

Single Cell Sorter Driven by Resonance Vibration of Cell Culture Substrate

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Abstract : The Research Goal: With the growing demand for regenerative medicine, an effective mass cell culture process is required. In a repetitive subculture process for proliferating cells, preparing single cell suspension which does not contain any cell aggregates is highly required because cell aggregates often raise various undesirable phenomena, e.g., apoptosis and decrease of cell proliferation. Since cell aggregates often occur in cell suspension during conventional subculture processes, this study proposes a single cell sorter driven by a resonance vibration of a cell culture substrate. The Method and the Result: The single cell sorter is simply composed of a cell culture substrate and a glass pipe vertically placed against the cell culture substrate with a certain gap corresponding to a cell diameter. The cell culture substrate is made of biocompatible stainless steel with a piezoelectric ceramic disk glued to the bottom side. Applying AC voltage to the piezoelectric ceramic disk, an out-of-plane resonance vibration with a single nodal circle of the cell culture substrate can be excited at 5.5 kHz. By doing so, acoustic radiation force is emitted, and then cell suspension containing only single cells is pumped into the pipe and collected. This single cell sorter is effective to collect single cells selectively in spite of its quite simple structure. We collected C2C12 myoblast cell suspension by the single cell sorter with the vibration amplitude of 12 $\mu\text{m-p}$ and evaluated the ratio of single cells in number against the entire cells in the suspension. Additionally, we cultured the collected cells for 72 hrs and measured the number of cells after the cultivation in order to evaluate their proliferation. As a control sample, we also collected cell suspension by conventional pipetting, and evaluated the ratio of single cells and the number of cells after the 72-hour cultivation. The ratio of single cells in the cell suspension collected by the single cell sorter was 98.2%. This ratio was 9.6% higher than that collected by conventional pipetting (statistically significant). Moreover, the number of cells cultured for 72 hrs after the collection by the single cell sorter yielded statistically more cells than that collected by pipetting, resulting in a 13.6% increase in proliferated cells. These results suggest that the cell suspension collected by the single cell sorter driven by the resonance vibration hardly contains cell aggregates whose diameter is larger than the gap between the cell culture substrate and the pipe. Consequently, the cell suspension collected by the single cell sorter maintains high cell proliferation. Conclusions: In this study, we developed a single cell sorter capable of sorting and pumping single cells by a resonance vibration of a cell culture substrate. The experimental results show the single cell sorter collects single cell suspension which hardly contains cell aggregates. Furthermore, the collected cells show higher proliferation than that of cells collected by conventional pipetting. This means the resonance vibration of the cell culture substrate can benefit us with the increase in efficiency of mass cell culture process for clinical applications.

Keywords : acoustic radiation force, cell proliferation, regenerative medicine, resonance vibration, single cell sorter

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