# ROBUST DIFFERENTIATION PROCEDURE INTO HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS AND THEIR PROPERTIES

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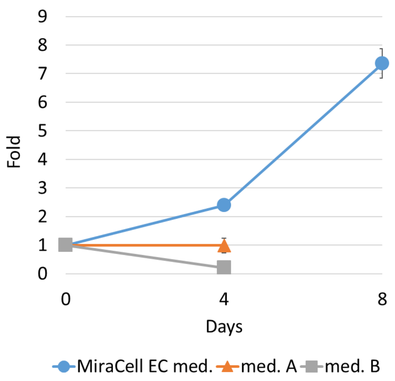
## Abstract

Primary human endothelial cells (ECs) such as HUVECs are widely used for vascularization study, various organoids formation or safety/toxicological test. However, primary ECs sometimes show uncontrollable lot-to-lot variances in these researches due to genetic diversity of derived donors, and thereby may lead to the difficulty of obtaining the reproducibility of the experiment. Human induced pluripotent stem cell-derived ECs (iPS-ECs) are expected to use for these research field as cell source having stable properties among manufacturing batches from single donor. Nevertheless, iPS-ECs have not been widely utilized in those experiments, since current iPS-ECs have some problems such as unstable differentiation from iPS cells or poor growth capacity. To overcome this, we have developed robust differentiation procedure into iPS-ECs in which continuous proliferation has been observed. We tested their ability of the procedure to differentiate into ECs from various iPS cell clones. All iPS-ECs from tested iPS cell clones showed more than 90% of both CD31 and CD144 positive population, which are typical endothelial cell surface markers. Then, these iPS-ECs were evaluated for their growth capacity for one month. Although all iPS-ECs exhibited almost similar endothelial cell-related genes and markers expression, there were big differences in the proliferation rate. The most proliferated iPS-ECs showed about one thousand fold expansion, even though one out of 6 iPS-ECs could little proliferate after differentiation (about a few fold expansion). In spite of the differences in growth capacity, all iPS-ECs retained both CD31 and CD144 positive population during observation period. We also confirmed whether iPS-ECs could be used for angiogenesis inhibition assay by observing the tube formation by measuring the fluorescence of calcein-labeled ECs. As a result, the inhibition of tube formation was observed in the addition of Wortmannin in a dose-dependent manner. Overall we have successfully developed iPS-ECs differentiation system. It can be overcome the lot variation problems of primary endothelial cells, making them suitable for various kinds of vascularization researches.

## Culture medium for iPSC-derived Endothelial Cells

Fold expansion




Commercial available medium for culturing primary HUVECs couldn't be proliferated iPSC-derived endothelial cells.

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The media formulation was optimized for iPSC-derived endothelial cells and newly developed medium was named "MiraCell™ EC medium".

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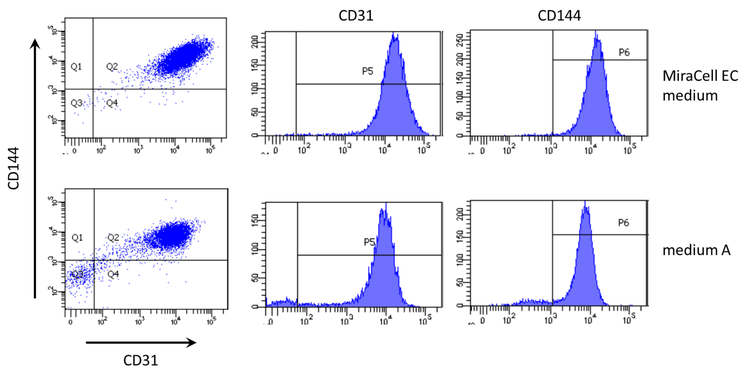
MiraCell™ EC medium, medium A or medium B were tested for their ability on expansion of iPSC-derived endothelial cells (iPS-ECs). MiraCell™ EC medium and medium A are commercially available, and medium B was prepared from M199 supplemented with several growth factors such as VEGF, EGF and FGF.

FCM analysis




iPS-ECs could be expanded in MiraCell™ EC medium, whereas cells didn't proliferate in medium A or B. At day4, cells in MiraCell™ EC medium or medium A were stained by anti-CD31ab and anti-CD144ab, which are endothelial cell marker, and then analyzed by flow cytometer. Although iPS-ECs in MiraCell™ EC medium were maintained both CD31 and CD144 expression, a little decrease of CD31 and CD144 expression were observed in cells cultured by medium A.

## MiraCell iPS Cell to Endothelial Cell Differentiation Kit

iPSC culture → mesoderm differentiation → Endothelial cell differentiation → expansion

DEF-CS® 500 Culture System

MiraCell™ iPS Cell to Endothelial Cell Differentiation Kit

MiraCell™ EC Culture Medium

We have developed "MiraCell™ iPS Cell to Endothelia Cell Differentiation Kit" that can be used for iPSC differentiation into endothelial cells by only medium change and subculture.

*Acknowledgement*

We really appreciate to members of Yamashita Lab. and iHeart Japan corporation for great discussion and comments.

