Application Note

FLEXcyte 96

Positive inotropic effects in human iPSC-derived cardiomyocytes can be triggered through a flexible pro-maturation environment.

High throughput Screening (HTS) scalable techniques with highly predictive cell models are needed to improve the expensive and time-consuming drug development process. Potentially dangerous consequences of side effects on the human heart make safety testing of heart related issues the main focus of pre-clinical drug development studies. However, one of the most commonly used gold standard technique for cardiac contractility measurements, the *ex vivo* Langendorff set-up, does not efficiently support modern drug development processes as it uses non-predictive animal models on a very low throughput level.

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) combine a number of features fostering the drug development process such as high predictivity, large scale applicability with high throughput potential, low ethical concerns and cost effectiveness. Yet, when cultured on overly stiff substrates like glass or plastic, the cells are placed under unnecessary stress due to the missing auxotonic physiological environment provided by a flexible substrate.

These unphysiological conditions lead to drastic transcriptional and metabolic deregulation in cardiomyocytes which affect the predictive value of this established cell model [1].

To bridge the gap of predictive contractility measurements and HTS analysis for drug development studies, innoVitro co-developed the FLEXcyte 96 (Figure 1) with Nanion Technologies as an add-on for the CardioExcyte 96 platform. With less than 10 µm in thickness and sophisticated surface modification, the polydimethylsiloxane (PDMS) membranes of the FLEXcyte 96 disposable plates offer physiological elasticity of native human heart tissue [2]. As a result hiPSC-derived CM behave in an in vivo manner and finally reach their full potential as a CiPA confirmed model for drug development processes [3] [4].

Beta-adrenergic agonist isoproterenol and L-type calcium channel agonist S-Bay K8644 are both well known for their positive inotropic effects on the human heart, although common iPSC-CM *in vitro* assays fail to display this physiological response by showing negative inotropic effects instead.

Here, we show that the auxotonic environment of the FLEXcyte 96 enables mature physiological responses of hiPSC-CMs on positive inotropic substances such as L-type calcium channel agonist S-Bay K8644, beta-adrenergic agonist isoproterenol and cardiac myosin activator omecamtiv mecarbil.

Figure 1. **FLEXcyte 96 device for the assessment of cardiac contractility under physiological mechanical conditions**

