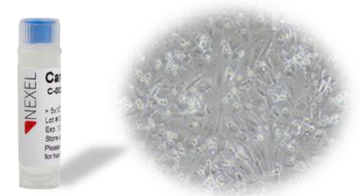
Application note

NEXEL

Measurment of calcium transient in cardiac spheroids of varied sizes formed from Cardiosight®-S



Introduction

One of the important topics in cardiovascular research is the study of calcium handling, due to its critical importance in the cellular functions. Even a small change in the intracellular calcium concentration can alter the function of the cell, with bigger impact to come in the downstream processes. For example, an increase in the intracellular calcium ions underlie cardiac contractile function, with the electrical depolarization of cardiomyocytes inducing and regulating changes in intracellular calcium concentration throughout the heart muscle. Therefore, measurement of calcium levels can be used to evaluate the efficacy and toxicity of compounds in human cardiomyocytes *in vitro*.

While 2D cell cultures are widely used across the industry for research and analysis uses, there is a distinct advantage given by employing 3D cell culture methods. Compared to 2D cardiac muscle cell cultures, 3D cardiac spheroid cultures mimic important features of human cardiac morphology, biochemistry, and pharmacology. Thus, offering a promising alternative to animal testing and standard cell cultures in terms of mechanistic insights and prediction of cardiotoxicity.

Unfortunately, there is yet to be a platform that allows a full extent of analysis on the electrophysiological features of 3D cardiac spheroids generated through the use of iPSC-derived cardiomyocytes. However, as an alternative, calcium transient assay can be carried out as a base experiment to reveal more information, which then may build up towards a full suite of electrophysiological experiments on 3D cardiac spheroids in the future. Hence, this application note describes a method for calcium transient analysis in 3D cardiac structures using cardiac spheroid constructed of Cardiosight®-S.

Workflow

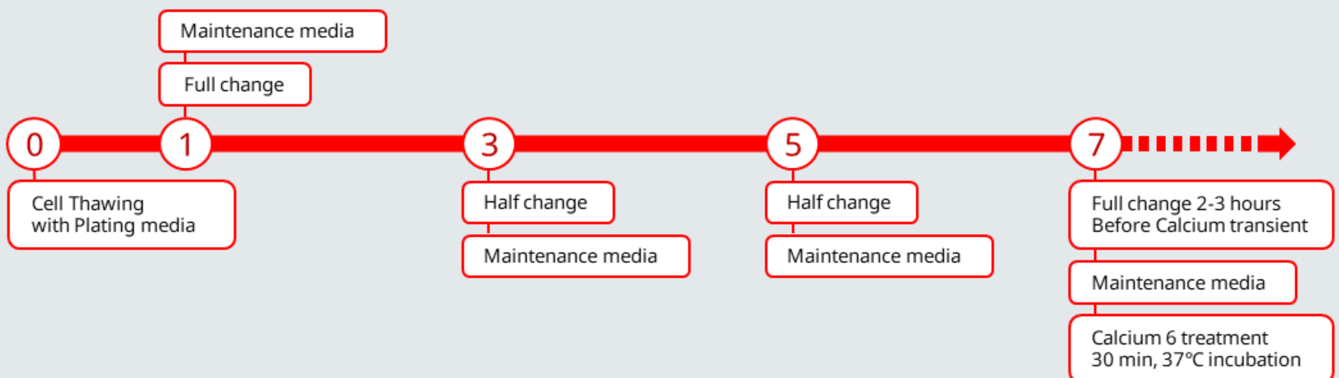


Table 1. Timeline of the Workflow from Day 0.

