Microelectronic Engineering xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Microelectronic Engineering

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

Blood plasma separation microfluidic chip with gradual filtration

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ARTICLE INFO

Article history:
Received 9 October 2013
Received in revised form 2 May 2014
Accepted 19 May 2014
Available online xxxx

Keywords:
Microfluidics
Plasma separation
Gradual filtration
Capture
Filter

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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http://dx.doi.org/10.1016/j.mee.2014.05.032
0167-9317/

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

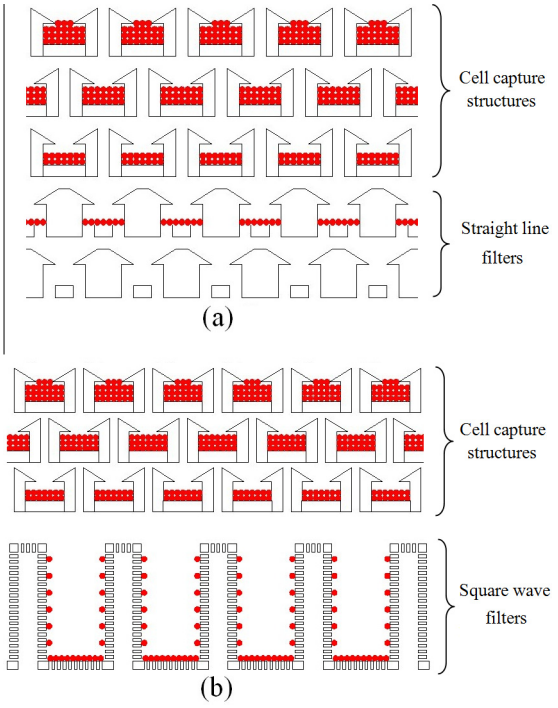

Fig. 1. Design of the chips with the front-end cell capture structures and the different back-end filters: (a) straight line filters and (b) square wave filters. The cell capture structures captured blood cells and the filters prevented blood cells from flowing through them. Some blood cells were captured 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-

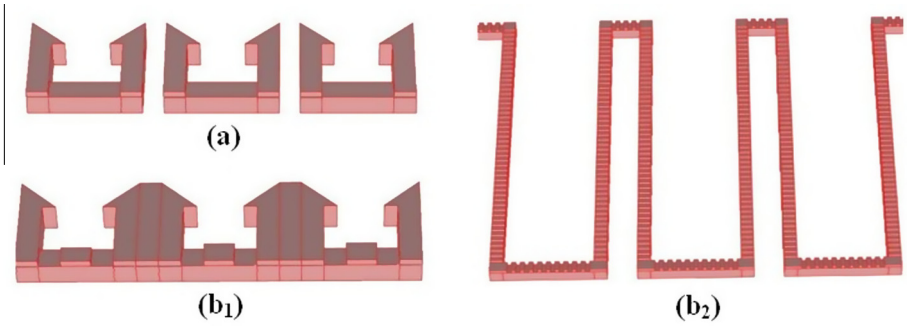

Fig. 2. Design of (a) cell capture structures, (b_1) straight line filters and (b_2) square wave filters. The cell capture structures are designed with two layers. The first layer consisted of pillars to create small gaps between the second layer and glass, and the second layer of cell capture structures was an array of U-shaped structures. Both the straight line and square wave filters are also two layers.

