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

12th FDSS Users Meeting
June 9th, 2016

pluriomics
CLOSE TO THE HEART

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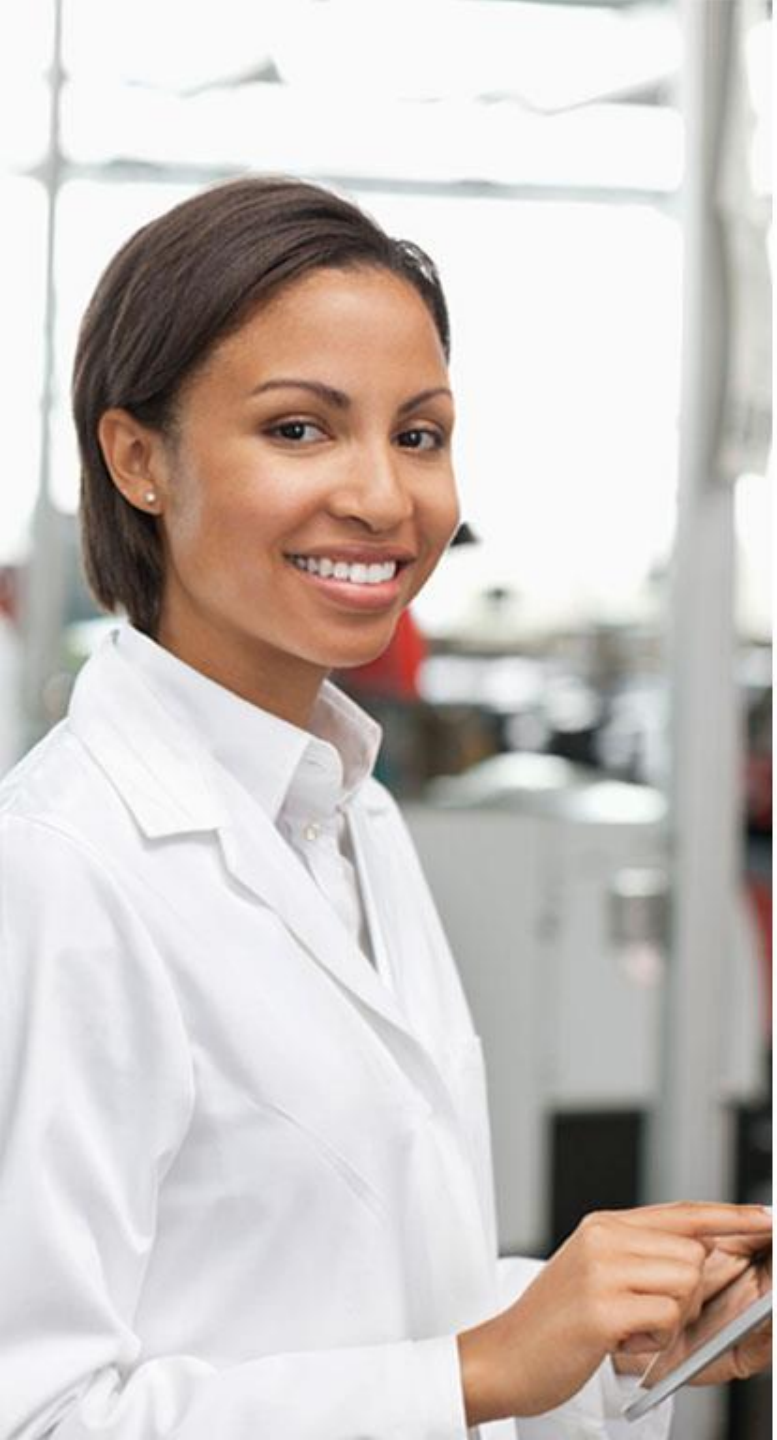


