pro-arrhythmic drugs (Table 2.1) was dissolved in DMSO at a concentration of 10 mM 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 xCELLigence® RTCA CardioECR instrument. Compound concentrations were increased by 3-fold (Figure 2.2a) for each separate recording step (Figure 2.2b). The data were analyzed using the xCELLigence® RTCA CardioECR Software to determine the compound effects on beat rate, field potential duration, sodium spike amplitude, and Cell Index.

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Drug</th>
<th>Expected effects on hiPSC-derived cardiomyocytes’ electrophysiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>hERG channel blockers (I_{Kr})</td>
<td>E4031, Dofetilide</td>
<td>Delay of the repolarization phase by blocking the hERG channel, resulting in prolonged FPD and ultimately arrhythmias</td>
</tr>
<tr>
<td>Sodium channel blockers (I_{Na})</td>
<td>Flecainide, Mexiletine</td>
<td>Decrease of the sodium spike amplitude by blocking sodium channels. Higher concentrations may also block potassium channels resulting in an increased FPD</td>
</tr>
<tr>
<td>Calcium channel blockers (I_{Ca,L})</td>
<td>Nifedipine, Diltiazem</td>
<td>Decrease of the FPD and CI amplitude by blocking calcium channels</td>
</tr>
<tr>
<td>β-adrenergic receptor agonist</td>
<td>Isoproterenol</td>
<td>Increased beat rate due to activation of β-adrenergic receptors, resulting in decreased FPD</td>
</tr>
<tr>
<td>Myosin II inhibitor</td>
<td>Blebbistatin</td>
<td>Inhibition of cardiomyocyte contraction, resulting in diminished CI amplitude. No effects on the field potential signal (ECR)</td>
</tr>
</tbody>
</table>

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

### A

<table>
<thead>
<tr>
<th>Compounds</th>
<th>t=0</th>
<th>t=30 min</th>
<th>t=60 min</th>
<th>t=90 min</th>
<th>t=120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>E4031</td>
<td>3 nM</td>
<td>10 nM</td>
<td>30 nM</td>
<td>100 nM</td>
<td>--</td>
</tr>
<tr>
<td>Dofetilide</td>
<td>3 nM</td>
<td>10 nM</td>
<td>30 nM</td>
<td>100 nM</td>
<td>--</td>
</tr>
<tr>
<td>Flecainide</td>
<td>300 nM</td>
<td>1 µM</td>
<td>3 µM</td>
<td>10 µM</td>
<td>--</td>
</tr>
<tr>
<td>Mexiletine</td>
<td>300 nM</td>
<td>1 µM</td>
<td>3 µM</td>
<td>10 µM</td>
<td>30 µM</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>3 nM</td>
<td>10 nM</td>
<td>30 nM</td>
<td>100 nM</td>
<td>300 nM</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>30 nM</td>
<td>100 nM</td>
<td>300 nM</td>
<td>1 µM</td>
<td>3 µM</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>3 nM</td>
<td>10 nM</td>
<td>30 nM</td>
<td>100 nM</td>
<td>300 nM</td>
</tr>
<tr>
<td>Blebbistatin</td>
<td>300 nM</td>
<td>1 µM</td>
<td>3 µM</td>
<td>10 µM</td>
<td>30 µM</td>
</tr>
</tbody>
</table>

### B

- Baseline measurement
- t = 0 min, addition of compounds
- Direct measurement
- t = 30 min, increase of compound concentration
- Direct measurement
- t = 60 / 90 / 120 min, increase of compound concentration
- Direct measurement

Figure 2.2 Cumulative Dose-Response Experiment. A: An overview of the compounds that were tested on Pluricyte® Cardiomyocytes in the given increasing concentrations. B: The experimental procedure for this study.
2.2 Results

**hERG potassium channel blockers** block the rapid component of the delayed rectifier outward potassium current ($I_{Kr}$), thereby delaying the repolarization phase, resulting in an increase in field potential duration and flattening of the repolarization peak. Additionally, literature shows that, at higher concentrations, blocking of the hERG channel leads to Torsade de Pointes (TdP)-like arrhythmias\(^1\,^2\). Similar findings are observed in Pluricyte® Cardiomyocytes. Figure 2.3 shows prolongation of the field potential duration (FPD) and flattening of the repolarization peak in Pluricyte® Cardiomyocytes, induced by the hERG potassium channel blockers E4031 and dofetilide. Furthermore, arrhythmias were observed occasionally at 30 nM E4031 and 30 nM dofetilide, and in all wells from 100 nM E4031 and 100 nM dofetilide.

**Sodium channel blockers** affect the depolarization phase of the field potential by blocking $I_{Na}$ channels, resulting in a decrease in sodium spike amplitude\(^1\). Figure 2.4 shows that sodium channel blockers flecainide and mexiletine indeed decrease the sodium spike amplitude in Pluricyte® Cardiomyocytes in a concentration-dependent manner. In addition, flecainide and mexiletine are known to block hERG ($I_{Kr}$) potassium channels\(^3\,^4\) which is also observed in Pluricyte® Cardiomyocytes by an increase in field potential duration.

