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Blood plasma separation microfluidic chip with gradual filtration

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ABSTRACT

Blood is one of the most crucial biological materials that can be used to diagnose diseases. In order to avoid the effects of blood cells for cell free plasma detection, the first step toward a blood test is the blood separation. We developed a microfluidic chip for blood plasma separation with gradual filtration, which consisted of front-end cell capture structures and back-end filters. Two types of filters were proposed: straight line filters and square wave filters. The cell capture structures and filters, fabricated on PDMS (polydimethylsiloxane), included two structural layers. The first layer consisted of pillars to create small gaps between the second layer and glass, which enabled the flow of the plasma through the capture structures while trapping the cells in the structures. The second layer was an array of U-shaped structures. The results showed that the separation efficiency of plasma enhanced with increased dilution factor and decreased height of the gap created by the pillar layer. The separation efficiency was only 20% under 2 μm gap and dilution factor 10 and increased to 91% under the gap of 1 μm height and dilution factor 50 in the chip with the straight line filters. The separation efficiency was close to 100% under the gap of 1 μm height and dilution factor 20 in the chip with the square wave filters.

1. Introduction

Blood is composed of two main components: blood cells and plasma. In practice, clinical diagnostics is often performed on cell free plasma rather than raw blood. In order to avoid the effects of blood cells, the first step toward the blood test is the blood separation [1]. The traditional method employed by laboratories and clinics uses centrifugation equipment, which is time-consuming and labor intensive, and requires a large blood sample volume. One approach to overcome these difficulties is to use microfluidics or "lab-on-a-chip" technology, which needs nano- to micro-liter of samples and can prevent possible contamination commonly seen in the process of off-chip plasma separation [2]. Since the demonstration of a micro-total analysis system (μ-TAS) in the early 1990s [3], microfluidics has been considered as a promising technology to miniaturize the conventional diagnostic equipment and technologies. It offers advantages of small volume, low cost, short reaction time and high throughput. Microfluidic technology has already been used in chemical and biological analysis [4,5], cell and gene analysis [6,7], drug research [8,9], protein engineering [10], material synthesis [11,12] and environmental monitoring [13].

Recently, many different methods relying on active energy sources were used to separate plasma from the whole blood on microfluidic chips. Nakashima et al. used dielectrophoresis and capillary forces to separate and extract plasma from whole blood [14]. They extracted 300 nl blood plasma from 5 μl blood at an applied AC voltage of 10 V and 1 MHz. The blood separation efficiency enhanced with increased applied voltage and reduced electrode gaps [15]. Unfortunately, the plasma will be contaminated by the damaged blood cells in a high electric field. White blood cells exhibit diamagnetic behavior and red blood cells exhibit diamagnetic or paramagnetic behavior [16]. Therefore, they will move due to the magnetic force in the applied magnetic field. Furlani presented a microfluidic system for separation of red and white blood cells in plasma using a magnetic method, which is composed of an array of integrated soft-magnetic elements [17]. In addition, the method can be used to separate plasma by controlling the movement of blood cells. Acoustic standing wave force generated by ultrasonic standing waves was also used to separate plasma from blood, in which cells in a channel move to the pressure nodes or the pressure anti-nodes of the standing wave field [18–20]. In generally, these methods using active energy sources require the applied energy sources.

The common way is size-dependent particle separation, which is based on the difference in dimensions between blood cells and

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plasma. Filters or meshes are usually used to block blood cells movement and allow for the collection of plasma in these devices. Di Carlo et al. designed a microfluidic chip with arrays of physical U-shaped hydrodynamic trapping structures to capture HeLa cells [21]. Chen et al. proposed a microfluidic chip for direct and rapid trapping of white blood cells from whole blood by arrays of two-layer capture structures [22,23]. But the methods only relying on cell capture structures can not capture cells completely. Aran et al. described a cross-flow filtration microdevice for the continuous extraction of blood plasma and the outlets of blood cells and plasma were set with high and low pressure to enhance the plasma separation efficiency [24]. The separation efficiency will decrease dramatically with increased pressure of plasma due to the subsequent functional unit integration with the outlet. Crowley and Pizziconi proposed a microfluidic chip for the separation of plasma from whole blood using these planar microfilters [25]. Shim et al. designed and fabricated a disposable on-chip whole blood/plasma separator using a silica bead-packed microchannel [26]. The plasma flowed through the pores between the beads but the blood cells were blocked by the beads. However, the designs might lead to blood cell clogging or jamming easily. Yang et al. proposed a microfluidic device for continuous, real time blood plasma separation, which was based on Zweifach–Fung effect [27]. In the plasma separation chip supported by the filtration mechanism, most plasma separation chips using filtration method only consist of filters. And the gradual filtration is a nice way to enhance the separation efficiency and alleviate efficiently clogging and jamming [28].

