Application Note

MakCell
Compact Automatic Cell Culture Apparatus

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

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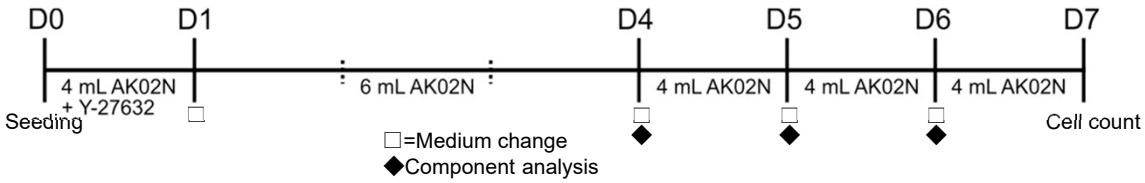
Analysis of medium component using MakCell's culture

In daily medium change during cell culture, it is important to remove cellular metabolites by aspiration and disposal of culture supernatant, and to supply substances that are reduced by metabolism. Especially in human stem cell culture, the concentration of lactic acid, a metabolite, is cytotoxic if it exceeds a threshold value. Therefore, it is important to remove lactate when changing the culture medium. Therefore, we performed medium change during iPS cell maintenance culture using MakCell, a desktop automated cell culture system, and compared the results with those of manual methods.
We analyzed and compared the medium before and after manual methods with MakCell' methods using a medium composition analyzer, as the lactate concentration, glucose concentration, and pH. And we observed the morphology of iPS cells at Day 7 before passaging and counted of cell number. To confirm whether the undifferentiated iPS cells is maintained when the culture medium is changed by MakCell for a period of 2 months, we continuously changed the culture medium of iPS cells by MakCell for 2 months (manual passaging only) and evaluated the gene expression levels of pluripotent markers using RT-qPCR.

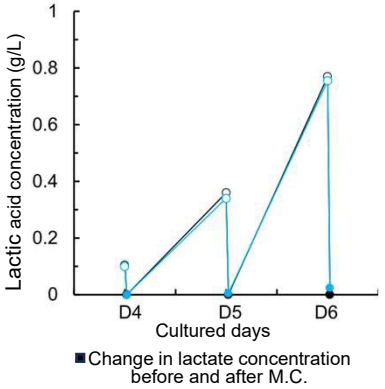
METHODS

Cell: Human iPS 253G1 (seeding density: 4.8×10^2 cells/cm^2)
Culture medium: StemFit AK02N (Ajinomoto), CultureSure Y-27632 (Wako)
Culture dish: 60 mm tissue culture dish (BD Falcon), iMatrix-511 (Nippi)
Medium component analysis: Vi-CELL MetaFLEX (Beckman Coulter)
Cell counting: Countess 3 (ThermoFisher)
RT-qPCR: StepOne Plus (ThermoFisher), TaqMan Gene Expression Assays (ThermoFisher)

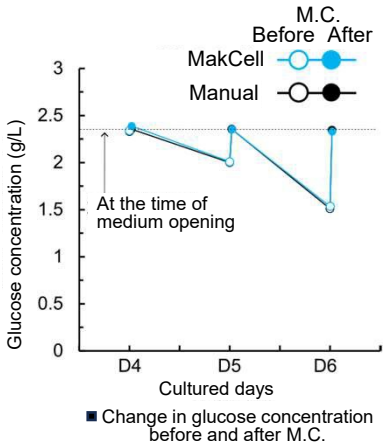
Schedule for medium changing and component analysis



RESULTS

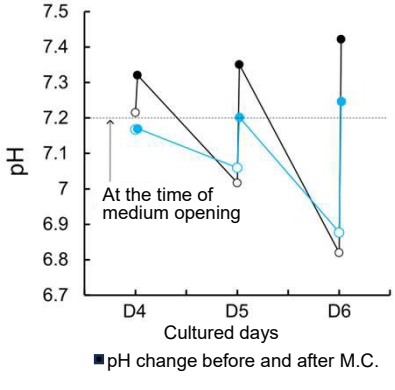


■Change in lactate concentration before and after M.C.



■Change in glucose concentration before and after M.C.

※M.C.=Medium change



■pH change before and after M.C.

The lactate concentration was zero after medium change, and the glucose concentration was at the same concentration as when the medium was opened, indicating that MakCell was able to aspirate medium and supply fresh medium in a manner comparable to manual medium change.
Manual medium change involves opening and closing the medium bottle each time, which decreases dissolved carbon dioxide and raises the pH of the supplied medium, but MakCell does not involve opening and closing the bottle, so the pH fluctuations of the supplied medium were suppressed.

