GENProtocols

Protocol

Removal of rare undifferentiated induced pluripotent stem cells (iPSCs) by multi-step negative sorting

TRANSLATIONAL MEDICINE ANALYTICAL TESTING

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Abstract

In the field of regenerative medicine involving ES or iPS cells, differentiated cells are often cultured to tissues and implanted to patients. However, there is an unneglectable problem that some remaining undifferentiated cells in the implanted tissue may cause tumors. Therefore, a complete removal of undifferentiated cells prior to implantation is important. For this purpose, we developed a novel technology called multi-step negative sorting, to completely remove undifferentiated cells. This technology also provides an absolute quantification of iPS cells as a quality control of cells for transplantation.

Introduction

Conventional cell sorters are typically used for purification of cells, but they are not suitable for use in regenerative medicine because those cells tend to be damaged during sorting. To overcome this inherent problem, we developed a microfluidic chip-based damage-free cell sorter, On-chip Sort (Fig. 1a). This sorter uses a disposable and sterile microfluidic chip (Fig. 1b) as the core of the technology. Target cells are isolated by pulse flow created by pneumatic actuation. Fig. 2 shows the method of multi-step negative sorting on On-chip Sort for complete removal of undifferentiated cells. This method works in the opposite way to conventional sorting methods. Sample is loaded on sample reservoir of the chip. Undifferentiated cells (unwanted non-target cells) are removed by formation of the pulse flows into the collection reservoir, while differentiated cells (target cells) flow into the waste reservoir, located downstream of the chip, where those cells are retained rather than being discarded like conventional cell sorters. Differentiated cells are recovered and reloaded on the sample reservoir to be sorted again for further purification. This process is repeated until all undifferentiated cells are removed. The method only requires sorting speed that corresponds to the detection rate of the rare cells. When the percentage of undifferentiated cell is 1% of the whole population, then this negative selection is 100 times faster than collecting differentiated cells using positive selection.

Reagents and Equipment

1. Disposable microfluidic chip
2. Cell mixture containing undifferentiated cells for sorting

Procedure

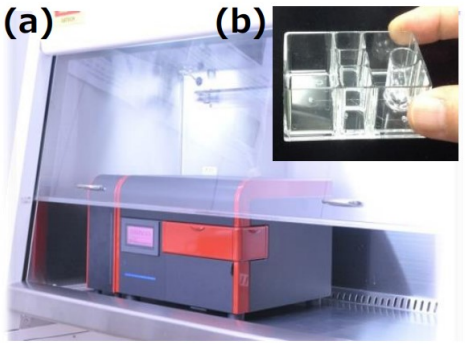

Fig. 1. On-chip Sort (a) and disposable microfluidic chip for cell sorting (b).

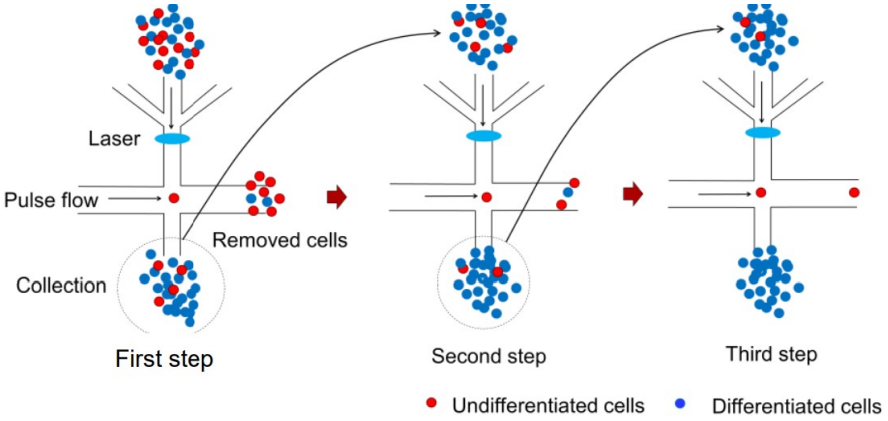

Fig. 2. Principle of multi-step negative sorting for complete removal of undifferentiated cells.

Undifferentiated cell removal by Multi-step negative sorting

The multi-step negative sorting method was applied to remove undifferentiated cells in the neural stem cell populations that have been differentiated from iPSCs (Fig. 3). The initial sample contained 1.2 x 106 singular cells dissociated from neurospheres, and 4% of those were undifferentiated cells. Anti-TRA-1-60 antibody (FITC) was used for staining of undifferentiated cells. All undifferentiated cells were removed after three sorts. Collected differentiated cells (5 x 105 cells) were cultured and confirmed viable (Fig. 3).

