Application Note

MakCell
Compact Automatic Cell Culture Apparatus

Data courtesy of Specially Appointed Associate Prof. Matsui, The University of Osaka.

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Early endoderm differentiation using MakCell

The purpose of this data is to show how automation and mechanization of medium change can reduce worker workload. We tested whether there is a difference between manual and automated methods in the appropriate timing of using various media and reagents to differentiation of iPS cells. Therefore, we were used MakCell, which can use three kinds od culture medium and can set some schedules for medium change.

We were installed in MakCell of three types of culture medium. and used to scheduling software and automatic differentiation induction by the device. Furthermore, we tested whether automatic differentiation induction is possible by introducing a wash sequence at the time of switching medium. As a control group, we compared the induction of early mesoderm differentiation by manual. As a method of differentiation, we added CHIR99021 from day 0 to day 2 and ITS-X from day 2 to day 6. We collected cells daily from the start of culture. And we analyzed the gene expression levels of those cells as pluripotent markers and mesoderm markers.

METHODS

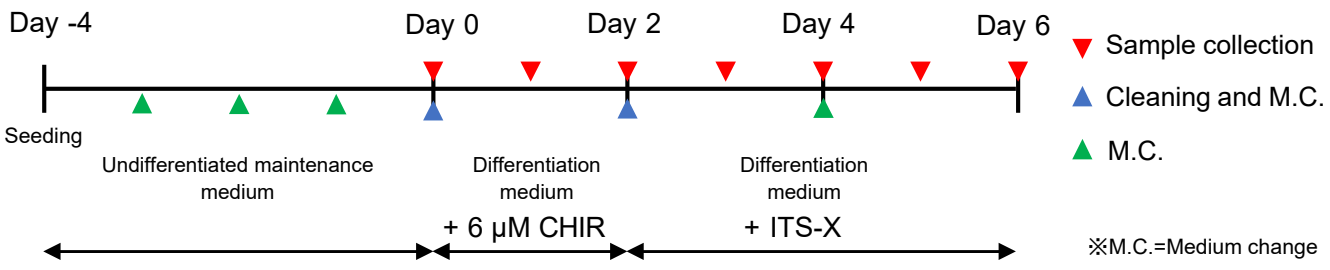
Cell: Human iPS 253G1 (seeding density: $8x10^5$ cells/well)
Human iPSCs maintained using CellPet 3D-iPS and CellPet FT, and we dispersed spheroid and seeded in 12-well plate.
Undifferentiated maintenance medium: AK02N (Ajinomoto)
Culture plate: 12 well plate (Falcon), iMatrix-511 (Nippi)
Differentiation medium: RPMI1640 (Wako), Ascorbic acid (Sigma), BSA (Wako), GlutaMAX (Gibco)
Reagent: CHIR99021 (Wako), ITS-X (Gibco)
Wash medium: differentiation basal medium
RT-qPCR: StepOne Plus (ThermoFisher), TaqMan Gene Expression Assays (ThermoFisher)

MakCell setting

Set 3 types of medium on Day 0

Bottle I : Wash medium (same as differentiation medium)
Bottle II : Differentiation medium + 6 μM CHIR99021
Bottle III : Differentiation medium + ITS-X
Supply tube: " 500 mL/1000 mL bottle supply tube set (low absorption)"

