Application Note FLEXcyte 96

Lot-to-lot robustness study of commercial human iPSC-derived cardiomyocytes

New approach methodologies (NAMs) employing human-based cell types hold great promise for improving drug attrition rates early in the drug development process[1]. Advantages of these approaches over current animal-based testing methods encompass functional as well as financial reasons, such as elimination of the animal-to-human translational gap and increased throughput for cost-efficient data generation.

Cardiac-related side effects display one of the major causes of high drug attrition rates, demonstrating the urgent need of the cardiovascular field for integrating human-relevant platforms to reliably analyse preclinical safety risks[2]. Commercial human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) represent an ideal cell model for this matter with constant availability of well characterized cardiomyocytes[3]. Nevertheless, consistent functional behaviour amongst different lots needs to be assessed adequately to prove this cell models' reliability for the use in preclinical drug development.

Here we demonstrate the robustness of hiPSC-CMs (iCell® Cardiomyocytes[2], FCDI) on the FLEXcyte 96 system, a NAM that mimics physiological human heart conditions with flexible membranes as substrates for the cells on a 96-well plate[4,5]. Cardiac contraction behaviour of ten hiPSC-CM lots was assessed before and after compound treatment with nifedipine and sotalol, not only demonstrating the reliability of this cell model, but also the robustness and combined power of hiPSC-CMs and the FLEXcyte 96 system.

Fig. 1

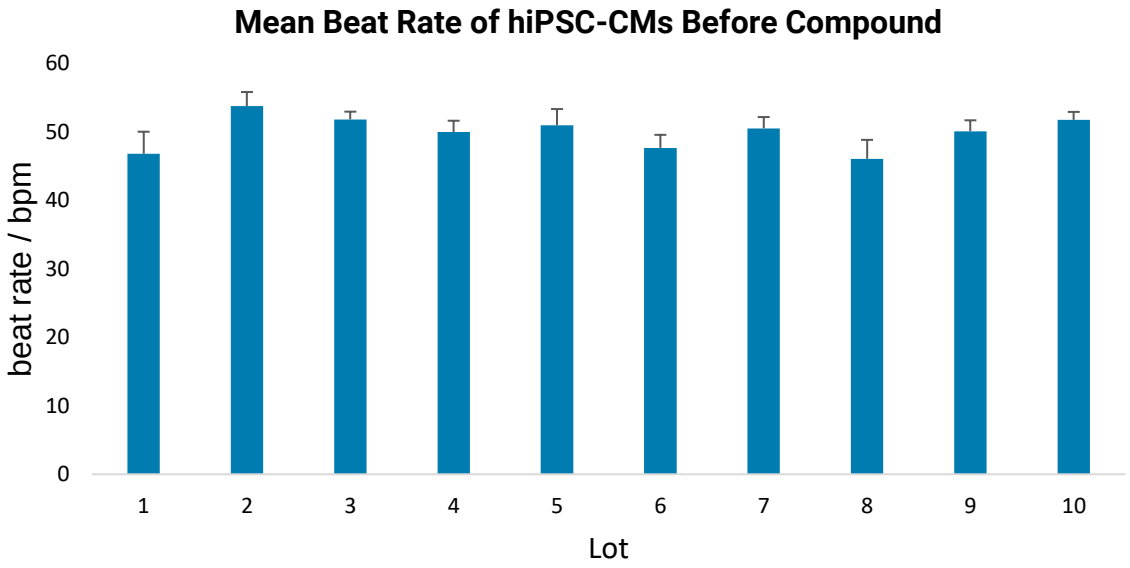

Figure 1. Mean beat rate of hiPSC-CMs (iCell® CM², FCDI) on FLEXcyte 96 plates before compound addition. Ten different lots collected over a time span of 1 year were assessed for beat rate after 6 days in culture. Bar graph shows the beat rate/bpm (beats per minute) of the lots with only minor fluctuations ranging from 47 bpm – 53 bpm.

