HTS of Ca$^{2+}$ Transients in Human iPS-derived Cardiomyocytes as a Predictive and Cost Effective Assay Early on in Drug Development

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Content

- Introduction into the production of Cor.4U cardiomyocytes
- Overview of established applications with Cor.4U cardiomyocytes
- HTS calcium transient assay in the Hamamatsu FDSS/μCell
  - Compound screening
  - Data analysis
- Outlook
Generation of Cor.4U human iPS derived Cardiomyocytes

- iPS generated according to Yamanaka (licensed from iPS Academia)
- Production system by Axiogenesis enables large lot sizes
- Tightly controlled differentiation results in minimal lot to lot variations
- Selection based purification technology results in pure cardiomyocytes
- Cryopreserved in various formats
- GFP positive and colourless varieties will be available
- Complete FTO for commercial use on the limited use label license
- Cryopreserved
- Fresh cultures on different substrates (cell culture flasks, 96/384 well plates)
Applications of Human Cor.4U Cardiomyocytes

- Automated Patch Clamp
- MEA
- Manual Patch Clamp
- Impedance-based Assay Systems
- Fluorescent Plate Reader Assays
- Mito- and Cytotox Assays

Human iPS-derived Cor.4U Cardiomyocytes

Immunostaining of Cor.At Cardiomyocytes: cardiac actinin and connexin 43; nuclei

[Diagram showing applications of Cor.4U Cardiomyocytes with various assay methods connected to the central image of the cells.]
Introduction

Recording of Calcium Transients in Cor.4U Cardiomyocytes
Excitation-Contraction Coupling

Many Channel Types contribute to the Action Potential

- Na⁺
- Ca²⁺ L-type
- Ca²⁺ T-type
- Na⁺/Ca²⁺-exchanger
- K⁺ I_to
- K⁺ I_Ks
- K⁺ I_Kr
- K⁺ I_Kur

Calcium is the messenger that integrates the electrochemical signals of the action potential with the molecular signaling pathways that regulate contraction.
Hamamatsu FDSS/μCell

- Dispenser head
- Disposable tips
- Assay plate
- Excitation light source
- Emission filter
- Camera lens
- Camera (sensor)
Plating Efficiency on 384 Well Plates

Fluo-4 Assay

Data was kindly provided by Dr. Thomas Licher, Sanofi Germany
## FDSS Parameters Analysed by the CalDio/Wave Checker Software

### Parameters:

1. **Peak Number** (total, BPM)
2. **P-P time [ms]** (Ave, Std, Max, Min)
3. **Ratio** (Ave, Std)
   
   \[ \text{Ratio} = \frac{(\text{AMP} + \text{RMP})}{\text{RMP}} \]
4. **AMP** (Ave, Std)
5. **RMP** (Ave, Std)
6. **Slope** (Ave, Std)
   
   - **Rising Slope**: Slope from bottom to peak
   - **Falling Slope**: Slope from peak to bottom
   
   0% - 10%, 10% - 90%, 20% - 80%, 30% - 70%
7. **Integration** (Ave, Std)
8. **PWD (10% - 90%) [ms]** (Ave, Std)

### Diagram:

- **(1)** Peak Number (total, BPM)
- **(2)** P-P time [ms] (Ave, Std, Max, Min)
- **(3)** P-P duration time
- **(4)** Ratio (Ave, Std)
- **(5)** RMP = Fluorescence intensity at the bottom peak
- **(6)** AMP = Peak Fluorescence count - RMP
- **(7)** Max_slope (Fluorescence counts/ms) same as upstroke slope (but bottom to peak)
- **(8)** MaxNeg_slope (Fluorescence counts/ms) same as downstroke slope (but peak to bottom)
- **(9)** APD 10 to 90
  - APD10
  - APD50 (Peak Width, FWHM)
  - PWD 90
Use our discoveries to advance yours

hERG/\(I_{\text{Kr}}\) blocker
Astemizole
hERG Blocker
FDSS μCell - Astemizole

5 min

Vehicle control

41 nM

370 nM
FDSS μCell - Astemizole

30 min

vehicle control

4.57 nM

13.7 nM
FDSS μCell - Astemizole

30 min

41 nM
123 nM
370 nM
Data Analysis with the Wave Checker Module

Astemizole
FDSS μCell - Astemizole

Wave Checker Module
FDSS μCell - Astemizole
FDSS µCell - Astemizole

export of text file format
FDSS µCell - Astemizole
FDSS μCell - Astemizole

Graph 1: Beating frequency [bpm] vs. concentration [nM]
- Baseline
- 5 min
- 10 min
- 15 min
- 20 min
- 25 min
- 30 min
- 35 min

Graph 2: Average falling slope [RFU/ms] vs. concentration [nM]
- Baseline
- 5 min
- 10 min
- 15 min
- 20 min
- 25 min
- 30 min
- 35 min
Dofetilide
hERG Blocker
FDSS μCell - Dofetilide

5 min

Vehicle control

24.7 nM

222 nM
FDSS μCell - Dofetilide

30 min

vehicle control

24.7 nM
FDSS μCell - Dofetilide

Graphs showing the effects of various concentrations of Dofetilide on beating frequency and average rising/falling slope in μCells.

- **Beating Frequency**:
  - Data points for each concentration (Control, 0.03, 0.1, 0.3, 0.91, 2.74, 8.23, 24.69, 74.07, 222.22, 666.67, 2000.00 nM) plotted over time (baseline, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min).
  - Graphs for baseline and different time points.

- **Average Rising/Falling Slope (RFU/ms)**:
  - Data points for each concentration over time.
  - Graphs for baseline and different time points.

The graphs illustrate how Dofetilide concentration affects the beating frequency and rising/falling slope in μCells, with distinct trends observed at different time intervals.
FDSS μCell - Dofetilide

![Graphs showing concentration vs. time for different average peak widths and baseline widths.](image)
FDSS µCell - Clustering of Compounds
Outlook
Outlook - Hamamatsu FDSS 7000EX

Camera for fast recording of fluorescence and luminescence.
Outlook

- Evaluation of different kinds of fluorescent calcium and membrane potential dyes for the Cor.4U Cardiomyocytes
- Establishment of cardiac cytotoxicity/viability assay
- Combination of different luminescence assays
  - viability
  - cell death detection
  - apoptosis
Dopaminergic and Sensory Neurons
Patch Clamp of hiPS-derived Dopaminergic Neurons

Manual current clamp recording of spontaneous action potentials

Manual voltage clamp recording of sodium currents

Data was kindly provided by PoreGenic GmbH, Rostock, Germany
Patch Clamp of hiPS-derived Peripheral Neurons

Manual voltage clamp recording of sodium currents

Data was kindly provided by PoreGenic GmbH, Rostock, Germany
Use our discoveries to advance yours

Health and Environmental Science Institute (HESI) Initiative

CiPA Initiative
(Comprehensive in vitro Pro-Arrhythmia Assay)
Stem Cell Working Groups - Newly Initiated

Following the workshop, the Cardiac Stem Cell Working Group formed and three subteams were created:

- Cytotoxicity
- Contractility
- Electrophysiology

To explore issues of sensitivity, reproducibility, and predictivity for safety endpoints.
Cardiac Stem Cell Working Group: Timeline

4Q 2013
• Subteam goals and objectives drafted
• Data collection initiated
• Link to CIPA project

1-2Q 2014
• Literature search conducted
• Laboratory study protocol planning

2-3Q 2014
• Laboratory study initiated
• Manuscript(s) drafting

2015
• Manuscript(s) drafting and submission
Thanks to:

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  Dr. Thomas Licher