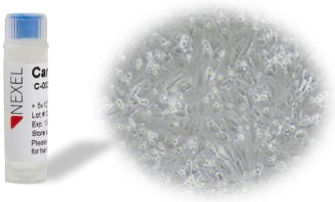
Application note

Methods for Simultaneously Measuring Drug-induced Extracellular Field Potential (EFP) and Impedance (IMP) Changes using Cardiosight®-S



Introduction

In the ongoing regulation changes, many institutions organizations are still conducting preclinical or toxicity tests in animal cells or models, with inconsistent results due to interspecies differences. Therefore, developing a new toxicity study model to replace the use of animals in the non-clinical study is of the utmost urgency.

NEXEL Co., Ltd. strives to provide high-quality human cardiomyocytes derived from induced pluripotent stem cells (iPSC-CMs). The Cardiosight®-S is a highly pure and electrophysiologically active population of cells, ensuring that researchers get a reliable product. These cardiomyocytes recover quickly upon thawing to form a synchronized monolayer of spontaneously beating cells.

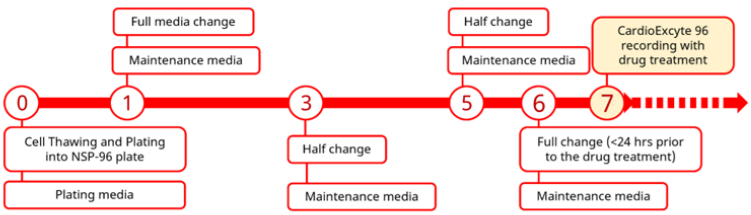
The CardioExcyte 96 (Nanion Technologies) is a fully automated hybrid system recording both contractility and electrophysiology of

Workflow

Cardiosight®-S (#C-002) and CMS-A media kit

Cell thawing and Maintenance

- Cells seeding with fibronectin coating
- 50,000 cells/well
- Full media change on Day 1 to maintenance media and half media exchange every 48 hrs



Note: An alternative workflow may be acceptable. Contact Nexel's Technical Support for more information.

Data recording and analysis

NSP-96 sensor plate with CardioExcyte 96 platform

- Possible to record the EFP and Impedance
- Parameter

1) EFP: Beat rate, Spike Amplitude, FPDcF, Beating Regularity CoV
2) Impedance: Beat rate, Amplitude, Rising time, Beating Regularity CoV

<CardioExcyte 96 platform>

<NSP-96 Type Standard: 0.6 mm>
(Cat #201002)

Caution: All experimental steps and results are optimized for **Cardiosight®-S** and **NSP-96 Type Standard: 0.6 mm plate**. NEXEL does not guarantee equivalent results unless you use the cells and plate described in this application note.

intact cardiomyocyte networks in a 96-well format. The extracellular field potential (EFP) and Impedance (IMP; contractility) measurements are performed at high resolution, are non-invasive, and are label-free.

In this Application note, we present data recorded using Cardiosight®-S (NEXEL Co., Ltd. Cat No. C-002) on the NSP-96 (Nanion Technology, #201002) with basic guidance on how to plate and culture. Cardiosight®-S cardiomyocytes can be successfully culture, maintained and recorded on CardioExcyte 96 NSP-96 plate for extended time spans, thereby showing the experimental flexibility.

Moreover, this provides basic instruction for compound treatment, data acquisition, and analysis, which suggests Cardiosight®-S for cardiac *in vitro* screening of variety of acute, sub-acute and chronic drug-induced effects or safety screening applications. This document should be used in addition to the Cardiosight®-S User Guide and CardioExcyte 96 Application protocol provided by NEXEL.

