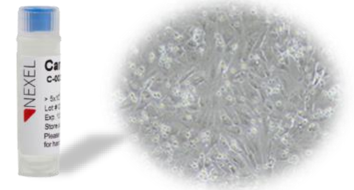
Application note

NEXEL

Introduction of foreign DNA into Cardiosight®-S through liposome-mediated transfection



Introduction

Transfection is the introduction of foreign DNA into a cell to assess gene expression, evaluate gene silencing, and for the analysis of recombinant proteins. Transfected cells enable the study of genes and their functions by either repression or overexpression and to produce recombinant proteins[1].

iPSC-derived differentiated cells are very difficult to transfect without a viral vector base[2]. However, if liposome-mediated transfection is possible, various disease models, acute or chronic, can be created within iPSC-derived differentiated cells without the need for CRISPR.

NEXEL Co., Ltd. strives to provide high-quality human cardiomyocytes derived from induced pluripotent stem cells (iPSC-CMs). iPSC-derived cardiomyocyte is in the spotlight as an alternative model for research on cardiac and vascular diseases that are difficult to model in laboratory or experimental animals.

In this application note, we established a highly efficient liposome-mediated transfection method for Cardiosight®-S (NEXEL Corporation), which could be applied towards promotion of cardiac repair[3], create cardiac disorder models, and complement preceding *in vivo* models[4].

Workflow

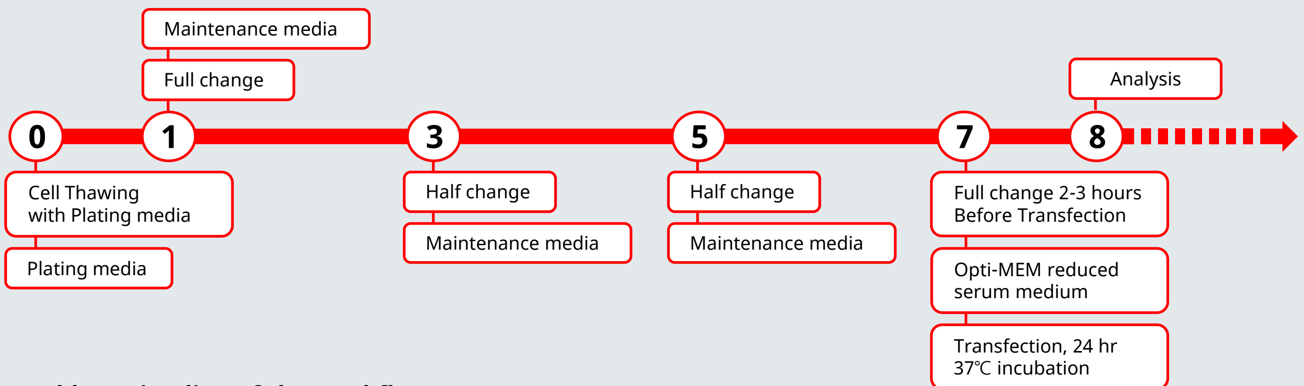
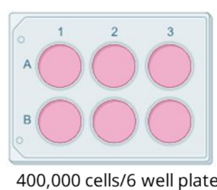
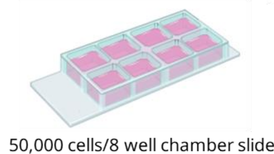


Table 1. Timeline of the Workflow From Day 0.