Fig. 2

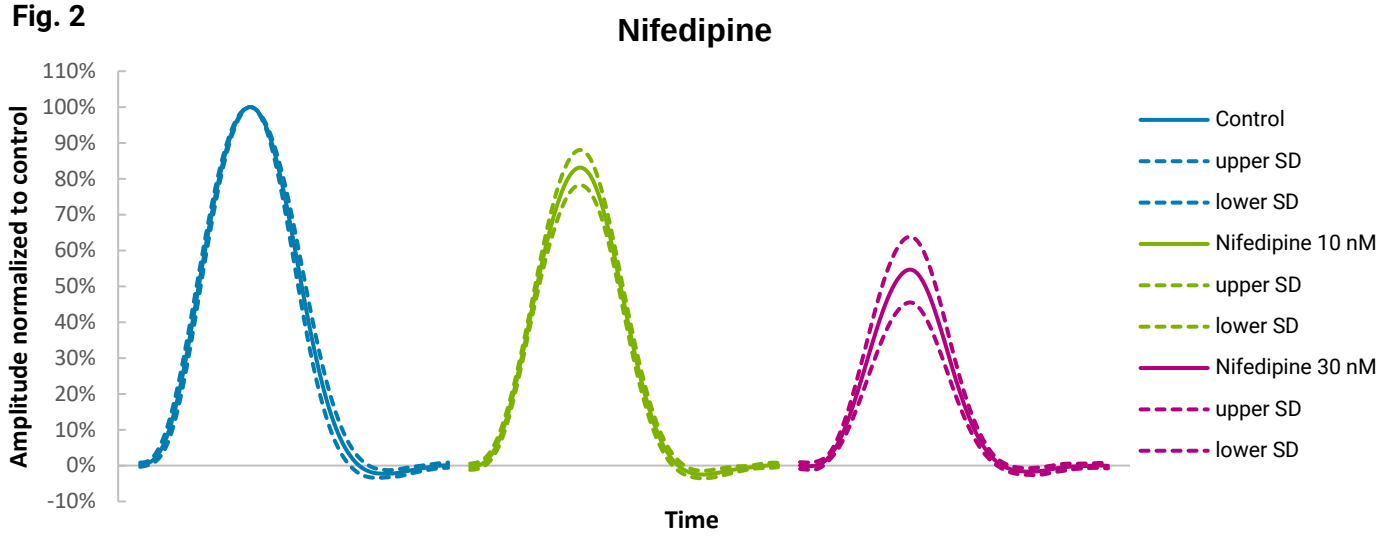

Figure 2. Amplitude of hiPSC-CMs (iCell® CM²) analyzed with the FLEXcyte 96 system after compound treatment with nifedipine. Control condition (blue) is normalized to 100%. Nifedipine concentrations of 10 nM (green) and 30 nM (pink) are shown. Dotted lines in respective colors represent standard deviations.

Results

Over a time course of one year, iCell® CM² vials from ten different lots were cultured on FLEXcyte-96 plates according to manufacturers' guidelines. The cells were maintained in culture for 6 days to allow proper syncytium formation. To analyze the stability of the general beating behaviour amongst the lots, contractility was assessed with the FLEXcyte 96 system. The data demonstrates a stable performance of each lot with similar beating behaviour ranging from 47 – 53 bpm (Fig.1).

After contraction analysis of iCell® CM², acute drug-induced effects were analyzed using two concentrations of gold standard compounds nifedipine and sotalol, respectively. Amplitude of contraction force and beat duration served as contractility-related parameters to determine the functional behaviour of hiPSC-CMs upon compound treatment. Treatment with calcium channel blocker nifedipine showed a concentration-dependent decrease in mean contraction amplitude from 1.0 (control) to 0.8 ± 0.05 upon 10 nM, and a decrease to approx. 0.5 ± 0.09 upon 30 nM nifedipine treatment.
The low variability in between lots is shown by the standard deviation not exceeding 10% (Fig. 2).