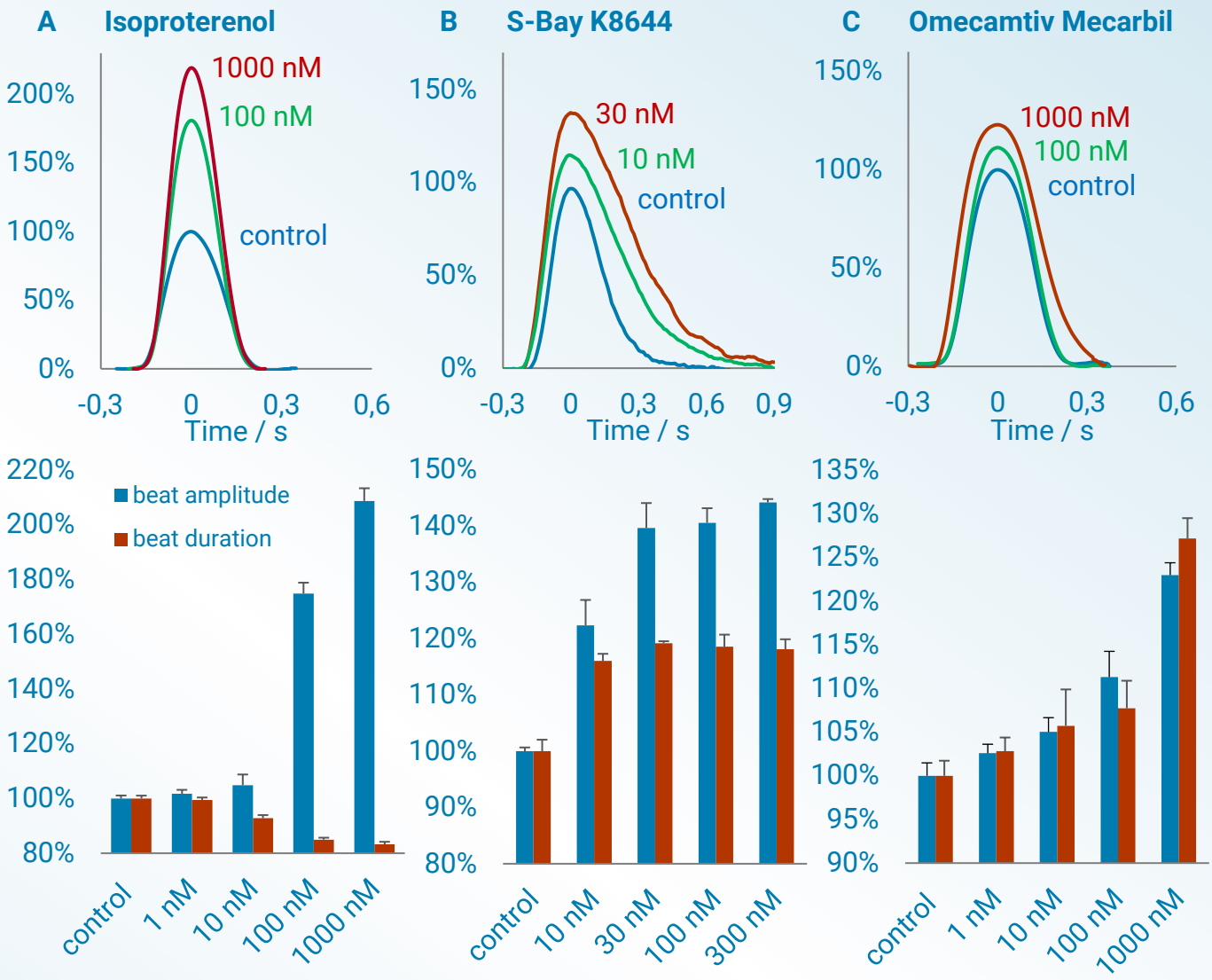

Figure 2. **Recorded beat shape and corresponding beat duration / beat amplitude analysis of hiPSC-CM cultured on FLEXcyte 96 well plates after treatment with isoproterenol (A), S-Bay K8644 (B) and omecamtiv mecarbil (C). A**. hiPSC-CM (Cardiosight®-S, Nexel) beat shape after treatment with 100 nM and 1000 nM isoproterenol in comparison to control. Beat amplitude and beat duration analysis of hiPSC-CM after treatment with increasing concentrations of isoproterenol (1 nM – 1000 nM) in comparison to control. **B**. hiPSC-CM (iCell® CM², FCDI) beat shape after treatment with 10 nM and 30 nM S-Bay K8644 in comparison to control. Beat amplitude and beat duration analysis of hiPSC-CM after treatment with increasing concentrations of S-Bay K8644 (10 nM – 300 nM) in comparison to control. **C**. hiPSC-CM (Cardiosight®-S, Nexel) beat shape after treatment with 100 nM and 1000 nM omecamtiv mecarbil. in comparison to control. HiPSC-CM beat amplitude and beat duration analysis of omecamtiv mecarbil treatment (1 nM – 1000 nM) in comparison to control.

Results

Figure 2 shows the dose-dependent effects of isoproterenol, S-Bay K8644 and omecamtiv mecarbil treatment on hiPSC-CM seeded onto FLEXcyte 96 well plates.

Isoproterenol increased the contraction amplitude in a dose-dependent manner to approx. 210% of control at 1 µM. At the same time, the durations of contraction and relaxation phase were shortened, indicating an increase in acceleration and deceleration of the contraction.

In contrast, S-Bay K8644 elicited a symmetry change in the beat shape. While the contraction phase was unaffected, the increase in intracellular calcium concentration resulted in a prolongation of the relaxation phase. As a result, the total duration of the contraction-relaxation-cycle was increased to 120% of control. The amplitude reached 150% of control at 30 nM. Omecamtiv mecarbil increased both the contraction amplitude as well as the duration of the contraction-

relaxation-cycle, indicating the positive inotropic effect based on activation of the myosin complex. The symmetry of the contraction-relaxation-cycle was not changed. The mature physiological responses of hiPSC-CM shown here are triggered by compounds acting at different target levels, underlining the pro-maturation effects of the FLEXcyte 96 which cannot be elicited with other assays commonly used for drug development purposes [5].

Methods

Human iPSC-derived cardiomyocytes were kindly provided by Fujifilm Cellular Dynamics International (Madison, WA, USA) and NEXEL (Seoul, Republic of Korea).
The cells were cultured on FLEXcyte 96 well plates according to manufacturers' guidelines in 200 µL maintenance medium per well. Cells were seeded approximately 6 days before compound treatment at 50k (Cardiosight®-S, NEXEL) or 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. For the experiments, 50 µL for the cell culture medium was removed and replaced with 50 µL medium containing 4x concentrated compound, resulting in the desired final compound concentration.
The CardioExcyte/ FLEXcyte Control software enables online analysis of contractility parameters. An adaptive signal detection algorithm extracts the positions and values of beating events. Besides beat intervals, amplitudes, rising and falling time, pulse witdhs are detected. Furthermore, integrals and arrhythmia are identified and quantified as well.

References

[1] Heras-Bautista et al., *Acta Biomater 89,* pp. 180-192, 2019.
[2] Gossmann et al., *Cell Physiol Biochem 38,* pp. 1182-1198, 2016.
[3] Blinova et al., *Cell Reports 24,* pp. 3582-3592, 2018.
[4] Bot et al., *JPTM 93,* pp. 46-58, 2018.
[5] Jiang at al., *Mol Cells 41,* pp. 613-621, 2018.

Notes

innoVitro GmbH
Artilleriestraße 2
52428 Jülich
Germany

FON +49 (0) 2461 3170561
FAX +49 (0) 2461 3173859
E-MAIL info@innovitro.de
Web www.innovitro.de