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A tunable micro filter modulated by pneumatic pressure for cell separation

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ABSTRACT

This study reports a new microfluidic-based filter for size-tunable separation of microbeads or cells. The filtration separation mechanism is based on the pneumatically tunable deformation of polydimethylsiloxane (PDMS) membranes, which block the fluid channel with a varied degree. This defines the dimensions of the open area of the fluid channel and thus determines the maximum diameter the microbeads or cells which can pass through. The proposed device incorporates pneumatic micropumps for automatic liquid handling. Another unique feature of this filter is an unclogging mechanism using a back-flush operating mode, by which a reverse-directional flow is utilized to flush the clogged filter zone. The separation performance of the proposed device has been experimentally evaluated. Results show that this developed device is able to provide precise size-dependent filtration, with a high passage efficiency (82–89%) for microbeads with sizes smaller than the defined void space in the filter zone. Also, the proposed separation mechanism is also capable of providing a reasonable filtration rate (14.9–3.3 μl/min). Furthermore, the separation of chondrocytes from a 30 μl suspension of enzymatically digested tissue is successfully demonstrated, showing an excellent cell passage efficiency of 93% and a cell viability of 96%. The proposed device is therefore capable of performing cell separation in situations where either the harvested specimen is limited or the sample cell content is sparse. It also paves a new route to delicately separate or to isolate cells in a simple and controllable manner.

1. Introduction

With this recent advent of bio-MEMS technology, microfabricated bio-analytical-systems, also referred to as micro-total-analytical-systems (μ-TAS), have created a new toolset to perform analytical tasks on the micro-scale. The real benefits behind miniaturized bio-analytical devices, compared to their macro-scale counterparts, include portability, decreased manufacturing costs, disposability, less time required of analysis, lower consumption of reagents and samples, and reduced production of potentially hazardous waste [1].

In order to realize the concept of a μ-TAS, microfluidic technology is usually used for transporting and manipulating minute amounts of fluids and/or biological samples through microchannels; therefore, allowing for the integration of various chemical and biochemical processes into fast and automated microflow systems. A μ-TAS is ideally capable of integrating multiple fundamental operations in a general bioassay such as sampling, transporting, mixing, reacting, separating and detection on a single microfluidic chip to achieve the goal of a so-called lab-on-a-chip (LOC). Among these functions, an on-chip separation device is a crucial component since it eliminates the need for costly and time-consuming off-chip separation and makes portable analytical instruments feasible.

In general for bioassays, separation broadly refers to the procedures undertaken to isolate or purify cells, biomolecules, or other targets of interest from specimen samples mainly for the acquisition or enrichment of targets, the removal of impurities or interfering constituents, the enhancement of product stability, or for sorting purposes. Separation strategies adopted in bio-analytical applications generally vary with the targets to be separated. Nevertheless, the basic sorting rationale is primarily based on distinctions in size, shape, density and other physical or biochemical features. For example, to isolate cells in a suspension or to separate particular cell species from a cell pool, centrifugation techniques are commonly used. In addition to using a centrifuge, various membrane-based filtration schemes are also used to perform a specific cell separation or sorting task [2–4]. Although these conventional approaches have a long history of application in a variety of bio-analytical protocols, these operations are usually time-consuming, costly, labor-intensive and, most importantly, not applicable when either the specimen volume is limited or the cell content in the sample is low. This is mainly because a small quan-

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Nomenclature

CCD	charge-coupled device
DEP	dielectrophoresis
DMEM	Dulbecco's Modified Eagle's Medium
EMV	electromagnetic valve
LOC	lab-on-a-chip
MEMS	micro-electro-mechanical-systems
PBS	phosphate buffered saline
PDMS	poly-dimethylsiloxane
SoC	system-on-a-chip
S-shape	serpentine-shape
TMP	trans-membrane pressure
UV	ultraviolet
μ-TAS	micro-total-analytical-systems

tity of isolated cells is difficult to handle and can easily get lost in a conventional cell separation process.

Hence, microfluidic technology has expanded the minimum handling threshold for various protocols by reducing the sample volumes required for analysis. Recently, several microfluidic systems have been intensively explored for various cell-based research applications such as for drug/toxin screening and testing [5], for studying cell physiology [6–7], for bioassays [8] or for disease diagnosis [9]. In such micro-scale operations, the separation or isolation of cells from a fresh specimen has been a technical challenge since the conventional techniques mentioned earlier may not be appropriate. This identifies an urgent need for a device with a proper size scaling in which cells can be adequately separated or isolated, particularly from a limited sample.