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

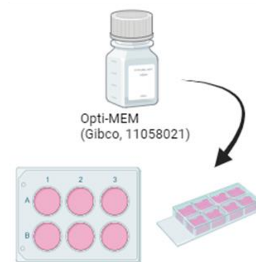


400,000 cells/6 well plate

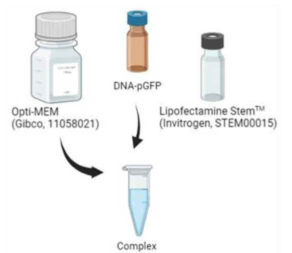


50,000 cells/8 well chamber slide

1) Media change with Opti-MEM

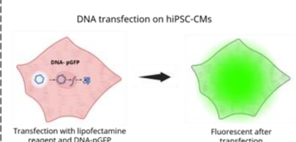


2) Prepare a complex for transfection



3) Transfection

4) Analysis



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

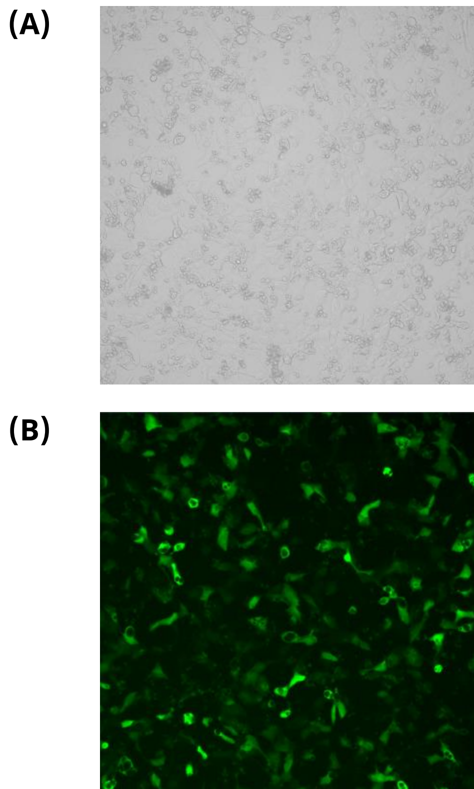


Figure 1. Cardiosight®-S transfected via Lipofectamine Stem.
Cells transfected with GFP-tagged plasmid were imaged using fluorescence microscopy. Image showing (A) bright-field and (B) GFP fluorescence.

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.
Cardiosight®-S were plated into a Matrigel-coated 6-well plate in CMS-A plating media at a density of 400,000 cells/well for Flow cytometry (FACs) analysis. And Fibronectin-coated 8-well chamber slide in CMS-A plating media at a density of 50,000 cells/well for a fluorescent microscope analysis.
The following day, the plating media was replaced with maintenance media, and maintained the cells by half volume media change every other day.

Transfection

Transfection was based on Lipofectamine Stem's (Invitrogen, #STEM00015) manual.
2-3 hours before transfection, replace media with Opti-MEM Red Serum Media no phenol red (Gibco, #11058021). Then incubate incubation at 37℃ to After mixing the LipoSTEM and pDNA in a ratio of 1.0:1.2, incubate at RT for 10 minutes (The ratio of complex to each plasmid DNA used needs to be optimized). After putting the prepared complex into the iPSC-CM to be 10% of the total media, incubate it for 24 hours.

Transfection efficiency measurement

The transfection efficiency of hiPSC-CMs was analyzed by flow cytometry (ACEA, NovoCyte) and Fluorescence Microscopy (Nikon, Eclipse Ti2).

The cells detached with 1X TrypLE were resuspended in DPBS. GFP expression in Control and Transfected cells was measured with flow cytometry and acquired data was analyzed with NovoExpress (ACEA).

Cardiosight®-S on an 8-well chamber slide uses a fluorescence microscope to determine transfection efficiency. Transfection images of the Cardiosight®-S are measured using a 10X Objective and taken twice using bright field and fluorescence laser (green). Two versions of the image are acquired: a bright field image to determine the number of cells seeded, and a fluorescence laser (green) image to determine the cells transfected.

Image J analysis

Image J analysis is described in Figure 2.
Calculate the area (%) of the plated cell from the bright field image of the Transfection cell (Figure 2. upper). And fluorescence laser image in the same location also calculates the area (%) (Figure 2. bottom). Among the plated cells, % of the cells expressing GFP were analyzed by Image J to quantify the efficiency of Transfection.

