MICROFLUIDIC COUNTERFLOW CENTRIFUGAL ELUTRIATION FOR CELL SEPARATION USING DENSITY-GRADIENT MEDIA

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ABSTRACT

This paper reports a microfluidic system for cell separation employing counterflow centrifugal elutriation (CCE), combined with the density-gradient media and the branching inlet structures. In conventional CCE, the balance of centrifugal force and fluidic force is gradually changed by increasing the flow rate and/or by decreasing the rotation speed, achieving the separation and recovery of cells based on size, shape, and/or density. In the presented system, both the centrifugal force and the driving force to transport fluids are generated by the device rotation at the same time. We successfully separated microparticles, and observed the retention behaviors of cells in the separation chamber. The pump-integrated rotor systems required for conventional CCE are dispensed with by employing the density-gradient media, which remarkably simplified the instruments and procedures.

KEYWORDS: Elutriator, Cell separation, Centrifugal microfluidics, Density gradient

INTRODUCTION

Separation or purification of particulate samples based on size, shape, and/or density is often a crucial step in biological, environmental, and industrial preparations. CCE is a powerful method for continuously separating cells, and has been applied to blood cell fractionation, stem cells purification, live/dead cells separation, synchronization of cell-cycle stages, and so on [1-3]. In conventional CCE systems, cells loaded into the separation chamber are subjected to the centrifugal force generated by the rotation of a rotor in an outward direction and to the fluid force pumped into the separation chamber in an inward direction. By gradually changing the balance of these two forces, cells are separated according to size, shape, and/or density. The conventional CCE systems therefore requires complicated instruments such as a rotor integrated with a flow rate-adjustable pump, and the devices cannot be disposable. Here we propose a simple microfluidic system of CCE employing density-gradient media to achieve particle/cell separation without changing the rotation speed.

THEORY

The principle of microfluidic density-gradient CCE is shown in Fig. 1. Unlike the conventional CCE, microchannels are formed on the PDMS plate two-dimensionally. First, particles suspended in a low-density liquid solution are loaded into the separation chamber by rotating the device, instead of by using pumping devices (Fig. 1 (a)). In the separation chamber, particles are subjected to the centrifugal force in an outward direction and to the fluid force in an inward direction, as in the case of conventional CCE. At a given rotation speed, centrifugal forces are higher at peripheral points of the chamber but lower at the central point, while the fluid forces are higher at the periphery, decrease along about 80% of the chamber length, but increase again near the central end due to the geometry of the separation chamber. Therefore, a particle with a specific size, shape, and density keeps a retention position where the two forces are balanced (Fig. 1 (b)). For a spherical particle, the effects of the centrifugal and the fluid forces can be estimated by calculating the terminal sedimentation velocity and by predicting the flow velocity, respectively. The terminal sedimentation velocity, U_s, is expressed by the Stokes' law as follows:

$$U_s = \frac{\rho_p - \rho_f}{18\mu} D_p^2 r_d \omega^2 \qquad (1)$$

where ρ_p and ρ_f are the densities of the particle and the fluid, respectively, μ is the fluid viscosity, D_p is the particle diameter, r_d is the radius of rotation, and ω is the angular velocity of rotation. In case of non-spherical samples such as dividing cells, the shape would also influence the sedimentation velocity. While the flow velocity of the rotation-driven

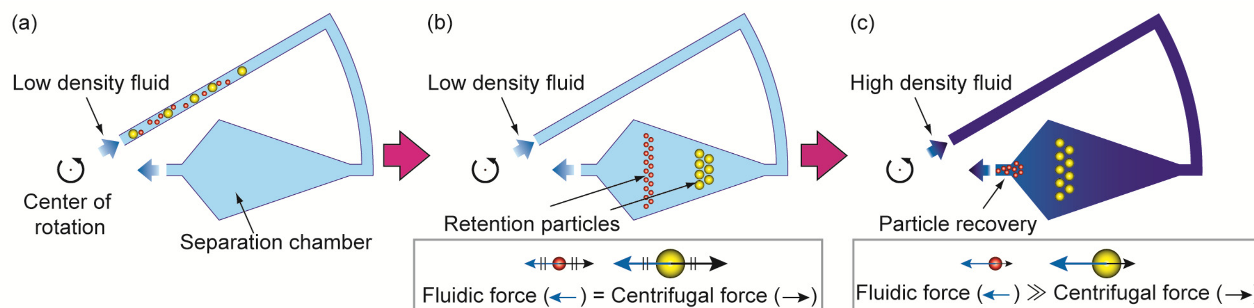

Figure 1: Principle of the microfluidic counterflow centrifugal elutriator. (a) Rotation-driven flow containing particles/cells is introduced into the microchannel. (b) In the separation chamber, particles remain at specific positions where the fluidic and centrifugal forces are balanced. (c) Particle recovery is achieved by gradually increasing the fluid density.

flow, U_f is expressed by the following equation:

$$U_f = \frac{Q_{total}}{S} = \frac{1}{2}\rho_f \omega^2 (r_2^2 - r_1^2) \times \frac{1}{R} \qquad (2)$$

where Q_{total} is the total volumetric flow rate of the rotation-driven flow, S is the cross-section area of the channel at a given point, r_1 and r_2 are the distances between the center of rotation and the inlet and outlet ports, respectively, and R is the hydrodynamic resistance of the whole channel. From equations (1) and (2), it is expected that the retention positions of particles would not be the function of the rotation speed, which suggests the robustness in the separation procedures. Also, the increase in the fluid density would shift the retention positions gradually toward the center of rotation (the outlet of the chamber), achieving the stepwise elution of particles (Fig. 1 (c)).

