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A lab-on-CD prototype for high-speed blood separation

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Abstract

Blood separation is the first step for subsequent blood tests in clinical diagnosis. Lab-on-a-chip technology provides an automatic, cost-effective and fast solution for a wide variety of blood analyses. The objective of this work is to design a new lab-on-CD microstructure capable of separating blood cells from the whole blood into different reservoirs directly. A CD platform including a microchannel network consisting of a straight main microchannel, a curved microchannel and a branching microchannel has been proposed. The merits of this design are its simple structure, less operating time and high separation efficiency because it utilizes multiple separation mechanisms, for instance, two centrifugal forces and Coriolis force. One centrifugal force is due to the system rotation; the other centrifugal force is due to the curvature of the specifically designed curved channel. In this work, systematical evaluation on the functionality and performance of such a design has been done. Ninety-nine per cent separation efficiency is achieved for diluted blood of 6% hematocrit.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

In practice, clinical diagnostics are often performed on cell-free serum or plasma instead of raw blood to avoid the effects of blood cells and cellular components, which may cause difficulties in analysis standardization. Therefore blood separation is important practically since it is the first step toward the blood test. Traditionally, blood separation is conducted in a laboratory using centrifugation equipment, and then the plasma is extracted out for biochemical analysis. Such a bench-top method is a kind of time and cost consuming, and labor extensive. With the development of lab-on-chip technology, chemical and biological experiments can be performed on all-in-one, automatic lab-on-chip devices, which require less reagent, smaller sample volume and a shorter assay turnaround time [1, 2]. On-chip blood separation of diluted blood or raw blood has been implemented by several groups using different methods, for instance, by Weigl and Yager using diffusion [3], by Moorthy and Beebe using a built-in porous filter [4], by Blattert *et al* utilizing a bent microchannel structure [5], by VanDelinder and Groisman using cross-flow filtration [6] and by Yang *et al* using the Zweifach–Fung effect [7].

Lab-on-CD is the main branch of lab-on-chip technology, which employs the inherent centrifugal force as the driving mechanism instead of traditional mechanical or electrokinetic pumping. With this simple method of propulsion, lab-on-CD could easily be adapted to numerous applications in the fields of chemistry and biotechnology [8–13]. Madou *et al* presented a polymer-based CD platform that realized functions of capillary valve, capillary metering, mixing and flow sequencing [8]. Riegger *et al* reported a novel method for determining blood hematocrit by visual inspection [9]. Kim *et al* realized a purely mechanical method of cell lysis on a CD platform, which is the first step toward nucleic acid analysis [10]. Berner *et al* reported an innovative flow switch on a CD platform, which is controlled by rotation frequency through the Coriolis force [11]. Park *et al* presented a phase change-based microvalve controlled by laser illumination [12]. Cho *et al* demonstrated DNA extraction from the whole blood on their

