Lot-to-lot consistency study of commercial human iPSC-derived cardiomyocytes regarding contractile properties

Bettina Lickiss[1], Matthias Gossmann[1], Peter Linder[1], Sonja Stoelzle-Feix[2], Ouissame Filali[3], Ravi Vaidyanathan[3] and Niels Fertig[2]

[1]innoVitro GmbH, Juelich, Germany, [2]Nanion Technologies GmbH, Munich, Germany and [3]FUJIFILM Cellular Dynamics International, Inc., Madison, WI, USA

ABSTRACT

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) serve as an ideal human-based cell model for assessing preclinical cardiac risk of new drug candidates. The combination of hiPSC-CMs and modern cell-based assay systems allows animal-free evaluation of the main cardiac endpoints (electrophysiological properties, calcium handling and contractility) that are addressed during preclinical drug development. Commercial hiPSC-CMs play an important role as stable source for preclinically involved cell-based assays, nevertheless stable lot-to-lot consistency is needed to assess potential hazardous side effects reliably.

Here we demonstrate the lot-to-lot consistency of commercial hiPSC-CMs (iCell® Cardiomyocytes², FCDI) functionally assessed with the FLEXcyte 96 technology regarding their contractile behavior upon treatment with gold standard compounds nifedipine and sotalol.

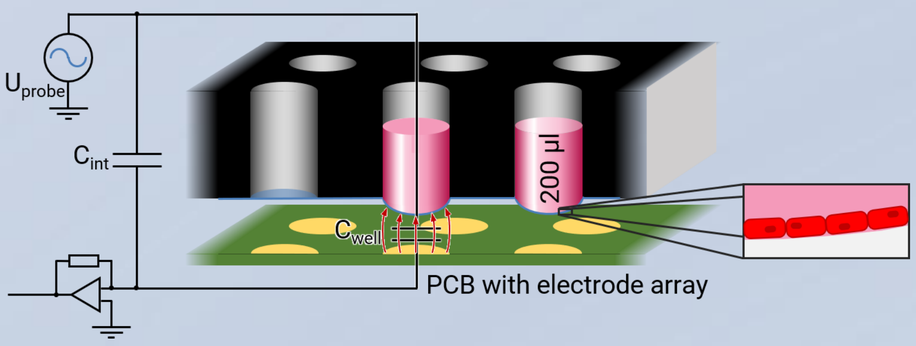
The cells were cultured on FLEXcyte plates for 6 days before compound treatment. A control measurement was performed before compound addition to evaluate general cardiomyocyte contraction behavior and compared among different cell lots regarding two parameters - contraction amplitude and beat rate. Cells were then treated acutely with two different concentrations of nifedipine and sotalol. Changes in amplitude or duration as well as beat rate were analysed and compared among different cell lots.

METHODS

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, an add-on system for the CardioExcyte 96 (Nanion Technologies).

In the FLEXcyte 96-well plate, the cells attach as monolayers to 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.1A).

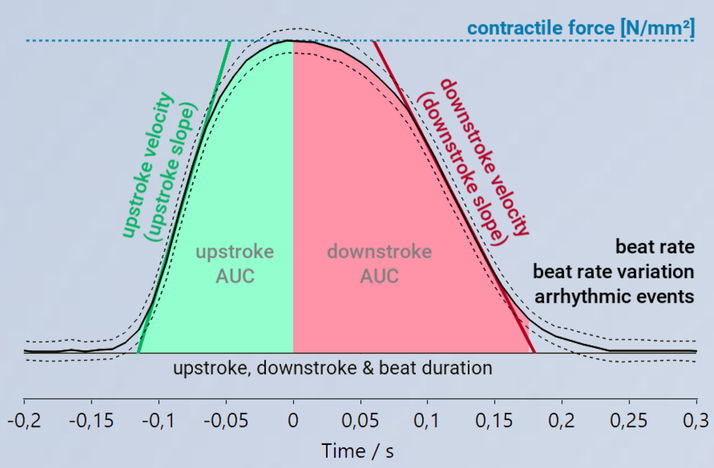
Figure 1.A



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. Parameters measured are (Fig.1B):

