RAPID CELL SEPARATION IN DOUBLE SPIRAL MICROFLUIDIC CHANNELS

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Summary Rapid cell separation and concentration has attracted significant attention because of its important applications in cell research and biomedical diagnostics. Here we propose a double spiral microfluidic platform with two symmetric bifurcated outlets that can continuously separate real biological samples based on size difference with throughput comparable to conventional techniques. The microfluidic device is first characterized with the binary mixture of 5- and 10-μm-diameter polystyrene beads at different flow rates controlled by a syringe pump. Using the same double spiral microfluidic device, we further separate 4T1 cells from the murine blood cells at a high throughput while achieving a concentration effect for 4T1 cells.

INTRODUCTION

Microfluidic methods, taking advantage of unique hydrodynamic effects under small length scales and low Reynolds numbers, are merging as potential tools to exceed the conventional separation techniques in terms of miniaturization, simplicity, and sensitivity [1-2]. Clever manipulation of the well-controlled laminar flow inside microfluidic channels provides many opportunities to separate different size cells into different streams. A particular geometries of microfluidic systems will induce additional hydrodynamic forces, which could be adopted to separate cells by size[3]. Another method is to design the passive spiral or double spiral microfluidic device that can separate particle or cell mixture by size based on the differential migration [4,5]. However, most passive-based cell sorters published so far have been focused on proof-of-concept demonstration by using specified particle mixtures or pre-conditioned biological samples, and hence lacking the biological sense.

Here we propose a double spiral microfluidic platform with two symmetric bifurcated outlets that can continuously separate real biological samples based on size difference with throughput comparable to conventional techniques. We develop a numerical model to track the particle trajectories in the Dean flows generated inside the curved geometries, by taking account of the effects of the key hydrodynamic forces on particles. After characterizing the separation performance using polystyrene beads, we further separate 4T1 cells from the murine blood cells at a high throughput while achieving a concentration effect for 4T1 cells.

NUMERICAL AND EXPERIMENTAL MTHODS

Numerical method

We designed a double spiral microchannel with 6-loop for each direction to particle/cell manipulation, as illustrated in Fig. 1. At a relatively high Reynolds number, a secondary cross-section flow (Dean flow) will develop in curved microchannels due to the action of centrifugal forces. Neutrally buoyant particles suspended in liquids are also subjected to drag force and lift force, which result in differential particle migration within the microchannel. A comprehensive understanding of the flow field and the particle behavior is crucial for a better design of particle/cell separation devices. Although substantial investigations have been done previously, the theory and the mechanism of particle dynamics, even within a seemly simple straight channel, are still far away from being conclusive. For a complex microfluidic system, numerical simulation has become an effective tool to quantitatively examine how different factors affect the particle dynamics. Here we present a Lagrangian particle tracking technique called Discrete Phase Model (DPM) to model the particle motion in the 3-D double spiral microchannel. In our experiments, the particle/cell concentration is very low. Therefore, it is reasonable to assume that presence of the particles does not significantly influence fluid properties and thus the flow field. The steady flow field is first obtained by solving the impressible Navier-Stokes equations. Subsequently, a particle trajectory is obtained by integrating the force balance based on Newton's second law of motion

$$\frac{d\vec{v}_P}{dt} = \frac{18\mu}{\rho_d a^2}\frac{C_D \mathrm{Re}_S}{24}(\vec{v}-\vec{v}_P) + \frac{\vec{g}(\rho_P-\rho)}{\rho_P} + \frac{1}{2}\frac{\rho}{\rho_P}\frac{d(\vec{v}-\vec{v}_P)}{dt} + \vec{F}_Z$$

Experimental method

The microfluidic device is fabricated using standard soft-lithography techniques with SU8 master mold on a silicon substrate. In the experiment of separating the particle mixture, the double spiral microchannels were first primed with deionized water. Binary mixture of polystyrene beads was then introduced to double spiral microchannels by using a syringe pump that was connected to microchannels through the inlet plastic tube. The flow rate inside the

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microchannels could be controlled by adjusting the parameters of the pump. Similarly, in the experiment for separating cells, the channels were first filled with PBS, followed by loading the mixture solution of 4T1 cells and murine blood cells via the pump. The assembled device was mounted onto the stage of a Leica DMI 6000 microscope. Fluorescent streak images were obtained by a CCD camera with exposure time of 5 s and superimposed using Adobe Photoshop.