EXPERIMENTAL

Microchannel designs are shown in Fig. 2. PDMS microfluidic devices were fabricated by using standard soft lithography and replica molding techniques. The depth of the microchannel is uniform, 25 µm. Each inlet and outlet port was partially covered by a lid of thin PDMS membrane in order to prevent the solutions from spilling out of the inlet/outlet ports during the device rotation. Before conducting separation experiments, the entire microchannel was filled with the buffer solution without containing particles/cells. Then the solution in the inlet port was replaced with the particle/cell suspension, and the device was rotated on a spinning device.

In the experiment, 3.0 and 9.9 µm polystyrene particles (ρ=1.05 g cm^{-3}) in 0.5% (w/v) Tween 80 aqueous solution (ρ=1.00 g cm^{-3}) were used to evaluate the effectiveness of the presented system. The density of the media was changed stepwise by introducing 10% (w/v) cesium chloride solution (ρ=1.07 g cm^{-3}) successively. For separating living cells, JM cells (human leukemia cell line) were used and their behaviors were observed; cells were labeled with Hoechst 33342 (blue) dye and suspended in PBS.

RESULTS AND DISCUSSION

Initially we used the PDMS microchannel shown in Fig. 2 (a), but the particle accumulations onto one sidewall of the separation chamber were observed because of the centrifugal alignment of the particles in the loading channel (Fig. 3 (a), (c)). To fully exploit the effect of the two forces in the separation chamber, microchannel with the branching structure was proposed as shown in Fig. 2 (b), where the introduced fluid is almost equally divided into two branches and then joined into the downstream just before the entrance of the separation chamber, achieving the focusing of particles onto the center of the chamber (Fig. 3 (b), (d)).

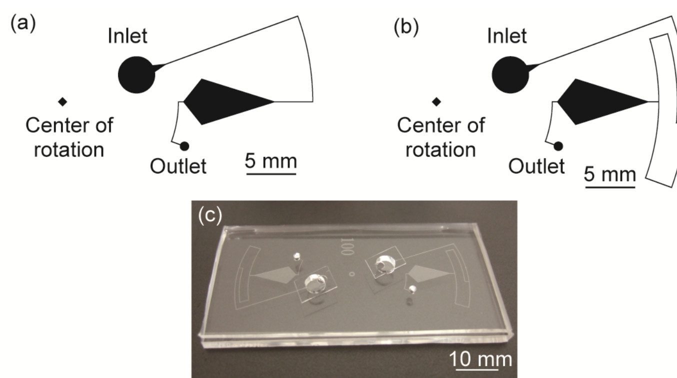

Figure 2: Microchannel designs (a, b), and photograph of the PDMS device (c).

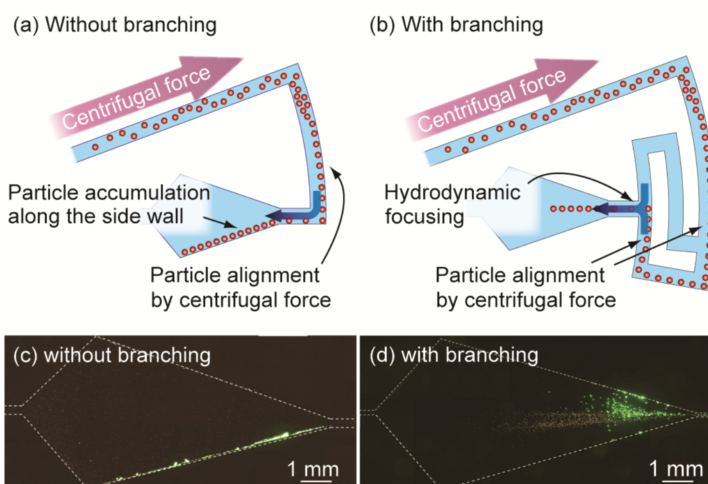

Figure 3: Schematic diagrams and micrographs showing the effect of the branching and recombining inlet channel. Without the branching channel (a, c), both 3.0 and 9.9-µm polystyrene particles were accumulated onto one sidewall in the separation chamber. While with the branching channel (b, d), particles were focused on the center of the chamber.

