A VACUUM-INTEGRATED CENTRIFUGAL MICROFLUIDIC CHIP FOR DENSITY-BASED SEPARATION OF MICROPARTICLES

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ABSTRACT

Here we present a new vacuum-integrated centrifugal microfluidic chip for the density-based separation of microparticles. A sample was loaded in a fluidic channel using the gas permeability feature of polydimethylsiloxane (PDMS) membrane between fluidic and control channels. Vacuum was applied from control channel to drive a density media and then the sample containing microparticles in the dead-end fluidic channel. Afterwards, the chip was disconnected from the vacuum and it was centrifugated. If the sample contains microparticles denser than the density media, the microparticles are sedimented at the end of the microfluidic channel so that these particles can be separated from remaining the lower density particles. With this approach, we separated 1.09 g/mL microparticles with 82,6% efficiency and 99% purity from 1.02 g/mL microparticles. Separated particles in the microfluidic chip can also be inspected under a microscope for further analysis. This simple approach offers high efficient density-based separation of microparticles with close densities.

KEYWORDS

Centrifugal microfluidics, density-based separation, microparticles, sample loading

INTRODUCTION

Cells have unique properties that can differ from each other such as size, shape, density and electrical charge [1]. Cell separation has significant importance in biomedical researches, clinical analysis, diagnostics, and monitoring. In density-gradient separation, cells are located according to their densities in a density-gradient medium. Although this principle has been used for cell separation, it is time consuming, high level of stress and concentrated solution can affect the cell viability. Density-gradient separation offers efficient and pure separation, but the discriminate separation of small densities particles are difficult [2]. Ficoll, Percoll, sucrose and dextran can be given as examples of density-gradient medium [3]. Ficoll allows the separation of peripheral blood mononuclear cells from other components by layering diluted blood on density-gradient medium and centrifuging. Erythrocytes and granulocytes move to the bottom and mononuclear cells remain above from the density-gradient medium [4]. Ficoll has been extensively used in cell separation but it requires high amount of blood sample to observe discrete separation and trained person to create density layer carefully [5].

Microfluidic adaptation of density-gradient separation has many advantages over conventional methods. Microfluidic based cell separation allows easy fluid transportation, minimum analysis time, and low amount of sample utilization. Also, staining of blood cells can be achieved in microfluidics with minimum human intervention and reduces the infection possibility [5]. Magnetic levitation based cell and/or microparticle separation takes place suspending particle in a paramagnetic solution [6]. When the magnetic force and buoyancy force act on the particle equilibrate, levitation occurs, and particles are levitated in a different height according to their densities. Magnetic levitation based cell separation offers point-of-care diagnostic [7]. Suspended microchannel resonators can measure single cell density with high precision and accuracy [8]. Requirement of two separate fluids in microcantilever and real-time imaging limits the utilization of suspended microchannel resonators for the cell separation [9].

In a microfluidic density-gradient centrifugation device using Ficoll, the separation of low-density polystyrene beads (1,02 g/mL) and high-density silicon dioxide beads (2.2 g/mL) was achieved with 99% separation efficiency. In this study, beads were tend to travel through outer wall of the microfluidic chip and densities of two beads are quite different [2]. A density-based separation was developed in a microfluidic chip based on Stokes equation. Separation of densities was achieved but at higher particle concentrations, clogging occurred in their loading channel [10]. A density-gradient medium based centrifugation microfluidic platform was also used for the peripheral blood mononuclear cell extraction. [11]. Furthermore, the extraction of 95.15% of leukocytes from whole blood was also conducted in a centrifugal microfluidic platform. [5]. However, sample loading is challenging in these devices since complex operation procedures with pumps and/or valves were required.

We designed a novel microfluidic chip for the density-based separation of microparticles with a simple sample loading approach using the gas permeability of PDMS membranes between fluidic and control channels. Density media and samples were loaded respectively in the dead-end fluidic channel by applying vacuum from the control channel. After disconnecting vacuum, the chip is centrifugated to separate microparticles based on their densities. With this design, the inlet of fluidic channel does not need to be blocked in order to eliminate spilling of the sample during centrifugation and microparticles denser than the density media was collected at the end of the fluidic channel. The separated particles can also be easily monitored under the microscope. This system allows microfluidic adaptation of density-gradient separation for discrete separation of microparticles have near densities. Therefore, we believe that our microfluidic method will offer new opportunities for density-based separation.

