Application Note

Damage-free sorting of fetal rat brain nerve cells

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Introduction

Flow cytometry is used to purify specific cell types from complex heterogeneous samples. Researchers are often encountering post-sorting changes to cells which hinder the downstream usage of the isolated cells. Therefore, the post-sorting effect on the growth of fragile cells such as nerve axons, collected from hippocampus of fetal rat brain, was investigated.

Methods

Fetal rat brain hippocampus cells were sorted using two types of sorters: conventional high-speed sorter (Sorter A) with a 70 µm nozzle, which uses the 'Jet-in-Air' separation method; and On-chip Sort which uses the 'Flow shift' method. After sorting on both sorters separately, a fraction of the cells were stained by Propidium iodide (PI) for immediate viability calculation, and the remaining fraction was cultured for seven days and growth was observed under microscopy.

Results

The viability of cells immediately after sorting was 77% and 80% for cells sorted using the conventional sorter (Sorter A) and On-chip Sort, respectively. The viability of cells without sorting was 82% (Fig. 1).

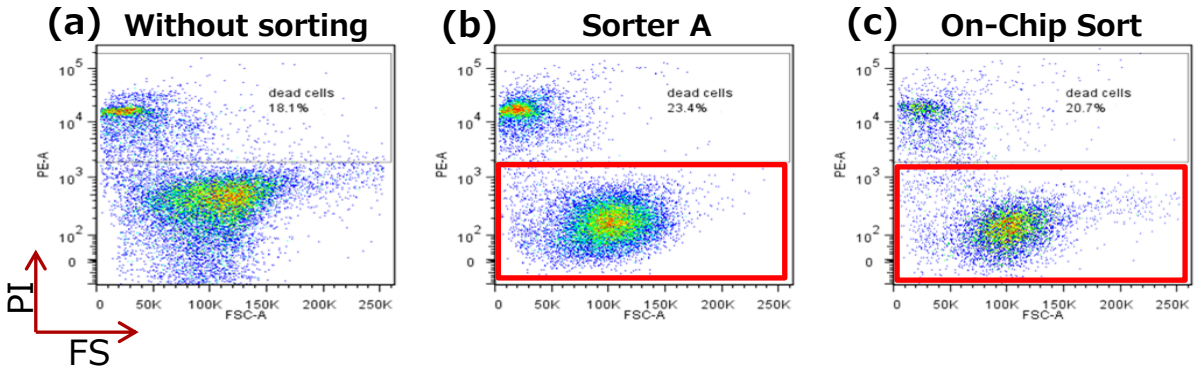

Fig. 1. PI vs. FSC density plots of fetal rat brain hippocampus cells without sorting (a), and sorted by conventional sorter A (b) and On-chip Sort (c). In (b-c), red boxes correspond to sorting gates.

Although the immediate post-sorting viability of nerve cells sorted using conventional sorter A and On-chip Sort were not too different to that without sorting (Fig. 1), the difference was apparent when cells were cultured over time (Fig. 2). Nerve cells sorted using On-chip Sort showed formation of axons after seven days of culture post-sorting (Fig. 2c) and displayed growth equivalent to that of the non-sorted sample (Fig. 2a). On the other hand, neurons sorted using the conventional cell sorter A were not viable and did not form axons even after seven days of culture (Fig. 2b).

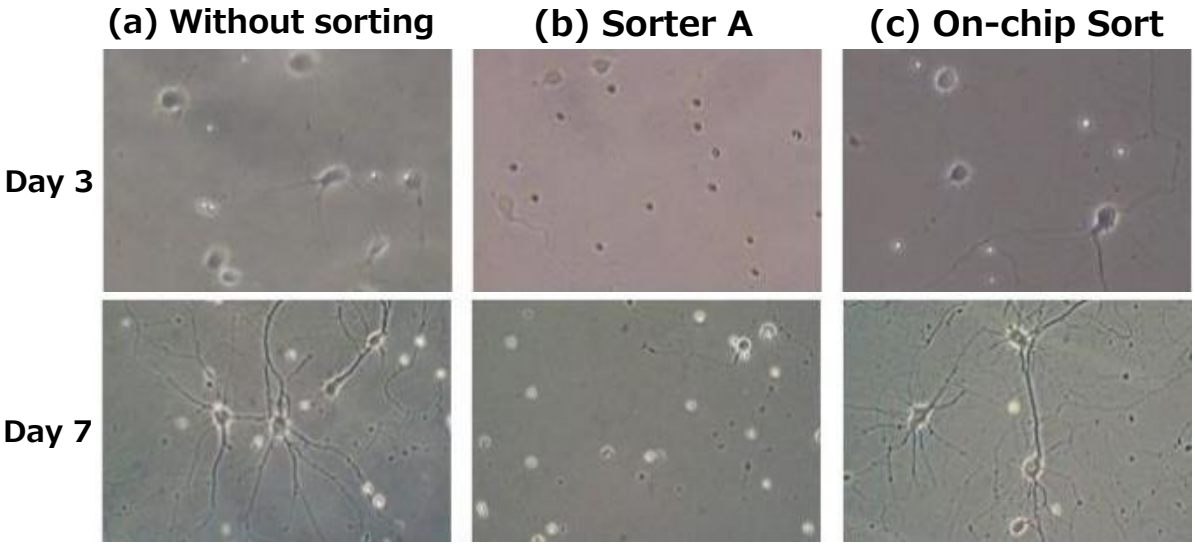

Fig. 2. Images of fetal rat brain hippocampus cells without sorting (a), and sorted by conventional sorter A (b) and On-chip Sort (c), and cultured for three and seven days.

Summary

In comparison to conventional cell sorter, neurons sorted on On-chip Sort were maintained viable over seven days of culture, at a condition comparable to unsorted cells. Therefore, On-chip Sort is a useful tool for sorting of fragile cells that are susceptible to damage.

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