High-throughput assessment of drug effects on calcium transients in human induced pluripotent stem cell (hiPSC) derived cardiomyocytes

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Abstract

There is a pressing need for predictive *in vitro* assays suitable for high-throughput screening (HTS) to detect cardioactive effects of compounds early in the drug discovery process. Human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes are a relevant *in vitro* model for this purpose.

We have recently developed fully functional hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes) which exhibit a relatively high level of maturity. This was demonstrated by an increased contraction profile, a highly organized sarcomere organization, as well as improved electrophysiological properties (negative resting membrane potential, well defined action potential plateau and rapid depolarization) as depicted in **Figure 1** [1] and **Figure 5**.

To assess acute cardiotoxicity, we developed a high-throughput drug screening assay by combining the FLIPR Tetra® (Molecular Devices) screening platform with Pluricyte® Cardiomyocytes and a fluorescent calcium dye. After optimization of the assay for application with 384-well plates, we investigated the impact of different cardioactive compounds (e.g. hERG-channel blockers, calcium channel agonists/antagonists and β-adrenergic agonists) on the calcium transients in Pluricyte® Cardiomyocytes. The results obtained with the FLIPR Tetra® screening platform were validated by comparing the calcium transient data to the data obtained by medium-throughput multielectrode array (MEA)/impedance assays.

We conclude that Pluricyte® Cardiomyocytes in combination with calcium flux assays on a high-throughput drug screening system provide a highly relevant *in vitro* assay to study cardiac safety of pharmaceuticals.

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

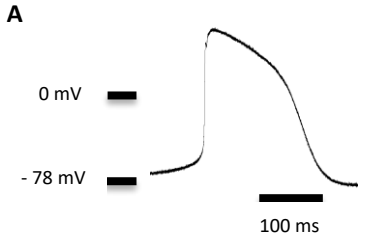

Figure 1. Characteristics of Pluricyte® Cardiomyocytes.
(A) A typical action potential of Pluricyte® Cardiomyocytes, demonstrating a low resting membrane potential and fast upstroke velocity.
(B) Pluricyte® Cardiomyocytes exhibit a high degree of ultra-structural organization of the sarcomeres as determined by immunofluorescence (Green: alpha actinin; Red: Myosin heavy chain 7).

Screening of drug-induced alterations of calcium transients in Pluricyte® Cardiomyocytes using the FLIPR Tetra® platform

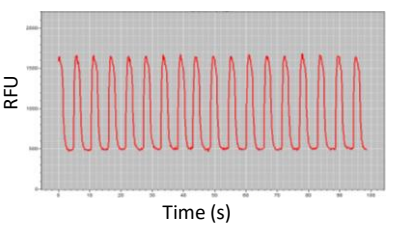

Figure 2. A representative calcium transient fluorescent signal of Pluricyte® Cardiomyocytes. Calcium transient data are obtained from Pluricyte® Cardiomyocytes treated with 0.25% DMSO (negative control) using the FLIPR Tetra® platform. The FLIPR Calcium 6 Assay Kit (Molecular Devices) was used to detect calcium-transients.

Row	[E4031] nM	[Bay K8644] µM	[Nifedipine] nM	[Isoprenaline] nM	[DMSO] %
A	10000	100	10000	1000	1
B	5000	50	5000	500	0.5
C	2500	25	2500	250	0.25
D	1250	12.5	1250	125	0.125
E	625	6.25	625	62.5	0.063
F	313	3.13	313	31.25	0.031
G	156	1.56	156	15.63	0.016
H	78	0.78	78	7.81	0.008
I	39	0.39	39	3.91	0.004
J	20	0.20	20	1.95	0.002
K	10	0.10	10	0.98	9.77E-04
L	4.9	0.05	4.9	0.49	4.88E-04
M	2.4	0.02	2.4	0.24	2.44E-04
N	1.2	0.01	1.2	0.12	1.22E-04
O	0.6	0.006	0.6	0.06	6.10E-05
P	0.3	0.003	0.3	0.03	3.05E-05

Table 1. Experimental set-up. The table shows the final test concentrations of different cardioactive compounds with increasing concentrations from bottom to top. Effects of these compounds on calcium transients in Pluricyte® Cardiomyocytes are presented in **Figure 3**.