EXPERIMENTAL METHODS

In this study, a microfluidic chip was fabricated using soft lithography process [12] The mold was printed using UV curable Formlabs Clear Resin printer in Formlabs Form 2 3D printer. After printing, the mold was washed

with isopropanol for 10 minutes to remove resin residues. PDMS was prepared as a mixture of elastomer and curing agent at 10:1 ratio. Then, the PDMS mixture was degassed and poured onto a mold, and then cured at 65 ˚C for 24 hours. Afterwards, cured PDMS was peeled from the mold, and bonded on the clean glass with oxygen plasma treatment at 100 W and 0.5 mbar for 2 min.

We used two different PDMS design to analyze sample loading and microparticle separation. Figure 1A shows the first PDMS design (PDMS chip 1) having 1 mm (width) × 10 mm (length) × 1mm (height) fluidic channel with a volume of 10 µL and a fork-shaped vacuum channel. As shown in Figure 1B, distances between fluidic and control channels were designed as 1 mm. For the density-based particle separation, we used a second microfluidic chip (PDMS chip 2) show in Figure 2A having high fluidic volume (500 µL). Two diamond shaped fluidic chambers was used to load the density media (Ficoll 400) and microparticles, respectively. The distance between the fluidic and control channels was set to 1 mm (Figure 2B).

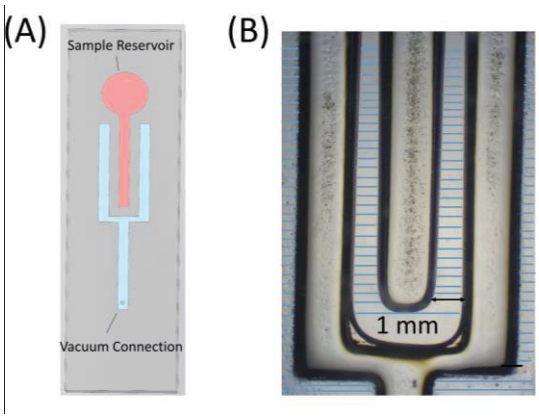

Figure 1: Microfluidic chip design. (A)Top view illustration of microfluidic chip; red channel represents a fluidic channel, blue channels represent control channels. (B) Micrograph of a microfluidic chip; Blue shaded regions show PDMS. Scale bar is 200 µm

To calculate the speed of sample loading, 10 µL red food dye solution was introduced to the sample reservoir of the PDMS chip 1, and the inlet of the vacuum channel was connected to the vacuum pump with a vacuum pressure of -0.88 bar. Time dependent profile of red food dye was investigated under an inverted microscope.

For the separation of 1.02 g/mL and 1.09 g/mL microparticles (Cospheric LLC, CA, USA) the density of Ficoll 400 was adjusted to 1.065 g/mL. Afterwards, 250 µL 1.065 g/mL Ficoll 400 solution was given into the sample reservoir of PDMS chip 2, the solution was withdrawn through the end of 250 µL first diamond shaped fluidic chamber. Then, 250 µL sample composed of Phosphate Buffered Saline (PBS) with 1% (w/v) Pluronic F-127 having 1.02 g/mL green fluorescent and 1.09 g/mL red fluorescent microparticles was introduced in the reservoir and withdrawn in the chip. Then, the microfluidic chip was placed into a centrifuge tube and centrifuged at 2000 rpm with different time period. We used the last 25% part of the second diamond-shaped chamber as the separation region of the chip. Fluorescence images were taken and separation efficiency and purity of fluorescent microparticles was calculated on the separation region.