Methods

Cell culture

Cardiomyocytes used in the study were manufactured and provided by NEXEL Co., Ltd. Cells were thawed and cultured according to the Cardiosight®-S User Guide and CardioExcyte 96 Application protocol. There are four types of CardioExcyte 96 plates depending on electrode size and the measurement strength of EFP and contractility. Cardiosight®-S were plated into a Fibronectin-coated NSP-96 (Nanion Technology, #201002) plate in CMS-A plating media at a density of 50,000 cells/well. Following day, the plating media was replaced with maintenance media and maintained the cells by half volume media change every 2 days (referred to the Work Flow, Cell thawing and Maintenance section). For drug treatment, it is necessary to exchange the full media of the wells with 200 µl maintenance media on 24 hours prior of recording.

Drug preparation

Four concentrations of Nifedipine were examined (Table 1). Nifedipine were dissolved in DMSO at 1,000x at the highest test concentration and then serially diluted in Cardiosight®-S maintenance media at the final concentration of 10x. To apply the compound to the cells, 10% of the media was removed and replaced with an equivalent volume of the 10x solution to arrive at the testing concentration with a final DMSO concentration of 0.1%. Drug solutions were prepared immediately before use.

CardioExcyte 96 Recording

The CardioExcyte 96 uses not only IMP measurement to study pharmacological effects on contractility but also coordinated ion channel activity through EFP recordings.[1] After checking that wells are beating in a synchronous manner, the baseline was recorded for 30 seconds with 10 minutes intervals from each well. Total baseline recording and interval time were 1 hour.

After recording the baseline, 10% of the media in each well will be replaced with the treatment solutions prepared at 10x the final concentration. Four concentrations of Nifedipine were added sequentially with 30 minutes of incubation time for each concentration. Electrical activity and impedance were recorded every 10 minutes for 30 seconds following exposure to drugs or negative control. The analysis was carried out through DataControl96 Software, provided by Nanion Technologies.

Table 1. Test concentration for CardioExcyte 96 experiment

Compound		Concentration			
Name	**Effect**	**Treat 1**	**Treat 2**	**Treat 3**	**Treat 4**
DMSO	Negative control	0.1 %			
Nifedipine	Ca^{2+} channel blocker	0.001 µM	0.01 µM	0.1 µM	1 µM

Results

Representative data

The CardioExcyte 96 uses not only ion channel activity through EFP recordings but also coordinated IMP measurement to study pharmacological effects on contractility.[1]

Figure 1 shows the steady signals of EFP and IMP recordings from Day 1, 3, 5, 7, and 14. Surprisingly, EFP showed a prominent T-wave from Day 1, which was small but can be easily auto-analyzed by DataControl 96 software.

In addition, IMP exhibited immature contraction force and shape until Day 5, but after that, it showed complete contraction signals as the systolic velocity accelerated. Taken together, it is confirmed that Cardiosight®-S produced a stable signal during the culture period for both EFP and IMP in CardioExcyte 96.

Compound response

Nifedipine, an L-type calcium channel blocker known to affect the EFP, calcium transient, and contractility, was tested. The representative traces of the EFP (Figure 2A) and IMP (Figure 2C) presented the overall signal changes upon Nifedipine treatment. The specific parameters for each signal were analyzed in Figures 2B and 2D.

As expected, 0.1% DMSO did not significantly change any parameters in EFP and IMP. Nifedipine exhibited similar responses in the EFP and IMP analysis, which was found to increase in beat rate in a dose-dependent manner. The spike amplitude and FPDcF in EFP were significantly decreased compared to the baseline.

Furthermore, Nifedipine diminished the contractile force (amplitude) and the systolic velocity (rising time velocity) in IMP, showing the impact of calcium channel inhibition on both EFP and IMP. However, there was no significant change in beating regularity CoV upon Nifedipine treatment.

