Improved functional maturation of hiPSC-derived ventricular cardiomyocytes *in vitro*: MEA based electrophysiological characterization

Vlaming MLH[1], Stevenhagen F[1], de Korte T[1], Langenberg K[1], Duńska MI[1], Tertoolen LG[2], Mummery CL[2], Grandela C[1], Braam SR[1]

[1]Pluriomics BV, Galileiweg 8, 2333 BD Leiden, The Netherlands

[2]Dept Anatomy and Embryology, Leiden University Medical Centre, 2300 RC Leiden, The Netherlands

marijn.vlaming@pluriomics.com

Abstract

In drug development it is important to assess cardiac safety of drug candidates. hiPSC-derived cardiomyocytes are increasingly used for this purpose, for example using MEA and/or impedance measurements to investigate drug effects on electrophysiology. 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 (prolonged APD50 and APD90) and gene expression patterns comparable to mature cardiomyocytes. 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 analysed by FACS, as well as by MEA analyses. FACS showed high expression of the ventricular marker MLC2V (>70%) and the cardiac marker TNNT2 (>90%). Furthermore, MEA analysis showed field potentials with a pronounced repolarization peak. This was in line with simulations predicting the cardiac field potential from action potential signals of adult ventricular cardiomyocytes. MEA and impedance analysis of the effects of the cardioactive compounds E4031, diltiazem, nifedipine, isoproterenol and blebbistatin 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® Cardiomyocyte characterization – electrophysiology and morphology

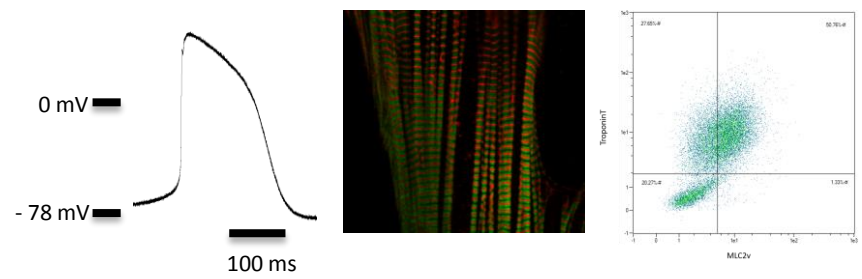

Fig. 1. Characterization of Pluricyte® cardiomyocytes using the perforated patch-clamp technique, immunofluorescence (Red: alpha actinin, green: troponin), and FACS analysis (vertical axis: troponin T, horizontal axis: MLC2V). Note the negative resting potential, fast upstroke velocity and high degree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

Translation of cardiomyocyte action potential signal to a field potential

Whereas for action potential (AP) data it is quite clear how these correspond to the electrophysiology of cardiomyocytes, the interpretation of extracellular field potential (FP) measurements obtained with MEA analysis is less straightforward, nor is it always clear how AP and FP data are related. Therefore, a simulation model that describes this interdependence between AP parameters and experimental FP data was developed (Fig 2).