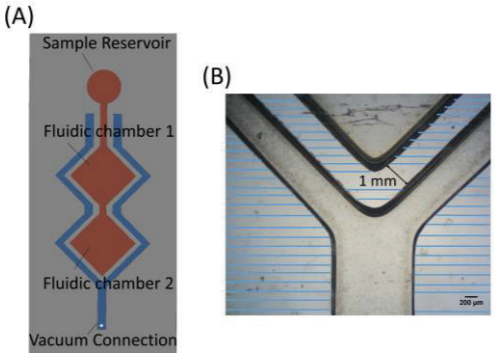

Figure 2: PDMS chip 2 design. (A) Top view illustration of microfluidic chip; red channel represents a diamond shaped fluidic chambers, blue channels represent control channels. (B) Micrograph of a microfluidic chip; Blue shaded regions show PDMS. Scale bar is 200 µm.

RESULTS AND DISCUSSIONS

Red food dye solution loading was observed in PDMS chip 1 (Figure 3). Our results revealed that food dye was filled completely in the fluidic channel for about 60 minutes with a vacuum applied through the control channels. On the other hand, 140 min was necessary to fill the fluidic channel in PDMS chip 2. Even the volume and the length of the PDMS chip 2 are 50-fold and 4-fold higher than the PDMS chip 1, the filling time of PDMS chip 2 is only 2.3-fold longer than the chip 1.

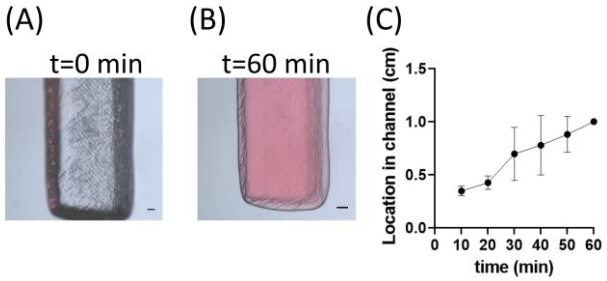

Figure 3: Time-dependent profile of the food dye loading in the microfluidic channel. (A) Micrographs of fluidic channel (A) before starting vacuum and (B) after 60 minutes of vacuum. (C) Reached location of the food dye in channel for different pumping time. Scale bar is 200 µm..

We conducted density-based microparticle separation in PDMS chip 2. We observed that 1.09 g/mL red fluorescent microparticles were started to accumulate and separated from 1.02 g/mL green fluorescent microparticles at the end of the diamond-shaped chamber 2 (Figure 4A). According to our results, separation of 1,09 g/mL microparticle was achieved with 82,6% efficiency and 99% purity from 1.02 g/mL microparticles with 15 min centrifugation (Figure 4B). By increasing centrifugation time, the efficiency was slightly increased. However, significant difference in efficiency and purity was not observed for different centrifugation time ($p < 0.05$).

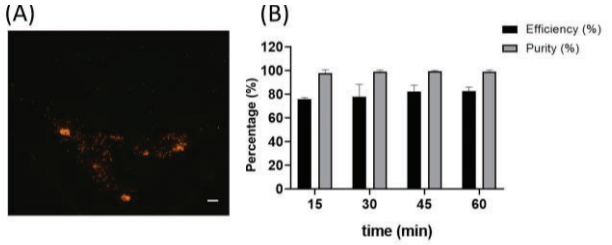

Figure 4: Density-based separation results. (A) Fluorescence micrograph of the microfluidic chip showing separation of 1,09 g/mL in the second chamber at 2000 rpm 15 min. Scale bar is 200 µm (B)Separation efficiency and purity of 1,09 g/mL microparticle

CONCLUSIONS

In this paper, we introduced a new vacuum-integrated centrifugal microfluidic chip for the density-based separation of microparticles. With easy sample loading process and centrifugation steps, we achieved high efficiency and purity in density-based microparticles separation. The device presented here allows also monitoring of separated particles under a microscope, which cannot be possible with traditional density-gradient separation methods. The only limitation of the device seems the long sample loading process that could be reduced by increasing contact area between fluidic and control channels and using high vacuum pumping conditions. Density is one of the unique properties to separate cells that can be used for therapeutics, clinical diagnostics and prognosis. So our device could bring new opportunities in density-based separation for different applications with its easy-to-use design.

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