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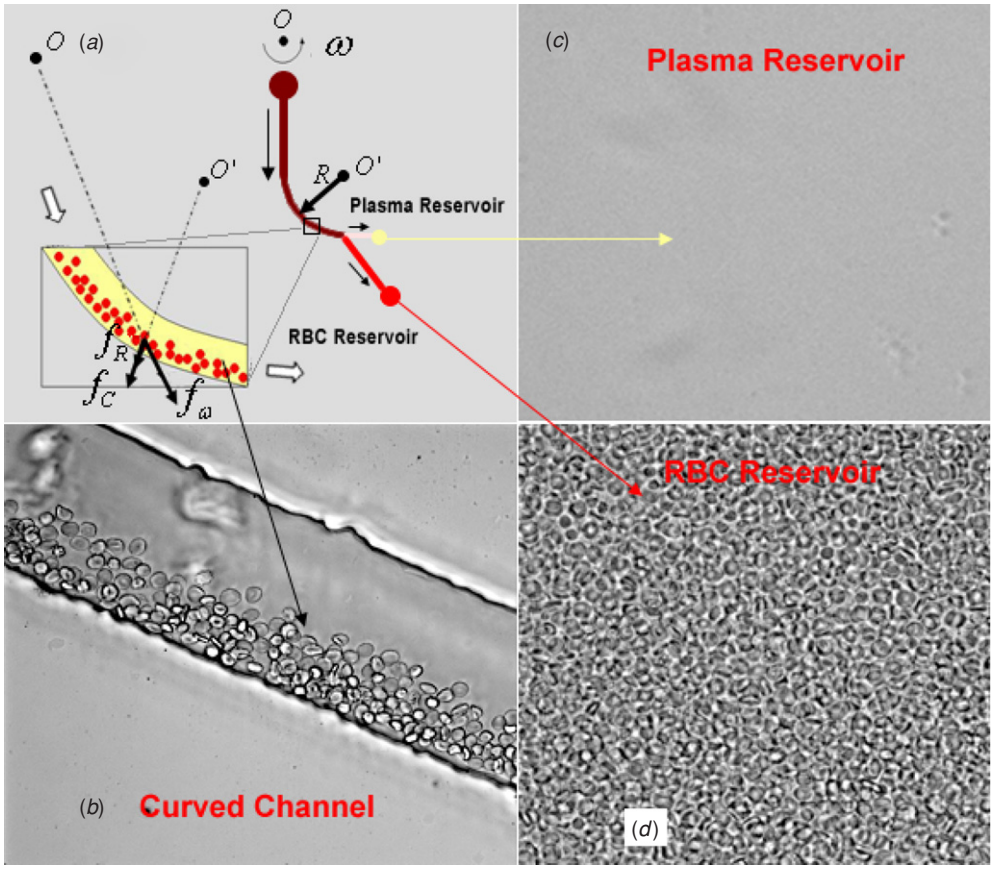

Figure 1. Separation mechanisms and separation results. (*a*) Schematic diagram of the microchannel network which is not drawn to scale, (*b*) flowing blood is separated in the curved microchannel, (*c*) nearly pure plasma flows into the plasma reservoir and (*d*) RBCs are accumulated in the RBC reservoir.

novel lab-on-CD devices [13]. In addition to pumping, the inherent centrifugal force can also be used for separation based on the variance of mass density among different components or phases. For instance, populations of cells that differ from each other by size and density upon exposure to the centrifugal force field can then be differentiated, manipulated and eventually partitioned from the whole sample. Using this centrifugation-based method for phase separation, Haeberle and his co-workers realized on-disk raw blood separation and plasma extraction through a decanting structure where the overflow of plasma drained off [14].

The objective of this work is to design a simple microchannel network on lab-on-CD for blood separation of tiny droplets of blood (typically ~1 μL). Differing from the traditional sedimentation method by which blood cells and plasma are separated into different phase layers but in one tube/reservoir, the present method makes blood cells and plasma flow separately into two microchannel branches simultaneously. Therefore subsequent blood tests can be conducted in the microchannel branch of plasma immediately after separation regardless of the amount of the blood sample. This merit could be very useful for fast, qualitative blood analysis for the purpose of prescreening pathogens. In order to enhance the quality of blood separation, four separation mechanisms are integrated into the microchannel network: the centrifugal force due to the CD rotating; the Coriolis force due to the system rotation; the centrifugal force due to the curved channel and the Zweifach–Fung effect. Here, the Zweifach–Fung phenomenon plays a minor role since it only affects blood cells at the bifurcation point of the forking microchannel. In the following sections, separation mechanisms, microfabrication, experimental design and results will be introduced.

2. Separation mechanisms

The microchannel network is schematically illustrated in figure 1(*a*). Three body forces (the centrifugal force f_ω due to the system rotation, the Coriolis force f_C as a function of the rotation angular frequency and velocity of liquid, and the centrifugal force f_R due to the curvature of the curved microchannel) are present in this rotating microchannel network. Their mathematical expressions are

$$f_\omega = r\rho\omega^2, \tag{1}$$

$$f_C = 2\rho\omega u, \tag{2}$$

$$f_R = \rho u^2/R, \tag{3}$$

where r is the distance from the rotation center to the point of interest, ρ is the density of the blood component to study, u is the velocity of the flow, ω is the rotation angular frequency and R is the radius of the curvature of the curved microchannel. In

the main channel between the inlet reservoir and the curved microchannel, the Coriolis force f_C is the only separating force. In the curved microchannel, the centrifugal force f_ω, the Coriolis force f_C and the second centrifugal force f_R all together contribute, at different degrees, to the separating process. Given the structure of the microchannel network, approximately, $f_\omega \propto \omega^2$, $f_C \propto \omega^3$, $f_R \propto \omega^4$ due to the fact of $u \propto \omega^2$. The following approximate analysis of these two major forces can be

$$\frac{f_C}{f_R} \cong \frac{2\rho\omega u}{\rho u^2/R} = \frac{2\omega R}{u}. \tag{4}$$

