Application of human-induced pluripotent stem cell derived cardiomyocytes in high-throughput screening assays for drug safety and efficacy testing

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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 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, ultra-structural 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** and **Figure 2**. [1]

To assess the potential of Pluricyte® Cardiomyocytes for application in HTS assays (i.e. FDSS/µCELL Functional Drug Screening- and FLIPR Tetra® High-Throughput Cellular Screening assays) we analyzed the effects of different cardioactive compounds (e.g. hERG channel blocker, calcium channel activator, β-adrenergic receptor agonist) on calcium transients and compared these data to well-established, medium-throughput multielectrode array (MEA)/impedance assays.

We conclude that Pluricyte® Cardiomyocytes combined with HTS-compatible assays form a highly relevant model to study pharmacological and toxicological responses of large numbers of drug candidates.

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

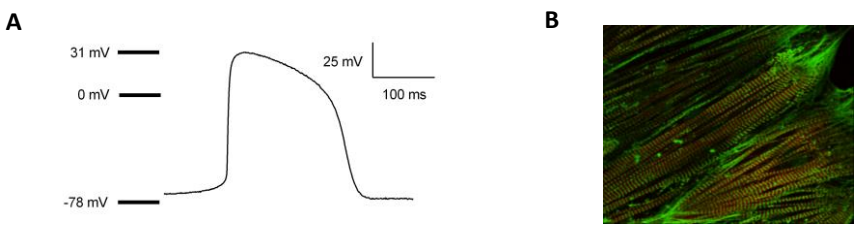

Figure 1. Characteristics of Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium.
(A) A typical action potential of Pluricyte® Cardiomyocytes, demonstrating a low resting membrane potential and fast upstroke velocity.
(B) Pluricyte® Cardiomyocytes exhibit a high degree of ultra-structural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

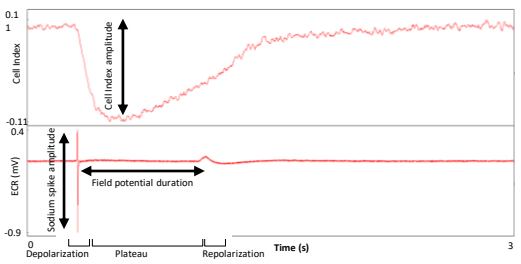

Figure 2. A typical waveform of a Pluricyte® Cardiomyocyte monolayer acquired through MEA/impedance analysis. Cell Index (i.e. impedance, a surrogate marker for contractility) signal (**top**) and ECR (i.e. field potential) signal (**bottom**) enable simultaneous assessment of cardiomyocyte contractility and electrophysiology in a medium-throughput manner. Data was obtained using the xCelligence® RTCA CardioECR instrument (ACEA Biosciences).

Validation of calcium transient data: Compound effects on the impedance and calcium transient fluorescent signals of Pluricyte® Cardiomyocytes

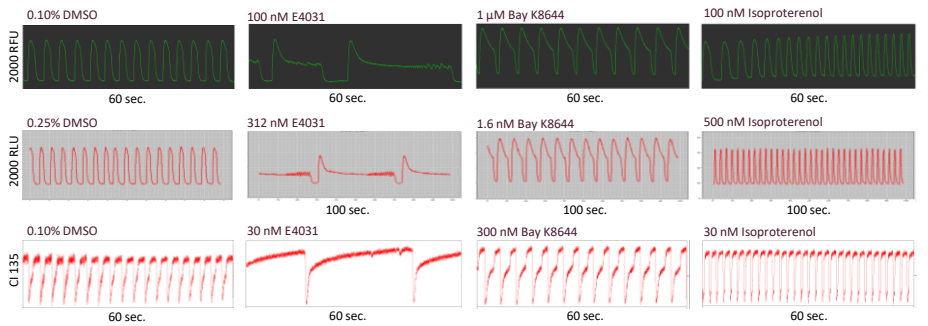

Figure 3. Pharmacological responses of Pluricyte® Cardiomyocytes to cardioactive compounds measured by calcium transient fluorescent signals and impedance signals.
Calcium transient data were obtained using Pluricyte® Cardiomyocytes in combination with the FDSS/µCELL Functional Drug Screening System (**upper panel**) and the FLIPR Tetra® High-Throughput Cellular Screening System (**middle panel**) using the Calcium 6 Assay Kit (Molecular Devices). Both systems display calcium transient patterns that are in line with the impedance signals measured using the xCelligence® RTCA CardioECR instrument (**lower panel**). Positive inotropic effects (Bay K8644), positive chronotropic effects (Isoproterenol) as well as proarrhythmic effects (E4031) were detected in both, calcium flux assays and impedance assays.

