Application Note

Cells: Cardiosight-S®
Tools: FLEXcyte 96

FLEXcyte 96 recordings in comparison to *in vivo* and *in vitro* cardiac muscle contraction via MUSCLEMOTION data

Nanion Technologies GmbH, Munich.
Human iPSC-derived cardiomyocytes kindly provided by Nexel.

Summary

In order to reduce cardiovascular safety liabilities of new therapeutic agents, there is an urgent need to integrate human-relevant platforms/approaches into drug development[1]. Optimizing baseline function of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) is essential for their effective application in models of cardiac toxicity and disease[2]. Here, hiPSC-CMs were cultured on flexible substrates using the FLEXcyte 96 system. The pro-maturation environment enables observation of inotropic and chronotropic compound effects, which are typically hard to detect with 2D monolayers on overly stiff substrates[3]. For example, the beta-adrenergic agonist isoprenaline, or isoproterenol, is well known for its positive inotropic effects on the human heart, although common hiPSC-CM *in vitro* assays fail to display this physiological response by this compound.

Sala *et al*. (2018)[4] developed a method that allows for a comparison of cardiac contraction measurements derived from different measurement approaches. The method, called MUSCLEMOTION, builds on previously existing algorithms, is fully automated and can be used on videos, image stacks, or image sequences loaded in the open-source image-processing program ImageJ[5]. It is an open-source, dynamic platform that can be expanded, improved, and integrated for customized applications. Dynamic changes in pixel intensity between image frames are determined and the output is expressed as a relative measure of movement during muscle contraction and relaxation. Here, we compare the previously depicted data sets from Sala et al. 2018[4] and compare it to FLEXcyte 96 data as recorded with Cardiosight-S® cardiomyocytes (Nexel).

Results

Mean beat contraction and velocity parameters for hiPSC-CMs grown on the flexible substrate of the FLEXcyte 96 were calculated and compared with beat parameters from the MUSCLEMOTION software program as previously published by Sala *et al*, 2018[4]. Figure 1A shows the contraction and velocity profiles of isolated hPSC-CMs, hPSC-CM monolayers, cardiac organoids, engineered heart tissue (EHTs) and adult rabbit cardiomyocytes. Figure 1B shows the contraction and velocity profiles of hiPSC-CM monolayers on the FLEXcyte 96. Interestingly, the contraction and velocity profiles are highly comparable to adult cardiomyocytes indicating a more mature phenotype of these cells.

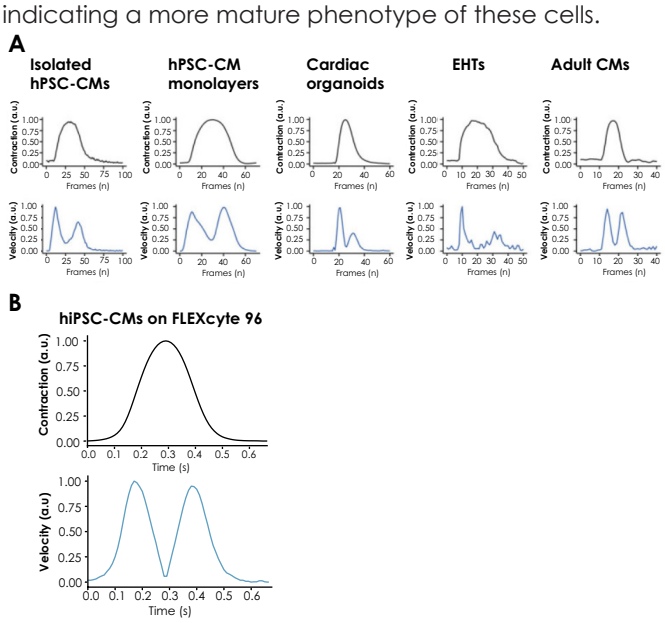

Figure 1: A Contraction (top) and velocity (lower) individual beat profile generated using MUSCLEMOTION for hPSC-CMs, hPSC-CM monolayers, cardiac organoids, engineered heart tissue (EHTs) and adult rabbit CMs (adapted from Ref. 4). **B** Contraction and velocity of mean beats of hiPSC-CMs recorded on the FLEXcyte 96.