The centrifugal force f_ω contributes extensively to the velocity u that relates to both of f_C and f_R. Blood separation mainly occurs in the curved microchannel, where the centrifugal force f_ω, the Coriolis force f_C and the second centrifugal force f_R all contribute to blood separation. This has been demonstrated in our computational fluid dynamics (CFD) simulation shown below. We found that these three forces are at the same order of magnitude in our experimental situations. Therefore all the three forces should be counted. As shown in equation (4), magnitude of each force also depends on geometrical parameters.

The working principle of the separation mechanisms is essentially based on the density differences among different blood components [15, 16]. The three forces contribute at different degrees, but work together to separate RBCs (red blood cells) from plasma before they enter the bifurcation microchannels. With a higher density than plasma, RBCs deviate from the center of microchannel and move toward the outer wall of the curved microchannel under the two centrifugal forces and the Coriolis force. Consequently, blood cells pile up and form a packed cell layer. As shown in figure 1(*b*), the whole blood is clearly separated into a blood cell layer and a plasma layer. Subsequently, the cells in the blood cell layer will flow forward along the outside wall and enter the cell channel. On the other hand, the plasma will flow into another branch channel extended along the inner wall of the curved channel. Figure 1(*c*) shows the plasma reservoir full of pure plasma and figure 1(*d*) shows the RBC reservoir full of packed red blood cells.

In addition to using the two centrifugal forces and the Coriolis force as settling sources, the Zweifach–Fung effect happens to be an additional separation mechanism. The Zweifach–Fung effect is characterized by the phenomenon observed in microcirculation that a blood cell at a bifurcation point of a branching blood vessel tends to enter the wider vessel branch of a larger flow rate [7, 17, 18]. Detailed analysis shows that the blood cell is subject to a net pressure force and a net shear force, both of which point to the wider daughter branch vessel [18]. In particular for the conjunction of the microchannel structure of this study, the branch channel where blood cells to enter is wider than that of the channel branch where plasma to flow. Since blood separation has been largely performed in the curved microchannel, the Zweifach–Fung effect is not a dominant mechanism, but can further enhance the efficiency of blood separation.

Besides the abovementioned geometrical parameters, many other factors may also affect the performance of blood separation such as hematocrit, density, temperature and viscosity of a blood sample, and smoothness and hydrophilicity of a channel surface.

3. Experimental design and method

The lab-on-CD device (figure 1(*a*)) was fabricated by soft lithography [19]. In this work, the pattern (figure 1(*a*)) was drawn using AutoCAD software. The photomask was printed onto a clear transparency film by a high-resolution photoplotter (CAD/Art Services Inc). A traditional photolithography technique was used to define a microstructured master on SU-8. Polydimethylsiloxane (PDMS) was then molded on this master. Grooves were molded into the PDMS structural material. A 1 mm diameter through a hole was drilled using a blunt needle at the location of the inlet reservoir of the PDMS layer in order to perfuse the blood. A 0.5 mm diameter through a hole was introduced to each outlet reservoir in order to balance the pressure. The microchannel network was formed after the featured structural material (PDMS here) was bonded to a glass coverslip slide. Bonding was performed at 80 °C temperature, and a small pressure (∼20 kPa) was applied to enhance the adhesion. The geometries of the microchannel network are: the inlet reservoir is of 2.5 mm in diameter and 40 μm in depth; the two outlet reservoirs are of 1.5 mm in diameter and 40 μm in depth; the straight part of the main channel is 8 mm and the radius of the curved channel R is 9 mm; the main channel cross section is 40 μm × 160 μm; the dimensions of the two branch channels are 40 μm × 100 μm for the RBC channel and 40 μm × 60 μm for the plasma channel in order to take advantage of the Zweifach–Fung effect.