**Calcium channel blockers** affect the resting phase between the depolarization and repolarization phase, resulting in a shortening of the field potential duration\(^1\). As shown in Figure 2.5 the L-type calcium channel blockers nifedipine and diltiazem indeed both reduce the field potential duration of Pluricyte® Cardiomyocytes in a concentration-dependent manner. In addition, by blocking calcium channels, nifedipine and diltiazem also affect Pluricyte® Cardiomyocyte contraction, shown by decreased Cell Index amplitudes (Figure 2.5).

**Drugs which do not affect ion channels directly** but affect cardiomyocyte contraction through other mechanisms, such as isoproterenol (a $\beta$-adrenergic agonist) and blebbistatin (an inhibitor of myosin II), may still cause changes in the field potential signal and/or the contractility of the cells, which can be observed using the ECR or CI measurement, respectively. Isoproterenol activates $\beta$-adrenergic receptors, resulting in increased beat rate and consequently a reduction in field potential duration\(^5\). Figure 2.6 shows that isoproterenol indeed had a concentration-dependent effect on the firing rate of Pluricyte® Cardiomyocytes, as well as on the absolute field potential duration.

As explained above, the impedance measurement can be used to assess the contractility of cells. Blebbistatin is an inhibitor of myosin II, resulting in impairment of contraction\(^6\), which is expected to be identified by a decrease in CI amplitude. As the compound does not interfere with ion channel function, the field potential is not expected to be affected. In this case study, this is clearly shown by the fact that increasing concentrations of blebbistatin lead to significant reductions of the CI amplitudes in Pluricyte® Cardiomyocytes, while the electrical field potential (ECR) remains unaffected (Figure 2.7).

Figure 2.8 provides an overview of the cardio-active compounds investigated in this study and their effects on beat rate, field potential duration and sodium spike amplitude (expressed in percentage of change compared to the baseline).
2.3 Concluding Remarks

Pluricyte® Cardiomyocytes are extremely suitable for safety pharmacology screening assays due to their unique strengths and characteristics (Introduction) including field potentials containing well-proounced depolarization and repolarization peaks.

In this case study, we assessed the effects of a set of cardio-active compounds on Pluricyte® Cardiomyocytes’ electrophysiology by ECR (electrical field potential) and CI (Cellular Index) measurements using the ACEA xCELLigence® RTCA CardioECR instrument. Pluricyte® Cardiomyocytes, cultured in Pluricyte® Cardiomyocyte Medium, showed expected pharmacological responses in a reproducible manner, which could be readily detected with the ACEA xCELLigence® RTCA CardioECR instrument. Therefore, we conclude that the combination of Pluricyte® Cardiomyocytes with the ACEA xCELLigence® RTCA CardioECR platform provides a highly suitable in vitro assay to study the cardiac safety profile of compounds at an early stage of drug development.

![Figure 2.3 hERG channel blockers. hERG potassium channel blockers E4031 (A) and dofetilide (B) increased the field potential duration and caused a reduction in the amplitude of the repolarization peak, as shown by an overlay of waveform averages (top panel A and B). Higher concentrations induced arrhythmias (lower panel A and B).](image)
Figure 2.4 Na⁺ channel blockers. By blocking the I\(_{\text{Na}}\) channels, mexiletine (A) and flecainide (B) reduced the amplitude of the sodium spike, as shown by an overlay of waveform averages (top panel A and B). Mexiletine and flecainide also block hERG (I\(_{\text{Kr}}\)) potassium channels, resulting in an increase in field potential duration (lower panels A and B).
Figure 2.5 L-Type Ca²⁺ channel blockers. Calcium channel blockers nifedipine (A) and diltiazem (B) reduced the field potential duration of Pluricyte® Cardiomyocytes, as shown by an overlay of waveform averages (top panels A and B). In addition, nifedipine and diltiazem reduced the Cell Index amplitude by inhibiting the influx of calcium needed for contraction (lower panel A and B).
β-adrenergic receptor agonist isoproterenol. Isoproterenol activates β-adrenergic receptors, resulting in increased beat rate and subsequently reduced field potential duration, as shown by an overlay of waveform averages (top panel) and multiple waveforms of a representative well (lower panels).
Figure 2.7 Myosin II Inhibitor blebbistatin. Blebbistatin inhibits myosin II, resulting in impairment of contraction. Blebbistatin reduced the Cell Index amplitude of Pluricyte® Cardiomyocytes (top panel) while the electrical field potential (ECR) remained unaffected (lower panel).
Figure 2.8 Overview of effects of cardio-active compounds on Pluricyte® Cardiomyocytes. Overview of different drugs and their effects on beat rate, field potential duration (time between the detected repolarization peak and the preceding sodium spike), sodium spike amplitude and Cell Index amplitude, expressed as percentage of change compared to the baseline. Mean ± SD, N= 4 wells for each condition.
3. References


Acknowledgement

This project has received funding from the European Union’s Horizon 2020 Research and Innovation Programme under grant agreement No 726513.