NEWLY OPTIMIZED MEDIUM FOR THE EXPANSION OF HUMAN PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS

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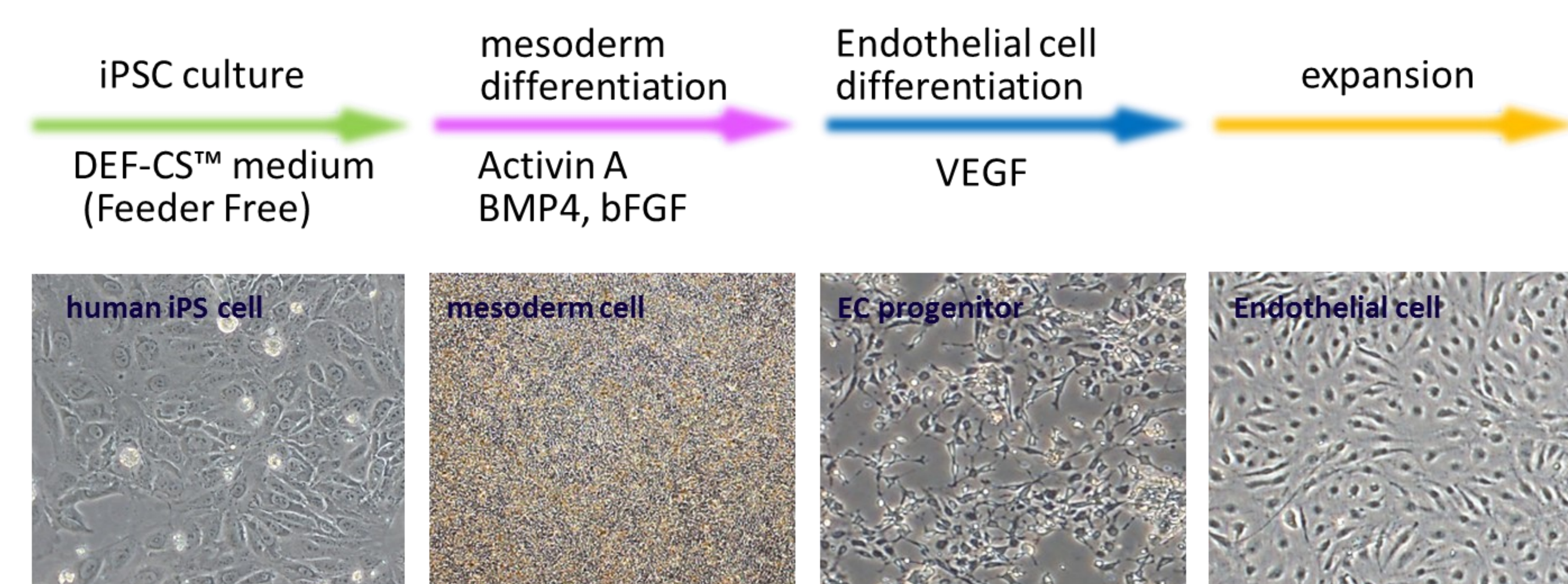
Abstract

Human endothelial cells (ECs) are widely used in the field of vascularization study such as drug discovery, safety/ toxicological test, tumor growth or tissue engineering including organ-on-a-chip technology. Although primary ECs like HU-VECs are generally used for these purposes, there are some drawbacks in utilizing primary cells because of the donor difference due to a wide variety of genetic background. To overcome this, human pluripotent stem cell-derived ECs (hPS-ECs) are expected to be supplied as stable cell source with similar properties and without the donor difference among manufacturing batches. We have already developed the differentiation method from pluripotent stem cells into ECs population having more than 95% of CD31 and CD144 positive cells, which are identified as endothelial cell. Our hPS-ECs also express CD34 in more than 95% of cells, suggesting immature phenotype. This is considered to be one of the appropriate features for tissue engineering study because ECs may be needed to be educated to fit in each tissue. However, it was difficult to obtain a large number of hPS-ECs by using commercially available medium for primary ECs due to poor proliferation capacity, even though many cells are required for tissue engineering. Thus, we have also developed the culture medium for hPS-ECs. As a result, it was observed that hPS-ECs show about 10-fold expansion in one week by newly optimized medium. The expanded hPS-ECs maintained ECs surface marker (CD31 and CD144) expression similar to the cells in pre-expansion. Furthermore, tube-like structure formation on matrigel was also observed after expansion, indicating an angiogenic capacity of hPS-ECs. Overall we successfully developed hPS-ECs culture system using newly optimized medium. The cells and medium can overcome the lot variation problems of primary endothelial cells, making them suitable for industrialization with mass cell production. Further, we are currently developing xeno-free medium for hPS-ECs to apply to the manufacture of regenerative medicine.