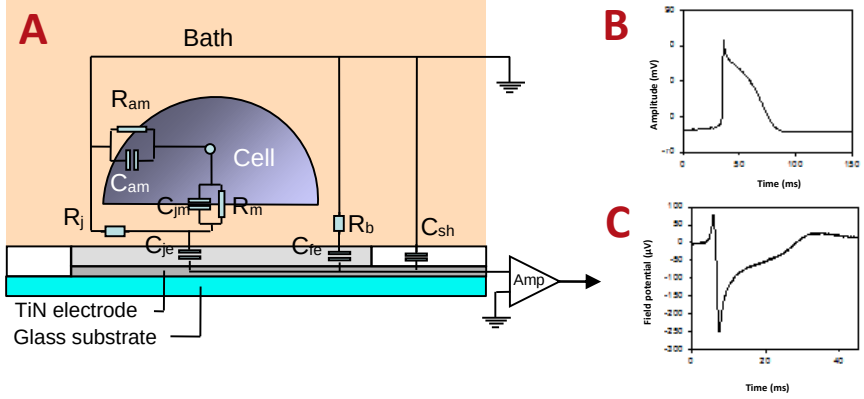

Fig. 2. Model describing the relationship between a FP signal and an AP of a cardiomyocyte. (A) The FP of a cardiomyocyte on a single MEA electrode was described as electronic equivalent circuit, where the main determinant of the circuit is the junction resistance of the basal cell membrane and the MEA electrode. This was described as a resistor-capacitance combination (Rj-Cje). Electronically this functions as a high pass filter and can be simulated by a Chebychev type (second order) high pass digital infinite impulse response (IIR) filter with half maximal cut-off frequency (Fc) of ~ 30 Hz. (B), measured AP (whole cell current clamp) of a spontaneous beating mouse E17.5 cardiomyocyte. (C), result of FP simulation after Chebychev IIR filtering (Fc = 30 Hz) of the AP in panel B. *R_{am}: apical cell membrane resistance to the bath. C_{am}: apical membrane capacitance to the bath. C_{jm}: cell membrane capacitance to junction R_m: membrane resistance to the junction. R_b: bath resistance C_{fe}: electrode capacitance via the bath resistor. C_{sh} : shunt capacitance of the electrode. R_j : junction resistance C_{je}: junction capacitance. I_p: injection point of the action potential (AP) in the simulation circuit. TiN: Titanium Nitrate.*

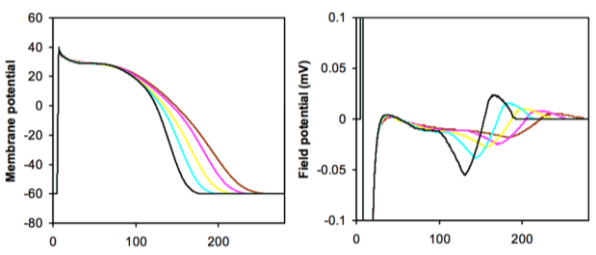

Fig. 3. Simulation of potassium current modulation using the model described in Fig. 2. In this simulation, triangulation of the AP signal (left panel) translates to clear changes in the repolarization peak shape of the FP signal (right panel).

Pluricyte® Cardiomyocyte characterization using MEA – Field Potential, Cell Index and Pacing

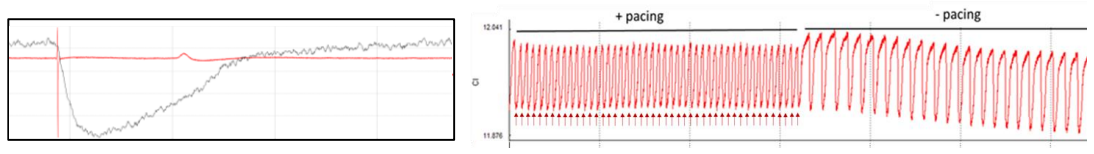

Fig. 4. Characterization of Pluricyte® Cardiomyocytes using the xCelligence CardioECR platform (ACEA Biosciences). Left panel: overlay of the field potential and cell index (CI) of a single waveform of Pluricyte® Cardiomyocytes. Right panel: cell index of Pluricyte® Cardiomyocytes during (+ pacing) and after (- pacing) pacing using the xCelligence CardioECR system. Pacing was performed for 60 sec at 1000 mV, 0.8 Hz. Arrows indicate the stimulation time points.

Compound activity testing with Pluricyte® Cardiomyocytes

Tests with different cardioactive compounds showed expected effects (Figs 5, 6, Table 1). As predicted by the simulation (Fig 3), addition of the hERG blocker E4031 led to a rightward shift and flattening of the repolarization peak (Fig 5A). Also treatment with other cardioactive compounds led to the effects expected for mature human cardiomyocytes (Fig 6, Table 1).