- Amplitude of Contraction Force (N/mm²)
- Beat Duration
- Upstroke and Downstroke Velocity
- Upstroke and Downstroke Area under Curve (AUC)
- Beat Rate
- Beat Rate Variations
- Arrhythmic Events

Figure 1B



RESULTS

Fig 3. Mean beat rate of 10 different hiPSC-CM lots before compound treatment

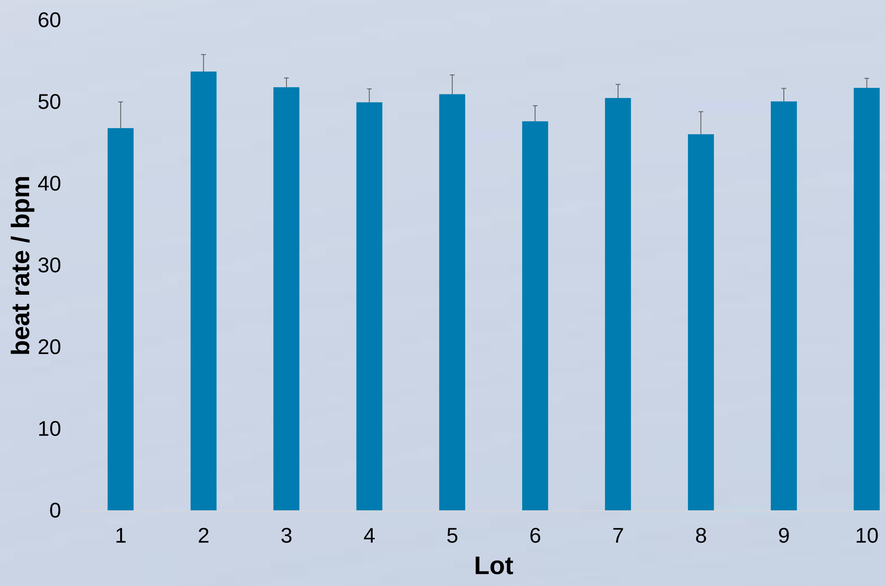


Figure 3. Comparison of 10 different iCell® Cardiomyocytes² lots regarding beat rate. Mean beat rate was assessed before compound addition 6 days after seeding on FLEXcyte 96 plates. Beat rate of ten lots varied between 47 – 53 beats per minute (bpm).