It has been demonstrated in the literature that micro-scale cell separation or isolation can be realized by adopting different mechanisms, including dielectrophoresis (DEP) [10–14], acoustic [15–19], magnetic-based [20–22], or microchannels with specific geometric or structural designs [23–30]. However, separating or isolating cells based on these mechanisms usually require some strict operating conditions, complicated fabrication processes, and costly or bulky equipment. Furthermore, these processes are highly technically demanding, which hinders their widespread use. For example, it has been demonstrated that the DEP force can be used to isolate white blood cells from diluted whole blood [14]. For that work, both a non-uniform electric field and a medium solution with specific electrical properties were required. Besides, exposure of the cells to an electric field could affect the physiology of cells, which might in turn complicate subsequent analysis of the cells. Acoustic forces generated by applied ultrasonic waves combined with microfluidic technology have also been used to separate microparticles and cells [19]. In that study, however, an ultrasonic wave generator is required, which may not be generally available at many biomedical laboratories. More importantly, the impact of ultrasonic waves on biological entities requires further investigation. This might be a concern when one intends to use this approach to manipulate living cells. Apart from these methods, the combination of antibody-conjugated magnetic beads with a specific magnetic field has been regarded as a promising strategy for cell sorting and separation [20–22]. For example, it has been demonstrated that CD15/45 antibodies-coupled to magnetic beads can be used to isolate leukocytes from a human whole blood sample in a microfluidic system [22]. Antibodies on the surface of the magnetic bead selectively bind to a specific surface antigen on the leukocytes and a complex is formed that can be separated from the whole blood suspension by an applied magnetic field. Although this technique has proven the feasibility of cell separation tasks, there are two major technical hurdles with this method; specifically, the availability of antibodies specific to the target cells to be separated, and a reliable method for conjugating the antibody to the magnetic bead. Alternatively, microchannels with specific geometric or structural design coupling with fine-tuned flow control in a microfluidic system can enable size-dependent separation of microparticles or cells in a continuous flow [23–30]. In this category, for example, a simpler approach is to construct multiple pillars with varied layouts in a microchannel to mimic a filter or a membrane so that the passage of microparticles of a particular size can be defined [31–33]. However, the fouling problem remains a technical challenge in these devices since the microbeads or cells become clogged in these filters and are difficult to be practically removed.

In order to easily carry out cell separation or isolation in a flexible manner, a new microfluidic-based filter integrating a filter zone, four serpentine-shape (S-shape) pneumatic micropumps and four reservoirs are designed and fabricated based on soft lithography of a poly-dimethylsiloxane (PDMS) elastomer [34,35]. The filtration mechanism is based on the pneumatically tunable deformation of the PDMS membranes in the filter zone, which partially block the fluid channel to various degrees. Then this defines the dimensions of the remaining passageway in the fluid channel and thus restricts the passage of the microbeads or cells to those less than a specific size. The other unique features of this device include the incorporation of pneumatic micropumps for automatic liquid handling and an unclogging mechanism using an intermittent back-flush operating mode. The performance of this microfluidic filter is evaluated using polystyrene microbeads with a size range from 5 to 20 μm in diameter. They are separated under a varied set of pneumatic pressures in the filter zone. Results showed that the presented device was able to provide highly size-dependent selectivity with high separation efficiency (82–89%) for the microbeads with their size smaller than the defined void space in the filter zone, whereas, with low separation efficiency (4–7%) another way around. Besides, the proposed mechanism was also capable of providing a reasonable filtration flux rate, allowing the filtration separation operation of tiny amount of sample in a couple of minutes. Furthermore, the feasibility of using the microfluidic filter platform for the separation of chondrocytes from the limited enzymatically digested tissue suspension was successfully demonstrated. The outcomes revealed that the proposed device was not only able to achieve an excellent cell separation efficiency of 93% but also offers a cell separation process with its cell viability as high as 96%. As a whole, because of the small scale the proposed device is particularly suitable to perform cell separation under the circumstance that either the specimen is limited or the cell content in a sample is sparse. Besides, due to the designed separation mechanism and the tunable characteristics of separation performance, the presented work opens a new route to separate cells (or microbeads) in an uncomplicated, flexible and cell friendly way.

2. Materials and methods

2.1. Design

The proposed micro filter integrates the functions of both a pneumatically tunable filter and a sample transportation mechanism so that the separation of cells or beads can be automatically performed. The layout of the micro filter is schematically shown as Fig. 1. It comprises one filter zone, four S-shape pneumatic micropumps, cross-microchannels for sample flow (vertical one) and washing buffer flow (horizontal one) as well as four reservoirs for sample loading, filtrate collection and washing buffer loading. As shown in Fig. 1, each individual microchannel is equipped with its own S-shape pneumatic micropump (indicated as 1–4) for both liquid delivery and flow control. Besides, the microchannel