Chronic cardiac contractility assessment of human iPSC-derived cardiomyocytes in a pro-maturation environment for preclinical cardiac safety and toxicity studies

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Abstract

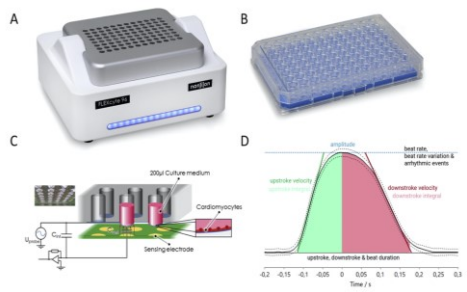
In pre-clinical drug development, cardiac contraction analysis of potential drug candidates is one of the crucial steps to ensure a successful and reliable transition to clinical stages. The use of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) continues to increase in the assessment of safety and toxicological side effects of newly developed compounds, due to their reproducibility and low ethical concern. However, their premature phenotype causes issues concerning non-physiological responses for preclinical drug development. Moreover acute testing within limited timescales (min to h) after compound application remains the primary application so far, partly due to the inability of common cell-based assays to analyze cellular behavior reliably over prolonged periods of time.

The aim of this study was to evaluate the applicability of hiPSC-CM contractility measurements for safety and chronic toxicological assessment using the high-throughput FLEXcyte 96 system. 15 kinase inhibitors and 3 anthracyclines with well-known cardiotoxic profiles were selected to evaluate the reproducibility of clinical data with the FLEXcyte system over a time span of five days. To underline the pro-maturation effect of the technology, previously shown treatment of hiPSC-CMs with positive inotropic compounds exhibited functionally mature cardiomyocyte responses. Additional immunostainings of pronounced filamentous actin further supports this pro-maturation effect on a phenotypic level.

Technology

The FLEXcyte technology is based on a special 96 well plate that contains high-precision, ultra-thin and hyper-elastic silicone membranes instead of stiff plastic surfaces as basis for human iPSC-CMs (Fig.1B). This FLEXcyte 96 plate is analysed in the FLEXcyte 96 device (Fig.1A), an add-on system for the CardioExcyte 96 (Nanion Technologies).

Figure 1. A-D FLEXcyte Technology



While being deflected by the weight of the culture medium, rhythmic contraction of the cardiomyocytes lifts the membranes in the 96-well upwards. These changes in deflection are quantified by means of capacitive distance sensing (Fig.1C). The unique Mean Beat Function of the software automatically visualizes the average beat of traces from one well per sweep, enveloped by the standard deviation. Additional parameters like amplitude, rising and falling times as well as beat duration are analysed via the obtained mean beat while the beat rate is examined separately (Fig.1D) (Gossmann et al., 2016, Gossmann et al., 2020).

Workflow

Human iPSC-CMs (iCell® CM², Fujifilm Cellular Dynamics) are cultured on FLEXcyte 96 well plates at 100k per well according to manufacturers' guidelines in 200 µL maintenance medium. Cells are seeded 6 days before compound treatment to allow proper monolayer and network formation. A final media change is conducted 4-6 hours before drug application. For the experiments, 50 µL of the cell culture medium is removed and replaced with 50 µL medium containing 4x concentrated compound, resulting in the desired final compound concentration. Measurements are performed between 1 and 5 days. (Fig.2)

