Application Note

MakCell
Compact Automatic Cell Culture Apparatus

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

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Cardiomyocyte differentiation from human iPS cells using MakCell

The "MakCell," a tabletop automated culture system, is a device that automatically performs medium change and contributes to the reduction of work time and holiday work required for the daily cell maintenance. In this note, induction of differentiation of stem cells, which is performed in regenerative medicine research, was performed with MakCell. These results show that MakCell reduces the workload by automating the complicated tasks required for differentiation induction experiments, such as the timing and frequency of the use of multiple types of culture media, and that MakCell is useful for the promotion of efficient and effective research.

We attempted to induce cardiomyocyte differentiation from human iPS cells by switching the medium containing inhibitors using three supply and discharge lines of MakCell. Four patterns of days of addition in GSK/Wnt inhibitor for multiple conditions were set, and differentiated conditions were compared by the positive rate of cTnT, a cardiomyocyte marker, at day 15 of differentiation.

METHODS

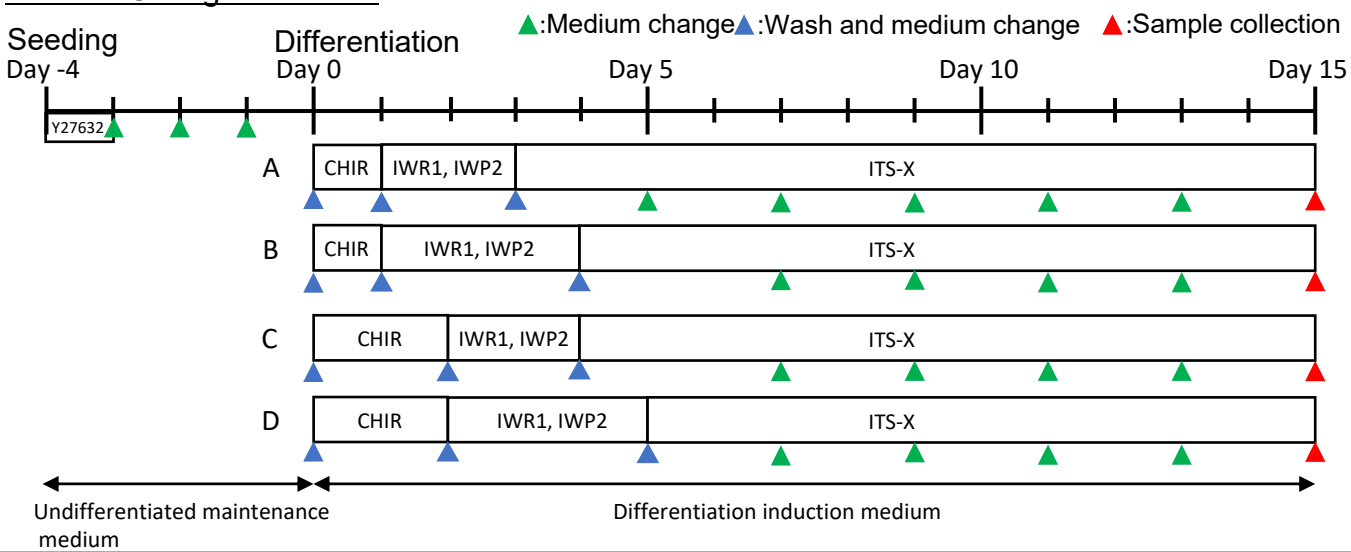
Cell type: Human iPS 253G1 (seeding density 8x10^4 cells/well), iPS spheroid expansion and maintenance culture using CELLFLOAT®CellPet 3D-iPS and CellPet FT, then enzymatically dispersed and seeded into the device
Undifferentiated maintenance medium: AK02N (Ajinomoto)
Culture plate: 12 well plate (Falcon), iMatrix-511 (Nippi)
Differentiation induction medium: RPMI1640 (Wako), Ascorbic acid (Sigma), BSA (Wako), GlutaMAX (Gibco)
Inhibitor reagent: CHIR99021 (Wako), IWR-1 (Sigma), IWP-2 (Sigma)
Additive reagent: Y-27632 (Wako), ITS-X (Gibco)
Wash medium: Same as differentiation induction medium
Flow cytometer: Attune Flow Cytometer (ThermoFisher)
Antibody: cTnT antibody (CT3, SantaCruz), α-actinin antibody (EA-53, Sigma)
Fluorescence-labeled secondary antibody (ThermoFischer)

