Compound effects on calcium transients in Pluricyte® Cardiomyocytes

using the Molecular Devices FLIPR Tetra® High-Throughput Cellular Screening System

Application note
Version 2.0
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1. Introduction

**Pluricyte® Cardiomyocytes strengths and characteristics**

Pluricyte® Cardiomyocytes are fully functional human-induced pluripotent stem-cell derived cardiomyocytes that are highly suitable for fluorescence-based calcium transient assays for cardiac safety screening. Pluricyte® Cardiomyocytes exhibit a relatively high level of maturity and present the following characteristics:

**Purity:**
- High purity of ventricular cardiomyocytes.
- Generated without any genetic modifications or purification/selection procedures.

**Electrophysiology:**
- Fast upstroke velocity and robust action potential amplitude.
- Low resting membrane potential (≤ -78 mV).
- Monolayer field potentials contain well-pronounced depolarization and repolarization peaks, enabling easy detection of field potential durations in multielectrode array (MEA) assays.
- Expected pharmacological effects of ion channel modulating compounds.

**Contractility:**
- Highly organized sarcomere structures.
- Strong contraction force.
- Relatively low beat rate (20-30 BPM).
- Clear presence of calcium transients, as shown by patch clamp, MEA and Ca2+-flux assays.

**Pluricyte® Cardiomyocytes**

In combination with the Molecular Devices FLIPR Tetra® High-Throughput Cellular Screening System, the Molecular Devices FLIPR Tetra® High-Throughput Cellular Screening System (FLIPR system) represents the next generation plate reader technology, providing access to high throughput detection of fluorescent and luminescent signals. This system is capable of simultaneous fluid dispensing and signal detection, and is therefore ideal for the fast detection of acute compound effects on calcium transients in Pluricyte® Cardiomyocytes. The combination of Pluricyte® Cardiomyocytes and the Molecular Devices FLIPR system contributes to the detection of potential cardiotoxic effects of compounds at high-throughput level (up to 384-well), which will support scientists in making decisions regarding the cardiac safety profile of drug candidates at early stages of preclinical drug development.
This application note describes the analysis of fluorescent dye-based calcium transients of Pluricyte® Cardiomyocytes upon compound treatment using the Molecular Devices FLIPR system. This demonstrates how Pluricyte® Cardiomyocytes, cultured in Pluricyte® Cardiomyocyte Medium, in combination with the Molecular Devices FLIPR system, provides a highly relevant in vitro model to study the cardiac safety profile of compounds at an early stage of drug development. For more data and information on how to use Pluricyte® Cardiomyocytes in combination with the FLIPR Tetra® High-Throughput Cellular Screening 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 www.ncardia.com, or contact us directly by e-mail (support@ncardia.com).
2. Assessment of effects of cardioactive compounds

on calcium transients of Pluricyte® Cardiomyocytes using the molecular devices FLIPR Tetra® High-Throughput cellular screening system

2.1 Calcium transients in Pluricyte® Cardiomyocytes measured with the FLIPR system

The FLIPR system is a cell screening system with an integrated dispensing head and imaging-based detector. The system allows simultaneous dispensing of test compounds onto Pluricyte® Cardiomyocytes incubated with a fluorescent calcium dye and real-time measurement of changes in fluorescent calcium transients at high temporal resolution in 96-, 384- or 1536-well plate formats. Signal peak parameters can be calculated based on a dynamic threshold and derivative analysis, and provide amplitude, time, frequency, and width values.

Intracellular calcium flux is important for cardiac contractility. Compounds that directly or indirectly affect calcium transients and/or cardiomyocyte beat rate could be identified using the FLIPR system. This makes the combination of the FLIPR system with Pluricyte® Cardiomyocytes extremely valuable for the high throughput evaluation of potential cardiotoxic effects of test compounds.

2.2 Experimental design to study acute effects of cardioactive compounds on the calcium transients of Pluricyte® Cardiomyocytes

