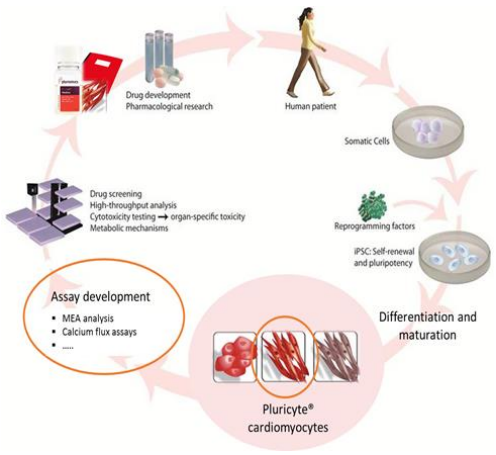
Assessment of proarrhythmic effects in Pluricyte® ventricular cardiomyocytes using the xCELLigence CardioECR platform

Vlaming MLH, Stevenhagen F, de Korte T, Langenberg K, Dunska MI, Grandela C, Braam SR
Pluriomics BV, Galileiweg 8, 2333 BD Leiden
marijn.vlaming@pluriomics.com

Abstract

In drug development it is important to assess cardiac safety of drug candidates. The CiPA (Comprehensive in vitro Proarrhythmia Assay) initiative is currently investigating cardiac safety screening assays, employing, amongst others, human-induced pluripotent stem cell (hiPSCs) derived cardiomyocytes. Although hiPSC-derived cardiomyocytes are an interesting model for (high-throughput) safety pharmacology studies, they are considered relatively immature compared to adult cardiomyocytes. We have recently developed a serum-free maturation medium (Pluricyte® Cardiomyocyte Medium, PCM) in which hiPSC-derived ventricular cardiomyocytes exhibit a relatively high level of maturity. This was demonstrated by an increased contraction profile, as well as electrophysiological properties and gene expression patterns comparable to mature cardiomyocytes [1]. To further investigate the (electro)physiology of hiPSC-derived cardiomyocytes cultured in PCM, and the potential of these cells for cardiac safety applications, Pluricyte® cardiomyocytes were characterised by MEA analysis using an xCELLigence RTCA CardioECR instrument. MEA analysis showed field potentials with a pronounced repolarization peak. Furthermore, the beat rate of the cells could be influenced by pacing. After addition of cardioactive compounds parallel field potential and impedance measurement showed relevant pharmacological responses of the Pluricyte® cardiomyocytes. We conclude that Pluricyte® cardiomyocytes cultured in PCM show improved maturity, and provide a highly relevant in vitro model to study cardiac safety and efficacy of compounds at an early stage of drug development.

Pluricyte® cardiomyocytes: application in drug development



Pluricyte® cardiomyocyte characterization – electrophysiology and morphology

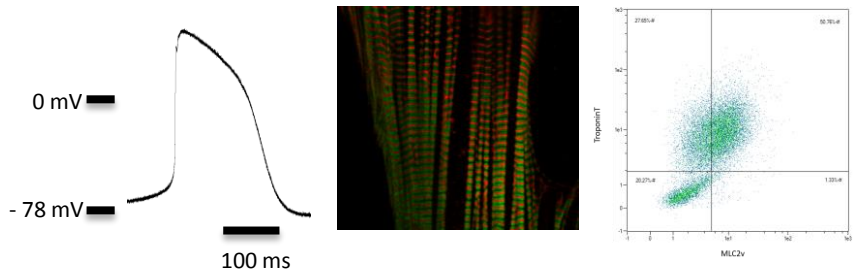


Fig. 1. Characterization of Pluricyte® cardiomyocytes using the perforated patch-clamp technique, immunofluorescence (Red: alpha actinin, green: troponin), and FACS analysis (vertical axis: troponin T, horizontal axis: MLC2V). Note the negative resting potential, fast upstroke velocity and high degree of ultra-structural organization of the cells.

