Sensors and Actuators B 190 (2014) 86–92

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Pneumatically controlled multi-level microchannel for separation and extraction of microparticles

Yoonkwang Nam [a], Minseok Kim [a], Taesung Kim [a,b,*]

[a] School of Mechanical and Advanced Materials Engineering, Ulsan National Institute of Science and Technology (UNIST), 50 UNIST-gil, Eonyang-eup, Ulsan 689-798, Republic of Korea
[b] School of Nano-Bioscience and Chemical Engineering, Ulsan National Institute of Science and Technology (UNIST), 50 UNIST-gil, Eonyang-eup, Ulsan 689-798, Republic of Korea

ARTICLE INFO

Article history:
Received 20 June 2013
Received in revised form 5 August 2013
Accepted 5 August 2013
Available online 30 August 2013

Keywords:
Microfabrication
Gray-scale photolithography
Multi-level microchannels
Separation
Extraction
Microparticles

ABSTRACT

Most microfluidic devices are fabricated by using standard photolithography technology so that they are typically limited to a single, uniform microchannel depth. In this work, we employ a polydimethylsiloxane (PDMS) gray-scale photomask (PGSP) developed in our previous work for fabricating multi-level microchannels (MLMs) that hold a high potential for enhancing the separation performance and efficiency of microparticles. Since the PGSP provides a series of multiple, uniform and precise filter gaps in a microchannel, we describe an MLM-integrated microfluidic device that not only filters but also accumulates microparticles by size such as polystyrene beads and yeast cells. In addition, we integrate a pneumatic pressure controller that manually manipulates the filter gaps to enable sequential extraction of the separated, accumulated microparticles from the device for additional post-analysis. Since the PGSP-based soft-lithography technology provides a simple but powerful fabrication method for MLMs, we believe that both the fabrication method and the separation and extraction device can be widely used for micro total analysis systems that benefit from MLMs.

1. Introduction

Microparticle Separation can be defined as the physical fractionalization and/or isolation of homogeneous microparticles from a heterogeneous mixture by size, shape, deformability, various intrinsic properties, etc. The microparticle separation is essential for applications in industry, medicine and biochemistry [1–3]; particularly, it has high potential for mammalian and bacterial cell separation for developments in biology [4] and microbiology [5]. While conventional particle separation methods mainly depend on membrane filtration, microfabrication technologies are demonstrating unprecedented capabilities in the separation of microparticles and cells [6,7]. In particular, microfluidic devices provide various methods for separation and filtration over wide ranges of microparticle sizes [8–12], shapes, and deformabilities [4,13].

Typically, microfluidic separation devices can be categorized into passive and active devices from the viewpoint of separation mechanisms. For passive methods, porous membranes [14,15], spiral channel networks [9], pillar structures [16,17], slanted microfluidic obstacles in a microchannel [11], non-Newtonian fluids [12], and inertial focusing [8,10,18] are used for microparticle separation and/or filtration. These methods determine the range of microparticle sizes to separate or filter at the design and fabrication step. Because of this, the dynamic range of separation of microparticles is relatively narrow and fixed, although passive separation is a continuous, high-throughput technique. For active methods of separation, various separation mechanisms such as electrical actuators [19], dielectrophoresis [20,21], acoustic waves [22,23], centrifugation [24,25], and magnetic forces [26,27] are used for the separation, sorting, and capture of microparticles. These methods allow more controllable separation and can function with a wider dynamic range of microparticle sizes although they require an additional external experimental setup.

Most passive devices for microparticle separation use physical trapping and penetration mechanisms of microparticles by and through filter gaps, respectively. However, more controllable techniques that actively adjust the filter gaps precisely and maintain the shape of them uniformly in microfluidic devices are required [28]. As mentioned earlier, the filter gaps in microfluidic devices are typically established at the design and fabrication step and are not easily controlled. To date, many passive devices have been designed and fabricated to separate microparticles on a chip

* Corresponding author at: School of Mechanical and Advanced Materials Engineering, Ulsan National Institute of Science and Technology (UNIST), 50 UNIST-gil, Eonyang-eup, Ulsan 689-798, Republic of Korea. Tel.: +82 52 217 2313; fax: +82 52 217 2409.
E-mail address: tskim@unist.ac.kr (T. Kim).

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http://dx.doi.org/10.1016/j.snb.2013.08.024

according to their size, but most of these separation devices were fabricated using standard soft-lithography technology, which limits the device to a single-level, uniform microchannel network.

In this work, we employed a simple but powerful microfabrication method that was developed in our previous work to form MLMs by combining a polydimethylsiloxane gray-scale photomask (PGSP) and standard soft-lithography technology [29]. The method provides a more convenient and compatible fabrication method with soft-lithography technology for producing high-aspect-ratio masters for PDMS replica devices. That is, the method allows the facile fabrication of a microfluidic device with different levels/heights but causes no significant hydrodynamic resistance in a microchannel, benefiting from multi-level features. We also employed a manual pneumatic pressure controller that independently raises the V-shaped filter barrier by attaching an individual PDMS chamber on top of each filter barrier to completely release the accumulated microparticles (Fig. 1(A)). We tested the MLM-integrated microfluidic device for its functionality in passively filtering and accumulating microparticles by the filter gaps based on their size. In addition, we demonstrated that it can sequentially release the accumulated microparticles along the microchannel by actively raising the filter barrier, which results in the sequential extraction of them from the MLM-integrated microfluidic device. Lastly, we demonstrated that the same method can be used for the separation, accumulation and extraction of yeast cells in grain-fermented alcohol by size for additional biochemical and microbiological assays requiring large concentrations such as the identification of microorganisms [30].