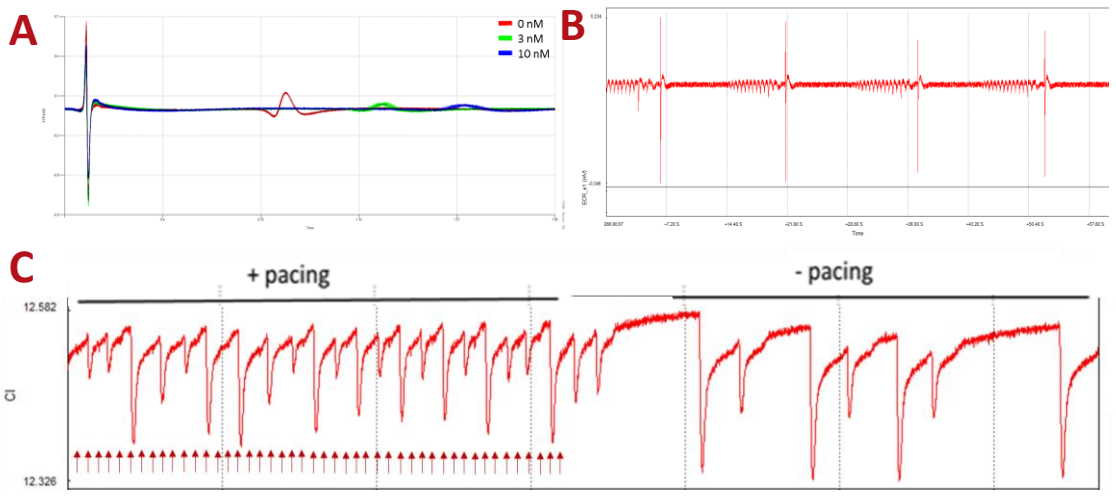

Fig. 5. Pharmacological response of Pluricyte® Cardiomyocytes (30,000 cells/well) to the hERG channel blocker E4031 determined with xCelligence CardioECR MEA analysis. A, prolongation of field potential duration. B, arrhythmias induced by E4031 (≥30 nM). C, cell Index (CI) of Pluricyte® Cardiomyocytes during (+ pacing) and after (- pacing) pacing in the presence of 10 nM E4031.

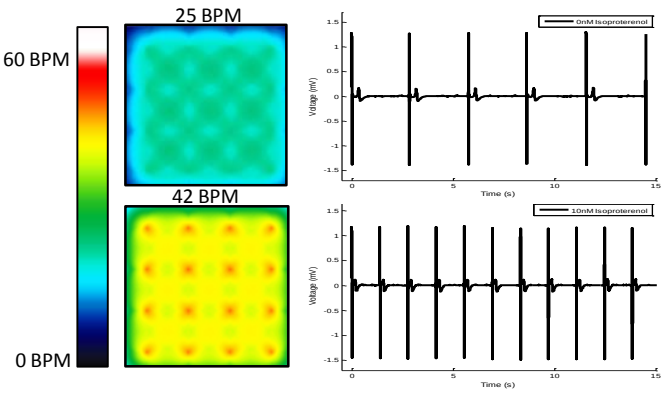

Fig. 6. Effect of 10 nM isoproterenol on beat rate of Pluricyte® Cardiomyocytes (40,000 cells/well) determined with a Maestro MEA system (Axion BioSystems). Left panel: beat rate of Pluricyte® Cardiomyocytes presented by a heatmap. Right panel: electric field potential measurements before and after addition of isoproterenol.

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 channel blocker	diltiazem	Decreased beat rate, decreased cell index amplitude
Calcium channel blocker	nifedipine	Decreased beat rate, decreased cell index amplitude
Sodium channel blocker	flecainide	Depolarization peak amplitude reduced, FP prolongation, decreased beat rate, arrhythmia
β-receptor agonist	isoproterenol	Increased beat rate
Myosin II blocker	Blebbistatin	Decreased cell index amplitude

Conclusions

Pluricyte® Cardiomyocytes show a mature, ventricular phenotype 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 different MEA systems. In addition, the beat rate of Pluricyte® Cardiomyocytes can be influenced by pacing. We conclude that the Pluricyte® Cardiomyocytes combined with relevant pharmacological assay platforms provide a highly useful tool to investigate proarrhythmic effects of (candidate) drugs *in vitro*. Furthermore, our simulations may be helpful with understanding the relationship between electrophysiology and MEA signals, and can be highly useful for prediction of cardiotoxic effects of candidate drugs.

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