MakCell Configuration

	Line1	Line2	Line3
Day -4 – Day 0	Undifferentiated maintenance medium	Differentiation induction medium + 6 μM CHIR99021	Differentiation induction medium (For cleaning)
Day 0 – Day 3	Differentiation induction medium + ITS-X	Differentiation induction medium + 6 μM IWR-1, 8 μM IWP-2	↓
Day 3 – Day 15	↓	-	↓

“500mL/1000mL bottle supply tube set (low adsorption)" is used for the supply tube set.

Medium Change Schedule



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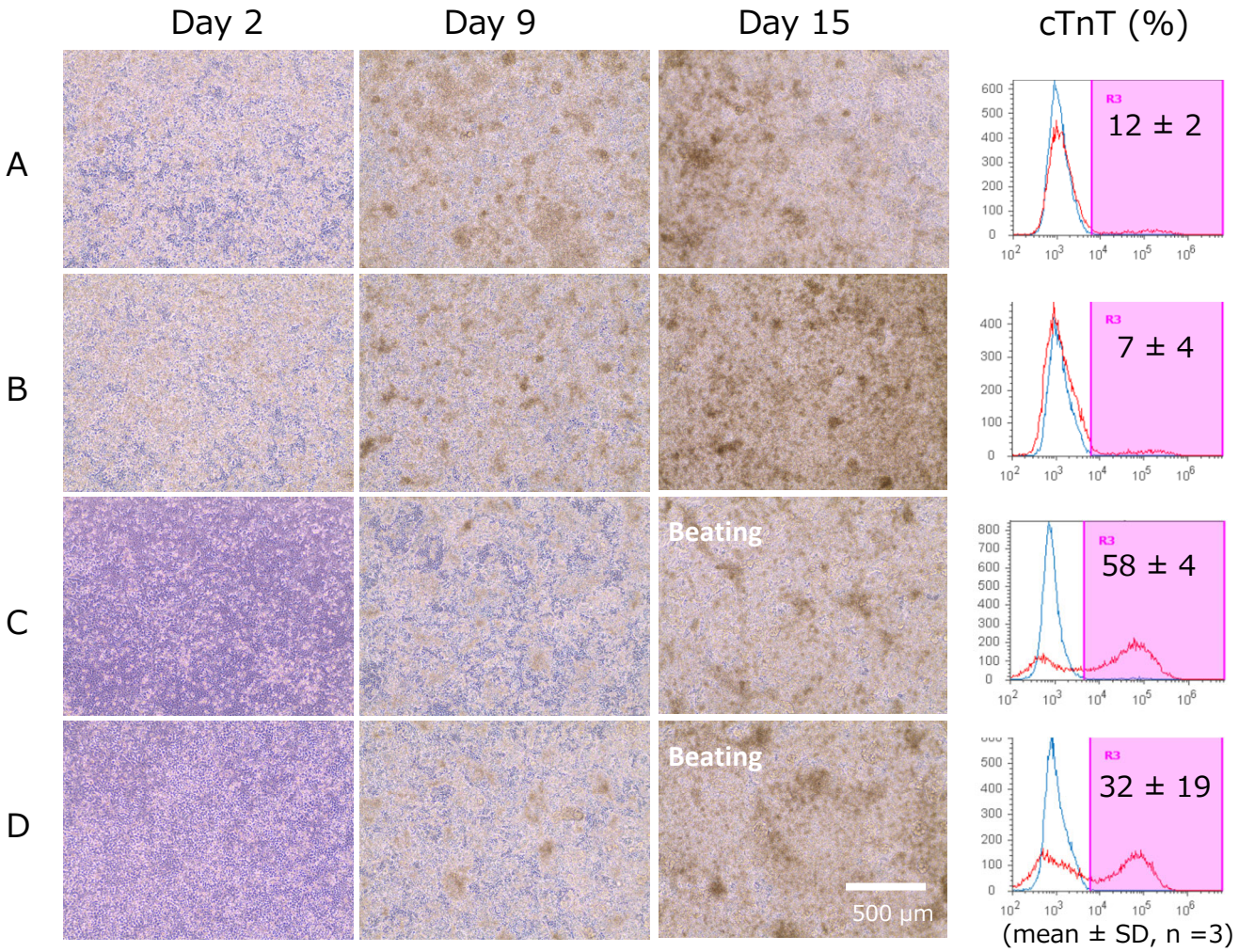
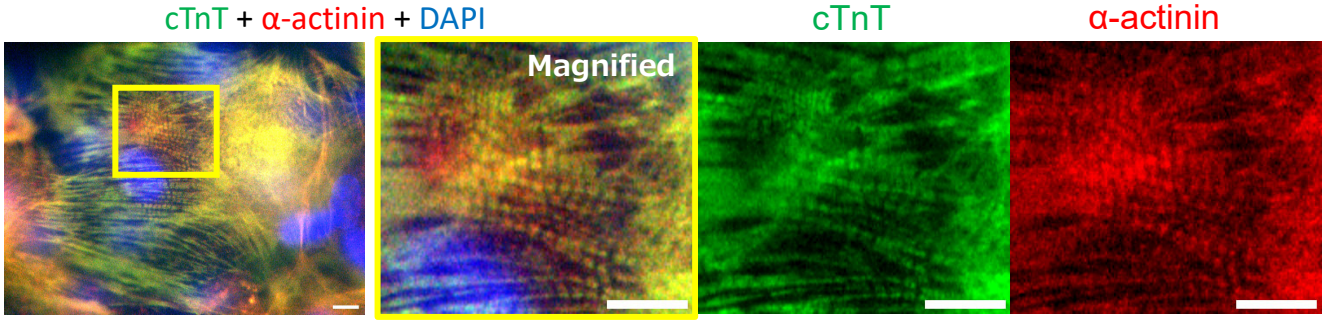
RESULT