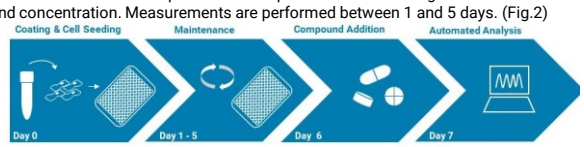
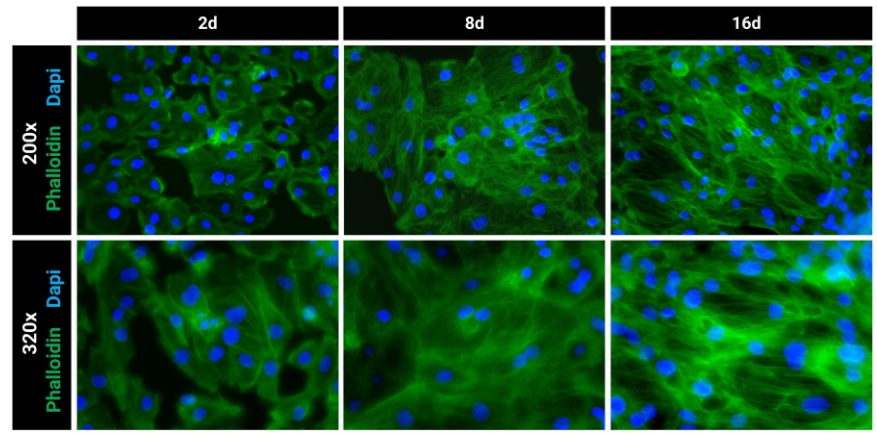


Figure 2. Workflow

Figure 3. Immunocytochemical staining of human iPSC-derived cardiomyocytes plated on FLEXcyte 96 plates



Human iPSC-CMs (iCell® CM², Fujifilm Cellular Dynamics) were cultured on FLEXcyte 96 well plates at 50k per well and stained for f-actin with Phalloidin (Alexa Fluor 488 Phalloidin, Thermo Fisher) and cell nuclei with DAPI (Invitrogen). Immunocytochemical stainings were performed 2, 8 and 16 days after plating to visualize filamentous actin formation over time in a physiological environment given by the FLEXcyte 96 well plates. The upper panel shows image sections at 200x magnification, the lower panel at 320x magnification.

Figure 4 A-D Chronic Cardiotoxicity assessment of human iPSC-derived cardiomyocytes with the FLEXcyte 96 technology