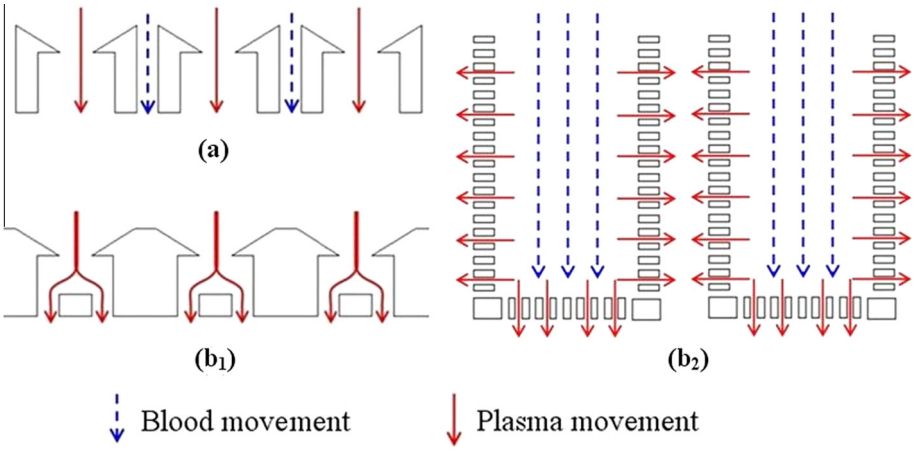

Fig. 3. Movement of blood cells and plasma at (a) cell capture structures, (b_1) straight line filters and (b_2) square wave filters. The residual cells that were not trapped by the front-end capture structures were flowed to the back-end filters through the spaces between the different structures in the same row (blue arrow). However, blood cells cannot pass through the filters and only plasma can flow through them when blood reached the back-end filters (red arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

strate, as shown in Fig. 4(a). A Cr/Cu seed layer was sputtered on the silicon wafer for the subsequent electroforming, as shown in Fig. 4(b). Then silicon wafer was spin coated with positive photoresist about 3 μm in thickness and patterned. Cu about 1–2 μm in thickness was electroformed to fabricate the pillars of the capture structures and filters, as shown in Fig. 4(c). The wafer was spin coated with positive photoresist about 20 μm in thickness and patterned again. Cu about 15 μm in thickness was electroformed to fabricate second player of the structures, as shown in Fig. 4(d). Then, PDMS prepolymer was poured over the mother mold, baked at 70 °C for 3 h, as shown in Fig. 4(e). After PDMS with the structures was released from the mother mold and punched through to function as inlets/outlet for sample injection and extraction, PDMS was bonded with a glass slide after oxygen plasma treatment, as shown in Fig. 4(f).

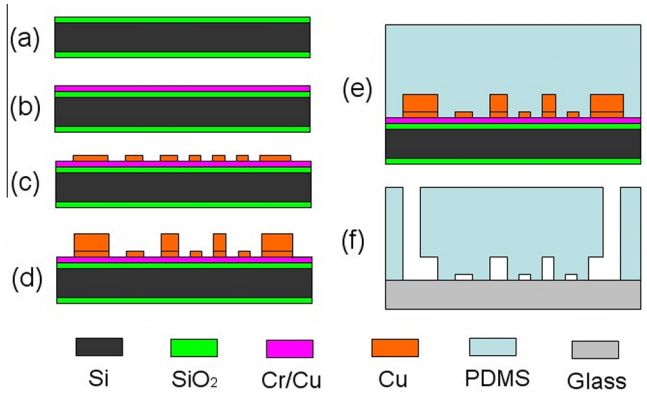

Fig. 4. Fabrication process of the chip. (a) A Si wafer; (b) sputtering a Cr/Cu seed layer; (c) electroforming Cu to fabricate the first layer (pillars); (d) electroforming Cu to fabricate the second layer; (e) transferring the structure to PDMS; (f) punching inlet/outlet and bonding PDMS and a glass slide.

2.3. Preparation of samples and reagents

Mouse blood was used to evaluate the effect of plasma separation from whole blood. The blood was collected into a tube containing heparin sodium. The concentration of blood cells was determined using a blood cell counting plate. The concentration of the blood cells in the original whole blood was about $9 \times 10^6/\mu l$. The mouse blood was diluted with different dilution factors (10, 20, 50) using phosphate buffered saline (PBS). Both the heparin sodium and PBS can further maintain the activity of blood cells.

2.4. Microfluidic system setup

The microfluidic system consisted of a PDMS chip, a syringe, a syringe pump and a microscope, as shown in Fig. 5.

