Tackling Chronic Compound Responses of hiPSC-CMs for Preclinical Cardiac Risk Evaluation: Defined Serum-Free Medium and Long-Term Culture on the FLEXcyte 96

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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, the obstacles of an immature phenotype as well as the need of serum-containing media for chronic assays raise concerns over non-physiological responses in preclinical drug development. To solve this issue, a variety of methods are currently being tested to foster hiPSC-CM maturation in vitro and to conduct chronic studies without the need of serum containing medium.

Here we assessed the effects of relatively defined medium (serum-free) and prolonged cell culture times on iCell® Cardiomyocytes² maturation with focus on functional contractile properties using the FLEXcyte 96 technology. Analysis of iCell Cardiomyocytes² contractile properties cultured in iCell Cardiomyocytes Serum-free Medium compared to being cultured in regular serum-containing iCell Cardiomyocytes Maintenance Medium showed similar results for most parameters and expected effects of gold standard compounds assessed over 5 days.

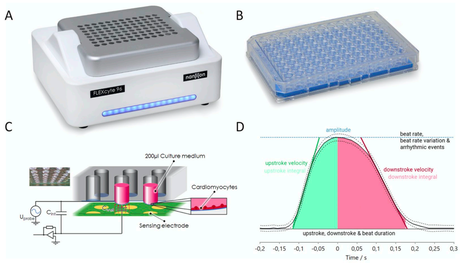
In addition, iCell Cardiomyocytes², cultured on FLEXcyte 96 plates and analyzed for 40 days, showed an increase in amplitude and a decrease in beat rate over time, indicating the pro-maturation effect of a physiological environment over time.

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. This FLEXcyte 96 plate is analysed in the FLEXcyte 96 device (Fig.1A), an add-on system for the CardioExcyte 96 (Nanion Technologies).

In the FLEXcyte 96-well plate (Fig.1B), the cells adhere as monolayers on flexible substrates. 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).

Figure 1. FLEXcyte 96 Technology



Methods: Workflow of iCell Cardiomyocytes² on FLEXcyte 96 System

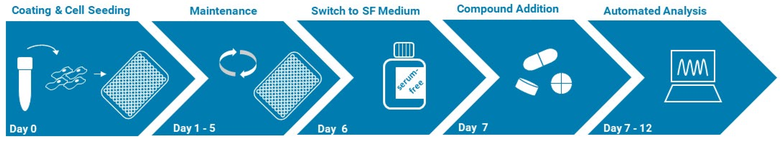


Figure 2. *Human iPSC-CMs (iCell Cardiomyocytes², 01434 FUJIFILM Cellular Dynamics, Inc.) were cultured on FLEXcyte 96 well plates at 100,000 cells per well according to manufacturers' guidelines in 200 µl serum containing maintenance medium. Cells were seeded and cultured for 6 days to allow proper monolayer and network formation. A final media change was conducted 24 hours before drug application for half the plate. For compound treatment, 50 µl of the cell culture medium was removed and replaced with 50 µl medium containing 4x concentrated doxorubicin or erlotinib, resulting in the desired final compound concentration. Measurements were performed for a maximum of 5 days.*

Results

Analysis of iCell Cardiomyocytes² Contractile Parameters Cultured in iCell Cardiomyocytes Serum-free Medium on the FLEXcyte 96 System.

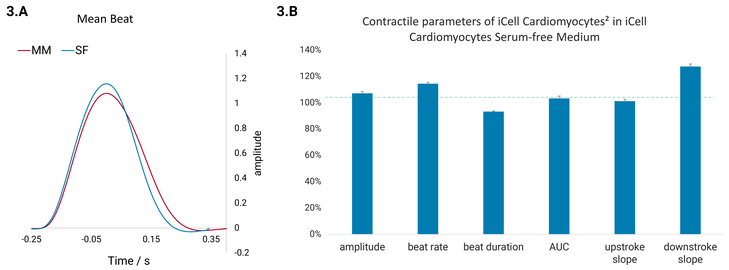
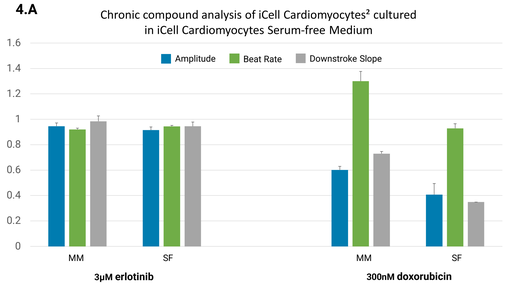
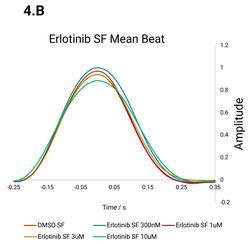


Figure 3. (A) *Comparison of mean beats are shown of iCell Cardiomyocytes² cultured in iCell Cardiomyocytes Serum-free Medium (SF) and serum containing iCell Cardiomyocytes Maintenance Medium (MM) 24 hours after medium switch.* ***(B)*** *A bar graph showing contractile parameters (amplitude, beat rate, beat duration, area under curve, upstroke and downstroke slopes) of iCell Cardiomyocytes² cultured in iCell Cardiomyocytes Serum-free Medium after 24 hours. Data is normalized to iCell Cardiomyocytes² cultured in iCell Cardiomyocytes Maintenance Medium.*

Chronic Compound Analysis of iCell Cardiomyocytes² Cultured in iCell Cardiomyocytes Serum-free Medium on the FLEXcyte 96 System.