Schedule for medium change and sample collection



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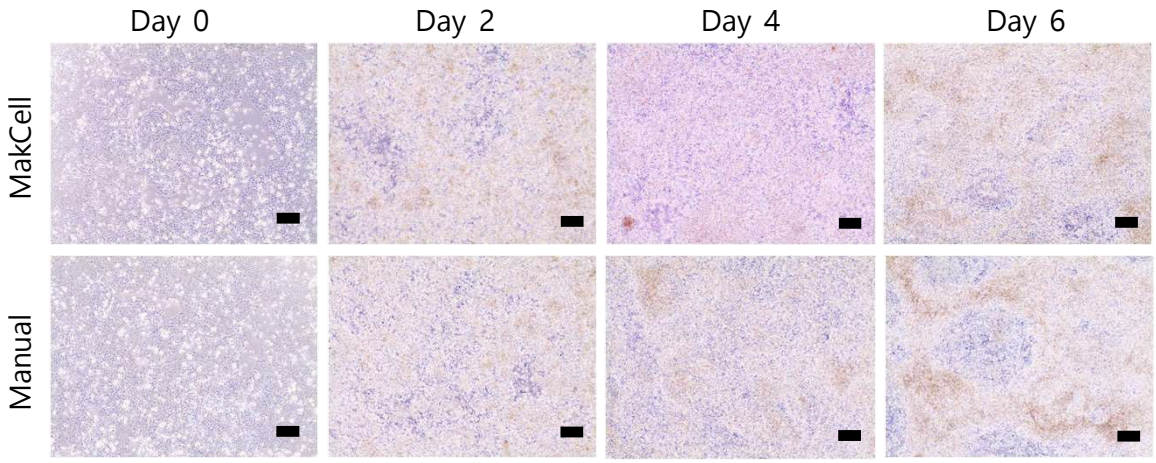
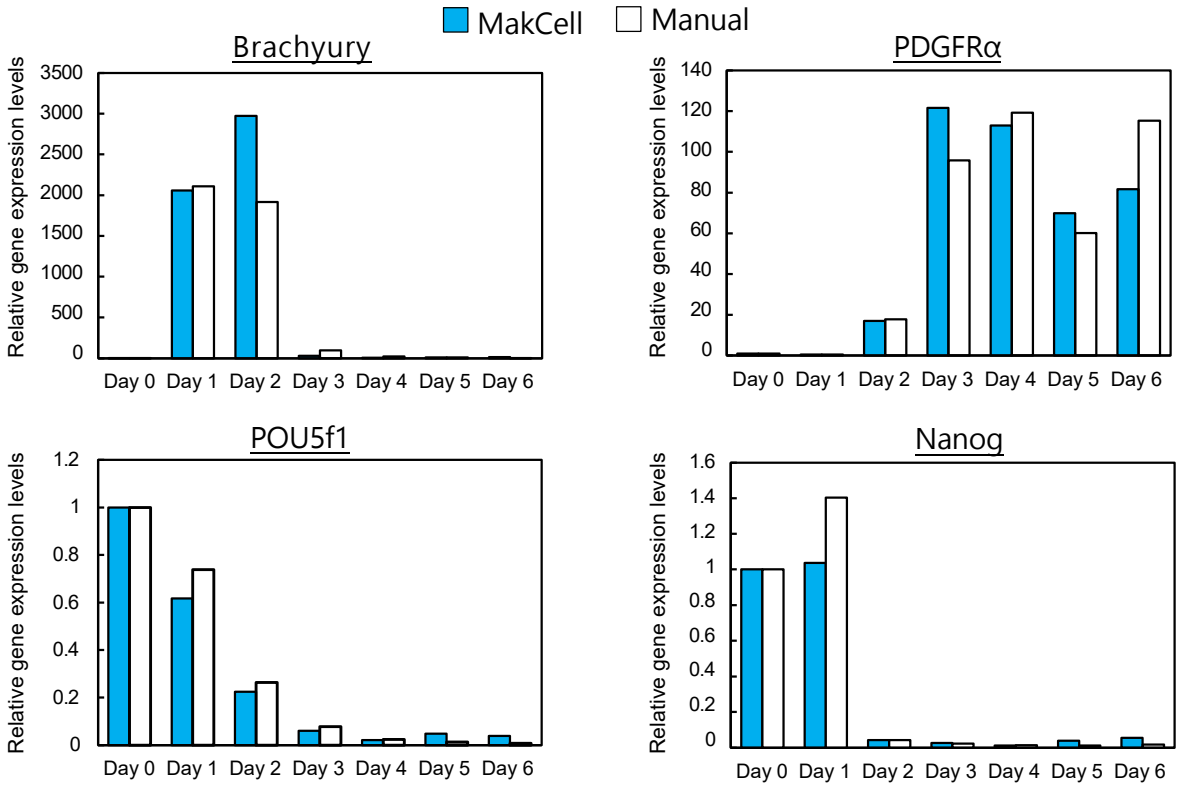
RESULTS



Microscopic images during differentiation (Bar = 200 μm)



Gene expression levels of pluripotent and mesoderm markers

We observed cell morphology in each condition and could not identify any differences. Similarly, the results of gene expression analysis did not confirm any differences. We detected Brachyury expression levels were increased on Day1 and 2, the same timing as the addition of CHIR99021, followed by a decrease. PDGFR alpha expression levels were found to increase from day 2. On the other hand, the expression levels of pluripotent markers decreased after day 2. These results suggested that hiPSCs were induced to differentiate to early mesoderm differentiation.

DISCUSSION

We tried to mesoderm differentiation of hiPSCs by switching between three culture medium using manual or MakCell. In the manual, pluripotent markers decreased, and mesoderm markers increased during the differentiated period. This result was similar when Makcell was used.
These results suggest that it is possible to automate differentiation induction using MakCell.

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