The chip was connected to the syringe by a tubing, and the blood was introduced into the chip at the inlet by the syringe pump. The flow rate of the blood was controlled by the syringe

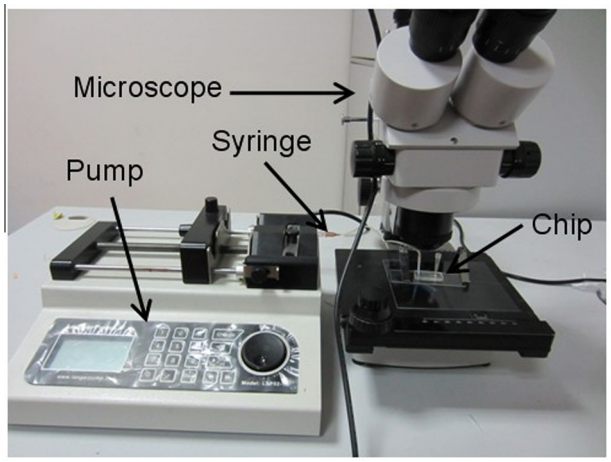

Fig. 5. Experimental setup for plasma separation from blood: a PDMS chip, a syringe, a pump and a microscope.

pump. The microscope was used to monitor and take images of plasma separation. The plasma was collected at the outlet and the concentration of the blood cells in collected plasma was counted by blood cell counting plates. Separation efficiency of plasma of blood cells can be calculated by the blood cells concentrations before and after separation.

3. Results and discussion

3.1. Mold characterization

The plasma separation chip was fabricated successfully using soft lithography. The chip contained cell capture structures and filters. The capture structures were two layers. The first layer (pillar layer) was fabricated about 1, 1.5 and 2 μm in height, respectively. The small gap created by the pillar layer was the key parameter to determine the separation efficiency. The filters also consisted of two layers. The straight line filters were about 22 μm in width. The square wave filters were about 5 μm in width. The cross-sectional areas of the space square wave filters was increased by about 9 times than it in the at the straight line filters. Cells with different sizes can be trapped by changing the height of the pillars during the first electroforming Cu easily.

3.2. Plasma separation

The key factor to evaluate the performance of the chip is separation efficiency (η) can be defined as [33]:

$$\eta = (C_{FC} - C_{PC})/C_{FC}$$

where C_{FC} is the cell concentration in the blood of the inlet and C_{PC} is the cell concentration in the plasma of the outlet.

Red blood cell is a discoid cell about 8 μm in diameter and about 2.5 μm in thickness, and white blood cell is a spherical cell 8–12 μm in diameter. In the chip, the blood entered from the inlet, flowing through the capture structures and filters, and exited at the outlet. Separation of plasma from blood in the chip was based on the size of the blood cells. So blood samples with different dilution were used in separation experiments on the chips with the different gaps in height (1, 1.5 and 2 μm) at 0.2 μl/min. The plasma collected from the outlet was about 5 μl. The results of the straight line filters with different dilution factors and gaps were shown in Fig. 6.

Separation efficiency enhanced with the increase of dilution factor in both straight line filters and square wave filters. The volume of the chip is about 0.18 μl. The separation efficiency