Pluricyte® Cardiomyocytes were cultured on a black wall, clear bottom 384-well culture plate in Pluricyte® Cardiomyocyte Medium for 8 days after thawing. Plating of the cells and refreshing medium was performed manually. On the day of the assay Pluricyte® Cardiomyocytes were treated with increasing concentrations of a set of cardioactive test compounds as described in Table 5.1. Compounds were dissolved in DMSO at a concentration of 10mM and diluted to 10x the final concentration in Pluricyte® Cardiomyocyte Medium. Pluricyte® Cardiomyocytes were incubated with the FLIPR Calcium 6 Assay Kit (Molecular Devices) according to the manufacturer’s protocol. Pluricyte® Cardiomyocytes were then treated with increasing concentrations of the test compounds (Table 5.2). Short-term effects on the fluorescent calcium transients of Pluricyte® Cardiomyocytes were measured by the FLIPR system, using the settings as described our user guide.
<table>
<thead>
<tr>
<th>Compound class</th>
<th>Compound</th>
<th>Expected effects in hiPSC-derived cardiomyocytes</th>
<th>Expected effects on fluorescent calcium transients in Pluricyte® Cardiomyocytes using the FLIPR system</th>
</tr>
</thead>
<tbody>
<tr>
<td>hERG channel blocker (I_{K_{Ca}})</td>
<td>E4031</td>
<td>Delays repolarization phase by blocking the hERG channel current, ultimately resulting in arrhythmias^{1,3}</td>
<td>Arrhythmic events at higher concentrations</td>
</tr>
<tr>
<td>Calcium channel agonist</td>
<td>Bay K8644</td>
<td>Enhances calcium transients by acting as an agonist of L-type Ca^{2+} channels^{2}</td>
<td>Increased calcium transient amplitude, increased average calcium transient peak width</td>
</tr>
<tr>
<td>Calcium channel antagonist (I_{Ca,L})</td>
<td>Nifedipine</td>
<td>Decreases calcium transients by acting as antagonist of L-type Ca^{2+} channels^{2}</td>
<td>Decreased calcium transient amplitude, diminishing of the calcium transients at high concentrations</td>
</tr>
<tr>
<td>β-adrenergic receptor agonist</td>
<td>Isoprenaline</td>
<td>Increases beat rate by activating β-adrenergic receptors^{3}</td>
<td>Increased number of peaks per minute</td>
</tr>
</tbody>
</table>

Table 2.1 List of cardioactive compounds and their expected effects on hiPSC-derived cardiomyocytes.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>10000</td>
<td>100</td>
<td>10000</td>
<td>1000</td>
<td>1*</td>
</tr>
<tr>
<td>B</td>
<td>5000</td>
<td>50</td>
<td>5000</td>
<td>500</td>
<td>0.5*</td>
</tr>
<tr>
<td>C</td>
<td>2500</td>
<td>25</td>
<td>2500</td>
<td>250</td>
<td>0.25*</td>
</tr>
<tr>
<td>D</td>
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<td>12.5</td>
<td>1250</td>
<td>125</td>
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</tr>
<tr>
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<td>6.25</td>
<td>625</td>
<td>62.5</td>
<td>0.063</td>
</tr>
<tr>
<td>F</td>
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<td>3.13</td>
<td>313</td>
<td>31.25</td>
<td>0.031</td>
</tr>
<tr>
<td>G</td>
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<td>1.56</td>
<td>156</td>
<td>15.63</td>
<td>0.016</td>
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<tr>
<td>H</td>
<td>78</td>
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<td>78</td>
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<td>I</td>
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<td>0.39</td>
<td>39</td>
<td>3.91</td>
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<tr>
<td>J</td>
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<td>20</td>
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<tr>
<td>K</td>
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<td>0.10</td>
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<tr>
<td>L</td>
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<td>0.05</td>
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<td>0.49</td>
<td>4.88E-04</td>
</tr>
<tr>
<td>M</td>
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<td>0.02</td>
<td>2.4</td>
<td>0.24</td>
<td>2.44E-04</td>
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<tr>
<td>N</td>
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<td>0.01</td>
<td>1.2</td>
<td>0.12</td>
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</tr>
<tr>
<td>O</td>
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<td>0.006</td>
<td>0.6</td>
<td>0.06</td>
<td>6.10E-05</td>
</tr>
<tr>
<td>P</td>
<td>0.3</td>
<td>0.003</td>
<td>0.3</td>
<td>0.03</td>
<td>3.05E-05</td>
</tr>
</tbody>
</table>

Table 2.2. Final test concentrations of each cardioactive compound decreasing from top to bottom of the plate.

*For experimental purposes in this case study DMSO concentrations exceed maximum recommended concentration of 0.1%.
2.3 Results

The results displayed below (Figure 2.1) were obtained in the FLIPR system directly after compound addition and thus indicate acute compound effects on Pluricyte® Cardiomyocytes for all tested concentrations. Figure 2.2 depicts typical calcium transient signals (beating patterns) of Pluricyte® Cardiomyocytes upon treatment with cardioactive compounds. The data were analyzed using ScreenWorks Peak Pro software (Version 4.0.0.30) in order to determine the compound effects on specific parameters, including peak frequency (beats per minute), peak amplitude and peak width diameter.