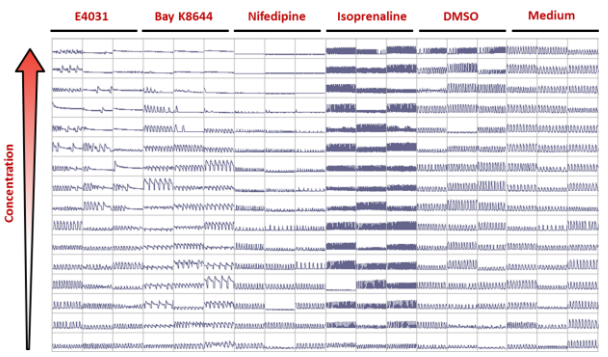

Figure 3. Screenshot of acute compound effects on the calcium transient signals of Pluricyte® Cardiomyocytes.
The measurement was performed directly after compound addition. The screenshot shows a 60 seconds time scale. We could observe:
Proarrhythmic effects: Increasing concentrations of E4031 (hERG channel blocker) resulted in arrhythmia-like events.
Positive inotropic effects: Bay K8644 (calcium channel agonist) caused increased peak amplitudes and increased peak widths.
Negative inotropic effects: Nifedipine (calcium channel blocker) reduced peak amplitudes.
Positive chronotropic effects: Isoprenaline (β-adrenergic receptor agonist) caused an increase in beat rate.

Quantification of drug-induced alterations of calcium transients in Pluricyte® Cardiomyocytes measured by the FLIPR Tetra® platform

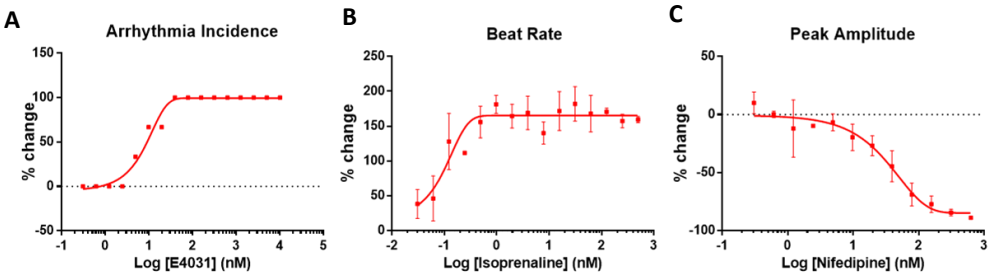

Figure 4. Concentration-response curves of cardioactive compounds determined by measuring very acute alterations in calcium transients of Pluricyte® Cardiomyocytes.
(A) Blocking of the hERG channel with increasing concentrations of E4031 resulted in arrhythmic-like events.
(B) The β-adrenergic receptor agonist isoprenaline induced an increase in beat rate.
(C) Increased concentration of L-type calcium channel blocker nifedipine reduced the calcium transient peak amplitude, eventually leading to complete diminishing of the signal.

Validation of calcium transient data: Compound effects on electrophysiology and contraction of Pluricyte® Cardiomyocytes

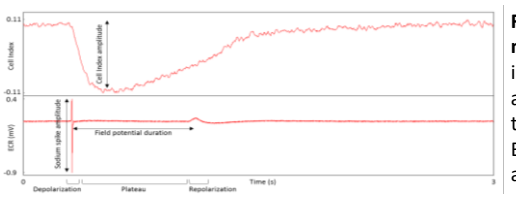

Figure 5. A typical waveform of a Pluricyte® Cardiomyocyte monolayer acquired through MEA analysis. Cell Index (i.e. impedance, a surrogate marker for contractility) signal (**top**) and ECR (i.e. field potential) signal (**bottom**) obtained using the xCelligence® RTCA CardioECR instrument (ACEA Biosciences). The field potential shows a robust sodium spike amplitude and a well-pronounced repolarization peak.