Application Note

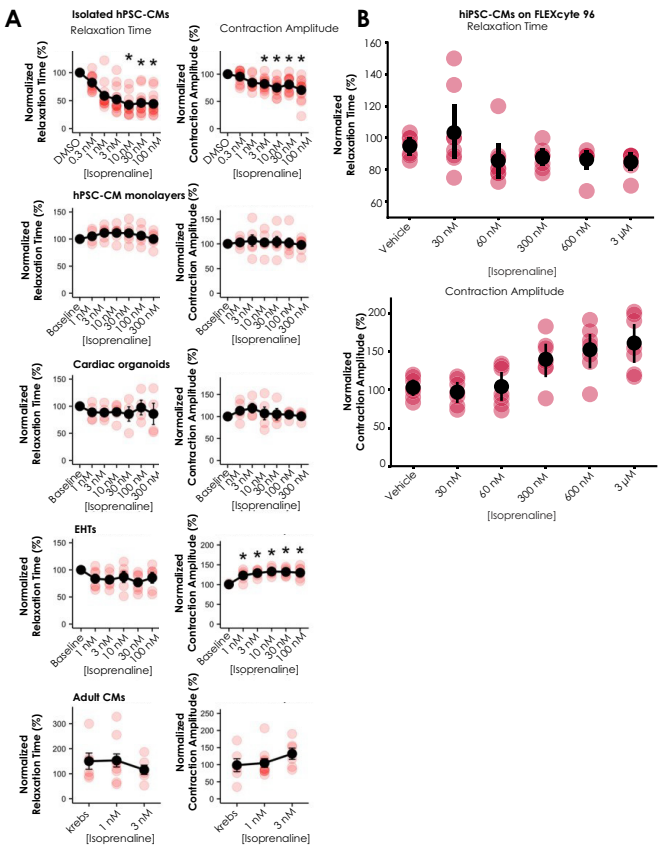

Figure 2: A Average concentration response curves (black) and single measurements (red) for isoprenaline for relaxation time (left) and contraction amplitude (right) generated using MUSCLEMOTION for hPSC-CMs, hPSC-CM monolayers, cardiac organoids, engineered heart tissue (EHTs) and adult rabbit CMs (adapted from Ref. 4). **B** Average concentration response curves (black) and single measurements (red) for isoprenaline for relaxation time (top) and contraction amplitude (lower) recorded on the FLEXcyte 96.

Average concentration response curves for relaxation time and contraction amplitude for isoprenaline, a well-known positive inotropic compound, for isolated hPSC-CMs, hPSC-CM monolayers, cardiac organoids, EHTs and adult rabbit cardiomyocytes obtained using the MUSCLEMOTION analysis of optical images are shown in Figure 2A. Average concentration response curves for relaxation time and contraction amplitude for isoprenaline obtained for hiPSC-CMs recorded on the FLEXcyte 96 are shown in Figure 2B. Importantly, a positive inotropic response is shown for contraction amplitude only in adult rabbit CMs and EHTs, whereas this increase in contraction amplitude can also be detected in hiPSC-CMs recorded on the FLEXcyte 96 (Figure 2B), albeit at high concentrations. A reduction in relaxation time is also seen in adult CMs and on hiPSC-CMs recorded on the FLEXcyte 96 indicating a more mature or adult-like profile of these cells when grown on the flexible substrate of the FLEXcyte 96.

In summary, the FLEXcyte 96 in combination with hiPSC-CMs can be used to investigate the effect of positive (and negative) inotropic compounds on parameters such as contraction amplitude, velocity and relaxation time. Moreover, hiPSC-CMs grown on the flexible substrate of the FLEXcyte 96 plates (FLX-96) display more adult-like contraction and velocity profiles compared with hiPSC-CMs grown on rigid surfaces. In this way, hiPSC-CMs can be used for more predictive assays for pro-arrhythmic effects.

References

1. Pang, L., *et al*. 2019. Circ. Res. 125: 855–867
2. Birket, M.J., *et al.* 2015. Cell Rep. 13(4): 733–745.
3. Stoelzle-Feix, S., *et al.* 2019. White Paper: https://www.nanion.de/en/products/flexcyte-white-paper-download.html
4. Sala, L., *et al*. 2018. Circ. Res. 122(3): e5–e16
5. Ribeiro, A.J., *et al*. 2015. PNAS. 112: 12705–12710.

Methods

Cells

We thank NEXEL for providing the hiPSC-CMs (Cardiosight-S®).

Contraction measurements

Contraction measurements were conducted according to Nanion's standard procedures for the FLEXcyte 96. Cardiosight-S® were cultured on FLX-96 plates for at least 6 days before compound addition. Compounds were applied in the external media. About 4-6 hours before drug application a final media exchange was performed, leaving 200 µl media in each well. For compound addition, 50 µl media was removed and replaced with 50 µl media containing the compound at 4x concentration, resulting in the desired final concentration. All signals were normalized to a group of control measurements on the same plate.

Nanion Technologies GmbH
Ganghoferstr. 70A
80339 Munich, Germany

phone +49 89 219 095-0
fax +49 89 218997960
www.nanion.de • info@nanion.de