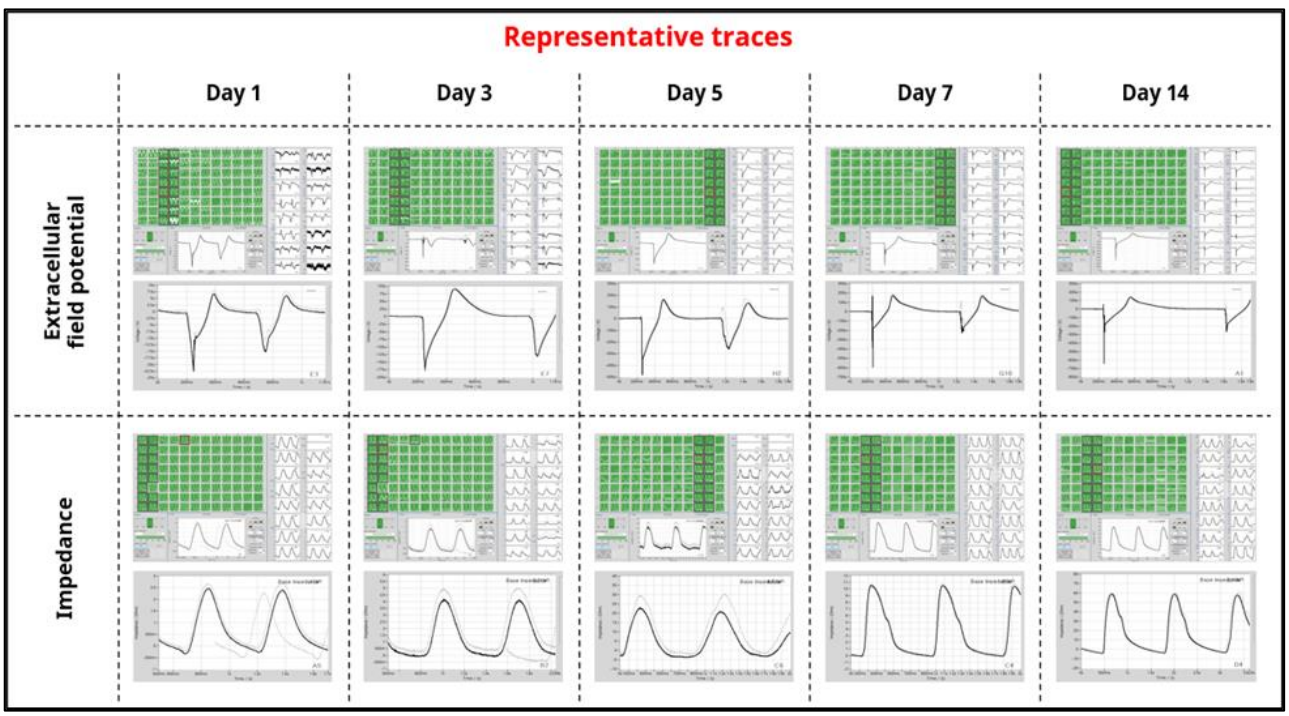


Figure 1. **Representative traces of extracellular field potential (EFP) and impedance (IMP) measured during the cultivation of Cardiosight®-S on CardioExcyte 96 system.** Upper traces showed that the representative tracks of EFP and IMP were captured from the CardioExcyte96 experimental view recorded on Day 1, 3, 5, 7, and 14 of culture. Lower traces presented the mean EFP and IMP representative trace from random selection of raw data.

