

# Towards prediction of acute and chronic drug effects using multi-parameter profiling of hiPSC-derived cardiomyocytes

De Korte T<sup>1</sup>, Wilbers R<sup>1</sup>, Langenberg K<sup>1</sup>, Nacken P<sup>1</sup>, Vlaming MLH<sup>1</sup>, Braam SR<sup>1</sup>

<sup>1</sup>Ncardia, Galileiweg 8, 2333 BD Leiden, The Netherlands

## Abstract

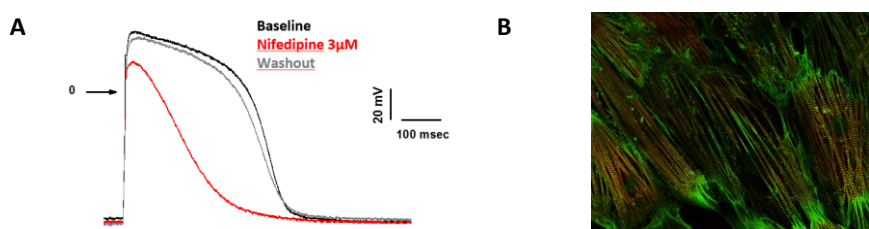
Standardization and validation of hiPSC-derived cardiomyocyte-based assays for *in vitro* cardiac safety studies are currently assessed by the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) consortium. Within CiPA, the acute effects of reference compounds (30±2 minutes after addition) are evaluated. However, besides acute effects on ion channels, especially chronic cardiotoxicity is a serious issue in the clinic.

In this study, we assessed both acute and chronic effects of a set of reference compounds on the electrophysiology and impedance of hiPSC-derived cardiomyocytes (Pluricyte® Cardiomyocytes).

While ion channel inhibitors, e.g. hERG-channel blocker E4031, clearly caused acute drug effects on the electrophysiology of cardiomyocytes, other compounds affected the electrophysiology or impedance only after longer incubation times. For example, lapatinib, a tyrosine kinase inhibitor significantly increased the impedance peak width after 16h (54%,  $P \leq 0.01$ ) to 24h (114%,  $P \leq 0.0001$ ) incubations. In addition, the antiprotozoal drug pentamidine did not cause any acute effects, but induced a field potential duration prolongation exclusively from 12 hours after treatment caused by disruption of hERG protein trafficking in Pluricyte® Cardiomyocytes.

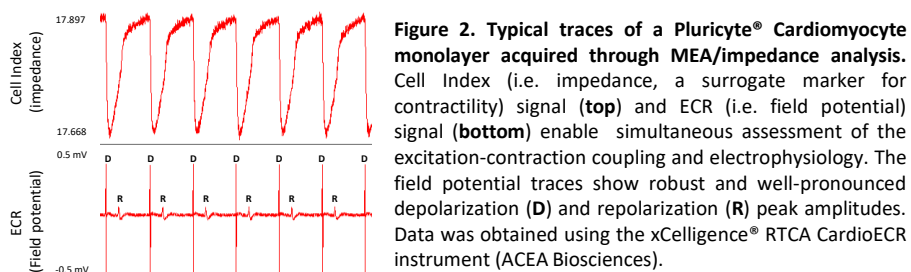
Taken together, our results show that studying both acute and chronic drug-effects in hiPSC-derived cardiomyocytes enables a better understanding of the wide range of drug-induced cardiotoxicities that can occur and will help to explain the cellular mechanisms behind late onset drug-induced cardiomyopathy.