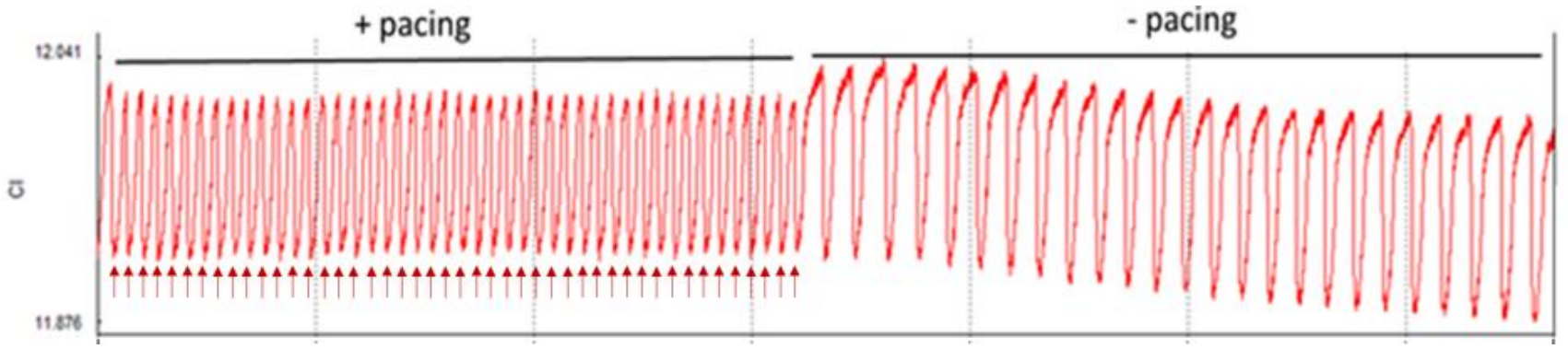
Outline – new assays

- 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

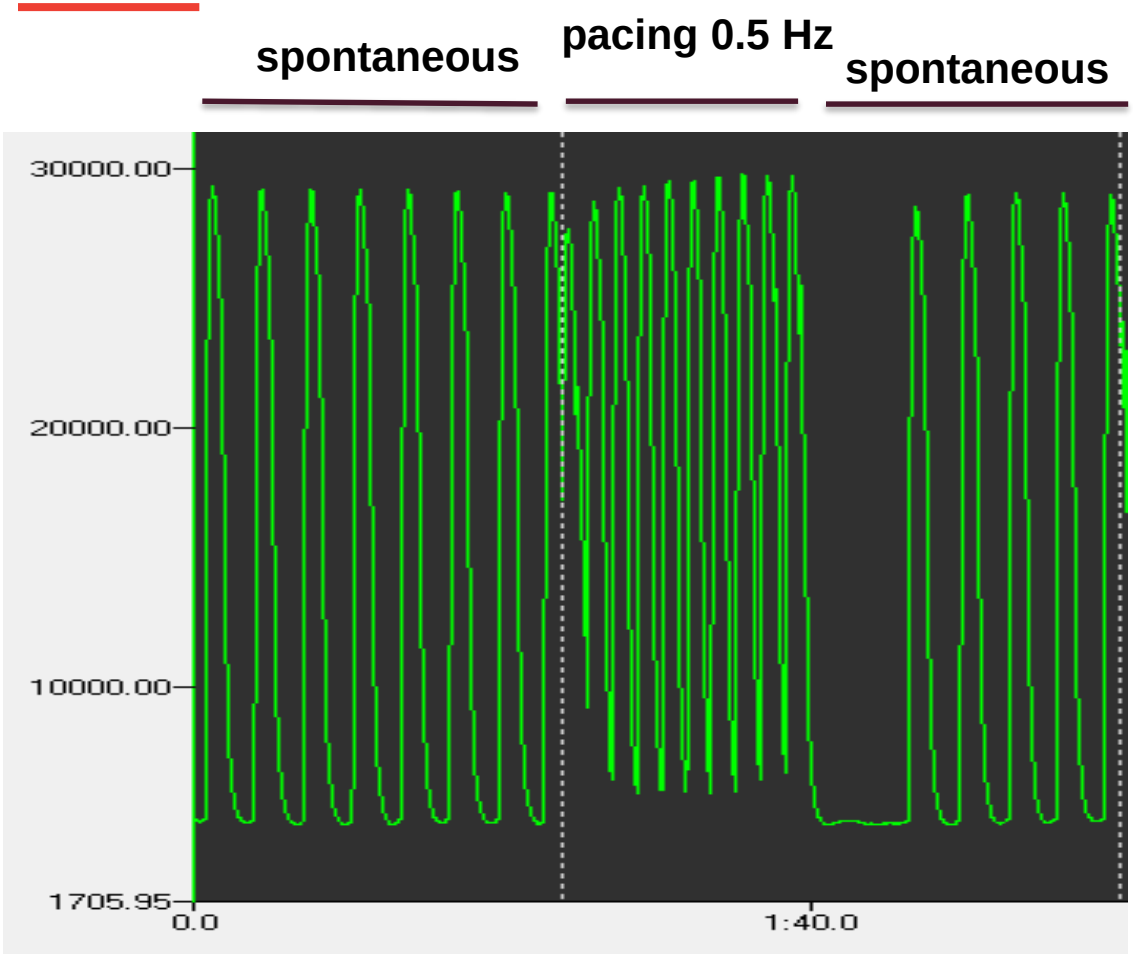
- More standardized
- Physiologically relevant beat rates
- Beat-rate dependent compounds
- Compounds effects isolated from beat rate
- Compatible with mature cells (no spontaneous beating)

