Novel assays to study drug effects in hiPSC-derived cells using the FDSS/µCell system

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Marijn Vlaming, PhD
VP Technology
marijn.vlaming@pluriomics.com
Outline – new assays

• Electric Field Stimulation (EFS) /pacing in hiPSC-derived cardiomyocytes
• Voltage Sensitive Dyes in hiPSC-derived cardiomyocytes
• Ca^{2+}-transient assays in hiPSC-derived smooth muscle cells
ELECTRIC FIELD STIMULATION (EFS)
Pacing cardiomyocytes

- Objectives for pacing:
  - Standardization of electrophysiology assays
  - Better predictivity of compound safety (or efficacy)
  - Increased biological relevance: adjusting beat rates along large physiologically relevant range
  - Investigation of beat rate–dependent compound effects

Pluricyte® Cardiomyocytes paced at 0.8 Hz, 1000 mV, CardioECR system
Pacing hiPSC-derived Cardiomyocytes

**Advantages**
- More standardized
- Physiologically relevant beat rates
- Beat-rate dependent compounds
- Compounds effects isolated from beat rate
- Compatible with mature cells (no spontaneous beating)

**Disadvantage**
- Pacing & readout both electrical → pacing artefacts

**EFS:** electrical stimulation with optical readout
Pacing Pluricyte® Cardiomyocytes with EFS

- **spontaneous**
- **pacing 0.5 Hz**
- **spontaneous**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Frequency (Hz)</td>
<td>0.5</td>
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<tr>
<td>Voltage (V)</td>
<td>5</td>
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<tr>
<td>Pulse width (ms)</td>
<td>10</td>
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<tr>
<td>Dispense Height (mm)</td>
<td>0.5</td>
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Compound effects/standardization of assays: Ryanodine (RyR2 blocker, negative inotrope) reduces calcium transient amplitude and increases peak width.

Data show that Pluricyte® CMs has a functional SR that plays an important role in E-C coupling.
Pacing using EFS – preliminary conclusions

- EFS provides useful option to separate electrical pacing from assay read outs
- EFS can help to standardize high throughput assays in hiPSC-derived cardiomyocytes
- Pacing Pluricyte® Cardiomyocytes with EFS at beat rates up to 0.5 Hz, higher frequencies and other pacing conditions to be tested/optimized
- Further studies to investigate compound effects to be performed
VOLTAGE SENSITIVE DYSES TO STUDY PLURICYTE® CARDIOMYOCYTE ELECTROPHYSIOLOGY USING THE FDSS SYSTEM
Membrane potential of Pluricyte®
Cardiomyocytes

Voltage sensitive dye FluoVolt to study changes of the Membrane Potential

Next step: testing compound effects with voltage sensitive dyes
Ca^{2+} flux assays with FDSS/μCell to study compound effects in hiPSC-derived smooth muscle cells
Pluriomics manufactures iPSC derived functional cell types and offers cell-based assay services.

- Smooth muscle cells
- Cardiomyocytes
- Endothelial cells

Assay development
- Electrophysiology
- Biochemistry
- Contraction

Drug development
Pharmacological research
Ca$^{2+}$ analysis of SMCs treated with GPCR agonists

**Endothelin-1**

- 20 nM ET-1
- 2 nM ET-1
- 0.2 nM ET-1
- 0.02 nM ET-1
- Control

**Angiotensin-II**

- 100 µM AT-II
- 10 µM AT-II
- 1 µM AT-II
- 100 nM AT-II
- 10 nM AT-II
- 1 nM AT-II
- 0.1 nM AT-II
- Control

**Graphs**

- **Endothelin-1** intensity over time (s)
- **Angiotensin-II** intensity over time (s)

**Concentration (µM)**

- ET-1
- AT-II
- Control

**Amplitude (au)**
Summary

- Besides “existing” Ca\(^{2+}\)-flux assays with Pluricyte\textsuperscript{®} Cardiomyocytes in the Hamamatsu FDSS/\(\mu\)Cell system, new assays will provide further opportunities for development and application of high-throughput multiparametric assays to study safety and efficacy of cardioactive compounds.
- The assays developed for cardiomyocytes, can also be used for other cell types, such as smooth muscle cells.
- Combining Pluricyte\textsuperscript{®} iPSC-derived cells with the FDSS/\(\mu\)Cell system contributes to:
  - More efficient, and therefore cost- and time-effective, decision making in early drug discovery & development
  - Reduction of animal experiments
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CONTACT:
support@pluriomics.com
www.pluriomics.com