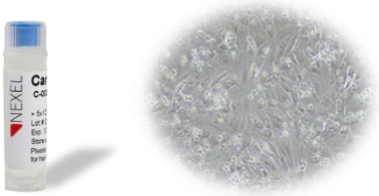
Application note

Impedance and Field Potential analysis of Cardiosight®-S using CardioECR system



Introduction

Drug-induced cardiac toxicities are major concerns of cardiac safety assessment. Pre-clinical tests, hERG assay and animal test (e.g., dog telemetry test), are reliable verification methods for drug safety. Cardiac toxicity is a primary reason that terminate drug development in the late pre-clinical or clinical development stage. At worst case, it can lead to drug withdrawal from market.[1] Recently, in vitro assay using human iPSC Cardiomyocytes (CMs) and techniques by Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative are developed for experimental approaches such as detecting drug-induced acute and chronic cardiac-electrophysiological changes or structural damages.[2]

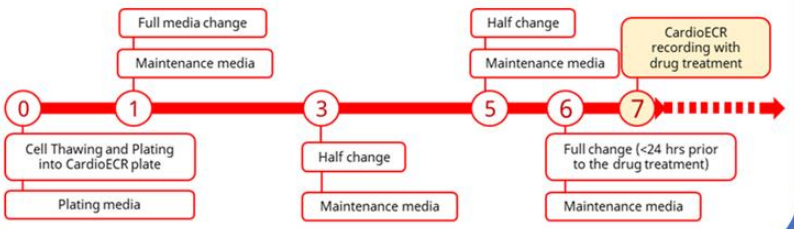
Cardiosight®-S is a human cardiomyocyte derived from induced pluripotent stem cells. All products go through a rigorous quality control tests that examine various parameters which include biological, biochemical, electrophysiological, functional, and Drug response test.

Workflow

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

Cell thawing and Maintenance

- Cells seeding with fibronectin coating
- 30,000 cells/well
- Full media change on Day 1 to maintenance media and half media exchange every 48 hrs



Data recording and analysis

E-Plate CardioECR 48 plate with CardioECR platform

- Possible to record the Field potential (FP) and Impedance (IMP)

- Parameter
1) FP: Beat rate, Spike Amplitude, FPD, Beating Regularity CoV
2) Impedance: Beat rate, Amplitude, Rising time, Beating Regularity CoV

<CardioECR platform>

<E-Plate CardioECR 48>

Abbreviation: human induced pluripotent stem (hiPS), Cardiomyocyte (CM), Field potential (FP), Field potential Duration (FPD), Field Potential Duration corrected by Fridericia's formula (FPDcf), Impedance (IMP), Amplitude (AMP)

NEXEL cardiomyocytes can be used in cardiac biological *in vitro* tests ranging from basic research to drug development or screening tests. These cardiomyocytes recover quickly upon thawing to form a synchronizes monolayer of spontaneously beating cells.

The xCELLigence RTCA CardioECR system is a label-free platform that combines field potential and impedance recording. This system allows for evaluation of viability, contractility, and electrical activity of cardiomyocyte. And this system collects both Field Potential and impedance together, and that data can be monitored.

NEXEL Cardiosight®-S used in combination with this technique form an excellent platform to predict cardiotoxic response and screen compound efficacy. Therefore, this application note describe how to incorporate and use Cardiosight®-S (NEXEL Co., Ltd. Cat No. C-002) on the RTCA CardioECR system.

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 xCELLigence RTCA CardioECR application protocol.

Cardiomyocytes were plated into a fibronectin-coated E-Plate Cardio 48 plate (Agilent, 300601110) in Cardiosight®-S plating media at a density of 30,000 cells/well. Following day, the plating media was replaced with maintenance media and cells were maintained by half volume media change every other day. (refer to the workflow, “Cell thawing and Maintenance” section).

For drug treatment, it is necessary to fully replace the media within the wells with 150 µl pre-warmed maintenance media 24 hours prior of recording.

Drug preparation

Four concentrations of Diltiazem was examined (Table 1). For stock solutions, Diltiazem was dissolved in DMSO at 1,000x of the highest test concentration, and then serially diluted to each test concentration. All stock solutions are at 1,000x. From there, it is diluted in Cardiosight®-S maintenance media to a 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 prepared drug solution to arrive at the testing concentration with a final DMSO concentration of 0.1%. Drug solutions were prepared immediately before use.

CardioECR Recording

Before cell seeding (thawing day), calibrate device and record default data following the device instruction. On Day 7, baseline were recorded in 30 seconds duration with a 1-hour interval. To treat the cells with compound, remove 20 µl from each well and quickly replenish with 20 µl of the prepared 10x compound solution to cell cultured E-plate. Gently mix by pipetting 2 times. Wait 10 minutes for the cells to stabilize and record the Drug treatment step. Example data were recorded 30 minutes after drug treatment and normalized to the baseline point before compound treatment. For detailed information, please refer to the Agilent user guide for xCELLigence RTCA CardioECR system.

