MICROFLUIDIC DEVICE FOR CONTINUOUS PARTICLE SEPARATION USING HYDRODYNAMIC FILTRATION

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Abstract

A size-dependent particle separation (classification) is one of the most important procedures in biochemical, environmental, or medical analyses and their applications. However, the smaller particle size or the smaller size difference makes the particle classification difficult. The Lab-on-a-Chip concept have encouraged the miniaturization of various separation or selection methods for small particles, including polymer beads, cells, and macromolecules. Microfluidic devices have a potential to facilitate rapid and precise particle manipulation, due to accurately fabricated structures close to particle sizes, in micrometer or sub-micrometer dimensions.

In our previous study, a new scheme for particle sorting, pinched flow fractionation (PFF), and that for separating and concentrating particles, hydrodynamic filtration (HDF), utilizing microfluidic devices have been proposed. In the latter method, simple introduction of particle suspension enables both size-dependent separation and concentration, simultaneously. However, the inevitable contamination of small particles into the concentrated large-particle fraction decreases the separation efficiency.

In this paper, the improved separation efficiency by employing flow splitting and recombining will be demonstrated, and the application of this method to blood cell separation. This microfluidic separation system will be useful due to its simplicity and accuracy.

Introduction

Particle classification according to their physical or chemical properties is one of the most important operations in the chemical or biochemical processes and also in the fields of biological research and medical care. There exist so many methods for the size-dependent separation of small particles. Filtration, centrifugation and sedimentation are frequently used techniques to classify particles suspended in liquid. Existing filtration methods are performed either in batch or continuous manner, and large-scale treatment can be easily achieved. However, when the particle size is much smaller, or when the difference in particle sizes is smaller, separation becomes difficult. Also, mesh clogging or membrane fouling is inevitable. Therefore, particle separation methods using microfluidic devices have also been proposed for the separation or selection of small particles, including polymer beads, cells, organelles, and biomacromolecules [1-4]. Usage of microfluidic devices facilitates rapid and precise particle manipulation, due to accurately fabricated microstructures nearly equal to particle sizes, in micrometer or sub-micrometer dimensions. On the other hand, as continuous particle processing is suitable for high-throughput treatment, a number of studies have been reported concerning continuous particle separation in microfluidic devices adopting dielectrophoresis [5-6], isoelectric focusing [7], acoustic waves [8-9], optical manipulation [10], and magnetic fields [11]. However, a system that does not require outer field controls or any moving parts is attractive and desirable for integration with other kinds of micro unit operations, such as particle pre-treatment, analysis, and further utilization.

In our previous study, a new scheme for particle sorting, pinched flow fractionation (PFF) [12], and that for separating and concentrating particles, hydrodynamic filtration (HDF) [13], utilizing

microfluidic devices have been proposed. In the latter method, simple introduction of particle suspension enables both size-dependent separation and concentration, simultaneously. However, the inevitable contamination of small particles into the concentrated large-particle fraction decreases the separation efficiency.

Principle

To solve the contamination issue, we proposed an improved scheme for particle separation, employing flow splitting and recombining. When the relative flow rate distributed to a side channel at a branch point is sufficiently low, particles cannot go through the side channel, even when they are flowing near the sidewall. However, when the relative flow rate is increased, the size limit of particles that can go through the side channel is increased. By combining these flow states, particle positions in the main channel can be precisely controlled. That is, as shown in **Fig. 1**. by repeating liquid flow removal from the main channel, and recombining into the downstream, particles are perfectly alignment onto one sidewall in the main channel Then, particles can be separated according to their size, by increasing the relative flow rates into the outlet channels.