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

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

Cardiac Spheroid Formation using Cardiosight®-S

V bottom 96well plate

- Cells seeding
- 3, 5, 10, 30, 50 x 10³ cells/well
- Adding media on Day 2 to maintenance media and half media exchange every 48 hrs

1)

300µl

FLIPR® Calcium 6 (Molecular Devices, R8195)

Dimethyl sulfoxide (Sigma aldrich, D2650)

(Dissolve a reagent)

2)

20X

Maintenance media (NEXEL, CM-002A)

FLIPR® Calcium 6 (Molecular Devices, R8195)

(Reagent dilution)

10X

Black 96well plate (Corning®, 3340)

FLIPR® Calcium 6 - Medium Complex

(Reagent treatment)

Required Consumables

Item	Vendor	Catalog number
Cardiosight® -S Cardiomyocytes	NEXEL	C-001 C-002
Cardiosight® -S Advanced Media	NEXEL	CMS-001A CMS-002A
FLIPR® Calcium 6 Assay Kit (Component A)	Molecular Devices	R8195
Dimethyl Sulfoxide (DMSO)	Sigma Aldrich	D2650
Corning® CellBIND® 96-well Flat Clear Bottom Black Polystyrene Microplates, with Lid, Sterile	Corning	3340

Method

Culture and formation of cardiac spheroids from Cardiosight®-S

The cardiomyocytes used in this study were manufactured and provided by NEXEL Co., Ltd. Cells were thawed according to the Cardiosight®-S User Guide. To form the various sized cardiac spheroid from the Caridiosight®-S, we referenced the Cardiac Spheroid Formation Using Cardiosight®-S application note.

For media changes, prepare the required amount of maintenance media and place it in the room temperature. at least 30 minutes before the media change to allow it to warm up. Add only culture medium on Day 2 and change the medium by half every other day until Day 7.

Calcium transient assay

On the day of the assay, suspend the spheroids in 200 µl of culture medium and transfer to a black 96-well plate. Reconstitute the Calcium 6 dye (Component A) from the kit according to the manufacturer's instructions. To dissolve this dye, we use Dimethyl sulfoxide (DMSO), which is made up to 200X and stored at -20℃ and dissolved as needed.

The Calcium 6 dye prepared as above is mixed with the culture medium in a 1:20 ratio. Then replace 20 µl of culture medium in each well with 20 µl of Calcium 6 and incubate at 37℃ for 30 minutes. After 30 minutes of incubation, use a Nikon laser scanning fluorescence microscope to measure intracellular calcium influx.

Calcium transient images are measured for 10 seconds using a 10X objective and quantitative analysis of the images is performed using NISElements AR software version 5.3 from Nikon. This allowed to measure calcium changes within the cardiac spheroids.

Results

Calcium transient assay

FLIPR® Calcium 6 dye was used to measure the calcium transient of spheroids.

Cardiac spheroids were made with cells ranging from 1,000 cells to 50,000 cells. In the larger sized spheroids, it became apparent that the center portion of the spheroids did not stain as well as the smaller sized spheroids, as same amount of calcium dye was added across all groups. Nevertheless, calcium transient measurements were able to be taken in all spheroid sizes.

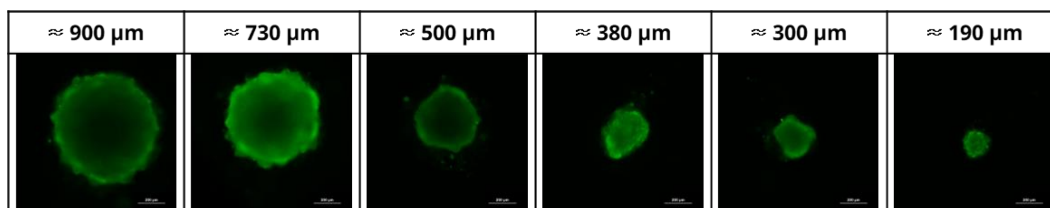

Figure 1. Measuring calcium transients in sized spheroids using Cardiosight®-S
Calcium transients were measured using Calcium 6 dye in cardiac spheroids sized according to the number of input cells. Results showed that extent of calcium dye penetration into the center of spheroid decreased as the spheroid size increased. However, the calcium transient could be measured regardless of the spheroid size and degree of calcium dye penetration. Approximate average size of cardiac spheroids made by seeding a certain amount of cells per well: 50,000 cells/well for ≈ 900 µm, 30,000 cells/well for ≈ 730 µm, 10,000 cells/well for ≈ 500 µm, 5,000 cells/well for ≈ 380 µm, 3,000 cells/well for ≈ 300 µm, and 1,000 cells/well for ≈ 190 µm.