Table 1. Test concentration for CardioECR experiment

Compound		Concentration			
Name	**Effect**	**Treat 1**	**Treat 2**	**Treat 3**	**Treat 4**
DMSO	Negative control	0.1 %			
Diltiazem	Ca^{2+} channel blocker	0.013 µM	0.13 µM	1.3 µM	13 µM

Results

Representative data

Each well of the CardioECR E-Plate has electrodes that collect impedance (IMP) and field potential (FP) data. Cell index present impedance (CI; impedance at time point n – impedance without cells/nominal impedance value) and viability of the attached cell.

Cardiosight®-S were cultured and recorded every 24 hr for 7 days (Figure 1). The cells started spontaneous beating the day after thawing, which showed fast and small representative traces of FP and IMP on Day 1 and 2. As the beating progressed gradually and systematically, it became stable, and the parameter value is measured above the QC criteria.

All parameters were appropriate based on the QC criteria within the Cardio ECR program. Data QC was conducted on a basis of 20/min for both Beat rate of FP data and CI, and 0.07 or more and 0.3 or more for spike amplitude and amplitude, respectively. Cardiosight®-S passed all applicable criteria by indicating the following values on Day 7.

CardioECR parameters of Baseline (Day 7) were stable with beat rate of 42 ± 3.9 bpm, Amplitude of ≥ 0.3, and FP Amplitude of ≥ 3. (data not shown)

Compound response

Diltiazem, an L-type Ca^{2+} channel inhibitor, is used in the treatment of hypertension and cardiac arrhythmias. In the *in vitro* contractility assay using cardiomyocytes, Diltiazem induced an increase in the beating rate and shortening in FPD. In addition, the measured values of FP and IMP are found to be very similar.

Little change was observed in the results of DMSO, the negative control of the experiment, in parameters such as Beat rate and Amplitude. However, significant decrease in a dose-dependent manner in spike amplitude and FPDcF in FP data for Diltiazem was detected when compared against the baseline (Figure 2. B). As for the IMP data of Diltiazem, it was found to decrease the contractile force (amplitude) and increase the beat rate, credited to the mode of action of diltiazem which inhibits the calcium influx during depolarization (Figure 2. C).

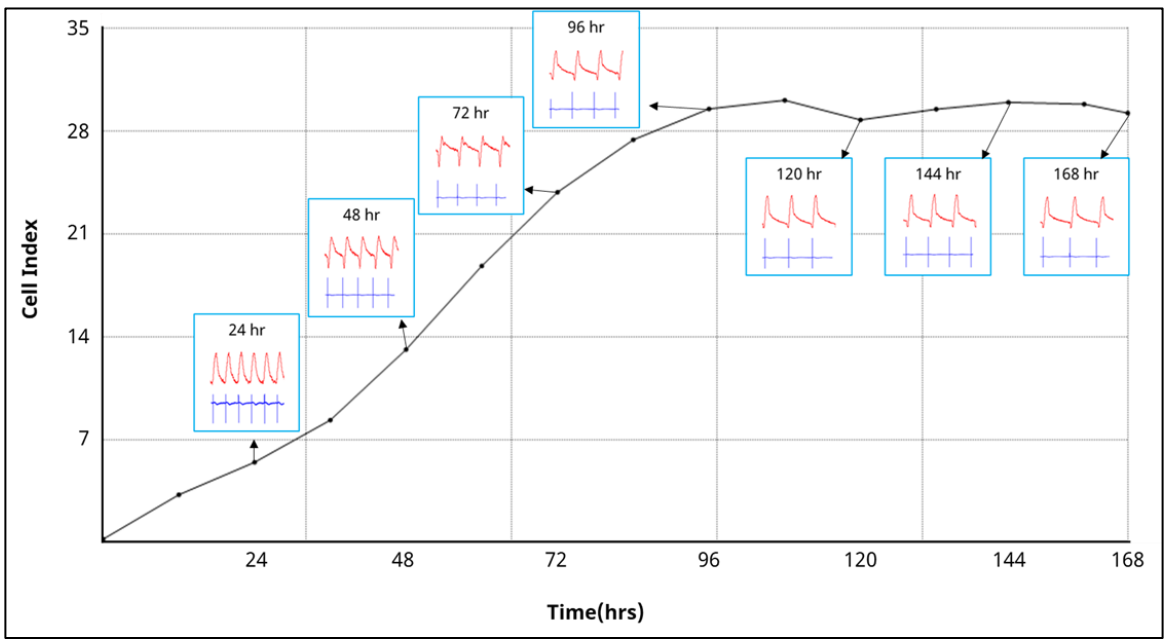


Figure 1. **The overall Cell index curve was recorded in real time after cell seeding.** Cell contraction (IMP; red trace) and field potential signal (FP; blue trace) were simultaneously measured along with the overall Cell index. Traces showed the representative tracks of FP and IMP captured from the CardioECR experimental view, recorded every 24 hours for 7 days of culture.