Disadvantage

- Pacing & readout both electrical → pacing artefacts

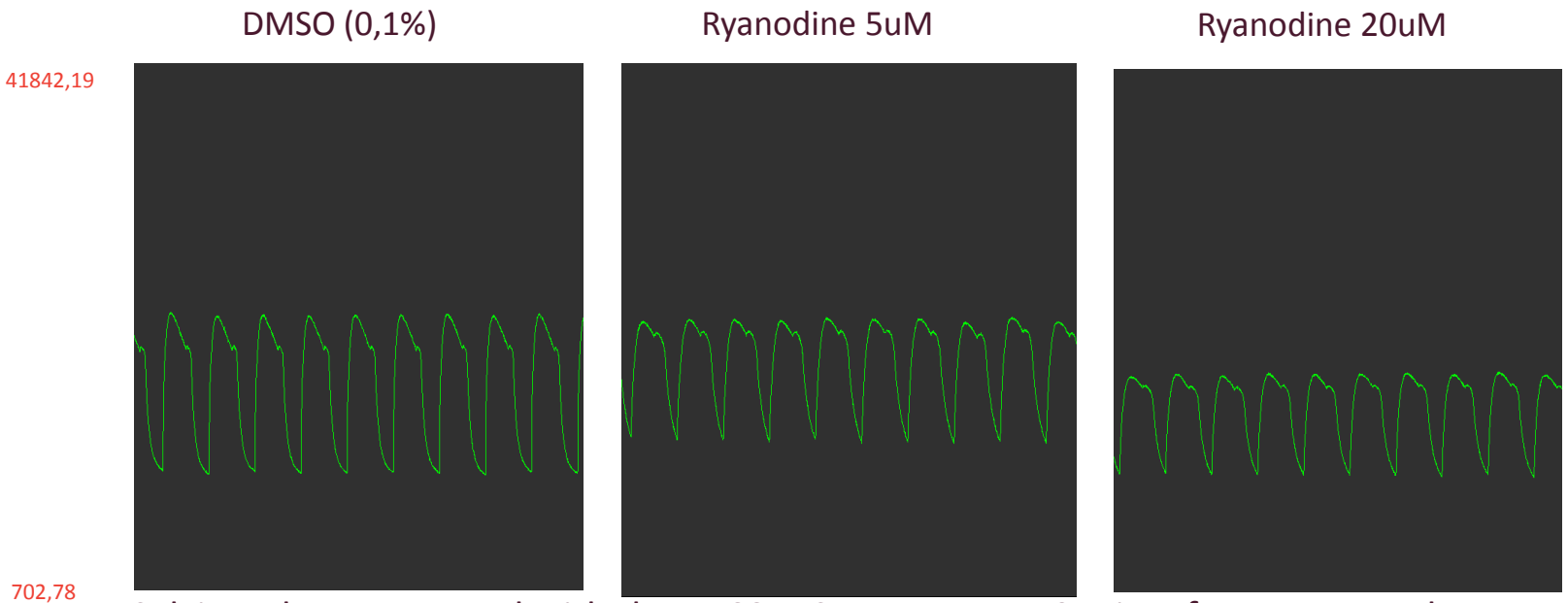
EFS: electrical stimulation with optical readout