## Human Pluricyte® Cardiomyocytes are fully functional and show a relatively high level of maturity



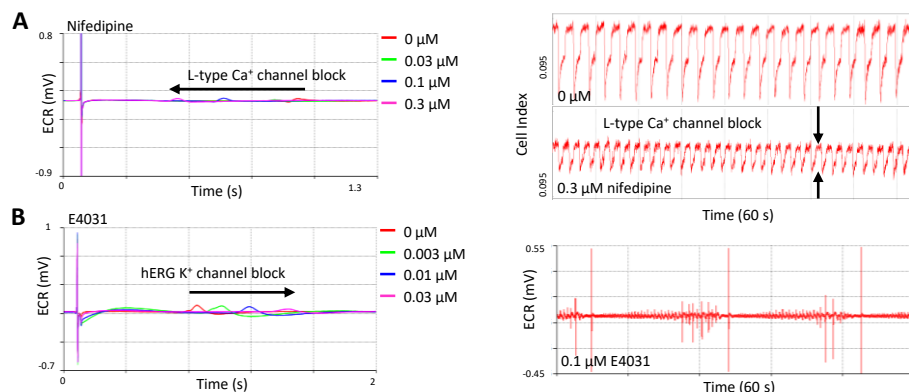
**Figure 1. Characteristics of Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium.**

**A:** A typical action potential of Pluricyte® Cardiomyocytes (measured by PhysioStim, Lautrec, France), demonstrating a low resting membrane (~-78mV) potential and fast upstroke velocity. As expected, L-type calcium channel blocker nifedipine decreased the APD20 (65%), APD90 (46%) and APA (6.2%). **B:** Pluricyte® Cardiomyocytes exhibit a high degree of ultra-structural sarcomere organization as determined by immunofluorescence (Green: alpha actinin; Red: myosin heavy chain 7).



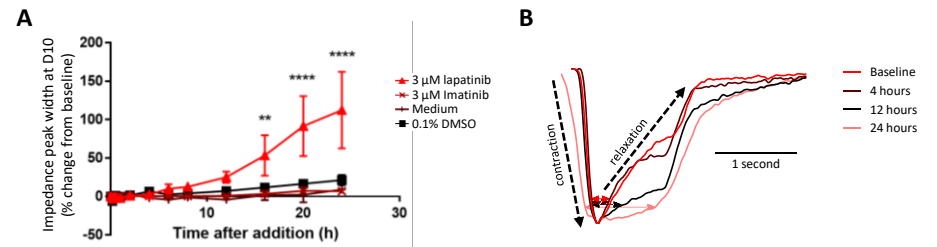
**Figure 2. Typical traces of a Pluricyte® Cardiomyocyte monolayer acquired through MEA/impedance analysis.** Cell Index (i.e. impedance, a surrogate marker for contractility) signal (**top**) and ECR (i.e. field potential) signal (**bottom**) enable simultaneous assessment of the excitation-contraction coupling and electrophysiology. The field potential traces show robust and well-pronounced depolarization (D) and repolarization (R) peak amplitudes. Data was obtained using the xCelligence® RTCA CardioECR instrument (ACEA Biosciences).

## Ion channel blockers E4031 and nifedipine induce acute effects on the electrophysiology of Pluricyte® Cardiomyocytes



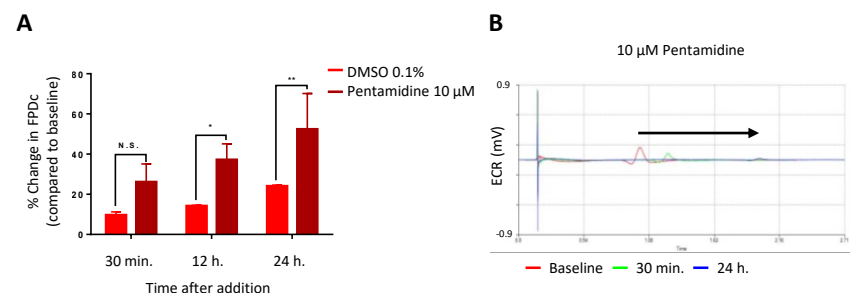
**Figure 3. Acute effects of E4031 and nifedipine on the electrophysiology and impedance of Pluricyte® Cardiomyocytes.** **A:** L-type calcium channel blocker, nifedipine, induced a clear decrease in field potential duration and Cell Index amplitude, which is line with the decrease in APD seen in Figure 1A. **B:** E4031, a hERG channel blocker, induced a concentration-dependent increase of the field potential duration and flattening of the repolarization peak. Arrhythmic-like events were observed at  $\geq 0.1 \mu\text{M}$ . Measurements were obtained using the xCelligence® RTCA CardioECR instrument (ACEA Biosciences).