RESULTS AND DISCUSSION

Presence of the secondary Dean flow and their extent in the spiral microchannel were confirmed by numerical simulations. Fig. 2 displays the trajectories of 5 μm diameter particles and 10 μm diameter particles. Due to page limit, we only present the results under two flow rates: 10 *ml/hr* and 40 *ml/hr*. As can be seen in Fig. 2(a) and (b), the larger 10 μm particles are focused into a thin stream while the smaller 5 μm particles still spread over a quite large part of the cross-section. These numerical results agree well with the experimental observations conducted in the same microchannel configuration and operating conditions (see Fig. 3 in the next subsection). We also plot the 3-D view of the trajectories of two types of particles in Fig. 2(c). The 10 μm particles (green color) actually were focused into two thin streams located at the upper and the lower inertial equilibrium positions while 5 μm particles were entirely entrained in the secondary flow circulations, which is consistent to the previous predictions in other references [3].
We first experimentally characterized the performance of the microfluidic cell sorter by separating the binary mixture of 5- and 10-μm-diameter polystyrene beads at a concentration of 1×10^6 beads/ml in deionized water. Fig. 3 shows the superimposed fluorescent images at the outlet for 5 μm (red line) and 10 μm (green line) beads at six different flow rates ranging from 10 to 60 *mL/hr*. To demonstrate that the double spiral microchannels are also capable of separating biological samples, we applied the same microfluidic platform to sort and concentrate 4T1 cells from murine blood cells at a mixture concentration of 2×10^6 cells/ml in PBS. The average size of the 4T1 cells is around 15 μm in diameter, significantly larger than the surrounding murine blood cells (erythrocytes are 6 μm and peripheral blood lymphocytes are 7-10 μm). Due to this large difference in cell size, we can simply differentiate 4T1 cells from blood cells by analyzing the bright-field microscopic images.

CONCLUSIONS

We have presented a double spiral microfluidic cell sorter that separates particles or cells at a high flow rate. Compared with the electrokinetic-based cell separation schemes, our pressure-driven cell separation method eliminates the need for external electric field and meanwhile realizes a high throughput cell separation.

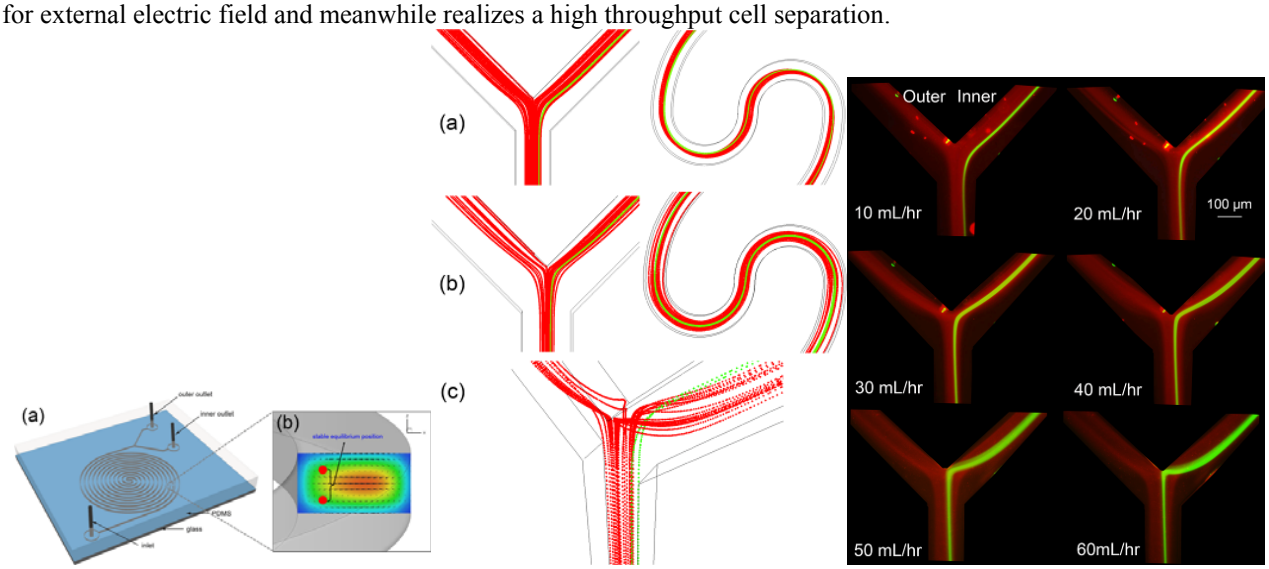

Fig. 1 Schematic of the double-spiral microchannel devices
Fig. 2 Numerical prediction of the praticle trajectories
Fig. 3 Experimental images of the particle focusing and separation

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