Figure 4. Mean Beat of compound effect – 10 lots

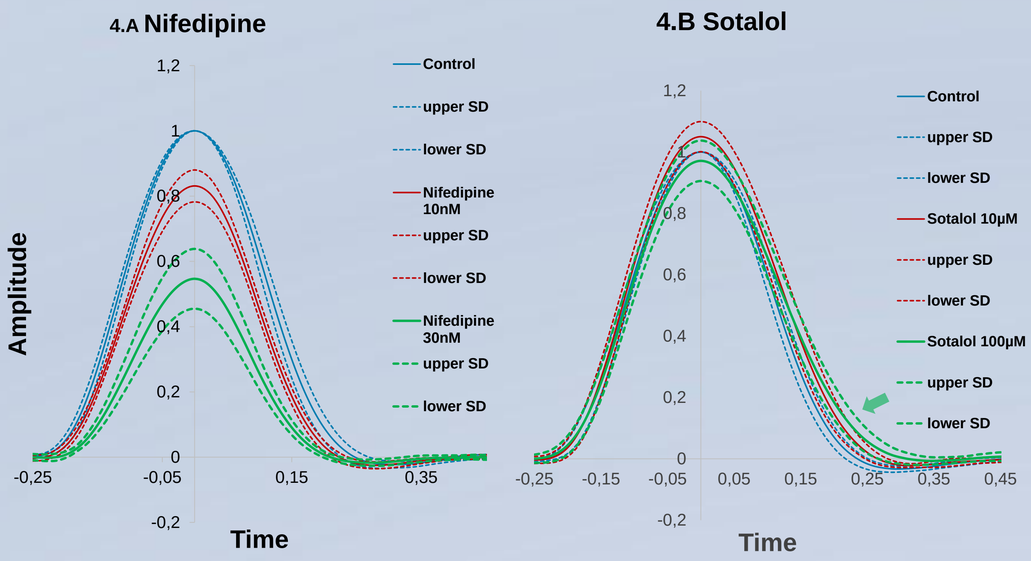


Figure 4. Changes in contraction amplitude (mean beat) after compound treatment with nifedipine (A) and sotalol (B). Contraction amplitude was assessed approx. 10 min after compound addition to iCell® Cardiomyocytes². Control condition is set to 1 on the y-axis and highlighted in blue. (A) Nifedipine concentrations of 10 nM and 30 nM were applied and contraction amplitudes of ten lots including standard deviations (dotted lines) were assessed with the FLEXcyte 96 technology. (B) Changes in contraction amplitude after sotalol treatment were also assessed under the same conditions. 10µM and 100µM sotalol were applied to ten lots of iCell® Cardiomyocytes². Blue arrow indicates compound-induced duration prolongation.

Fig 5. Effect of nifedipine and sotalol on beat rate and contraction duration - 10 lots

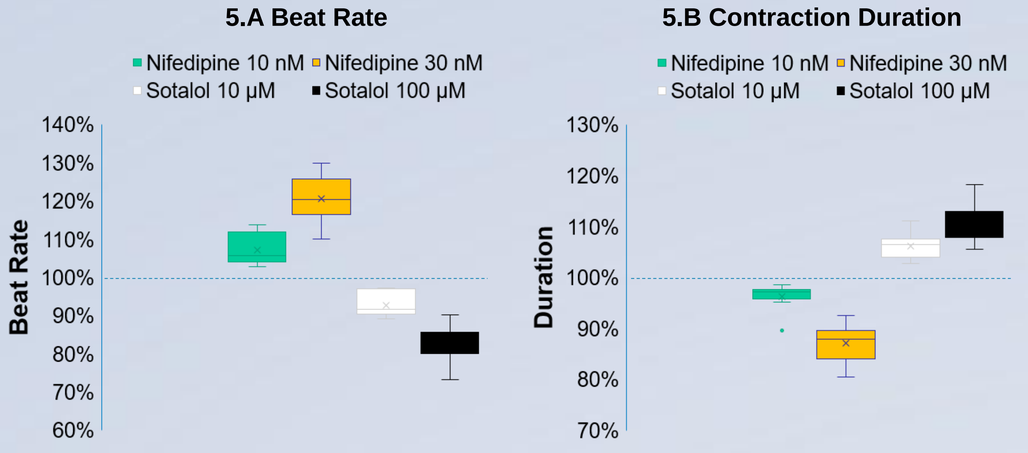


Figure 5. Mean beat rate (A) and contraction duration (B) of iCell® Cardiomyocytes² (ten lots) after compound treatment with nifedipine and sotalol. Box plots are shown for both parameters. Colours encode: Blue=nifedipine 10nM, orange=nifedipine 30nM, grey=sotalol 10µM and yellow=sotalol 100µM. Control condition is represented by dotted line at 100%.

Summary

- As an important source for cell-based assays involved in preclinical cardiac risk assessment, iCell® Cardiomyocytes² need stable lot-to-lot consistency.
- Comparison of ten different lots regarding beat rate before compound addition demonstrates stable conditions between 47 - 53 bpm.
- Treatment of the cells with calcium antagonist nifedipine showed an expected concentration-dependent decrease in mean amplitude, increase in beat rate as well as decrease in contraction duration with low variation among the lots demonstrated by the standard deviations.
- Treatment of the cells with beta blocker sotalol showed also expected effects with a sotalol-induced duration prolongation but no significant effect on amplitude, as well as a concentration- dependent decrease of beat rate and increase of contraction duration. Low variations of standard deviations also underline the consistency among the tested lots.

Conclusions

- The analysis and comparison of ten iCell® Cardiomyocytes² lots regarding contractile properties using the FLEXcyte 96 technology allowed for testing the functional reliability of this commercial cell line.
- The pre- and post-pharmacological assessment with the FLEXcyte 96 technology using gold standard compounds demonstrated expected effects with insignificant variation as depicted by the standard deviations.
- Hence, the data shown here proves the combined robustness of the iCell® Cardiomyocytes² cell line and the FLEXcyte 96 technology for preclinical cardiac risk assessment.