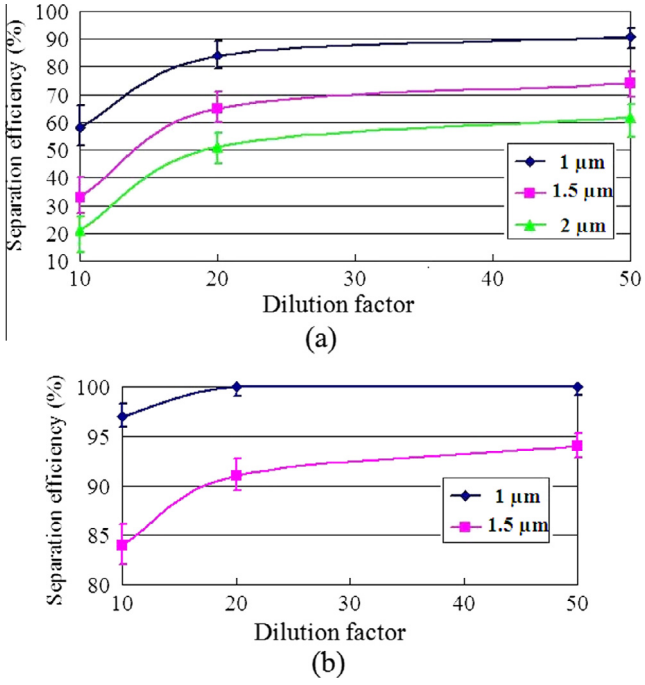

Fig. 6. Separation efficiency related to the gap height and dilution factors: (a) straight line filters, (b) square wave filters. Separation efficiency enhanced with the decrease of the gap height and the increase of dilution factor.

changed drastically when the dilution factor is smaller than 20 and became weakly dependent on dilution factor when the diluted factor is more than 20. This varying trend can also be seen from Ref. [34]. In general, plasma and blood cells constitute with ~60% and 40% volume fractions in blood, respectively [35]. The number of the blood cells trapped in the chip increased and fewer cells have the chance to pass through the filters with the dilution factor decreasing. Therefore, separation efficiency of plasma increased with the increase of dilution factor.

Separation efficiency of plasma enhanced with decreasing height of the gap created by the pillar layer. The gap width was the key parameter to determine the separation efficiency. Red blood cells can pass through the 2 μm gap easily because they are 2.5 μm in thickness. So the separation efficiency of the gap in 2 μm was the lowest in the three gaps (2 μm, 1.5 μm and 1 μm). With more and more blood cells were blocked by the filters, the pressure subjected to the blood cells became larger. Therefore, blood cells could flow through the gaps by changing shape under the fore of pressure. However, blood cells flow-through the gap decreased with decreasing the height of the gap.

Although the separation efficiency was enhanced to 91% under the gap of 1 μm height and dilution factor 50, it did not reach 100% due to large number of red cells. When some blood cells reach the straight line filters, the space of plasma flowing is blocked by the cells. So it becomes more difficult with blood cells increasing at the straight line filters. Optimization is still needed to further enhance the separation efficiency. The chip with square wave filters (1 μm and 1.5 μm height) was also tested using the blood, as shown in Fig. 6(b). The blood cells cannot flow through the gap (blue ring) of the capture structures and they flowed to the filters through the space between the different capture structures (red arrow), as shown in Fig. 7(a). Plasma flowed through the filters more easily at the upper than at the bottom because cells continued to accumulate at the bottom of the filters, as shown in Fig. 7(b).

Most blood cells were blocked by the first row square wave filters. Only very few blood cells escaped and they were blocked by the second row square wave filters. The separation plasma volume