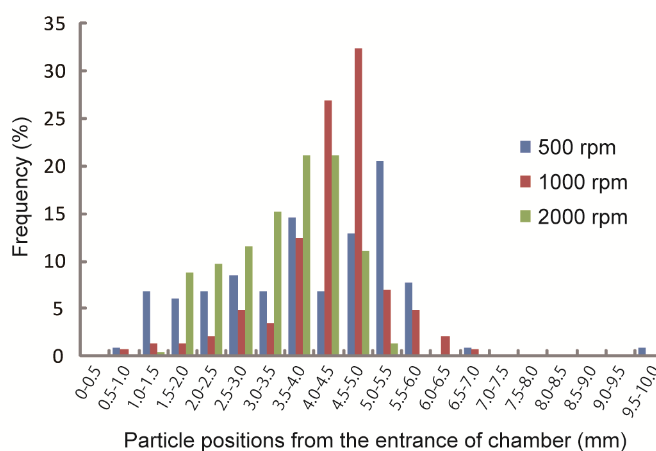

Figure 4: Retention positions of 9.9 µm particles in the separation chamber when the rotation speed was 500, 1000, or 2000 rpm. The mean ± SD particle positions were 3.4 ± 1.5, 3.8 ± 0.9, or 3.0 ± 0.9 mm, respectively.

Then we examined the retention positions of particles when the microdevice was rotated at 500, 1000, or 2000 rpm, by using the branching microchannel. The retention positions were not significantly affected by the rotation speed (Fig. 4), which was consistent with the theoretical estimation above and indicates the robustness of the presented system.

In the experiment using polystyrene particles, we successfully demonstrated the separation of 3.0 and 9.9 μm particles after 20 min of rotation at 1000 rpm (Fig. 5 (a)-(c), (f)). In addition, the remaining 9.9 μm particles were recovered by replacing the medium with 10% (w/v) cesium chloride solution and rotated for additional 8 min at 1000 rpm (Fig. 5 (d), (e), (g)).

Finally, we separated JM cells by using the presented system (Fig. 6). After 15 minutes of rotation at 1500 rpm, the cells with different sizes retained at different positions in the separation chamber (Table 1). This result suggests the applicability for the separation of living cells. We believe that the presented system would achieve high resolution fractionations with simplified instruments and procedures.

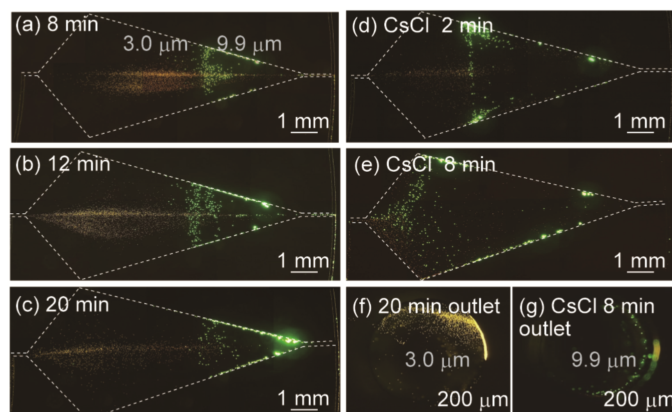

Figure 5: Fluorescence images of particles when the device was rotated at 1000 rpm. Each photograph shows the separation chamber or outlet at a rotation period as described. (a)-(c), (f) are the images when the surfactant solution was used as the media, while (d), (e), (g) are those when 10% (w/v) CsCl solution was introduced following (c).

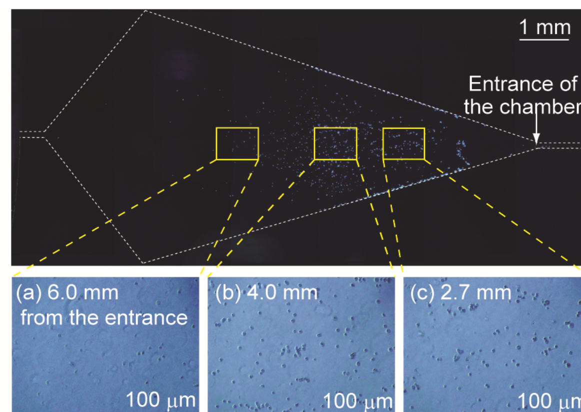

Figure 6: Micrographs of the separated JM cells in the chamber, after rotating at 1500 rpm for 15 min. Cells were labeled with Hoechst 33342.

Table 1. The size distribution of cells at three different points in the separation chamber. The average cell sizes were 8.7 ± 2.5, 14.3 ± 2.7, and 16.1 ± 4.0 μm for the points 6.0, 4.0, and 2.7 mm from the entrance, respectively. (a), (b), (c) correspond to those in Fig. 6.

Distance from the entrance (mm)	Cell diameter (μm)					
	0-5	5-10	10-15	15-20	20-25	25-
(a) 6.0	8%	68%	23%	3%	0%	0%
(b) 4.0	0%	5%	54%	38%	3%	0%
(c) 2.7	0%	6%	38%	42%	11%	4%

CONCLUSIONS

We have developed a microfluidic device for counter-flow separation of particles/cells, which significantly simplifies the conventional CCE systems and procedures. In the presented system, the PDMS devices are inexpensive and disposable, as well as the separation mechanism is simple and robust, which are the essential factors for providing actually useful tools both for the clinical and research fields. The presented system would be highly effective for separating or purifying a small amount of precious biological samples.

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