A 0.5 μL blood sample was introduced into the inlet reservoir by using a pipetter, and then the lab-on-CD was placed on a spin processor (Laurell Technologies Corporation, PA) for the subsequent separation experiment. The spin processor can generate an angular frequency up to 10 000 rpm. The so-called burst frequency [20] in this study was observed to be about 1500 rpm. All experiments in this study were conducted at an angular frequency of 1550 rpm. After the angular frequency reached 1550 rpm, we stopped the spin processor in about 1 s. Based on our observation, blood separation could be finished within 1 s after the angular frequency reaches 1550 rpm. Separation at larger angular velocities (e.g. 1650 rpm) has been tested. No noticeable increase in separation efficiency was observed. The spin processor can only reach a higher angular frequency by step increments. Blood separation actually has been finished shortly after the angular frequency reaches the 'burst frequency' [20], before the angular frequency reaches a higher value. Thus no remarkable improvement was observed for a higher angular frequency than the burst frequency.

The whole human blood was drawn from a healthy donor and pretreated with the anticoagulant EDTA. Before processing, the blood was diluted by PBS (phosphate buffer saline) to a designated hematocrit level.

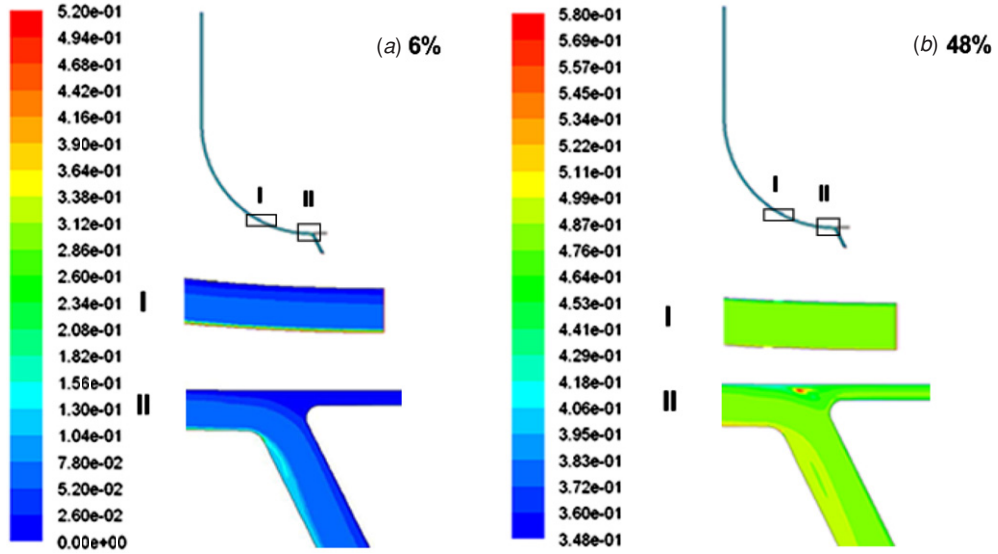

Figure 2. Simulation of blood separation in the proposed microchannel network for blood samples of (*a*) 6% hematocrit and (*b*) 48% hematocrit, respectively, at an angular frequency of 1550 rpm. The color bars represent the scale of RBC concentration.

4. CFD simulations

Due to the two-phase nature of blood and complexity of the geometry, CFD simulation is essential at the primary stage of the system design. Blood separation in the proposed microchannel network (figure 1(*a*)) was simulated in a rotating frame using the Eulerian multiphase model of the commercial software Fluent 6.3.26. The Eulerian multiphase model is a built-in standard module of Fluent specially designed for interpenetrating multiphase fluids. Details of the use of this model can be found in Fluent's user manual. Hydrostatic pressure of the inlet reservoir due to rotation has been taken into account. Transient 2D simulations were carried out with fixed-pressure boundary conditions at the inlet and outlet and no-slip boundary condition at the channel walls. The blood flow is assumed to consist of two phases only, plasma and red blood cells (RBCs). Physical properties of plasma and RBC are adopted from [21–23]. The phase of RBC is treated as granular flow with particles of 8 μm in diameter. Six per cent hematocrit and 48% hematocrit blood samples are simulated.

