Application Note

Cells:
EBiSC hiPSC-cardiomyocytes
Tools:
CardioExcyte 96

Monitoring the effect of Triiodothyronine (T3) and Dexmethasone (Dex) on the calcium handling property of hiPSC-cardiomyocytes

Nanion Technologies GmbH, Munich.
Bayer AG. Hamamatsu Photonics.

Summary

Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) provide new avenues for disease modelling, drug discovery and cell therapy. However, structural and functional immaturity of these cells, poses a great challenge[1]. Therefore, there is a highly unmet need for hiPSC-CMs with a mature phenotype. Recently, it has been reported that triiodothyronine (T3) and dexamethasone (Dex) play significant roles in hiPSC-CMs maturation, by promoting the structural T-tubule development[2], enhancing the electrophysiological maturation[3] and improving calcium handling ability.

Testing for maturity of hiPSC-CMs, demands a holistic approach and use of various techniques to monitor functional readout of those cells. Exceptional benefit is seen in multiplexing various assays, and obtain valuable information from exactly the same cells. Here, we have tested pro-maturation effects of T3 and Dex on EBiSC hiPSC-CMs, and monitored electrical activity, contractility and calcium transients. By using NSP-96 transparent plate, we were able to conduct these measurements on the same cell population, by multiplexing calcium imaging recordings using FDSS/µCELL, after CardioExcyte 96 recordings have been performed.

Results

Electrophysiological properties hiPSC-CMs seem to be altered by T3 and Dex treatment. Apart from changed expression of genes encoding relevant cardiac ion channels, shortening of action potential duration (APD) in those cells has been observed[2]. Here, we have used electrical field potential (EFP) recordings, to investigate the effects of T3 and Dex on population of hiPSC-CMs forming a functional monolayer. By using transparent plates, we were able to corelate those signals with calcium transients.
Figure 1 shows EFP recordings performed on NSP-96 transparent CardioExcyte 96 plates. EFP recordings are widely used as surrogates of action potential recordings, where the field potential (FPD) duration – time from the sodium peak to the T-wave – corresponds to the APD in action potential recordings. We could observe that T3 and Dex induced shorter FPDs in EBiSC hiPSC-CMs, which is consistent with the published results that T3 and Dex could shorten the APD in hiPSC-CMs[2]. Immediately after the electrical activity of the hiPSC-CMs was recorded, in the NSP-96 transparent plates, cells were loaded with calcium indicator dye (Calcium 6) and calcium transients have been recorded using FDSS/µCELL.

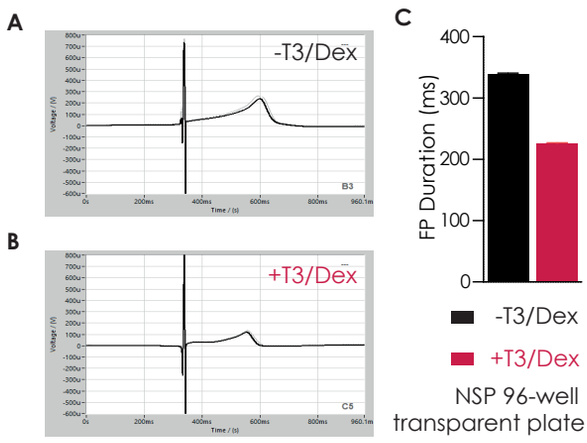

Figure 1. *Effect of T3 and Dex on the EFP signals of EBiSC hiPSC-CMs. Figure shows a typical EFP signal recorded from EBiSC hiPSC-CMs on NSP-96 transparent plates, (***A***) without and with (***B***) T3 and Dex treatment.* **C.** *Bar graph summarizes the shortening effect of T3 and Dex on FPD values. Values represent mean ± SEM.*