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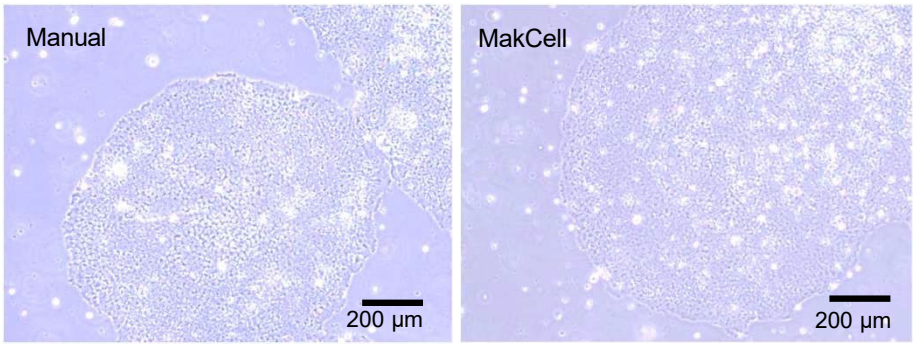
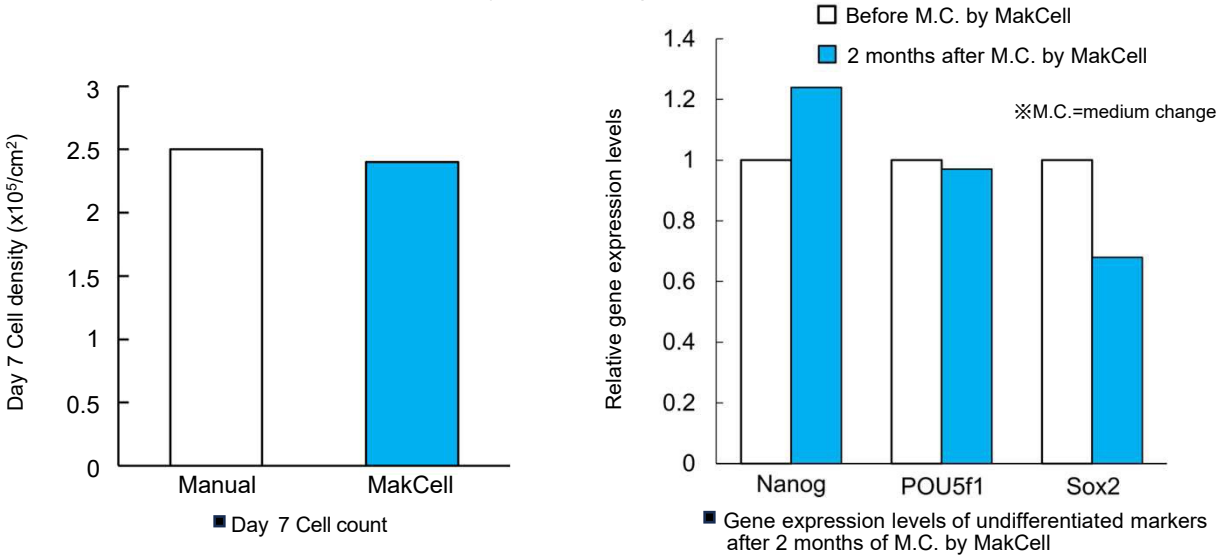


Day 7 Cell Image



Microscopic observation of iPS cells on Day 7 showed no morphological differences between iPS cells grown in manual medium change and those grown in medium change by MakCell.
The cell counting results on Day 7 showed no difference in the proliferation rate of iPS cells that were medium-changed manually and those that were medium-changed by MakCell. Using the gene expression levels of pluripotent markers in iPS cells before the start of culture medium change by MakCell as a standard, we examined the gene expression levels of iPS cells after 2 months of culture medium change using MakCell by RT-qPCR, and found that the undifferentiated iPS cells was maintained.

DISCUSSION

MakCell was able to aspirate medium and supply fresh medium without any difference in morphology or proliferation rate of iPS cells compared to manual medium change.
The undifferentiated nature of iPS cells was maintained even after 2 months of medium change using the MakCell, indicating that the MakCell is a stable medium change device.

■Reference: Comparison of work time

	Manual	MakCell
Work Contents	・Culture medium dispensed into a centrifuge tube 5 min/time ・Medium heating 15 min/time ・Medium change 30 min/time ・Medium change 4 times	・Preparation of tubes, medium, and nozzles 30 min. ・program setup 5 min.
Work Time Total	200 minutes (3 h 20 min.)	35 minutes

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