Results

An experiment was performed in advance to determine the transfection efficiency in Cardiosight®-S. Five different conditions of LipoSTEM to pGFP were tested: 0.8:1.0, 0.8:1.2, 0.8:1.4, 1.0:1.2, and 1.0:1.4 (LipoSTEM:pGFP ratio). The highest transfection efficiency was achieved with the LipoSTEM:pGFP ratio of 1.0 to 1.2, respectively. Based on this result, the volume of transfection agents was scaled up and cells were seeded in 6-well plates for flow cytometry and 8-well chambers for fluorescence imaging.

Transfected Cardiosight®-S was removed from the 6-well plate and its transfection efficiency was measured using flow cytometry. All data were gated based on light-scattering properties. GFP expression was gated relative to negative control. Results showed an average of 0.17% in the control and an average of 40.38% in cells transfected with pGFP.

Furthermore, the transfection efficiency of cells cultured in an 8-well chamber was measured using

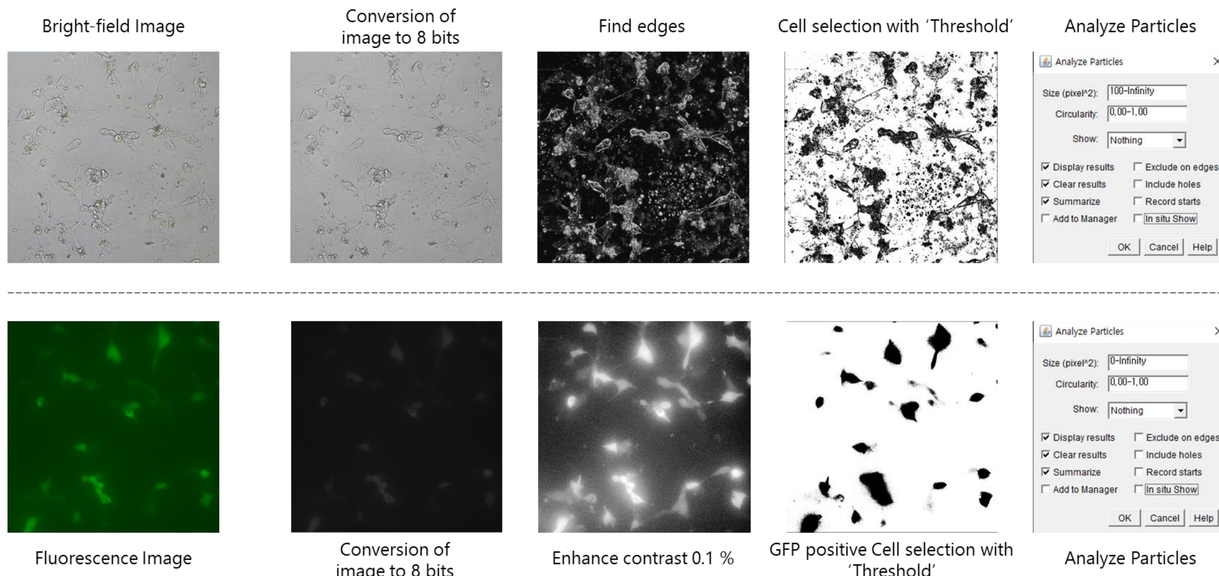


Figure 2. **Analysis process of transfection efficiency with Image J in fluorescence images.**
Both bright-field and fluorescence images are required for analysis of transfected cells. The **bright-field image** was converted to 8 bits for analysis, had edges of the cells highlighted with the "Find edges" function, and selected the area taken up by cells by using the "Threshold" function to calculate the area. **The fluorescence image** was likewise converted to 8 bits, had the GFP expression brightened through the "Enhance Contrast" function, and the area was calculated the area taken up by cells using the "Threshold" function. Each area was analyzed with the "Analysis particle" function, and the area of GFP expression compared to the area of bright-field was calculated to determine the efficiency of transfection.

fluorescence microscopy, with the aid of the Image J program. Images of bright-field and fluorescence images (n=3) were analyzed to quantify the transfection efficiency, and it revealed that transfected cells account for 38.5% of total cells on average.