Figure 2 shows profiles of RBC concentration for blood separation at an angular frequency of 1550 rpm after the two-phase flow reaches a steady-state condition. Simulation results show that RBCs are concentrated in a region near the outside channel wall. Six per cent hematocrit blood can achieve a better separation efficiency than that of 48% hematocrit blood. The simulation result of 6% hematocrit blood in the bifurcation area clearly shows that pure plasma flows into the plasma branch microchannel, and blood cells flow into the other branch microchannel. These simulation results are consistent with our experimental observations, which demonstrated that the present design does work well.

In addition, we particularly analyzed the ratio among the three forces (the centrifugal force f_ω, the Coriolis force f_C and the second centrifugal force f_R) in the middle of the curved channel for the case shown in figure 2(*a*), which is $f_\omega{:}f_C{:}f_R = 823{:}399.3{:}168.1$. The ratio shows that all of these three are at the same order of magnitude in our experimental situations; therefore, they have comparable contribution to the blood separation.

5. Result and discussion

The separation efficiency η is a key factor to evaluate the performance of blood separation, which is defined as

$$\eta = (C_{FC} - C_{PC})/C_{FC}, \tag{5}$$

where C_{FC} is the cell concentration in the inlet reservoir and C_{PC} is the cell concentration in the plasma reservoir. Cell concentration was determined by cell counting.

5.1. Effect of blood cell concentration (hematocrit) and temperature on the separation efficiency

As shown in figure 3, the separation efficiency for blood of different hematocrits at different temperatures was determined. Since multiple separation mechanisms are integrated into it, this device results in a higher separation efficiency compared with the 90% separation efficiency for blood of 5% hematocrit reported by the pioneering work [5]. In this study, 99% separation efficiency has been achieved for blood of 6% hematocrit. Such a high separation efficiency is similar to the >99% separation efficiency obtained by other lab-on-chip blood separation methods on either diluted blood [7] or raw blood [14]. As hematocrit increases, the separation efficiency decreases. The separation efficiency of blood of 12% hematocrit is 90%, and the separation efficiency of blood of 24% hematocrit is 78%. For the raw blood used in this study of 48% hematocrit, the separation efficiency reduces to 65%. Since the blood of a lower hematocrit has a lower viscosity, blood flows faster, and the Coriolis force f_C and the second centrifugal force f_R are larger. In addition, interactions among blood cells in the blood of lower hematocrit become weaker, which also largely enhances the separation performance.

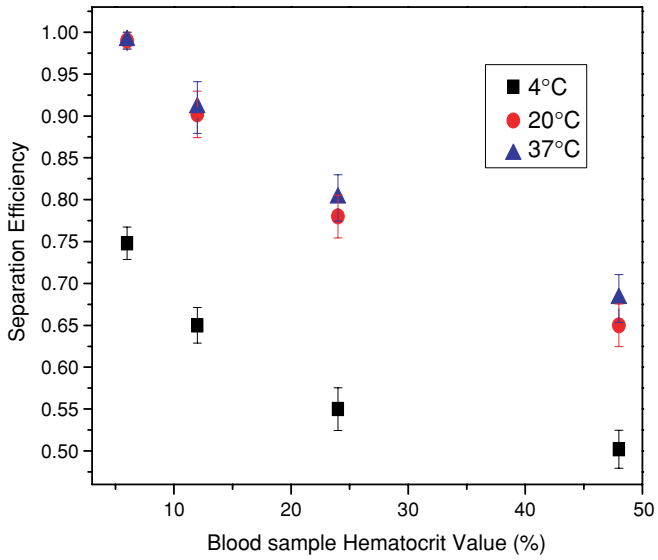

Figure 3. Blood separation efficiency for blood of different hematocrits at different temperatures. The error bars represent standard deviations of at least five measurements.