Pacing Pluricyte® Cardiomyocytes with EFS



Frequency (Hz)	0,5
Voltage (V)	5
Pulse width (ms)	10
Dispense Height (mm)	0,5

Compound effects/standardization of assays: Ryanodine (RyR2 blocker, negative inotrope) reduces calcium transient amplitude and increases peak width



Calcium data generated with the FDSS µCELL system, 10 min. after compound addition (incl. Pacing)

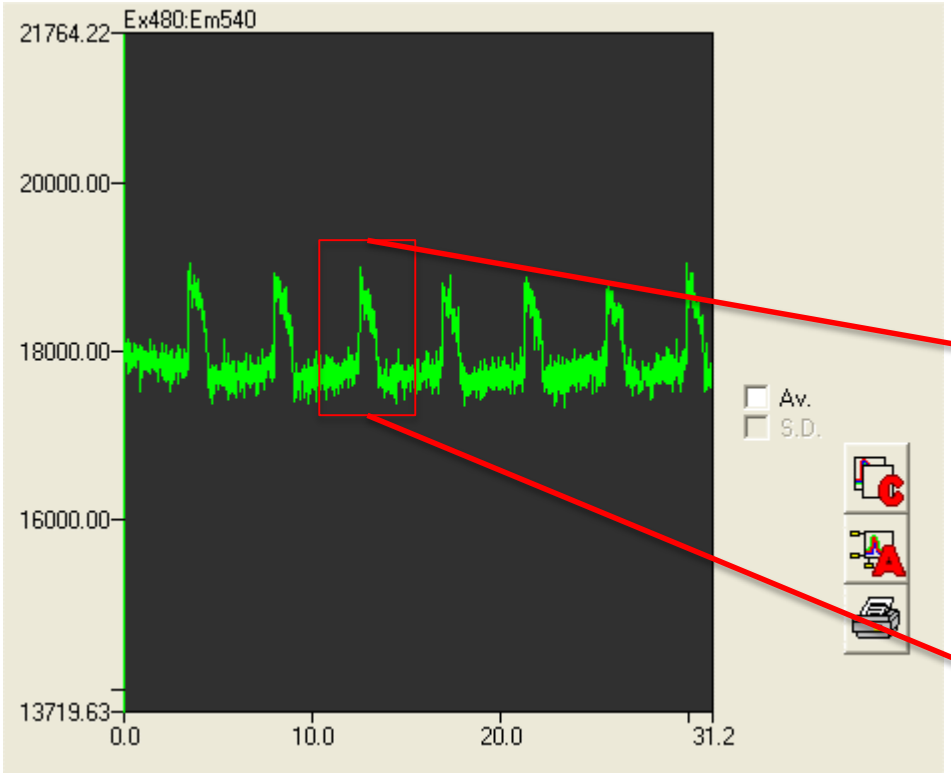
- Data show that Pluricyte® CMs has a functional SR that plays an important role in E-C coupling.