While analysis through fluorescence microscopy has an advantage of being able to visually identify the expression of GFP but has a disadvantage that only a small portion of the entire well is analyzed. On the other hand, flow cytometry can quantitatively measure the number of cells that express GFP, but cannot visually confirm the GFP fluorescence. Therefore, both methods were used to confirm the transfection efficiency. The resulting transfection efficiency were determined at near-identical 38.5% and 40.38% with fluorescence microscopy and flow cytometry, respectively.

Conclusion

Cardiosight®-S provides an in vitro test system that is used to study the function of genes. The results above demonstrate that Cardiosight®-S can be successfully transfected with plasmid DNA

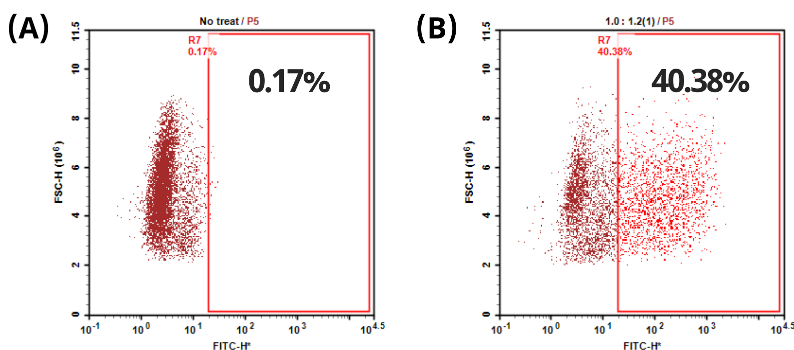


Figure 3. **Representative flow cytometry results of transfection efficiency.**
Transfection efficiency measured by flow cytometry after transfecting Cardiosight®-S with GFP-tagged plasmid. The control (A) was measured at 0.17%, while the group transfected with GFP tagging plasmid (B) was measured at 40.38%.

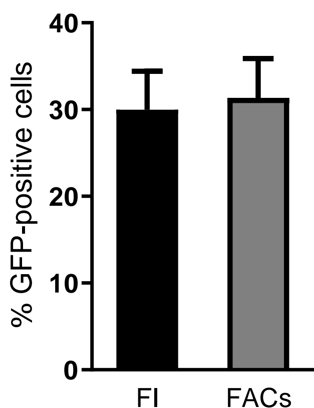


Figure 4. Comparison of transfection efficiency measured by fluorescence microscopy and flow cytometry.
Image of transfected cells cultured in an 8-well chamber slide were taken using fluorescence microscope and analyzed through Image J. The resulting transfection efficiency was determined at 38.5%. In addition, the transfection efficiency of cells cultured in a 6-well plate was measured through flow cytometry and was determined at 40.38%. The difference between two values of different analysis methods is not significant.

using lipofectamine as genetic carrier. In addition, the ability to transfect cells will become the cornerstone of future experiments that will dive into disease models, which often involves up- or down-regulation of specific gene or group of genes.

Exploring disease models with primary heart tissue is difficult due to its innate nature of non-renewable tissue. Therefore, the availability of human-derived pluripotent stem cell-derived cardiomyocytes can expand the horizon with experiments that can be carried out with the highest similarity to the characteristics of a primary human cardiomyocytes. With confidence, the transfection ability of Cardiosight®-S will make down- or up-regulation of certain targets possible, which will allow further exploration of cardiomyocytes in various fields.

Reference

1. Ruedel, A.; Bosserhoff, A.K. Methods in Cell Biology, 2012; Volume 112, p. 544.
2. Tan, S., Tao, Z., Loo, S. et al. Non-viral vector based gene transfection with human induced pluripotent stem cells derived cardiomyocytes. Sci Rep 9, 14404 (2019).
3. Pagliari, S.; Romanazzo, S.; Mosqueira, D.; Pinto-do-O, P.; Aoyagi, T.; Forte, G, Med. Chem. 2013, 20, 3429–3447.
4. Mosqueira, D.; Smith, J.G.W.; Bhagwan, J.R.; Denning, C. Trends Mol. Med. 2019, 25, 775–790

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.