Pluricyte® cardiomyocyte characterization – Field Potential and Cell Index

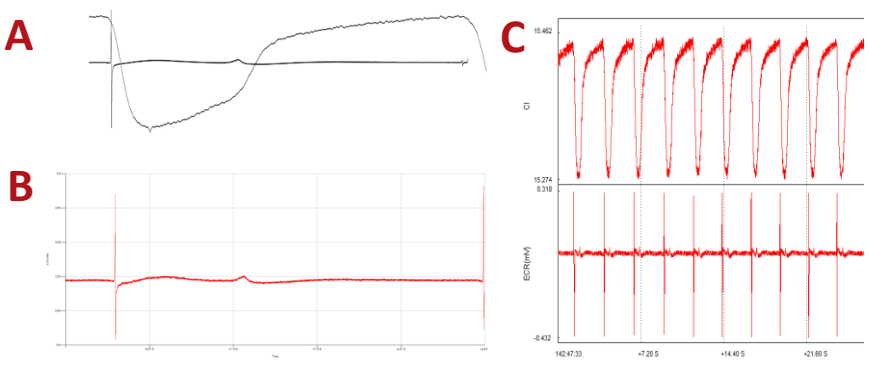


Fig. 2. Characterization of Pluricyte® cardiomyocytes using the xCelligence CardioECR. A, overlay of average waveforms of Field Potential and Cell Index signals. B, single waveform of Pluricyte® cardiomyocyte field potential. C, Parallel measurements of Cell Index (upper panel) and Field Potential (lower panel) of Pluricyte® cardiomyocytes.

Compound testing on xCelligence CardioECR with Pluricyte® ventricular cardiomyocytes

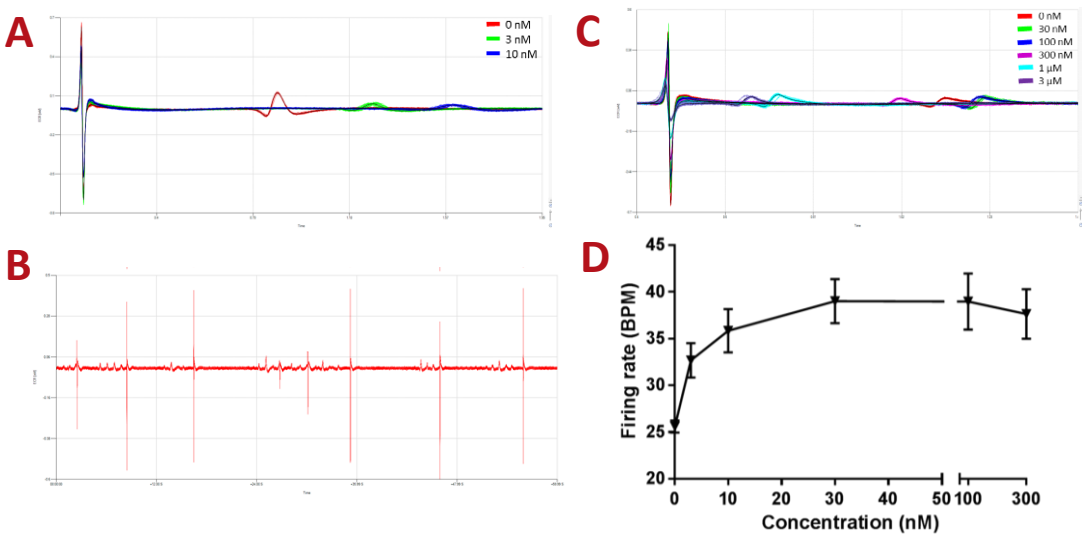


Fig. 3. Pharmacological response of Pluricyte® cardiomyocytes (30,000 cells/well) to E4031 (A, B), diltiazem (C), and isoproterenol (D) determined with xCelligence CardioECR field potential analysis. A, prolongation of field potential duration and triangularization. B, arrhythmias induced by E4031 (≥30 nM). C, shortening of field potential duration by diltiazem. D, Increased beat rates of Pluricyte® cardiomyocytes in response to increasing concentrations isoproterenol (0-300 nM) (n=3, mean ±SD).