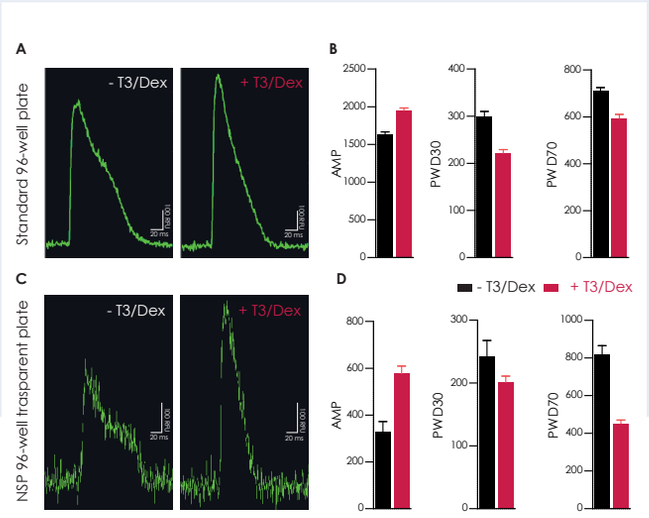

Figure 2: *Comparison of calcium transients, recorded from standard imaging and NSP 96-well plates.* **A** *and* **C** *depict raw traced recorded from these plates, with (right) and without (left) T3 and Dex treatment. Bar graphs summarize the effects of T3 and Dex on amplitude, PW 30 and PW 70 durations as recorded on standard imaging 96-well plates (***B***) and NSP 96-well plates (***D***). Values represent mean ± SEM.*

As control for calcium imaging recordings, standard 96-well imaging plates were loaded with dye as well, and recorded in parallel. Figure 2 shows the effect of T3 and Dex on the calcium signals of hiPSC-CMs recorded from standard 96-well imaging plate and NSP-96 transparent plate. Recordings performed on standard normal 96-well imaging plate clearly shows that T3 and Dex application increased the amplitude of calcium signals, and also accelerated calcium transient kinetics of treated hiPSC-CMs. Similar effects of T3 and Dex on the calcium handling properties of hiPSC-CMs have been observed using NSP-96 transparent plate, as well. Calcium imaging using NSP-96 transparent plates, confirmed that T3 and Dex increased calcium amplitude and induced a faster calcium decay in hiPSC-CMs.

In conclusion, by using NSP-96 transparent CardioExcyte96 plate, we were able to perform multiplex recordings of calcium transients and electrical activity from exactly the same hiPSC-CMs. Our results confirmed previous findings, that T3 and Dex influenced electrical activity of hiPSC-CMs. Moreover, we could also show that T3 and Dex improved the calcium handling ability of the same hiPSC-CMs. Increased amplitude and a faster decay of calcium signals have been observed in cells treated with T3 and Dex in both NSP-96 transparent plate and control standard 96-well imaging plate, indicating that NSP-96 transparent plates can be used to successfully deliver information of multiple readouts, crucial for monitoring maturation of hiPSC-CMs.

References

1. van den Berg C.W. et al. 2015. Development 142: 3231–3238.
2. Parikh S.S. et al. 2017. Circ. Res.; 121: 1323-1330
3. Wang L. et al. 2021. J Mol Cell Cardiol; 161:130-138

Methods

Cells

We thank Bayer AG for providing iPSC-derived EBiSC cardiomyocytes, and Hamamtsu for providing analysis software for this collaborative work. We thank Dr. Wenying Xian for performing experiments, analyzing data and her assistance with writing the document. This work has received support from the EU / EFPIA / Innovative Medicines Initiative 2 Joint Undertaking (EBiSC2 grant n°821362).

Impedance and contractility measurements

EBiSC hiPSC-CMs have been seeded on the NSP-96 transparent plates with density of 5k cells/well , and formed a synchronously beating monolayer 5 to 7 days after plating. 100nM T3 and 1µM Dex have been applied to the cardiomyocytes to induce their maturation. Impedance and EFP measurements were conducted according to Nanion's standard procedures for the CardioExcyte 96. Calcium imaging measurements were conducted according Hamamtsu´s standard procedures for the FDSS/µCELL, using Calcium 6 as an indicator for 20 minutes at the incubator and then started the calcium imaging with high speed settings. Data was analyzed with DataControl 96 software and FDSS/µCELL software.