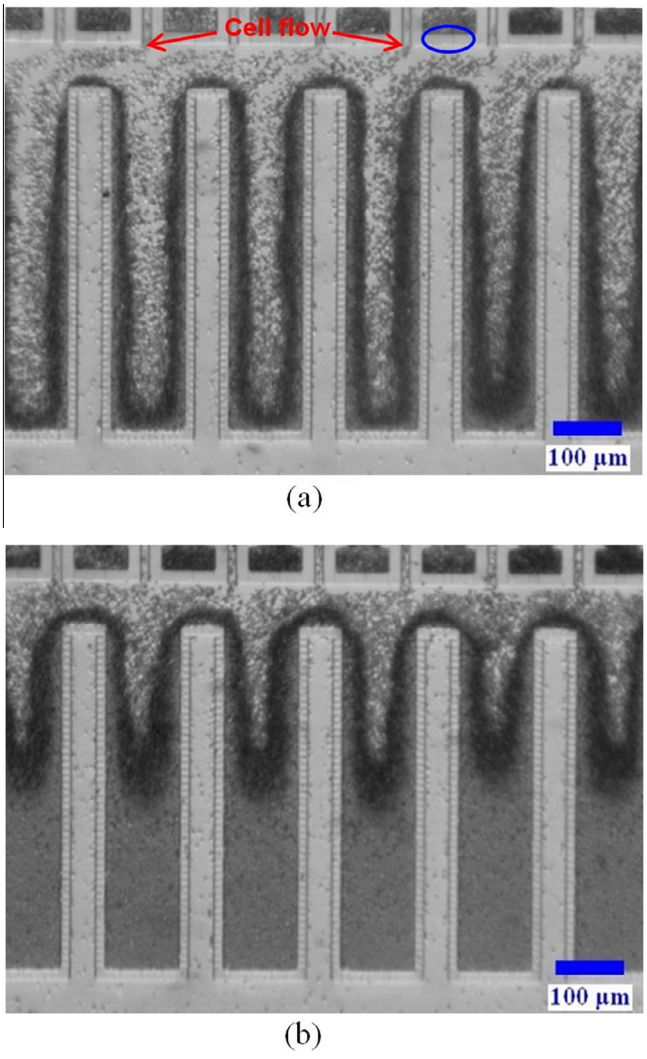

Fig. 7. Blood cells in the chip with the square wave filters after different time: (a) 20 min and (b) 35 min. Blood cells cannot flow through the gap (blue ring) and they flowed to the filters through the space between the different capture structures (red arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

collected from the outlet was increased to 10 μl and the plasma separation efficiency was close to 100% after two rows of filters.

For the square wave filters, the gap is about 5 μm × 1 μm (W × H) and is smaller than the gap 22 μm × 1 μm (W × H) of the straight line filters. Compared with the straight line filters, plasma can flow through the upper space of the square wave filters after the bottom space of the square wave filters is also blocked. It indicated the chance of plasma flowing through the gap was further increased. This resulted in that more plasma passed through the filters. So the separation efficiency of the chip with square wave filters is high than it with straight line filters.

In order to relieve and avoid clogging or jamming using gradual filtration mechanism, some rows of cell capture structures were designed in this chip. So some blood cells were captured before they reached the filters, but plasma can pass through the captures structures. This decreased the number of cells at the filters and increased the volume of plasma collected from the outlet.

Filtration is based on particle size differences makes it easy to separate the liquid phase from a suspension, so that it is a common and nice method for blood separation applications [36,37]. Moorthy et al. demonstrated that separation of blood cells from serum by the porous filter is comparable to traditional centrifuge techniques [38]. Aran et al. described a cross-flow filtration microdevice with 200 nm membrane pore size for the continuous extraction of blood plasma (100% cell-free). In order to enhance the plasma separation efficiency, the outlets of blood cells and plasma were set with high and low pressure [24]. Crowley et al. proposed a transverse-flow microfilter microfluidic device for the isolation of plasma from whole blood and demonstrated separation efficiency of these microfilters is approximately three times higher than it of microporous membranes under similar conditions [25]. Shim et al. designed a silica bead packed microchannel to separate plasma from whole blood [26]. However, the pores between the beads were easily to be blocked. The separation microchip in this paper is integrated cell capture structures and filters. The gradual filtration can enhance the separation efficiency and alleviate efficiently clogging and jamming [28].

4. Conclusions

We developed a microfluidic chip for blood plasma separation with gradual filtration. The chip contained cell capture structures and filters to capture blood cells and separate plasma from the whole blood. Both cell capture structures and filters consisted of two layers. The first layer was pillars layer, which created small gaps between the second filter layer and glass. Plasma can pass through the gap but blood cells were blocked due to larger volume. The chip was fabricated on PDMS (polydimethylsiloxane) using soft lithography. We fabricated two different filters (straight line filters and square wave filters), which were about 22 μm and 5 μm in width, respectively. Whole blood diluted to different factors (10, 20, 50) was used to test the separation efficiency. The separation efficiency of plasma enhanced with increasing dilution factor and decreasing the height of the gap created by the pillar layer. 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. We will further integrate an optical fiber was embedded into the chip through the channel enabling rapid and *in situ* plasma detection on-chip.

Acknowledgments

This work was financially supported by Ministry of Science and Technology of China (No. 2010CB933901) and Science and Technology Innovation fund of SJTU – University of Michigan.

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Please cite this article in press as: J. Chen et al., Microelectron. Eng. (2014), http://dx.doi.org/10.1016/j.mee.2014.05.032