Assessment of proarrhythmic effects in Pluricyte® ventricular cardiomyocytes using the xCELLigence CardioECR platform

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Abstract

In drug development it is important to assess cardiac safety of drug candidates. The CIPA (Comprehensive in vitro Proarrhythmia Assay) initiative is currently investigating cardiac safety screening assays, employing, amongst others, human-induced pluripotent stem cell (hiPSC) derived cardiomyocytes. Although hiPSC derived cardiomyocytes are an interesting model for (high-throughput) safety pharmacology studies, they are considered relatively immature compared to adult cardiomyocytes. We have recently developed a serum-free maturation medium (Pluricyte® Cardiomyocyte Medium, PCM) in which hiPSC derived cardiomyocytes exhibit a relatively high level of maturity. This was demonstrated by an increased contraction profile, as well as electrophysiological properties and gene expression patterns comparable to mature cardiomyocytes [1]. To further investigate the (electro)physiology of hiPSC-derived cardiomyocytes cultured in PCM, and the potential of these cells for cardiac safety applications, Pluricyte® cardiomyocytes were characterized by MEA analysis using an xCELLigence RTCA CardioECR instrument. MEA analysis showed field potentials with a pronounced repolarization peak. Furthermore, the beat rate of the cells could be influenced by pacing. After addition of cardioactive compounds parallel field potential and impedance measurement showed relevant pharmacological responses of the Pluricyte® cardiomyocytes. We conclude that Pluricyte® cardiomyocytes cultured in PCM show improved maturity, and provide a highly relevant in vitro model to study cardiac safety and efficacy of compounds at an early stage of drug development.

Pluricyte® cardiomyocytes: application in drug development

Pluricyte® cardiomyocyte characterization – electrophysiology and morphology

![Image](image1.png)

**Fig. 1.** Characterization of Pluricyte® cardiomyocytes using the perforated patch-clamp technique, immunofluorescence (Red: alpha actin, green: troponin), and FACS analysis (ventricle axis: troponin T, horizontal axis: Myosin II). Note the negative resting potential, fast upstroke velocity and high degree of ultrastructural organization of the cells.

Pluricyte® cardiomyocyte characterization – Field Potential and Cell Index

![Image](image2.png)

**Fig. 2.** Characterization of Pluricyte® cardiomyocytes using the xCELLigence CardiECR. A, overlay of average waveforms of Field Potential and Cell Index signals. B, single waveform of Pluricyte® cardiomyocyte field potential. C, Parallel measurements of Cell Index (upper panel) and Field Potential (lower panel) of Pluricyte® cardiomyocytes.

![Image](image3.png)

**Fig. 3.** Pharmacological response of Pluricyte® cardiomyocytes (10,000 cells/well) to E4031 (A, B), diltiazem (C), and isoproterenol (D) determined with xCELLigence CardiECR field potential analysis. A, prolongation of field potential duration and triangulation. B, arrhythmics-induced by E4031 (300 nM). C, shortening of field potential duration by diltiazem. D, Increased beat rate of Pluricyte® cardiomyocytes in response to increasing concentrations isoproterenol (0-300 nM). (n=3, mean±SD).

**Table 1.** Compound effects on Pluricyte® cardiomyocytes determined using the xCELLigence CardiECR system.

<table>
<thead>
<tr>
<th>Compound Class</th>
<th>Name</th>
<th>Observed effect on Pluricyte® cardiomyocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na+ blocker</td>
<td>Dextrose</td>
<td>TP prolongation, arrhythmia</td>
</tr>
<tr>
<td>K+ blocker</td>
<td>E4031</td>
<td>TP prolongation, arrhythmia</td>
</tr>
<tr>
<td>Calcium blocker</td>
<td>Diltiazem</td>
<td>TP shortening, decreased cell index amplitude</td>
</tr>
<tr>
<td>Calcium blocker</td>
<td>Nifedipine</td>
<td>TP shortening, decreased cell index amplitude</td>
</tr>
<tr>
<td>Sodium blocker</td>
<td>Flunarizine</td>
<td>TP amplitude reduced, PP shortening, arrhythmia</td>
</tr>
<tr>
<td>β-receptor agonist</td>
<td>Isoproterenol</td>
<td>Increased beat rate</td>
</tr>
<tr>
<td>Myosin II blocker</td>
<td>Blebbistatin</td>
<td>Decreased cell index amplitude</td>
</tr>
</tbody>
</table>

Conclusions

Pluricyte® cardiomyocytes cultured in PCM show improved maturity, and provide a highly relevant in vitro model to study cardiac safety and efficacy of compounds at an early stage of drug development. Pharmacological responses to various cardioactive compounds could be assessed in detail using the xCELLigence CardiECR platform. In addition, the beat rate of Pluricyte® cardiomyocytes can be influenced with the pacing function of the xCELLigence CardiECR platform. We conclude that the Pluricyte® cardiomyocytes combined with the xCELLigence CardiECR platform provide a highly useful tool to investigate proarrhythmic effects of candidate drugs in vitro.

References


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