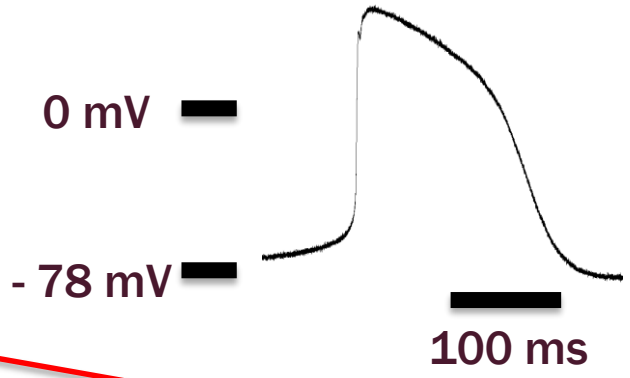
Pacing using EFS – preliminary conclusions

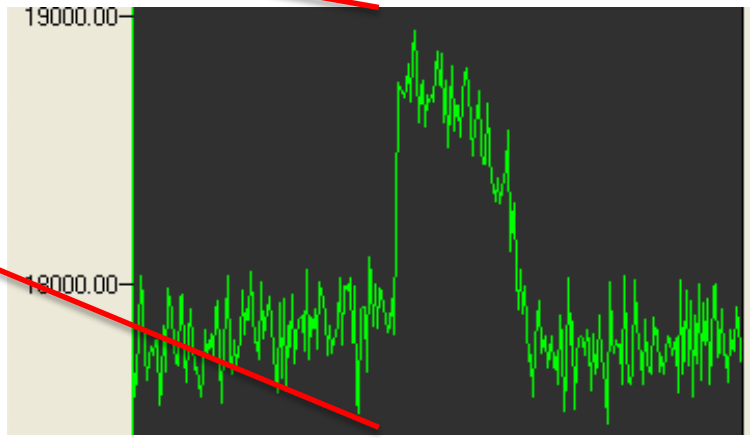
- EFS provides useful option to separate electrical pacing from assay read outs
- EFS can help to standardize high throughput assays in hiPSC-derived cardiomyocytes
- Pacing Pluricyte® Cardiomyocytes with EFS at beat rates up to 0.5 Hz, higher frequencies and other pacing conditions to be tested/optimized
- Further studies to investigate compound effects to be performed