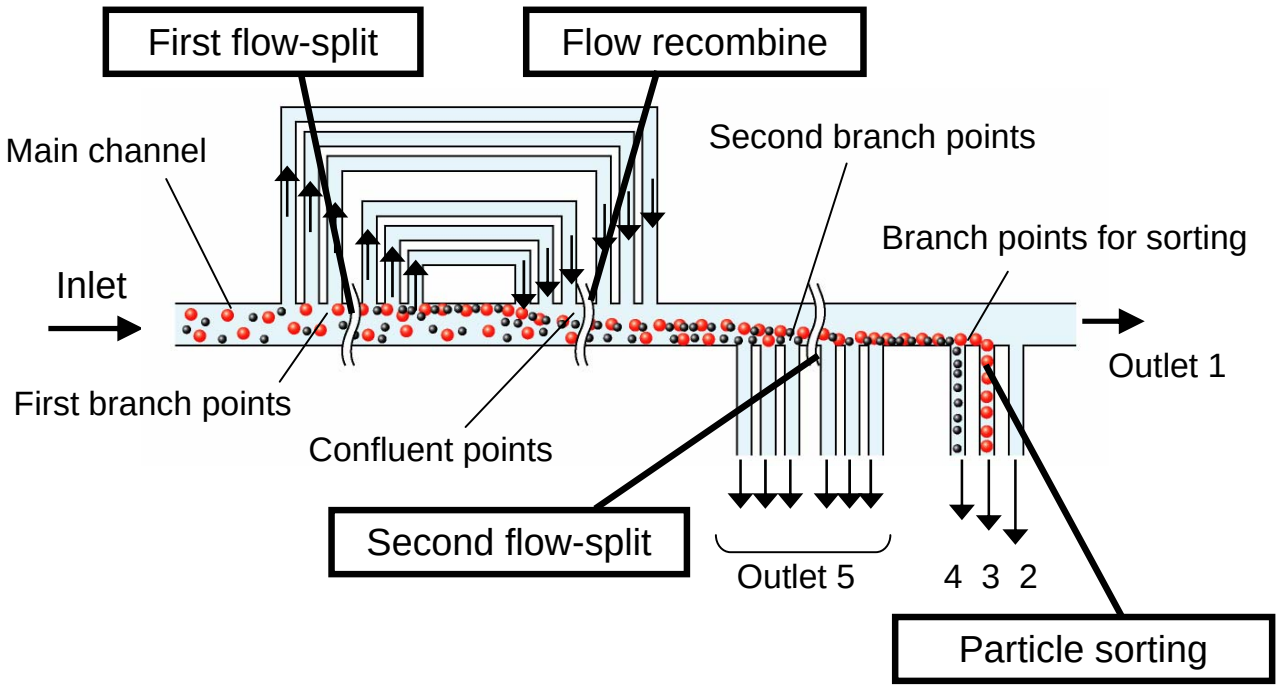

Figure 1. Principle of particle separation. By repeatedly splitting flow from the main channel (first flow-split) and re-combining (flow recombine), particle positions can shift toward one side wall. Then by repeatedly splitting flow through the side channels on the other sidewall of the main channel (second flow-split), particles are perfectly aligned on one sidewall and collected from outlets 1-4 according to their sizes (particle sorting).

Experimental

PDMS-glass hybrid microdevices were fabricated using usual soft lithography and replica molding method. These microdevices were designed regarding the microchannel network as a resistive circuit. For example, the device has one inlet, three outlets, 80 first and second side channels, respectively. Theoretically, ~34% of the liquid flow is split through the first side channels, while ~80% of the liquid flow is withdrawn through the second side channels. Also, it was expected that particles smaller than ~6 μm would be recovered from Outlet 3, while cells with diameter of 6 ~ 20 μm would be collected from Outlet 2.

Blood cells are mainly composed of erythrocytes and leukocytes, whose shapes and sizes are entirely different. Also, since the population of leukocytes is ~1/700 that of erythrocytes, usual filtration can not be employed for preparation of leukocytes. Separation of blood cells is essential for various research and clinical scenes, so we tried to separate these cells as a model. Whole blood was diluted (× 10) using PBS, and continuously introduced into the microchannel using a syringe pump with a flow rate of 20 μL/min. The recovered cells were immobilized and stained on glass slides.

Results and Discussion

Cells recovered from each outlet were stained with Wright-Giemsa solution. As a result, 99.97% of the introduced erythrocytes were collected from outlet 3, while ~98% of leukocytes were collected from outlet 2. The concentration of leukocytes in Outlet 2 was increased 30-fold compared to the initial value. From these results, the potential of this microdevice for blood cell separation was successfully shown.

Conclusions

Particle separation was conducted using the microfluidic devices based on the novel separation principle. Blood cells were successfully separated by this separation scheme. This microfluidic separation system will be useful due to its simplicity and accuracy.

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