# NAM-Related Robustness Analysis of Commercial hiPSC-Cardiomyocytes in the Light of Preclinical Cardiac Risk Assessment

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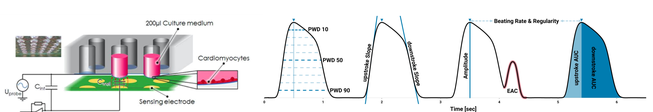
## Introduction

- Commercial human iPSC-derived cardiomyocytes (hiPSC-CMs) play an important role as a stable source for new approach methodologies (NAMs) in preclinical cardiac risk assessment.
- To address potential hazardous side effects reliably, lot-to-lot robustness and the possibility to assess drug responses without the presence of serum is needed.
- To analyze the lot-to-lot consistency of commercial hiPSC-CMs (iCell Cardiomyocytes$^2$, FCDI) as well as the effects of a defined serum-free medium on these cells, a functional readout of contractile properties (FLEXcyte 96 Technology) was chosen.
- Gold standard compounds nifedipine and sotalol were used for the robustness study on 10 different cell lots. Erlotinib and Doxorubicin served as test compounds for the serum-free medium study.
- Contractile changes were analyzed regarding contraction amplitude, beat rate and downstroke slope.

## Method

- The FLEXcyte technology is based on a special 96 well plate that contains ultra-thin and hyper-elastic silicone membranes as basis for human iPSC-CMs (Fig 1.A). This FLEXcyte 96 plate is analyzed in the FLEXcyte 96 device (Nanion Technologies, Germany).
- Rhythmic contraction of cardiomyocyte monolayers lift the membranes in the FLEXcyte 96 plate upwards. These changes in deflection are quantified by means of capacitive distance sensing (Fig.1A).
- Parameters analyzed are contractile force (mN/mm$^2$), rising and falling times, AUC as well as peak width duration (PWD) 10 - 90 and beat rate (Fig.1D) (Gossmann *et al.*, 2016 and 2020).

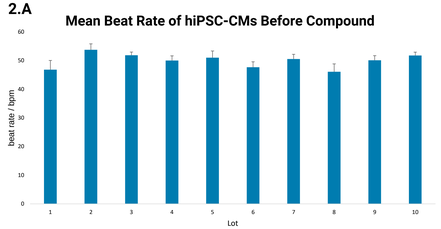
**1.A** **1.B**



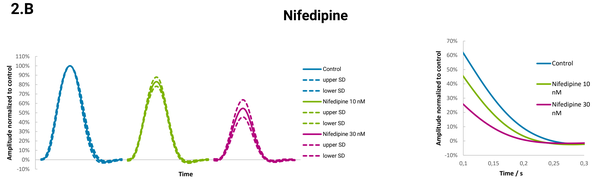

## Results

### Lot – to – Lot Consistency Study of hiPSC-Cardiomyocytes

**2.A**




**2.B** **Nifedipine**




**2.C** **Sotalol**

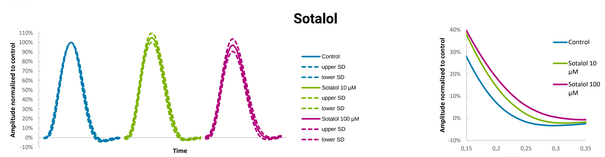
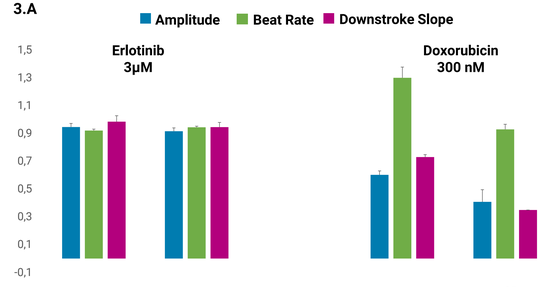


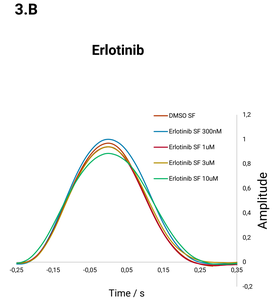

**Figure 2. A** Comparison of 10 different iCell® Cardiomyocytes$^2$ lots regarding beat rate Mean Beat Rate was assessed before compound addition 6 days after seeding on FLEXcyte 96 plates. Beat rate of ten lots varied between 47 53 beats per minute (bpm). **B,C** Amplitude of hiPSC-CMs (iCell® CM$^2$) analyzed with the FLEXcyte 96 system after compound treatment with nifedipine and sotalol. Control condition (blue) is normalized to 100%. Nifedipine concentrations of 10 nM (green) and 30 nM (pink) are shown as well as sotalol concentrations of 10 µM (green) and 100 µM (pink). Dotted lines in respective colors represent standard deviations.

### Serum – Free Medium Study of hiPSC-Cardiomyocytes

**3.A**




**3.B** **Erlotinib**




**3.C** **Doxorubicin**

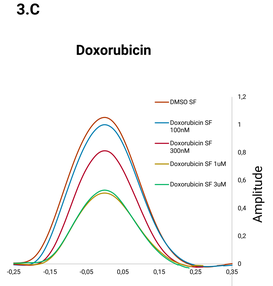



**Figure 3. A** Bar graph of iCell® Cardiomyocytes$^2$ cultured in iCell® Cardiomyocytes serum-free medium (SM) on FLEXcyte 96 plates and treated with gold standard compounds Erlotinib (3 µM) and Doxorubicin (300 nM) for 5 days. Depicted bar graph results show amplitude, beat rate and downstroke slope reactions after 48 hours. Data is normalized to iCell® Cardiomyocytes$^2$ cultured in iCell® Cardiomyocytes Maintenance Medium (MM) before compound addition. **B, C** Comparison of mean beats are shown of iCell® Cardiomyocytes$^2$ treated with different concentrations of erlotinib (300 nM - 10 µ M) and doxorubicin (100 nM - 3 µM) in iCell® Cardiomyocytes serum-free medium. Data represents mean beat of iCell® Cardiomyocytes$^2$ reactions 24 hours after compound treatment.

## Summary

- Pre-compound conditions of ten different cell lots showed a similar beat rate ranging between 47 – 53 beats per minutes (bpm).
- Cells treated with calcium antagonist nifedipine showed a concentration-dependent decrease in mean amplitude as well as decrease in contraction duration with low variation among the lots demonstrated by the standard deviations.
- Cells treated with beta blocker sotalol showed induced duration prolongation but no significant effect on amplitude. Low variations of standard deviations also underline the consistency among the tested lots.
- Robust results were also obtained with the serum-free study showing comparable compound-induced reactions (erlotinib and doxorubicin) of hiPSC-CMs cultured in either serum-containing or serum-free medium.

## Discussion

- Both studies demonstrate the stable performance of commercial hiPSC-CMs regarding lot – to – lot variability as well as culture conditions with or without serum-containing medium.
- Contractile property evaluation with the FLEXcyte 96 technology offers the unique possibility to analyze cardiac contraction behaviour under physiological conditions in a 96-well format (Gossmann *et al.*, 2016 and 2020).
- The data shown here proves the combined robustness of the iCell® Cardiomyocytes$^2$ cell line and the FLEXcyte 96 technology for preclinical cardiac risk assessment.

Gossmann M. et al. Journal of Pharmacological and Toxicological Methods. 2020
Gossmann M. et al. Cell Physiol Biochem. 2016