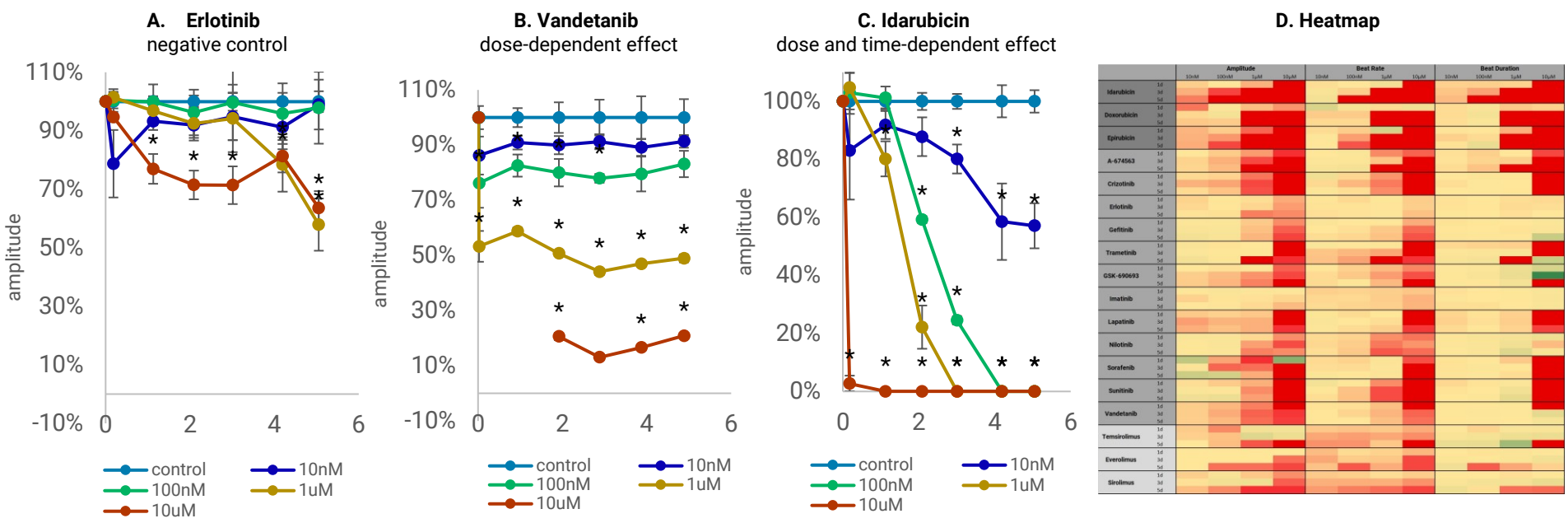


Figure 4 A-C. Chronic cardiotoxic effects of kinase inhibitors and anthracyclines. Amplitude of hiPSC-CMs (iCell® CM2, FCDI) cultured on FLEXcyte 96 well plates after treatment with kinase inhibitors erlotinib (A), vandetanib (B) and anthracycline idarubicin (C). Graphs show dose and time-dependent effects on hiPSC-CMs over the five days incubation span. Graphs represent mean ± SEM. Asterisks represent statistical significance with $p < 0.05$ (*) or $p < 0.01$ (**) (Wilcoxon-Mann-Whitney test, n = 4).
Figure 4 D. Heatmap of chronic cardiotoxic effects of kinase inhibitors and anthracyclines. hiPSC-CMs analysed after 1 d, 3 d and 5 d of compound treatment on the FLEXcyte 96. Shown parameters are amplitude, beat rate and beat duration. The heat map colours indicate increasing effects (green) of hiPSC-CMs, stable conditions (yellow) as well as decreasing reactions (red) up to ceasing effects (deep red). Erlotinib, imatinib, everolimus, sirolimus and temsirolimus are known compounds with low cardiotoxic potential and served as negative control. Anthracyclines are highlighted in dark grey, TKIs in grey and mTOR inhibitors in light grey.

Results

In total, 15 kinase inhibitors and 3 anthracyclines were analysed for chronic cardiotoxic side effects using human iPSC-CMs on the FLEXcyte 96. Known cardiotoxic anthracyclines such as doxorubicin and epirubicin show expected toxic effects, ranging from the reduction in contractility at nanomolar concentrations to ceased beating at micromolar concentrations (Fig.4D deep red). Negative controls with known low cardiotoxic risk such as erlotinib, imatinib, everolimus, sirolimus and temsirolimus only showed toxic side effects at super-therapeutic concentrations in a time-dependent manner (Fig.4D).

Erlotinib, generally regarded as non-cardiotoxic, had a minor dose and time-dependent effect on hiPSC-CMs only at concentrations in the micromolar range, probably based on general rather than cardiac-specific functional toxicity (Sharma et al., 2017) (Fig.4A). Vandetanib, with known cardiac safety issues (black box FDA cardiotoxicity warning), showed a dose-dependent effect on the contractility of hiPSC-CMs from 2 h of incubation, most probably due to its QT-prolonging properties (Lee et al., 2018) (Fig.4B).The effect of idarubicin, an anthracycline chemotherapy agent, was both time and dose-dependent with progression profiles of different concentrations (Fig.4C).

Functionally adult-like reactions of hiPSC-CMs upon positive inotropic compounds (e.g. isoproterenol, S-Bay K8644 and omecamtiv mecarbil) cultured on physiological FLEXcyte plates were shown before (Gossmann et al., 2020) and underlined here with a phenotypic characterization via immunostainings for f-Actin that show a more pronounced actin filament organisation of hiPSC-CMs over time when plated on FLEXcyte 96 plates.

Conclusion

The pro-maturation effect of the FLEXcyte system on hiPSC-CMs on functional (Gossmann et al., 2020) and phenotypic level in combination with chronic cardiotoxic progression profiles of anthracyclines and TKIs assessed, indicate the suitability of the FLEXcyte technology for (sub)chronic safety and toxicity evaluation of new drug candidates.
The combination of human iPSC-CMs and the FLEXcyte 96 technology allows for cardiac risk assessment using a predictive human cell model on a high-throughput format. The FLEXcyte technologies' comprehensive goal on a larger scale is to advance translational studies for contractile cardiotoxicity, replace/minimize animal use in drug development, and reduced risk of adverse cardiac side effects in clinical trials.

References

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Sharma et al., 2017 High-throughput screening of tyrosine kinase inhibitor cardiotoxicity with human induced pluripotent stem cells.
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