Endothelial Cell differentiation



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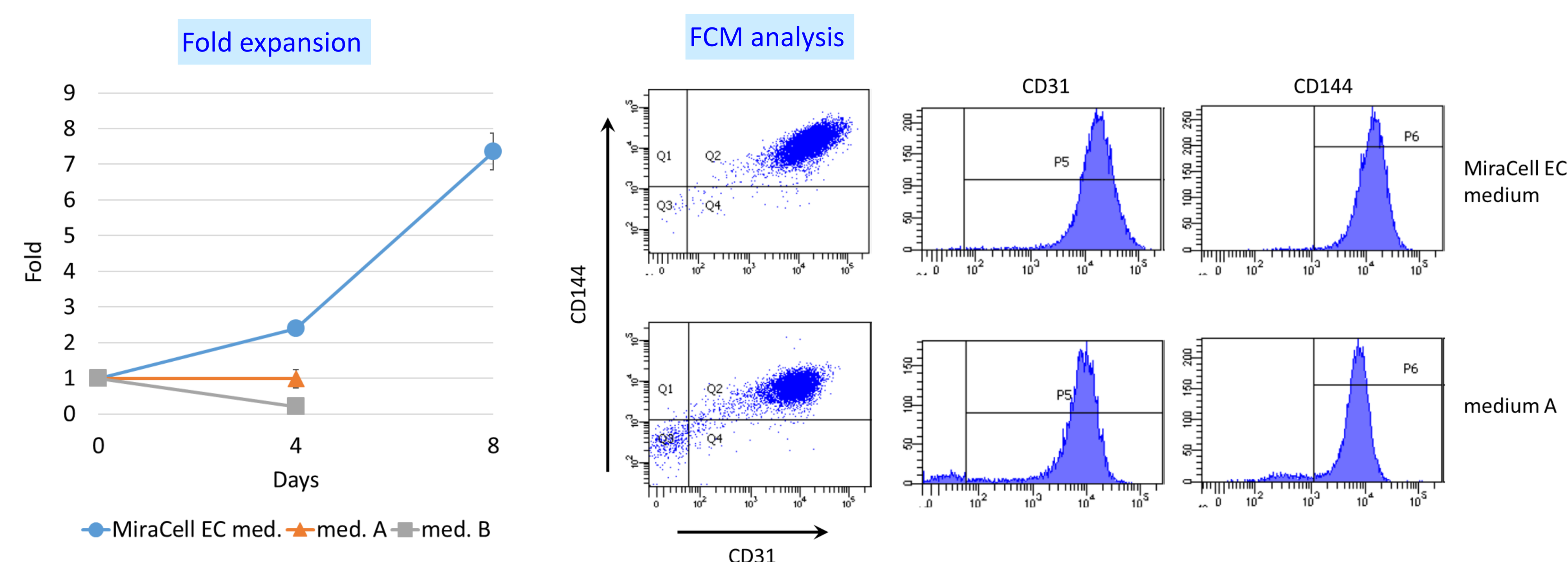
Human iPS cells were cultured in DEF-CS™ medium and then differentiated into endothelial cells according to previous report with some modifications regarding to culture medium, coating matrix, seeding density and so on. Although iPS cells were cultured with conditioned medium of mouse embryonic fibroblasts (MEF-CM) on matrigel, we changed to DEF-CS™ feeder-free culture system and made master cell bank (MCB) for stable cell processing. We have already launched iPSC-derived endothelial cell product as "MiraCell™ Endothelial Cells (from ChiPSC12)".