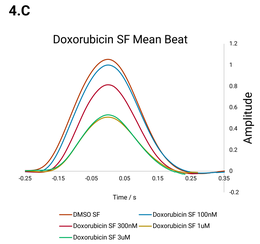


Figure 4. (A) *Bar graph of iCell Cardiomyocytes² cultured in iCell Cardiomyocytes Serum-free Medium (SF) on FLEXcyte 96 plates and treated with gold standard compounds Erlotinib (3µM) and Doxorubicin (300nM) for 5 days. Depicted bar graph results show amplitude, beat rate and downstroke slope reactions after 48 hours. Data is normalized to iCell Cardiomyocytes² cultured in iCell Cardiomyocytes Maintenance Medium (MM)* ***(B-C)*** *Comparison of mean beats are shown of iCell Cardiomyocytes² treated with erlotinib (300nM – 10µM) and doxorubicin (100nM – 3µM) in iCell Cardiomyocytes Serum-free Medium. Data represents mean beat of iCell Cardiomyocytes² reactions 24 hours after compound treatment.*

Analysis of Maturity Indicators Amplitude and Beat Rate of iCell Cardiomyocytes² Cultured on FLEXcyte 96 Plates Over 40 Days.

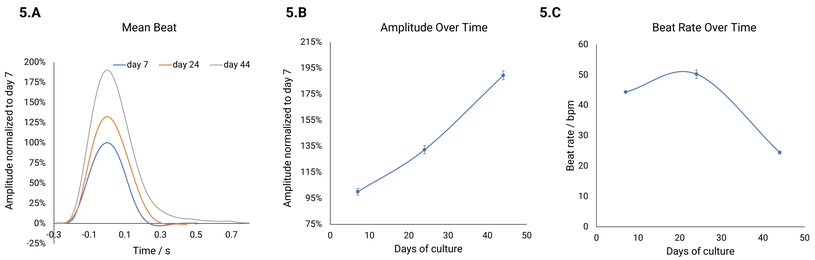


Figure 5. (A) *Graph shows mean beat comparison of iCell Cardiomyocytes² cultured for 7, 24 and 44 days on FLEXcyte 96 plates. Data is normalized to amplitude on day 7. (B) Graph shows rising amplitude of iCell Cardiomyocytes² over a time span from 7 – 44 days in culture. Data is normalized to amplitude on day 7. (C) Graph shows beat rate of iCell Cardiomyocytes² cultured with Geltrex on FLEXcyte 96 plates over a time span of 7 – 44 days.*

Summary

- iCell Cardiomyocytes Serum-free Medium for chronic cardiotoxic studies and prolonged cell culture times of iCell Cardiomyocytes² on FLEXcyte 96 plates to promote further maturation *in vitro* were successfully assessed.
- A defined iCell Cardiomyocytes Serum-free Medium was successfully assessed using iCell Cardiomyocytes², with a goal to find a solution for scientific issues arising from working with serum or serum free culture media; the presence of serum has the potential to alter the uptake and relative potencies of certain compounds, while lack of serum eliminates a protein source necessary for cardiomyocytes to keep stable conditions over prolonged cell culture times.
- iCell Cardiomyocytes Serum-free Medium did not alter most contractile properties of the cells assessed with the FLEXcyte 96 technology.
- An increase in the downstroke slope, referring to the relaxation phase of the contraction was detected, indicating a higher sensitivity towards intracellular calcium levels.
- Cardiosafe (erlotinib) and cardiotoxic (doxorubicin) compound treatment using iCell Cardiomyocytes² showed expected human relevant reactions over a time span of 5 days, indicating an ideal serum-free medium composition to keep the cells stable for chronic cardiac risk assessment.
- Additionally, by applying known maturation promoting cues, such as time and a physiological environment (flexible membranes of the FLEXcyte 96 plates), one can reliably increase the amplitude and decrease the beat rate of iCell Cardiomyocytes² over time. Both which are well known indicators for cardiomyocyte maturation (Yuxuan et al., 2020).

Conclusions

The displayed adult-like hiPSC-CM contractile parameters upon prolonged cell culture time further underline the known Gossmann et al., 2020) pro-maturation effect of the physiological environment created by the flexible membranes and the technologies potential for long-term cultures of human iPSC-CMs.

Serum-free chronic cardiotoxic compound analysis of iCell Cardiomyocytes² assessed on the FLEXcyte 96, demonstrates the combined power of iCell Cardiomyocytes² and the FLEXcyte technology for (sub)chronic safety and toxicity evaluation of new drug candidates.

References

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Yuxuan G., William T. P. Circulation Research. 2020;126:1086–1106