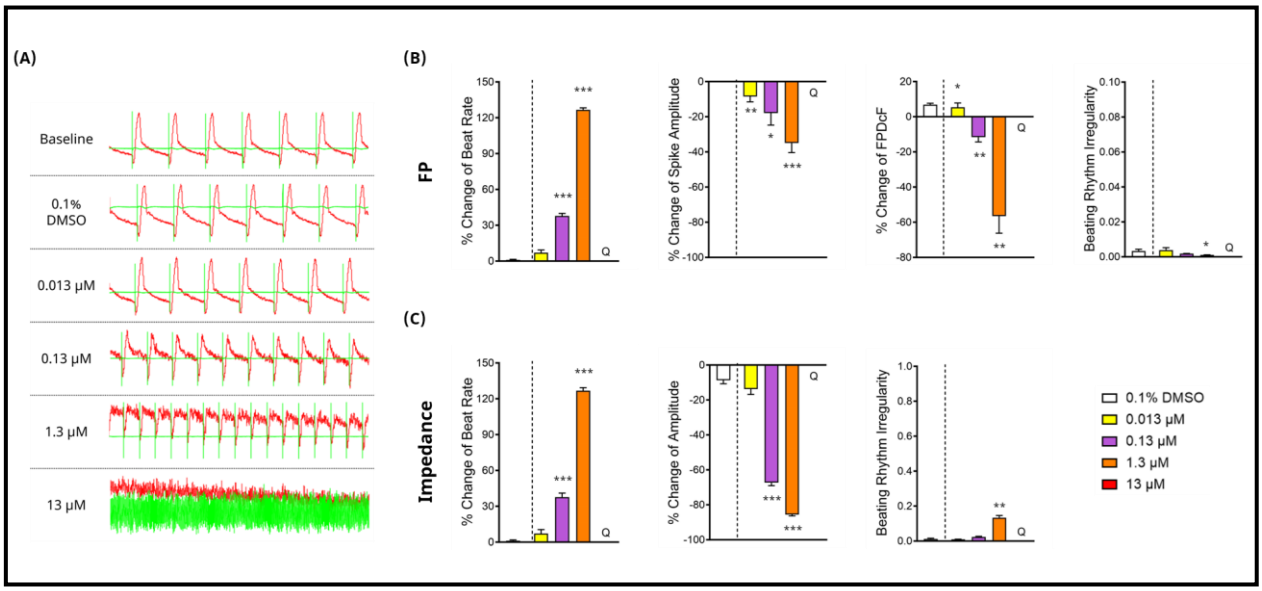


Figure 2. Cardiosight®-S were treated with Diltiazem. (A) Diltiazem was treated at concentrations of 0.013, 0.13, 1.3 and 13 μM for 30 minutes each and recorded for 30 seconds. FP and IMP traces were collected before and after drug addition. (B) **Effect of Diltiazem on cardiomyocyte Field Potential.** Trace overlay of FP exposed to increasing concentration of Diltiazem and the drug effect on FP beat rate, spike amplitude, FPDcf, and beating rhythm irregularity. (C) **Effect of Diltiazem on cardiomyocyte Impedance.** Trace overlay of impedance signals exposed to increasing concentration of Diltiazem and the drug effect on IMP beat rate, amplitude, and beating rhythm irregularity. (n=3), Bar graph represents mean ± S.E.M. *: p<0.05, **:p<0.01, ***:p<0.001 vs. Ctrl (0.1% DMSO).

The analysis of beating irregularity was difficult due to the proximity of 3rd concentration value to the noise signal filter threshold.

Conclusion

The combination of Cardiosight®-S and xCELLigence RTCA CardioECR System provides an insight into the cellular reaction to the compound treatment through various parameters. Spontaneous changes in field potential in iPSC-CMs represent response to ion channel blocker Diltiazem, causing shortening of FPDcF and increase of beat rate. With the use of hiPSC-derived Cardiomyocyte, Cardiosight®-S, spontaneous contraction and relaxation, and stable parameters such as beat rate and amplitude can be confirmed.

In conclusion, Cardiosight®-S, in combination with the CardioECR system, can effectively evaluate effects of compound by measuring IMP signal and FP signal. Cardiosight®-S can provide a constant and sensitive measure of reactivity to the drug. so, the potential as iPSC-CM to be used in trials to replace and develop nonclinical trials in the future is very high.

Reference

1. Mamoshina P., 2021. *Cell Rep. Med*. 2021 Mar 16; 2(3): 100216
2. Blinova, K et al., Cell Rep. 2018 Sep 25; 24(13): 3582-3592.

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

- Email: technical_support@nexel.co.kr
- Phone: +82-2-2088-8886

For questions about **xCELLIGENCE RTCA CardioECR system**, please contact Agilent Technical Support at

- HQ for general enquiries: Website: www.agilent.com
- Technical Support: informatics_support@agilent.com

Caution: Since all experimental steps described in this application note are optimized for Cardiosight®-S, results cannot be guaranteed when carried out with different cells. NEXEL recommends the use of media and reagents listed in the application note, otherwise results may not be replicable and further technical support may be difficult.