Temperature is also a key factor because it affects blood viscosity, and hence blood separation efficiency as well. In general, the viscosity of blood increases as temperature decreases. In order to study the temperature effect on separation efficiency, experiments are implemented for blood samples of different hematocrits at different temperatures, 4 °C, 20 °C and 37 °C, which represent the real temperatures of blood samples for three common cases. For instance, refrigerator-stored blood samples are normally at 4 °C; room temperature-stored blood samples are at 20 °C and freshly-drawn blood samples are at the body temperature, 37 °C. In practice, blood samples were heated up to 37 °C in a water bath. Experimental results are shown in figure 3. As expected, separation efficiency increases as temperature increases due to the decrease of viscosity. For a given hematocrit, the viscosity variation between a 4 °C blood sample and a 20 °C blood sample is larger than the viscosity variation between a 20 °C blood sample and a 37 °C blood sample [24]. Accordingly, separation efficiency reduction from a 20 °C blood sample to a 4 °C blood sample is more than that from a 37 °C blood sample to a 20 °C blood sample.

5.2. Plasma recovery rate

The plasma recovery rate is another important factor for blood separation. A higher plasma recovery rate means less blood sample is needed for blood tests. The plasma recovery rate also depends on the hematocrit of blood samples. The plasma recovery rate was measured only for the blood samples of 6% and 12% hematocrit because high purity plasma was not obtained for blood samples of higher hematocrit in this study so far. The plasma recovery rate is defined here as the ratio between the volume of liquid phase in the plasma reservoir and the volume of liquid phase of the total blood sample introduced. The former was determined as follows: collecting the liquid phase in the plasma reservoir of tens of experiments, measuring the volume of the collected liquid phase and taking the average as plasma recovery for this device. The latter was determined by excluding the volume occupied by RBCs according to the known hematocrit of the blood sample. The plasma recovery rates are 21.6% ± 2.2% for 6% hematocrit blood and 24% ± 2.6% for 12% hematocrit blood at 20 °C. The separation efficiency monotonically increases as the hematocrit decreases, but the plasma recovery rate does not. It is because the liquid phase of the 6% hematocrit blood sample flowing into the RBC reservoir is relatively more than that of the 12% hematocrit blood sample. Our recovery rate is comparable to other blood separation methods, for instance, 20% for 5% hematocrit blood and 25% for 7% hematocrit blood obtained by the plasma skimming method [7].

5.3. Discussion

The present method has so far achieved 99% separation efficiency for 6% hematocrit blood only. It is worthmentioned that diluted blood is also suitable for blood testing. For example, blood, plasma or serum are often diluted to certain ratios in order to produce color reactions in colorimetric methods. Although there is no dilution step involved in practice before whole blood specimens are placed into blood analyzers for most of blood testing, some of blood testing is performed on diluted plasma or serum if the concentration of the substance to be detected is high, for example, blood testing of rheumatoid factors. In this study, blood was diluted outside the disk before separation. The dilution step can also be performed on the disk if one adds a diluent reservoir on the disk and designs a dilution step in the flow sequencing [25]. The dilution ratio can also be precisely determined during the on-disk testing if other lab-on-CD techniques such as metering [25] and measuring blood hematocrit [9] are adopted. On the other hand, there are increasing applications of blood testing for prescreening purposes. These measurements are qualitative analyses where diluted blood is well suitable and the exact dilution ratio is not required.

The innovative overflow method of lab-on-CD proposed by Haeberle and his co-workers [14] enables metering and the separation of 5 μL whole blood in less than 20 s at 40 Hz rotation angular frequency. Interesting facts of the present method are the short operating time and the small amount of blood. For example, a 0.5 μL blood sample works in the present design. In this design, flowing blood is separated in the curved microchannel, and plasma directly flows into the plasma reservoir. Blood is separated within 1–2 s. There is no specific requirement for the amount of blood. Therefore this method provides flexibility for drawing the blood sample, and for qualitative blood analysis, in particular, prescreening of pathogens.

6. Conclusions

In this simple but effective lab-on-CD design, two centrifugal forces and one Coriolis force work together to separate blood cells from plasma. The separation efficiency monotonically increases as the hematocrit decreases. Blood temperature

also significantly affects the separation efficiency. This device demonstrated that RBC separation efficiency up to 99% and about 22% plasma recovery rate have been constantly achieved for the diluted blood of 6% hematocrit. This simple microchannel structure can easily be integrated into various fully functional lab-on-chip devices for blood diagnostics.

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