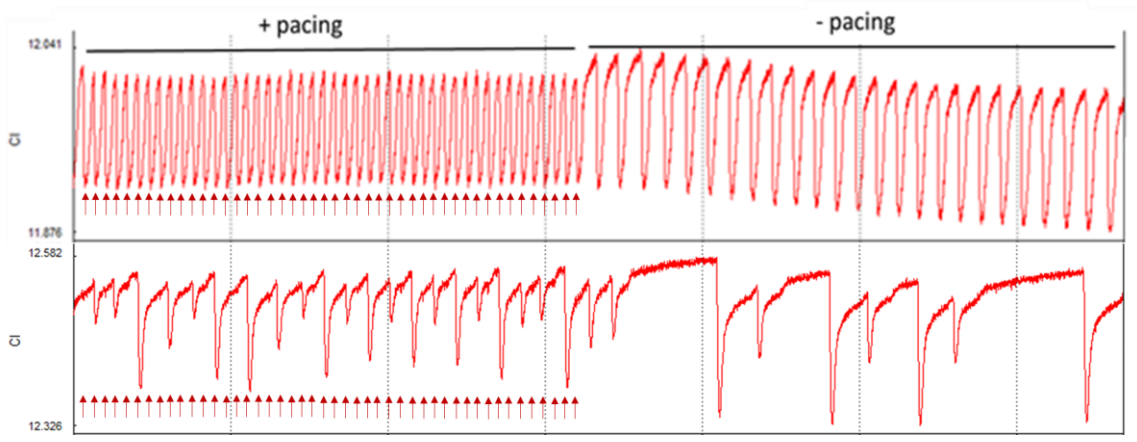


Fig. 4. Pacing of Pluricyte® cardiomyocytes using the xCelligence CardioECR system. Upper panel: Cell Index (CI) of Pluricytes during (+ pacing) and after (- pacing) pacing. Lower panel: Cell Index(CI) of Pluricytes during (+ pacing) and after (- pacing) pacing in the presence of 10 nM E4031. Pacing was performed for 60 sec at 1000 mV, 0.8 Hz. Arrows indicate the stimulation time points.

Table 1. Compound effects on Pluricyte® cardiomyocytes determined using the xCelligence CardioECR system.

Compound class	Name	Observed effect in Pluricyte® cardiomyocytes
hERG blocker	dofetilide	FP prolongation, arrhythmia
hERG blocker	E4031	FP prolongation, arrhythmia
Calcium blocker	diltiazem	FP shortening, decreased cell index amplitude
Calcium blocker	nifedipine	FP shortening, decreased cell index amplitude
Sodium blocker	flecainide	FP amplitude reduced, FP shortening, arrhythmia
β-receptor agonist	isoproterenol	Increased beat rate
Myosin II blocker	Blebbistatin	Decreased cell index amplitude

Conclusions

Pluricyte® cardiomyocytes cultured in PCM show improved maturity, and provide a highly relevant in vitro model to study cardiac safety and efficacy of compounds at an early stage of drug development. Pharmacological responses to various cardioactive compounds could be assessed in detail using the xCelligence CardioECR platform. In addition, the beat rate of Pluricyte® cardiomyocytes can be influenced with the pacing function of the xCelligence CardioECR platform. We conclude that the Pluricyte cardiomyocytes combined with the xCelligence CardioECR platform provide a highly useful tool to investigate proarrhythmic effects of (candidate) drugs *in vitro*.

References

[1] Ribeiro MC et al., Functional maturation of human pluripotent stem cell derived cardiomyocytes in vitro--correlation between contraction force and electrophysiology. Biomaterials (2015) 51:138-50.

Acknowledgments

This work was supported by the Netherlands Genomics initiative, NIRM and Eureka-eurostars.

pluriomics