2. Materials and methods

2.1. Reagent, microparticle and yeast

A dye (Allura red AC, Sigma–Aldrich, Korea) was diluted with distilled water to confer proper concentrations. A phosphate buffered saline (PBS, Sigma–Aldrich, pH = 7.4) solution and a 1% Pluronic surfactant (F-127, Sigma–Aldrich) were used for the experiments. The purchased microparticles (Spherotech, Korea) were 5.4 μm, 9.2 μm, and 12.0 μm in diameter. Yeast cells were obtained from a Korean traditional rice wine called Makgeolli (pH = 4.5–5.0). The solution was prepared by centrifuging 1 mL of Makgeolli at 1000 rpm for 30 s and then adding 0.5 mL of the supernatant solution to 0.5 mL of the PBS solution for the experiment.

2.2. Fabrication of microfluidic photomask and device

The device fabrication process is found in our previous work that describes photomask fabrication and MLM fabrication [29]. In this work, we fabricated three filter barriers in series in a single microchannel and the filter gaps are 12 μm (V_1), 8 μm (V_2) and 4 μm (V_3) from the inlet to the outlet, respectively, as illustrated in Fig. 1(A). In short, the fabrication process consists of three steps. First, a microfluidic photomask (75 μm deep and 100 μm wide) was fabricated by using standard soft-lithography technology as used in our previous work [31]. The PDMS mold was bonded using an oxygen plasma treatment with a cover glass under 50 sccm of O_2 at 50 W for 5–10 s (Cute-MP, Femto Science, Republic of Korea) as illustrated in Fig. 1(B). Second, the negative photoresist (SU-8, 2050, MicroChem, Newton, MA, USA) was spin-coated on a glass wafer at 500 rpm for 5 s and 3500 rpm for additional 30 s (to obtain a layer approximately 20 μm thick). It was then pre-baked for 5 min on a hot plate and the PGSP prepared in the above-mentioned step was attached to the backside of the glass wafer. Next, the glass wafer was exposed to a collimated UV light using a mask aligner (MA6, SUSS MicroTec, Germany) through the PDMS photomask to form a multi-level SU-8 master for PDMS replica devices. Lastly, the microfluidic chambers were fabricated in PDMS in the same manner as the first soft-lithography process and then attached to the top of the MLM-integrated device by using the same oxygen plasma treatment. The chambers were separately connected to a 3 mL syringe via an air-tight tube so that the pressure in the chamber was individually manipulated manually (see Fig. 1(C)). Because the top chamber layer (5 mm) is much thicker than the MLM layer (20 μm), a negative pressure easily raised the filter barrier. In this manner, each filter barrier was raised and lowered by manually controlling the pneumatic pressure in the chambers. Fig. 1(C) portrays a real image of the integrated device including the MLM and the pressure chambers used for the separation and extraction experiment.

2.3. Experimental procedure and data analysis

The separation channels were rinsed with PBS and then coated with a Pluronic surfactant (F-127, 1% in PBS) to minimize non-specific binding between the polystyrene microparticles and the glass/PDMS surfaces. The residue of the surfactant in the microchannels was gently rinsed with PBS (about 200 μL) for approximately 1 h. Polystyrene microparticles were agitated in a sonicator (5510E-DTH, Bransonic, USA) and then injected into the separation channel via a silicon rubber tube. A microscope (IX71, Olympus, Japan) equipped with a CCD camera (Clara, Andor Tech, CA, USA) and Metamorph 7.7 (MDS Analytical Technologies, Sunnyvale, CA) was used to take images of the microparticles and the yeast. The image processing and quantification of the polystyrene microparticles and yeast cells were performed using Metamorph and Image J (NIH, USA). The results were plotted using Origin 8.0 (OriginLab, Northampton, MA, USA).

3. Results

3.1. Filtration and accumulation of microparticles with an MLM

We tested the filtration of three microparticles (5.4 μm, 9.2 μm, and 12.0 μm) using an MLM-integrated device that had three filter barriers as shown in Fig. 2(A) The microparticles that crossed the line a–a′ were continuously filtered at each V-shaped barrier and accumulated over time as shown in Fig. 2(B). Because the first filter gap (V_1) was approximately 11 μm, the 12.0 μm microparticles were filtered while the smaller microparticles penetrated the first filter gap and crossed the line b–b′. When the smaller microparticles confronted the second filter gap (V_2) that was approximately 8 μm, the 9.2 μm microparticles were filtered in the same manner as the first filter gap. Only the 5.4 μm microparticles still flowed along the microchannel and crossed the line c–c′ until they met the third filter gap. Because the third filter gap (V_3) was approximately 4 μm, the 5.4 μm microparticles were filtered and continuously accumulated. The filtration efficiency (η) is defined as follows:

$$\eta = \frac{N_{\text{before}} - N_{\text{after}}}{N_{\text{before}}} \times 100\,(\%) \quad (1)$$

where N_{before} is the total number of microparticles flowing along a microchannel before a filter gap and N_{after} is the number of microparticles flowing after the filter gap. For example, the efficiency of the first gap was calculated by counting the number of microparticles crossing the line a–a′ before the filter gap and that crossing the line b–b′ after the filter gap when a homogenous mixture of 12.0 μm microparticles were loaded and observed for 20 min; the efficiency of the first filter was 89%. In the same manner, the efficiencies of the second and third filter gaps were calculated by loading 9.2 μm and 5.4 μm microparticles, resulting in efficiencies of 85% and 94.5%, respectively.