Microscopic images of the differentiation induction process, and Flow cytometric analysis of cTnT positivity on Day 15



Immunofluorescence staining of iPS-derived cardiomyocytes (Bar = 10 µm)

Using three medium lines of MakCell, cardiomyocyte differentiation was induced from the maintenance culture of undifferentiated iPS cells while replacing the culture medium bottles. The cTnT-positive rate at day 15 of differentiation was evaluated by varying the number of days of incubation for GSK inhibition and Wnt inhibition in induction of cardiomyocyte differentiation, respectively. Variation between each wells (3 wells) was low in conditions A-C. The results of Condition C showed a cTnT positivity rate of approximately 60%, and some beating ones were identified. Cells were detached from the well where beating was confirmed on day 15, reseeded onto a cover glass, and upon performing immunofluorescence staining, a sarcomere structure composed of cTnT and α-actinin was observed.

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DISCUSSION

- We investigated the conditions of incubation time for each inhibitor in the induction of cardiomyocyte differentiation using MakCell.
- We switched the culture medium containing small molecule inhibitors and showed that cardiomyocyte differentiation can be induced from iPS cells. .
- The MakCell was shown to be a device that can be used to automate the exchange of multiple types of media in differentiation induction.
- In about three-week differentiation induction experiment, human labor was required only twice, on Day -4 and Day 0, for the preparation of tubes, medium, and nozzles, indicating that the MakCell is a device that can significantly reduce labor time in experiments that require frequent medium changes, such as differentiation induction experiments.

Reference: Comparison of working hours

	Manual	MakCell
Work Contents	・Medium centrifuge tube dispensing 5 min/times ・Medium heating 15 min/times ・Wash and medium change 15 min/times ・Number of media changes 13 times	・Preparation of tubes, media, and nozzles 30 min ・Preparation count 2 times (Day -4, Day 0) ・program settings 20 min
Work Time Total	455 min (7 h 35 min)	80 min

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