In this paper, we developed a gradual filtration separation microchip with cell capture structures and filters. The front-end cell capture structures captured blood cells and the back-end filters prevented blood cells from flowing through them. In addition, two types of filters were designed and fabricated: straight line filters and square wave filters. The cell capture structures and filters, fabricated on PDMS (polydimethylsiloxane), included two layers. The first layer consisted of pillars to create small gaps between the second layer and glass, which enabled the flow of the plasma through the capture structures while trapping the cells in the structures. The chip could easily be adapted to different cell sizes by changing the height of the first layer (pillars). The chip not only avoided the problem of clogging or jamming, but also enhanced the separation efficiency.

2. Materials and methods

2.1. Design and principle

With the development of advanced microfabrication technologies, hydrodynamic method provides a new research tool for cell capture [29]. In order to avoid the problem of clogging or jamming, we designed cell capture structures and filters to capture blood cells and separate plasma from the whole blood, as shown in Fig. 1.

The front-end cell capture structures are some arrays of U-shaped structures in our previous study [22,23]. Two types of back-end filters (straight line filters and square wave filters) were designed to capture blood cells completely at the back-end of different chips. The straight line filters were changed from the front-end capture structure, as shown in Fig. 1(a). In addition, the gap was divided into two smaller gaps. This could decrease the chance of blood cells to flow through the filter. The square wave filters were designed to a square wave arrangement, as shown Fig. 1(b).

Both the cell capture structures and filters included two layers, as shown in Fig. 2. The first layer consisted of pillars to create small gaps between the second layer and glass, which enabled the plasma to flow through the capture structures but kept the cells

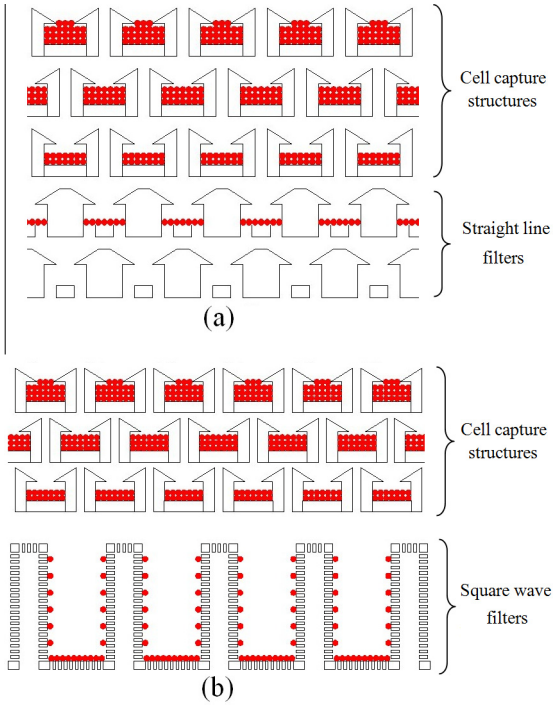

Fig. 1. Design of the chips with the front-end cell capture structures and the different back-end filters: (a) straight line filters and (b) square wave filters. The cell capture structures captured blood cells and the filters prevented blood cells from flowing through them. Some blood cells were captured by the front-end cell capture structures, and the residual cells that were not trapped were flow to the back-end filters. But only plasma can flow through the filters and blood cells cannot pass through them.

in the structures. The second layer of cell capture structures was an array of U-shaped structures, as shown in Fig. 2(a). The straight line filters and square wave filters are shown in Fig. 2(b_1) and (b_2), respectively. Different sizes of cells can be captured easily by changing the height of the pillars during fabrication.

The movements of blood cells and plasma at the cell capture structures and filters were different, as shown in Fig. 3.

Some blood cells were captured by the front-end cell capture structures and the plasma flowed through the gap between the cell capture structures and glass. But the cell capture structures cannot capture all blood cells in the blood. The residual cells that were not trapped by the front-end capture structures were flowed to the back-end filters through the spaces between the different structures in the same row, as shown in Fig. 3(a). But only plasma can flow through the filters and blood cells cannot pass through them when blood reached the back-end filters, as shown in Fig. 3(b_1) and (b_2). The designed of the front-end cell capture structures can also capture cells and prevented cells reaching the filter directly. So the capture structures decrease the number of blood cells at the filters and increase the amount of separated plasma. So the separation microchip integrated with cell capture structures and filters can further enhance the separation efficiency compared with the chips only with filters.

2.2. Chip fabrication

The microfluidic chip was fabricated with biologically compatible PDMS material [30,31] using soft lithography [32], as shown in Fig. 4.

The inverse structures were patterned to constitute the mother mold for the following replication steps. Firstly, a 480 μm thick double-side polished oxidized silicon wafer was chosen as the sub-