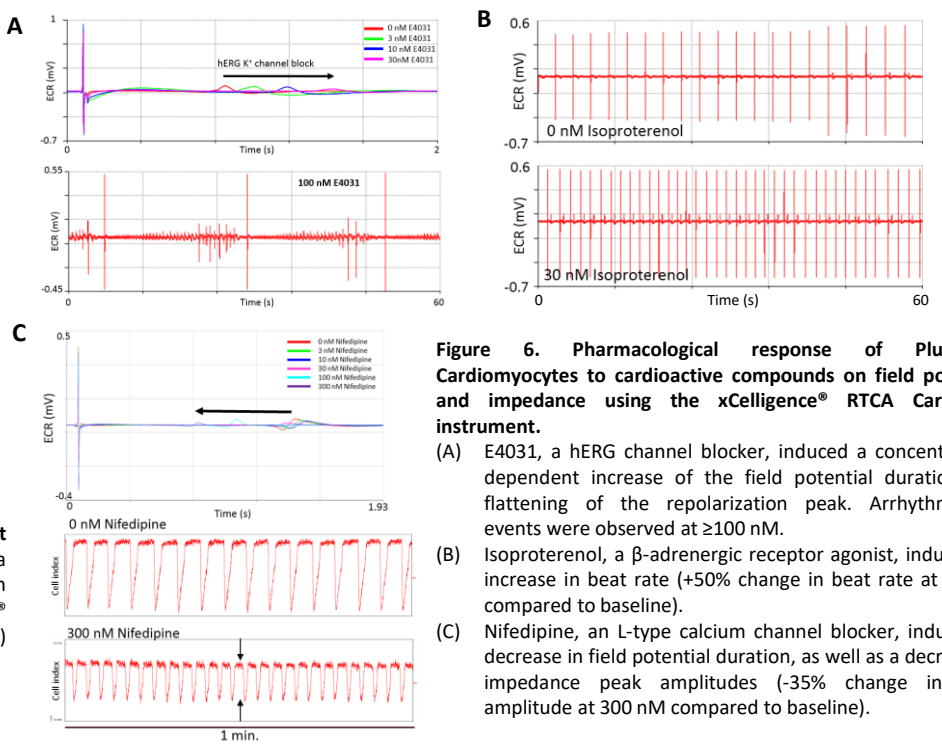

Figure 6. Pharmacological response of Pluricyte® Cardiomyocytes to cardioactive compounds on field potential and impedance using the xCelligence® RTCA CardioECR instrument.
(A) E4031, a hERG channel blocker, induced a concentration-dependent increase of the field potential duration and flattening of the repolarization peak. Arrhythmic-like events were observed at ≥100 nM.
(B) Isoproterenol, a β-adrenergic receptor agonist, induced an increase in beat rate (+50% change in beat rate at 30 nM compared to baseline).
(C) Nifedipine, an L-type calcium channel blocker, induced a decrease in field potential duration, as well as a decrease in impedance peak amplitudes (-35% change in peak amplitude at 300 nM compared to baseline).

Concluding Remarks

- Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium show a ventricular, relatively mature phenotype.
- Pluricyte® Cardiomyocytes can be efficiently used to detect **positive and negative chronotropic and inotropic effects, as well as proarrhythmic effects of test compounds on cardiomyocytes in a high-throughput calcium flux assay.**
- The calcium transient data showed a profile that was in line with the compound effects observed in MEA and impedance assays.
- Calcium-transient analysis in Pluricyte® Cardiomyocytes using the FLIPR Tetra® platform provides a robust high-throughput screening method, which can be used to study cardiac safety of pharmaceuticals and which supports the use of these assays for efficacy screenings.

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 project has received funding from the *European Union's Horizon 2020 research and innovation program* under grant agreement No 726513.