<table>
<thead>
<tr>
<th>E4031</th>
<th>Bay K8644</th>
<th>Nifedipine</th>
<th>Isoprenaline</th>
<th>DMSO</th>
<th>Medium</th>
</tr>
</thead>
</table>

![Figure 2.1](image.png)

**Figure 2.1.** Screenshot of acute compound effects on the calcium transient fluorescent signals of Pluricyte® Cardiomyocytes. The screenshot shows a 30 seconds time scale. The concentration of each compound increases from bottom to top as indicated in Table 2.2 and each concentration was analyzed in triplicate. DMSO and Pluricyte® Cardiomyocyte Medium (right columns) were used as negative controls. The measurement was performed directly after compound addition.
hERG potassium channel blockers block the rapid component of the delayed rectifier outward potassium current (Ikr), thereby delaying the repolarization phase of the cardiomyocyte action potential. At higher concentrations, blocking of the hERG channel may lead to TdP-like arrhythmias\textsuperscript{1,3}. Figure 2.1 shows the effect of hERG channel blocker E4031 on the calcium transient fluorescent signal in Pluricyte\textsuperscript{®} Cardiomyocytes. As Figure 2.3 depicts, arrhythmia incidence quickly increases to 100% at 39nM.

L-type calcium channel agonists increase the calcium influx during the plateau phase\textsuperscript{2} (the phase between the depolarization and repolarization phase of a cardiomyocyte action potential). Moreover, as calcium is important for cardiomyocyte contraction, activation of the L-type calcium channel may lead to changes in cardiomyocyte contractility. Figure 2.1 shows how treatment of Pluricyte\textsuperscript{®} Cardiomyocytes with the L-type calcium channel agonist Bay K8644 resulted in increased fluorescent calcium transient amplitude, increased peak width, and reduced peak frequency.

L-type calcium channel antagonists inhibit the calcium influx into cardiomyocytes during the plateau phase (the phase between the depolarization and repolarization of a cardiomyocyte action potential). As calcium is important for cardiomyocyte contraction, inhibition of the L-type calcium channel may lead to decreased contractility\textsuperscript{2}. Figure 2.1 shows how treatment of Pluricyte\textsuperscript{®} Cardiomyocytes with increasing doses of nifedipine results in gradually decreasing fluorescent calcium transient amplitudes, which was confirmed in the concentration-response curve (Figure 2.3).

β-adrenergic receptor agonists, such as isoprenaline, activate the β-adrenergic receptor\textsuperscript{3}, resulting in an increased cardiomyocyte beat rate and subsequently stimulation of intracellular calcium transients. Figure 4 shows how treatment of Pluricyte\textsuperscript{®} Cardiomyocytes with isoprenaline resulted in increased fluorescent calcium transient peak frequency, and decreased peak width. The concentration-dependent effect of isoprenaline on beats per minute (BPM) is shown in Figure 2.3.

![Figure 2.2](image_url). Typical calcium transient signals (beating patterns) of Pluricyte\textsuperscript{®} Cardiomyocytes upon treatment with cardioactive compounds. Representative calcium transient signal of Pluricyte\textsuperscript{®} Cardiomyocytes treated with indicated cardioactive compounds at the concentration of 312nM E4031, 1.56µM Bay K8644, 19nM nifedipine and 500nM isoprenaline. 0.25% DMSO is shown as negative control.
Figure 2.3. Concentration-response curves of the cardioactive compounds on Pluricyte® Cardiomyocytes. A number of concentration-response curves are displayed that can be used to determine the EC50 of cardioactive compounds. As shown for E4031, the arrhythmia incidence shows a clear concentration-dependent response. Clear concentration-response curves can also be observed for Pluricyte® Cardiomyocytes treated with nifedipine (peak amplitude), isoprenaline (beat rate) and high concentrations of DMSO (beat rate). The responses displayed are relative to the baseline measurement, performed before compound addition.

2.4 Concluding Remarks

In this case study, we assessed the effects of a set of cardioactive compounds on calcium transients in Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium. Intracellular calcium transients occur as a consequence of cardiomyocyte excitation and are crucial for cardiac contractility. Here, we show that cardioactive compounds, known to directly or indirectly affect calcium transients could be identified using Pluricyte® Cardiomyocytes in combination with the FLIPR system from Molecular Devices. This kinetic plate reader enables real-time detection of changes in fluorescent calcium transients in Pluricyte® Cardiomyocytes at high temporal resolution in 96- and 384-well plate formats. The high-throughput compatibility of this platform makes this assay exceptionally suitable for screening potential cardiotoxic effects of novel drug candidates at early stages of drug development.

Acknowledgement

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3. REFERENCES

Acknowledgement

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