## The tyrosine kinase inhibitor, lapatinib, alters the excitation-contraction coupling in Pluricyte® Cardiomyocytes after long term incubation



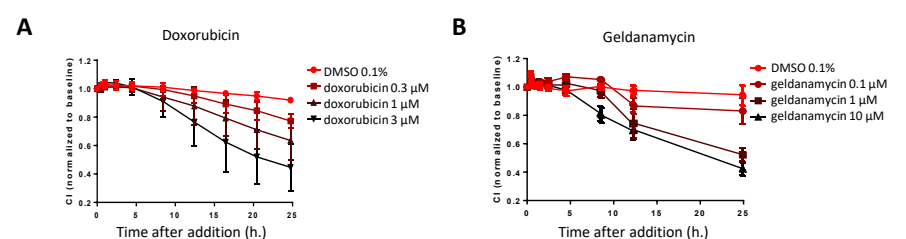
**Figure 5. Long-term effect of lapatinib on the impedance peak width of Pluricyte® Cardiomyocytes.** **A:** Unlike imatinib, lapatinib induced an increase in impedance peak width (D10) after 16h (54%,  $P \leq 0.01$ ) to 24h (114%,  $P \leq 0.0001$ ) post-addition, suggesting a long-term effect on the relaxation time of the cardiomyocytes. **B:** Overlay of impedance waveforms showing an increase in peak width in Pluricyte® Cardiomyocytes after long-term incubation with 3  $\mu\text{M}$  lapatinib. 1 waveform represents the average of all wave forms recorded during one measurement of 60 seconds. Data was obtained using the xCelligence® RTCA CardioECR instrument (ACEA Biosciences). All error bars represent SDs. Significance values indicated are the result from Dunnett's multiple comparisons test, after performing two way ANOVA. \*\*  $P \leq 0.01$ , \*\*\*\*  $P \leq 0.0001$ .

## The hERG trafficking inhibitor, pentamidine, induces an increase in field potential duration of Pluricyte® Cardiomyocytes after long term incubation



**Figure 6. Long-term effect of pentamidine on the field potential duration of Pluricyte® Cardiomyocytes.** **A:** Pentamidine showed no acute effects on the hERG channel, but after 24 hours of incubation, it significantly increased the field potential duration by disrupting hERG protein trafficking. **B:** An overlay of field potential traces show an increase in field potential duration and flattening of the repolarization peak of Pluricyte® Cardiomyocytes after 24 hours incubation with 10  $\mu\text{M}$  pentamidine. Data was obtained using the xCelligence® RTCA CardioECR instrument (ACEA Biosciences). All error bars represent SDs. Significance values indicated are the result from Dunnett's multiple comparisons test, after performing two way ANOVA. \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ .

## Doxorubicin and Geldanamycin affect Pluricyte® Cardiomyocyte cell viability after long-term incubation



**Figure 7. Long-term effect of doxorubicin and geldanamycin on the cell viability of Pluricyte® Cardiomyocytes.** Since changes in cell morphology and adhesion are reflected by changes in impedance, impedance can be used as a label-free readout for long-term cell viability. **A:** The anticycline, doxorubicin, induced a concentration- and time-dependent decrease in cell viability from 12 hours after addition. **B:** Geldanamycin, an ER-to-Golgi traffic inhibitor, induced a time-dependent decrease in cell viability at 1  $\mu\text{M}$  and 10  $\mu\text{M}$  from 12 hours after addition. Data was obtained using the xCelligence® RTCA CardioECR instrument (ACEA Biosciences). All error bars represent SDs.

## Concluding Remarks

- Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium show a ventricular, relatively mature phenotype.
- The typical long-term effects of anti-cancer drugs and (hERG) trafficking inhibitors are observed in Pluricyte® Cardiomyocytes by measuring the impedance, field potential or cell viability in a long-term, label-free and real-time manner.
- Studying both acute and long-term cardioactive effects in hiPSC-derived cardiomyocytes using a multi-parameter approach could provide a more detailed cardiotoxicity profile of (novel) compounds. This approach will further improve safety pharmacology decision-making at an early stage of drug development.

## Acknowledgments

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