Culture medium for iPSC-derived Endothelial Cells



Three culture medium 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. 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 me-

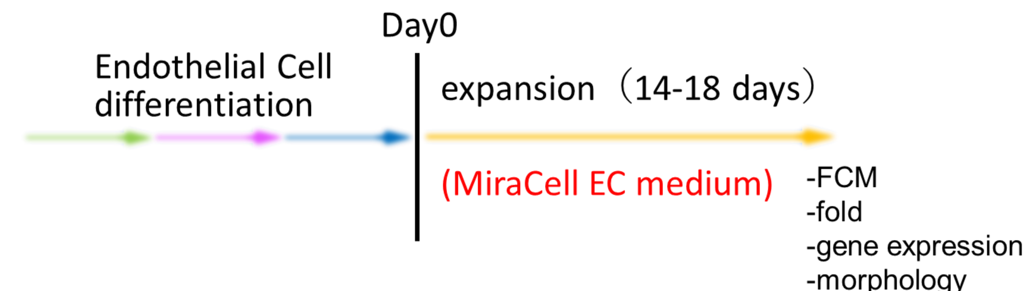
Endothelial Cell differentiation from various iPS cell lines

Donor information of each iPS cell line

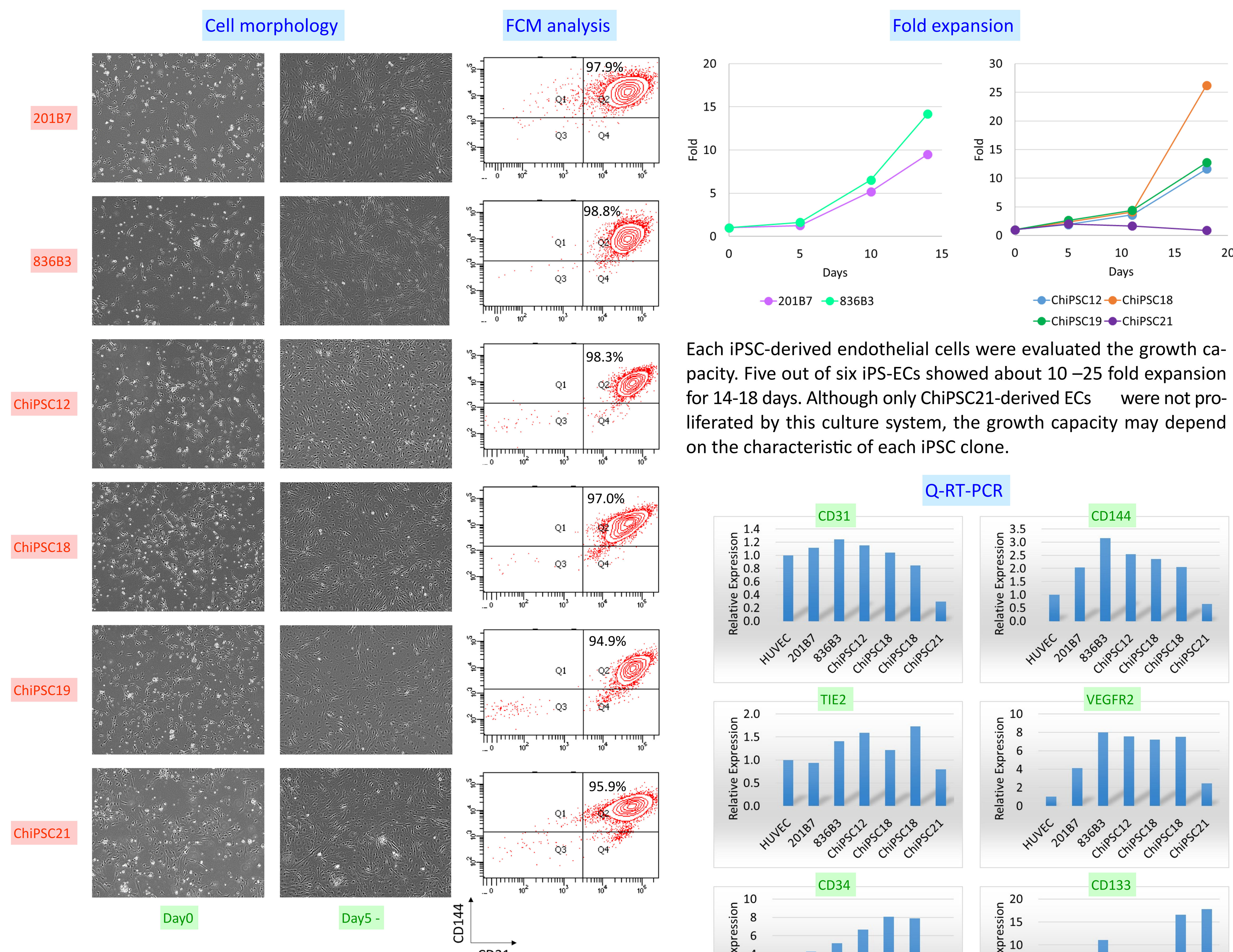
iPS cell line	Age	Gender	Karyotype		Race
201B7	30s	Female	46	XX	Caucasian
836B3	30s	Female	46	XX	
ChiPSC12	24	Male	46	XY	Caucasian or North African
ChiPSC18	32	Male	46	XY	
ChiPSC19	23	Male	46	XY	
ChiPSC21	26	Male	46	XY	