Additionally, assessing the effect of nifedipine on the beat duration further demonstrates the functional consistency of iCell® CM², as a concentration-dependent shortening of the beat duration[6] was observed for 10 nM and 30 nM nifedipine, respectively (Fig. 3).

Fig. 3

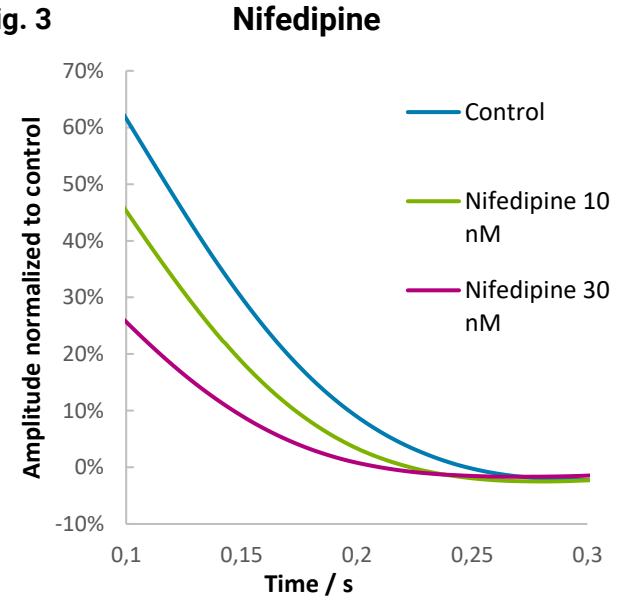

Figure 3. Nifedipine effect on iCell® CM² downstroke duration. 10 nM (green) and 30 nM (pink) nifedipine concentrations are shown compared to the control (blue). Higher concentrations of nifedipine lead to a shortening in beat duration. (Extract of downstroke duration is depicted).

Fig. 4

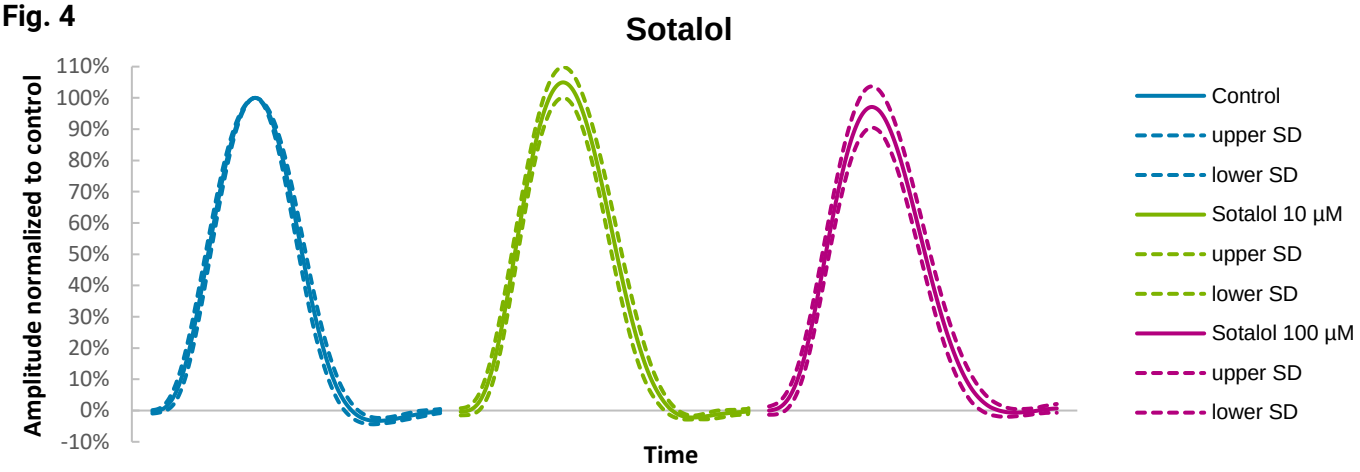

Figure 4. Amplitude of iCell® CM² treated with sotalol. Mean amplitudes are shown after 10 µM (green) and 100 µM (pink) sotalol treatment compared to the control condition (blue) set to 100%. Dotted lines in respective colors represent standard deviations.