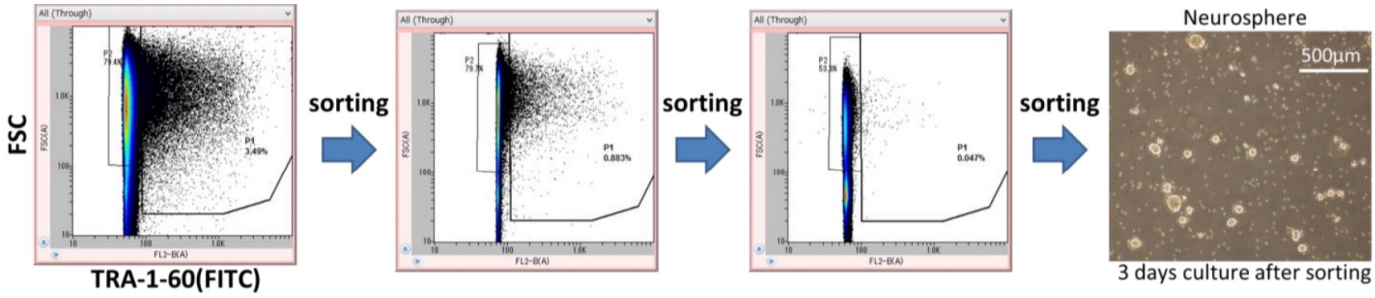

Fig. 3. Removal of undifferentiated cells by multi-step negative sorting.

Performance of iPSC removal from 10^8 differentiated cells

The efficiency of removing rare undifferentiated cells from a high cell concentration of differentiated cells was evaluated using sample containing 10^4 cells of iPSCs spiked in 5 x 10^7 cells of Molt4 cell line (Fig. 4). Figure 5a shows the change in the number of iPSCs after each sort. The iPSCs were eliminated at a rate of 85% per sort. iPSCs (104 cells) were completely removed in six sorting steps (total of 60 min). The total collection efficiency of Molt4 cells was roughly 60% (Fig. 5b). The recovery rate can be increased to 92% if another set of multi-step sorting is carried out on the removed portion of the sample. The flow rate of Molt4 cells in this condition was about 100,000 cells/s, and conventional cell sorters are not applicable of processing such large number of cells in the same sort duration.

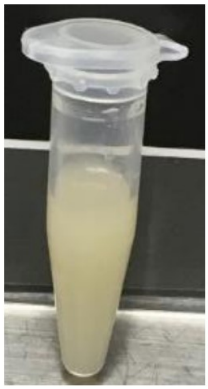

Fig. 4. Sample containing 10^4 iPSCs spiked in 5 x 10^7 Molt4 cells.

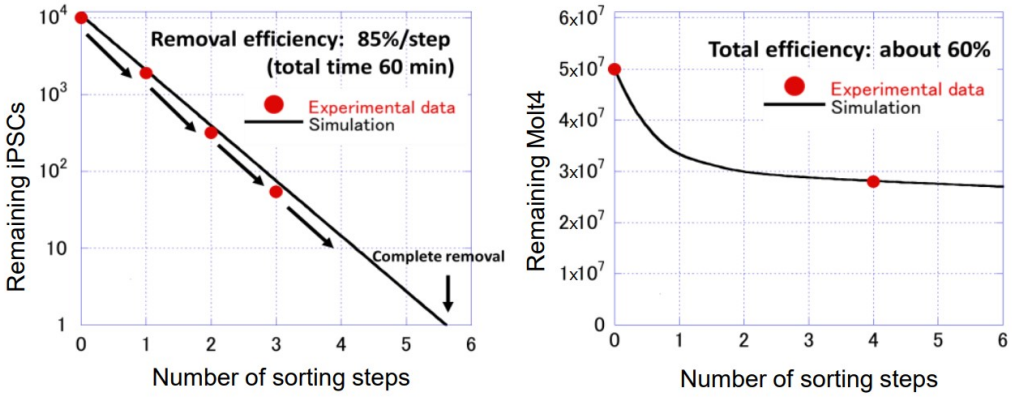

Fig. 5. (a) Change in iPSC count per sorting step. (b) Change in Molt4 count per sorting step.

Time Taken

1 hour

References

Not available