Automated iPS cell sorting by using CELL HANDLER™

Introduction

The Facility for iPS Cell Therapy (FiT), as a part of the CiRA foundation, manufactures inducible pluripotent stem cells (iPS) for regenerative medicine research. In the manufacturing process, selection of high quality iPS cells plays an important role. This is accomplished entirely by manual operation, which requires an excessive amount of time and labor. CELL HANDLER™ is capable of imaging and recognizing cells one by one, in addition to accurately dispensing single cells and colonies. In this report, we describe an automated method for isolating single cells and colonies of iPS cells by using CELL HANDLER™.

Experiment 1

Isolation of iPS single cells using CELL HANDLER™.

[Purpose]

A) Confirmation of iPS single cell isolation capability (success rate, operation time)
B) Evaluation of viability and colony formation of iPS cells under various tissue culture conditions

[Method]

Three types of iPS cells were prepared by subjecting iPS cells to different incubation periods, ranging from 5 to 7 days. To obtain 1 cell per grid, 6,000 cells per well were seeded in 6-well microgrid plates. Seeded cells were transferred into 96-well plates by CELL HANDLER™ to be cultured for 9 days. Growth of colonies was monitored (Figure 1).

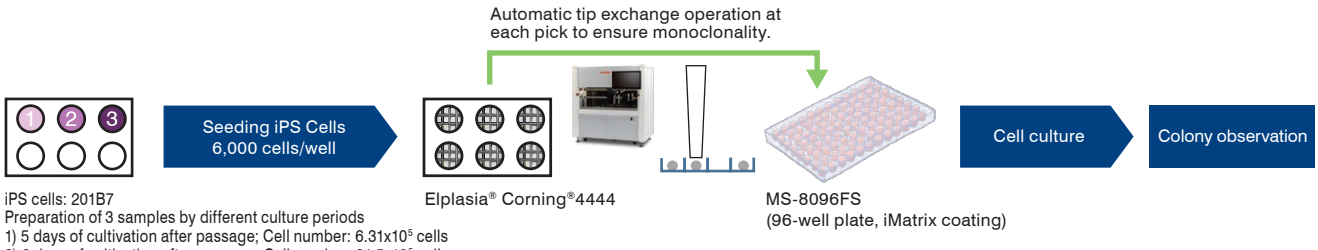

Figure 1. Workflow of Experiment 1.

[Results]

A) Single cells were selected and sorted by CELL HANDLER™ after seeding iPS cells in microgrid plates at a concentration of 6,000 cells/well (Figure 2). Monoclonality was ensured by avoiding cell pick up from non-target grids and replacing tips after each cell pickup. Furthermore, the success rate of isolation was 100%, and the transfer time of 96 cells was about 30 minutes, despite tip changes after each cell isolation (Table 1).

B) A high engraftment rate of around 70% was achieved after iPS cell isolation. No significant difference in viability and colony formation were demonstrated between the culture conditions of the sample cells (Day 5, Day 6, and Day 7 after passage) (Table 2, Figure 3).

<Preparation> - Equipment start-up and cell preparation: Approx. 10 min (including waiting for cell seeding and settling)
<CELL HANDLER™> Picking time of 96 well - Taking pictures of source cells: Approx.14 min - Cell selection: Approx. 10 min (manual selection) - Cell pick-up: Approx. 30 min (tip auto-exchange for each picking)* - Taking pictures of destination cells: Approx. 7 min *With no tip exchange setting, the time can be reduced to about 5 min.
<Completion of work> - Clean up: Approx. 5 min

Table 1. Time required for experiment tasks.

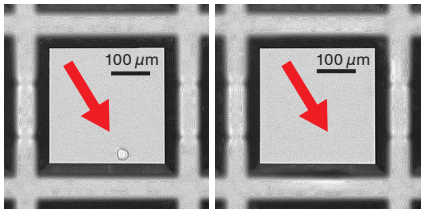

Figure 2. Image before and after cell isolation.

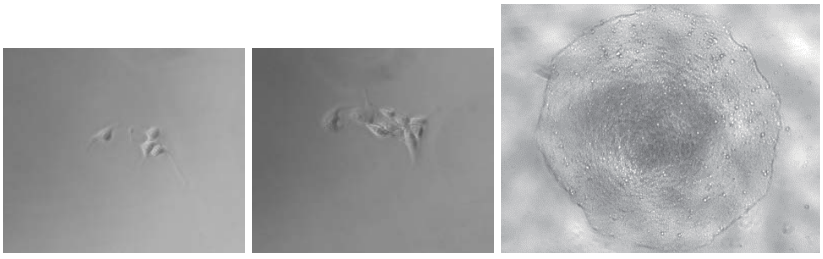

Figure 3. Cell expansion progress after isolation.