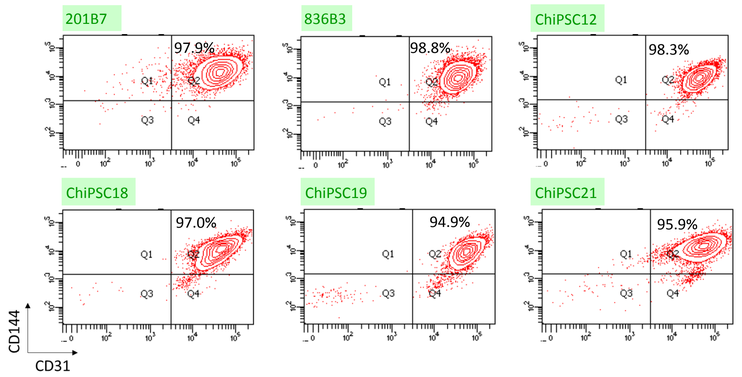
## Endothelial Cell differentiation from various iPS cell lines

Donor information of each iPS cell line

| iPS cell line | Age | Gender | Karyotype | | Race |
|---|---|---|---|---|---|
| 201B7 | 30s | Female | 46 | XX | Caucasian |
| 836B3 | 30s | Female | 46 | XX | |
| ChiPSC12 | 24 | Male | 46 | XY | Caucasian or North African |
| ChiPSC18 | 32 | Male | 46 | XY | |
| ChiPSC19 | 23 | Male | 46 | XY | |
| ChiPSC21 | 26 | Male | 46 | XY | |

201B7/836B3
Takahashi K. et al., Cell, 2007 Nov 30, 131(5):861-72,
Nakagawa M., Koyanagi M. et al., Nature Biotechnology, 2008 Jan: 26(1): 101-6
ChiPSC12/18/19/21
commercially available from Takara Bio Inc.

FCM analysis of iPS-ECs

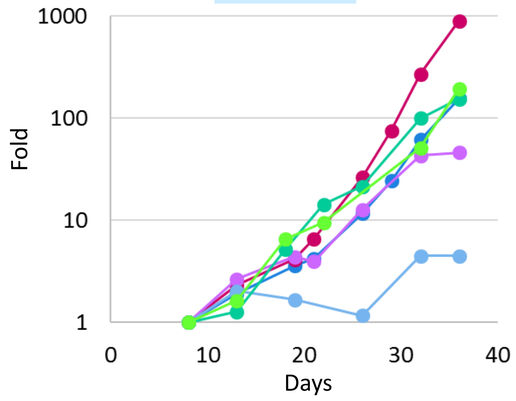



Our endothelial cell differentiation procedure and newly developed iPS-EC culture medium, "MiraCell™ EC medium" were used in several iPS cell lines. After endothelial cell differentiation, cells were cultured in MiraCell™ EC medium for 14 - 18 days.

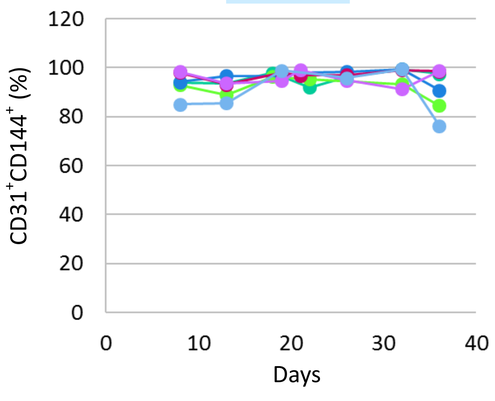
At day14 (201B7, 836B3) or day18 (ChiPSC12, 18, 19, 21), endothelial cell marker (CD31 and CD144) expression was analyzed by flow cytometer. More than 94% of double positive cells were observed in all iPS-ECs.

## Long-term culture of iPS-ECs

Fold expansion



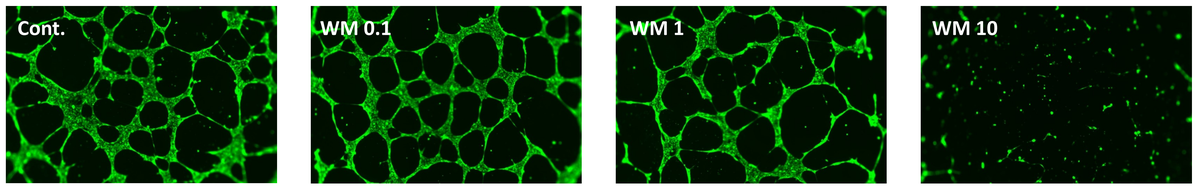

FCM analysis



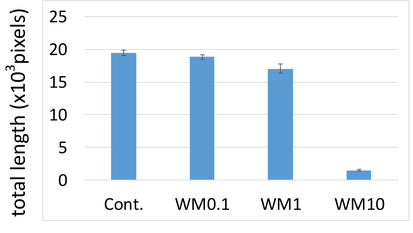

Each endothelial cells differentiated from various iPS cell lines was cultured in MiraCell™ EC medium for more than 1month. Each iPSC-derived endothelial cells except for ChiPSC21-derived ECs showed continuous proliferation during cultivation period. Endothelial cell surface markers, such as CD31 and CD144, were highly maintained in all tested iPS-ECs, even though growth rate in each iPS-ECs was largely different.

## In vitro angiogenesis inhibition assay

Tube formation




Total length




iPSC-derived endothelial cells were seeded on matrigel to test the tube formation ability. Wortmannin (WM), PI3 kinase inhibitor, was used to confirm the inhibition of tube formation in this assay. After overnight cultivation, cells were stained by calcein-AM, and then observed the total length of tube using ImageJ software.

Tube formation by iPS-ECs was observed, and the formation was inhibited by Wortmannin in dose-dependent manner. The total length of tube in quantitative measurement was also confirmed to be decreased by the addition of Wortmannin.

## Summary

✔Robust differentiation method into endothelial cells from various iPS cell lines was established.

✔iPS-ECs produced by our method showed long-term (>1 month) proliferation using MiraCell™ EC medium ,and also maintained endothelial cell surface marker expression during cultivation period.

✔iPS-ECs could be used for angiogenesis inhibition assay.