VOLTAGE SENSITIVE DYES TO STUDY PLURICYTE® CARDIOMYOCYTE ELECTROPHYSIOLOGY USING THE FDSS SYSTEM

Membrane potential of Pluricyte® Cardiomyocytes





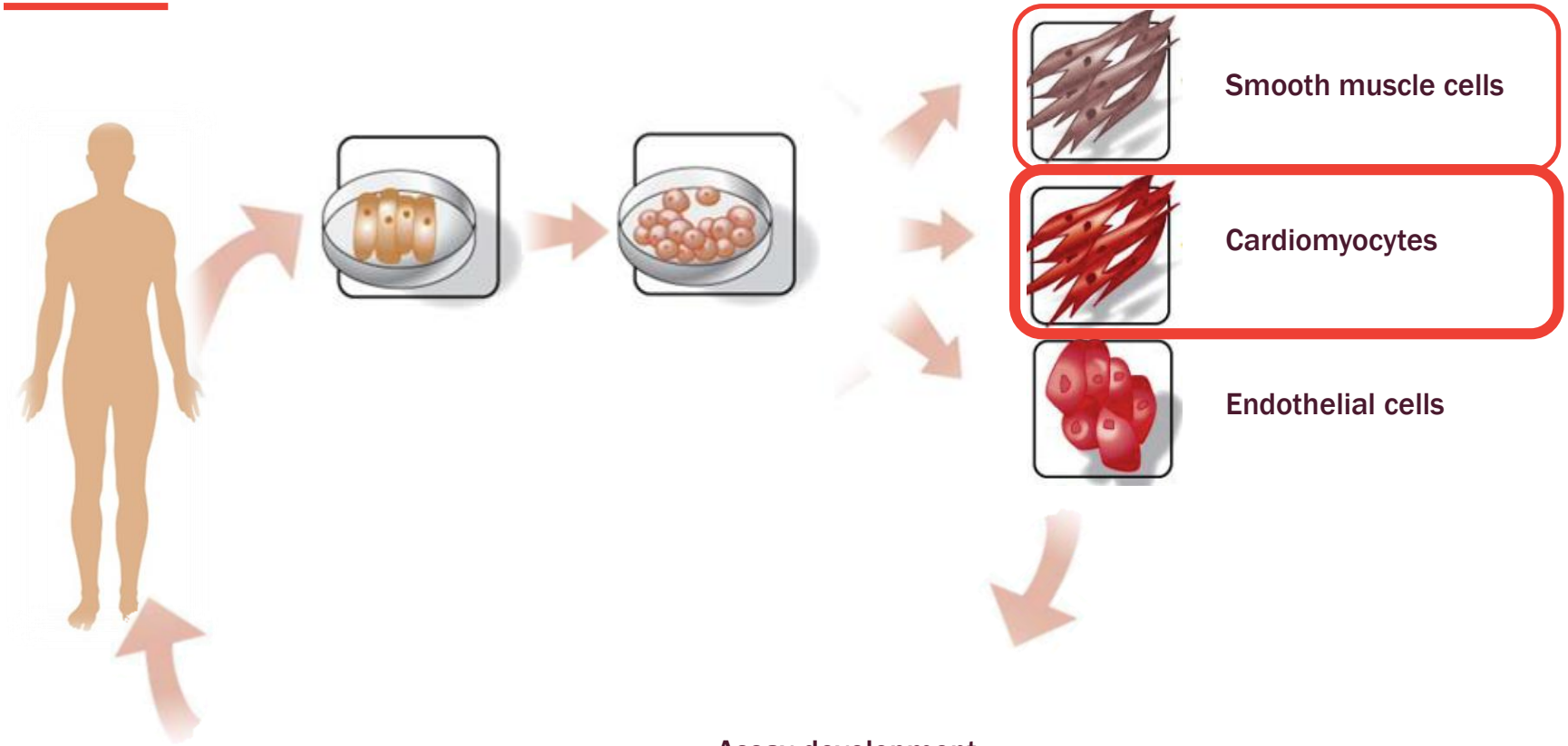


Voltage sensitive dye FluoVolt to study changes of the Membrane Potential

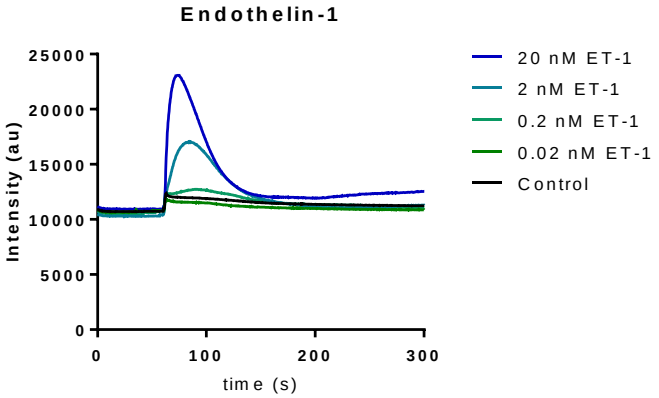
Next step: testing compound effects with voltage sensitive dyes