Sample1 (Day 5 after passage)	Sample2 (Day 6 after passage)	Sample3 (Day 7 after passage)
36/48 well (75%)	35/48 well (73%)	31/48 well (65%)

Table 2. Percentage of cell viability and colony formation of isolated iPS cells by CELL HANDLER™.

*Image and data provided by FiT.

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Experiment 2

Isolation and replication of adherent iPS cell-derived colonies by using CELL HANDLER™.

[Purpose]

Verification of the isolation and replication efficiency (isolation success rate and viability) of adherent iPS cell-derived colonies by using CELL HANDLER™.

[Method]

Single colonies were formed by seeding single iPS cells in each well of a 96-well plate (N=8). The formed colonies were divided into two sections and collected for replication by using CELL HANDLER™ (Figure 4). The colonies were cultured for 5 days, and their growth conditions were monitored.

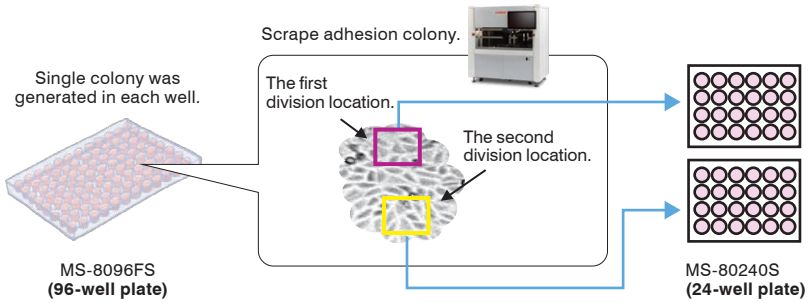

Figure 4. Workflow of Experiment 2.

[Results]

Replication (N=2) was performed on 8 colonies and successful replication of all colonies was achieved. After 5 days of culturing, new colony formation was observed for all replicated colonies (Figure 5).

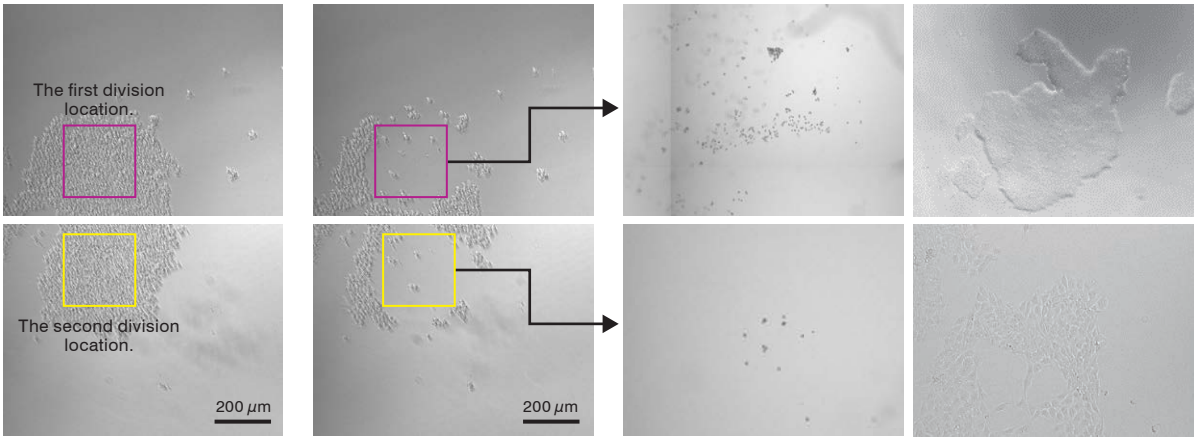

Figure 5. Images before and after colony isolation and during culture of isolated colonies.

[Summary]

The use of CELL HANDLER™ enables the selection and isolation of iPS single cells more reliably and faster compared to manual methods. Our results demonstrate that various culture conditions can be evaluated in a shorter time. CELL HANDLER™ supports a highly efficient establishment of monoclonal iPS cell-derived colonies. Furthermore, CELL HANDLER™ can replicate iPS cell colonies with a high success rate. Taken together, these results show that iPS cell culture expansion and iPS cell research using CELL HANDLER™ is more successful and efficient than existing methods. It is expected that CELL HANDLER™ will become an integral platform for iPS cell-based experiments in the near future.

Acknowledgement

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