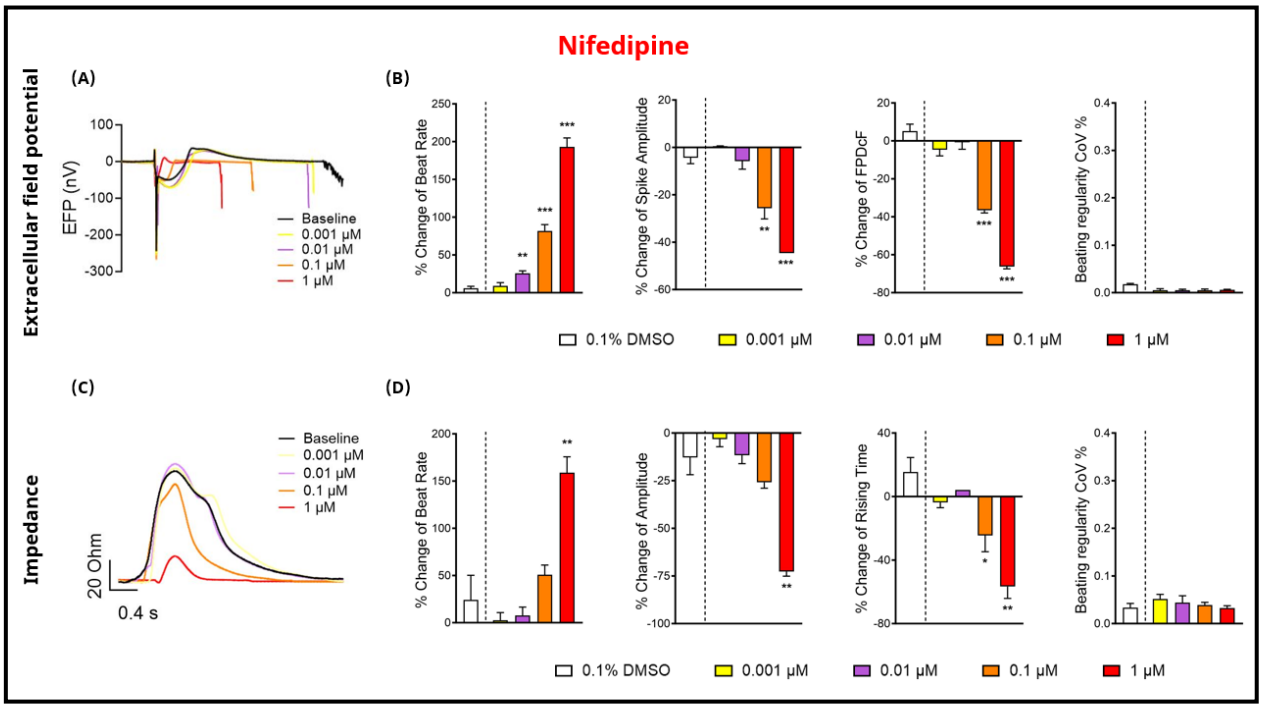


Figure 2. Effect of Nifedipine (I_{Ca-L} blocker) on Cardiosight®-S extracellular field potential (EFP) and impedance (IMP) measured with CardioExcyte 96 System. Nifedipine was treated at concentrations of 0.001, 0.01, 0.1, and 1 μM for 30 minutes each and recorded for 30 seconds. **Effect of Nifedipine on cardiomyocyte EFP.** Trace overlay of EFP exposed to increasing concentration of Nifedipine and the drug effect on EFP beat rate, spike amplitude, FPDcF, and beating regularity CoV (A, B). **Effect of Nifedipine on cardiomyocyte IMP.** Trace overlay of impedance signals exposed to increasing concentration of Nifedipine and the drug effect on IMP beat rate, amplitude, rising time velocity, and beating regularity CoV (C, D). (n=3), Bar graph represents mean ± S.E.M. *: p<0.05, **:p<0.01, ***:p<0.001 vs. Ctrl (0.1% DMSO).

Conclusion

Cardiosight®-S provides an *in vitro* test system that recapitulates native human cardiac myocyte physiology and function while the CardioExcyte 96 system provides a non-invasive, label-free, and high-throughput platform for monitoring cardiomyocyte electrical and contractile activity. These results highlight the ease of use with which robust and relevant data can be generated on the human cardiomyocyte EC process. The stable signals with the physiological shape of traces enable automated analysis and the predictive evaluation of compound effects on different parameters of the EFP and IMP result, which in turn extends the possibilities of the said Cardiosight-S to be used as a model for the drug development process.

Reference

1. Corina, T., et al., 2018. Journal P.T. J Pharmacol Toxicol Methods. 2018; 93: 46–58

For questions about culturing our **Cardiosight®-S**, please contact NEXEL's Technical Support at

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- Phone: +82-2-2088-8886

For questions about Nanion's **CardioExcyte 96 platform**, please contact Nanion Technical Support at

- HQ for general enquiries: info@nanion.de
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