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.

Experimental scheme



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



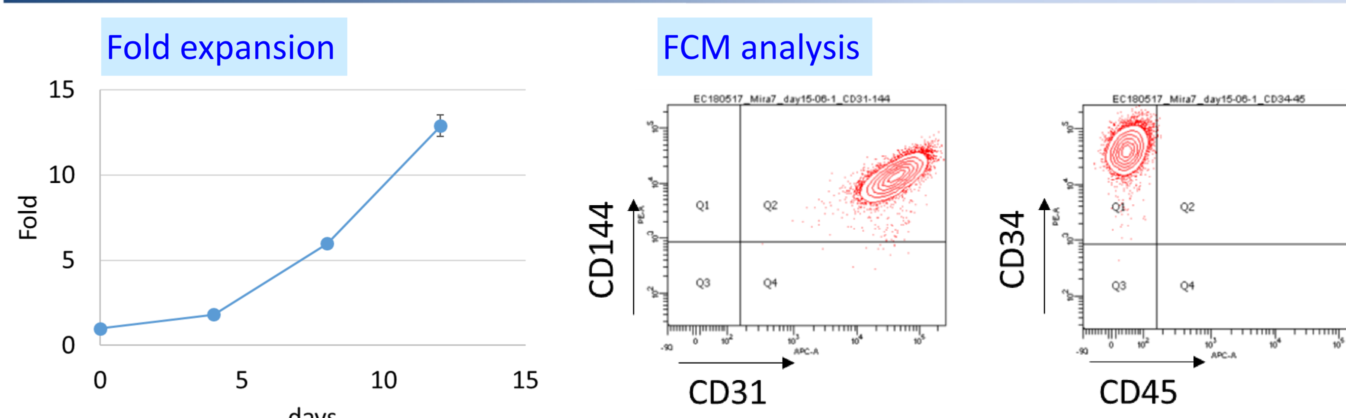
Each iPSC-derived endothelial cells were evaluated the growth capacity. Five out of six iPS-ECs showed about 10 –25 fold expansion for 14-18 days. Although only ChiPSC21-derived ECs were not proliferated by this culture system, the growth capacity may depend on the characteristic of each iPSC clone.

Each iPSC-derived endothelial cells at the beginning of expansion (day0) showed narrow and branched morphology. Homogeneous endothelial cell-like morphology was observed in all clones 5 days after culturing by MiraCell™ EC medium.

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.

Endothelial cell markers such as CD31, CD144 and Tie2 were expressed on each iPSC-derived endothelial cells as equal to HUVECs. On the other hand, the expression levels of CD34 and CD133 were much higher in iPS-ECs compared to HUVECs, suggesting iPS-ECs produced by our method might also have the potential of early-stage endothelial cells.

Xeno-free medium



The formulation of MiraCell™ EC medium was changed to it using xeno-free components. Fold expansion was almost equal to original MiraCell™ EC medium. Further, the expanded cells showed the expression of EC marker (CD31, CD144) and early-stage EC marker (CD34) but not lymphocyte marker (CD45) as similar to the cells expanded by

Summary

✓ MiraCell™ EC medium was newly developed for culturing iPSC-derived endothelial cells due to poor proliferation capacity in conventional medium for primary endothelial cells.

✓ iPS-ECs produced by our culture system using MiraCell™ EC medium showed more than 94% of CD31 and CD144 double positive population and higher expression of endothelial-cell progenitor markers, CD34 and CD133.

✓ Xeno-free formulation has been also developed for therapeutic application of regenerative medicine.

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