The effect of hERG channel blocker sotalol on ten iCell® CM² lots was also carried out with focus on amplitude, beat duration and respective standard deviations. As drug-treatment occurs in serum-free buffer, insignificant fluctuations of the mean contraction amplitude at 10 µM and 100 µM sotalol were observed, presumably as a result of a reduced/missing ß-adrenergic pre-stimulation. The low standard deviations not exceeding 10 % demonstrate once more the robust reaction window of the cells (Fig.4).

Fig. 5

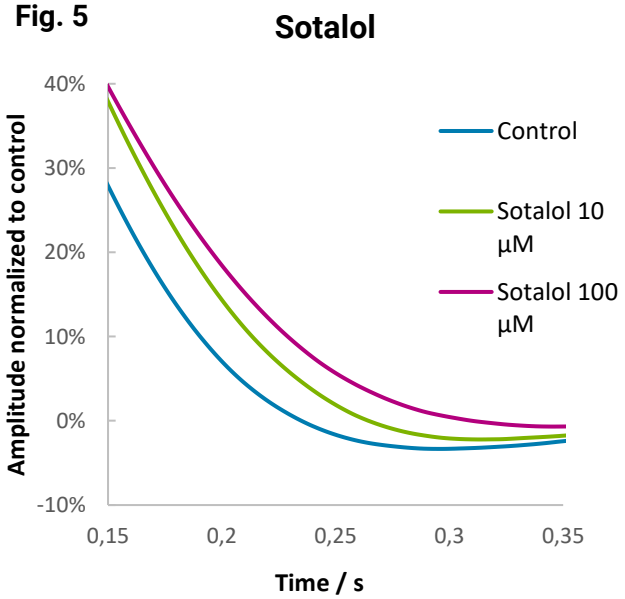

Figure 5. Sotalol effect on iCell CM² downstroke duration. 10 µM (green) and 100 µM (pink) sotalol concentrations are shown compared to control (blue). Higher concentrations of sotalol lead to a prolongation in beat duration. (Extract of downstroke duration is depicted).

The known torsadogenic risk potential of sotalol[7] could be shown with the analyzed beat duration after 10µM and 100 µM treatment. Here, a concentration-dependent duration prolongation was detected representing the contractile response to QT-prolongation (Fig.5).
Together, the data demonstrates the robustness of this cell-based assay system for reliable preclinical cardiotoxicity assessment, uniting cost effective high-throughput analysis with human-based cardiomyocytes.

Methods

iCell® CM² were cultured on FLEXcyte 96 well plates in 200 µL maintenance medium per well. Cells were seeded 6 days before compound treatment at 100k (iCell® CM², FCDI) per well to allow proper monolayer and network formation. A final media change was conducted 4-6 hours before drug application.

Measurements were performed acutely over a period of 20 min after compound addition. The CardioExcyte / FLEXcyte Control software enables direct analysis of contractility parameters. An adaptive signal detection algorithm extracts the positions and values of beating events. Beat intervals, amplitudes, rising and falling time, pulse widths are detected as well as integrals and arrhythmia.

References

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[4] Gossmann et al., J Pharm Tox M. 105, 106892, (2020).

[5] Lickiss et al., *J. Vis. Exp*. 188, (2022).

[6] Guo et al., Cell Physiol Biochem. 27 (5), 453-62 (2011).

[7] Ando et al., J Pharm Tox M. 84, 111–127, (2017).

Notes

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