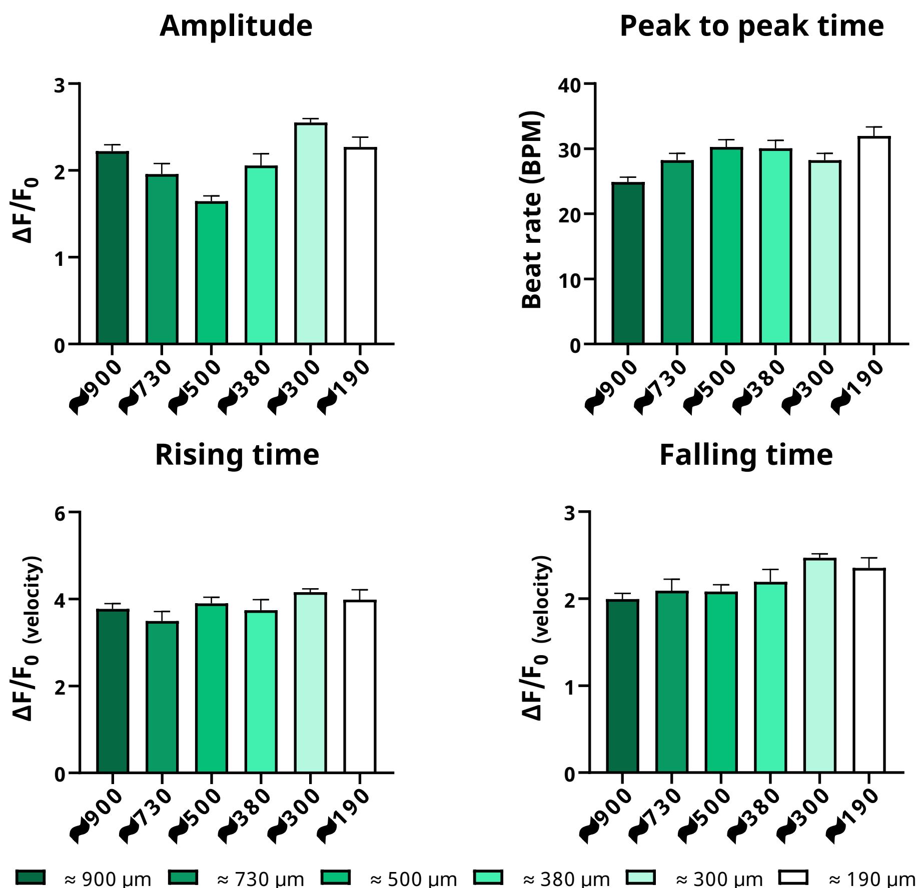

Figure 2. Calcium transient analysis of cardiac spheroids
After measuring the calcium transient according to the size of the cardiac spheroid using a fluorescence microscope, four parameters were analyzed: amplitude, peak to peak time (beat rate), rising time, and falling time. All four parameters showed similar values, with no tendency to increase or decrease in proportion to the size of the cardiac spheroids created.

Representative calcium transient traces were used to determine differences in various parameters such as amplitude, peak to peak time, rising time, and falling time based on spheroid sizes.

In all parameters, no significant differences were observed between groups, regardless of spheroid sizes.

Conclusions

Calcium transient within various sizes of cardiac spheroids created with Cardiosight®-S was taken with fluorescent microscopy. It can be observed that results of measured parameters, such as amplitude, peak to peak time, rising time, and falling time, were similar with no significant differences and no tendency to increase or decrease regardless of the spheroid size.

Therefore, it can be presented that the application of 3D cardiac spheroid with calcium-detecting dye was successfully demonstrated to measure calcium ion, one of the most important ions that plays a critical role in cardiomyocytes.

Considering the result of current application, it could be expected that 3D cardiac spheroid platform can evolve from a simple use in cellular function evaluation to a more intricate practice in drug evaluation and/or cardiotoxicity in the near future.

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

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.