High-throughput screening of drug-induced alterations in calcium transient fluorescent signals of Pluricyte® Cardiomyocytes using the 384-well FLIPR Tetra® screening system

Row	[E4031] nM	[BayK 8644] µM	[Nifedipine] nM	[Isoproterenol] 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: E4031 (hERG channel blocker); Bay K8644 (calcium channel agonist), Nifedipine (calcium channel blocker); Isoproterenol (β-adrenergic receptor agonist) with increasing concentrations from bottom to top. Effects of these compounds on calcium transients in Pluricyte® Cardiomyocytes are presented in **Figure 4**.

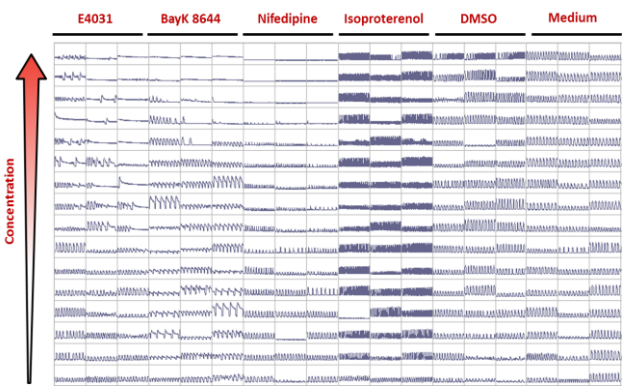

Figure 4. Screenshot of acute compound effects on the calcium transient fluorescent signals of Pluricyte® Cardiomyocytes in 384-well format.
The measurement was performed directly after compound addition. The screenshot shows a 60 seconds time scale. In line with the 96-well screening platforms, we could observe:
Proarrhythmic effects: Increasing concentrations of E4031 resulted in arrhythmia-like events.
Positive inotropic effects: Bay K8644 caused increased peak amplitudes and increased peak widths.
Negative inotropic effects: Nifedipine reduced peak amplitudes.
Positive chronotropic effects: Isoproterenol caused an increase in beat rate.

Quantification of drug-induced alterations of calcium transient fluorescent signals in Pluricyte® Cardiomyocytes using the 384-well FLIPR Tetra® screening system

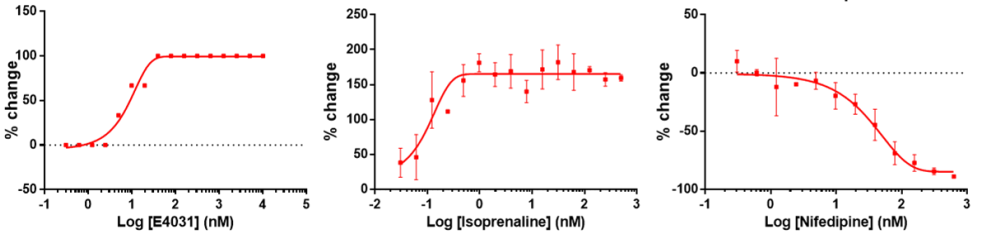

Figure 5. Concentration-response curves of cardioactive compounds determined by measuring very acute alterations in calcium transients of Pluricyte® Cardiomyocytes using the 384-well FLIPR Tetra® screening system.
(A) Blocking of the hERG channel with increasing concentrations of E4031 resulted in arrhythmic-like events.
(B) The β-adrenergic receptor agonist Isoproterenol 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.

Concluding Remarks

- Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium show a ventricular, relatively mature phenotype.
- The calcium transient data using the 96-well as well as the 384-well calcium flux assays could successfully capture the relevant cardioactive effects of the measured test compounds and showed a profile that was in line with the compound effects observed in impedance assays.
- Pluricyte® Cardiomyocytes can be efficiently used to detect positive chronotropic and inotropic effects, as well as proarrhythmic effects of test compounds in a high-throughput calcium transient assay.
- HTS-compatible assays in combination with Pluricyte® Cardiomyocytes form highly useful tools to study pharmacological responses of large numbers of drug candidates. Implementation of such tools in drug efficacy studies could further improve the predictivity of early phase drug 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

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Stem cell experts