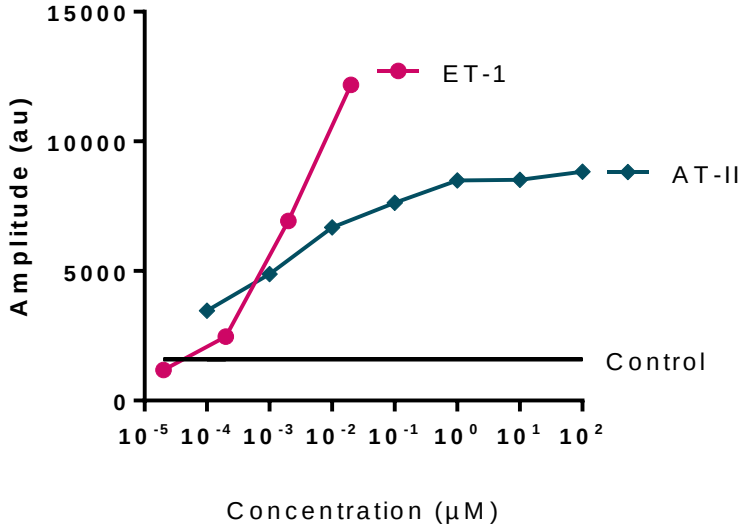
Ca^{2+} flux assays with FDSS/µCell to study compound effects in hiPSC-derived smooth muscle cells

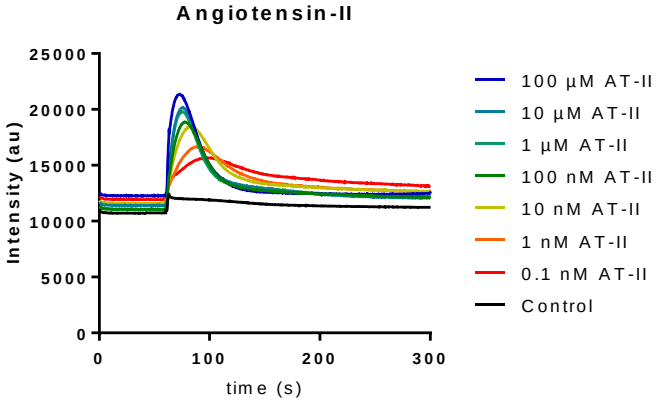
Pluriomics manufactures iPSC derived **functional cell types** and offers **cell-based assay services**

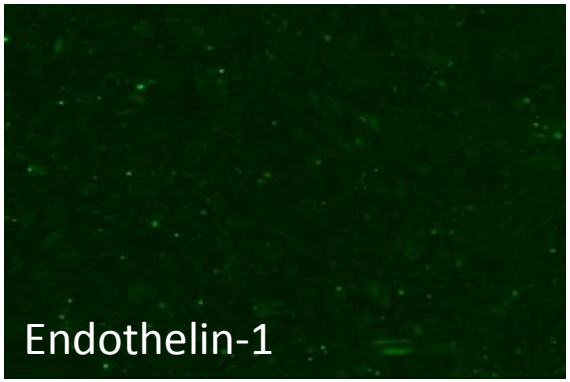


Ca^{2+} analysis of SMCs treated with GPCR agonists









Summary

- Besides “existing” Ca^{2+}-flux assays with Pluricyte® Cardiomyocytes in the Hamamatsu FDSS/µCell 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 cells types, such as smooth muscle cells

- Combining Pluricyte® iPSC-derived cells with the FDSS/µCell system contributes to:
  - More efficient, and therefore cost- and time-effective, decision making in early drug discovery & development
  - Reduction of animal experiments

Acknowledgements

Hamamatsu Photonics

Jean Marc D'Angelo
Emmanuel Pirson
Thomas Niedereichholz

Pluriomics BV

Peter Nacken
Fleur Stevenhagen
Rene Wilbers
Tessa de Korte
Arie Reijerkerk
Stefan Braam

Part of this work was performed within the CRACK-IT project InPulse, sponsored by NC3Rs and GlaxoSmithKline.

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