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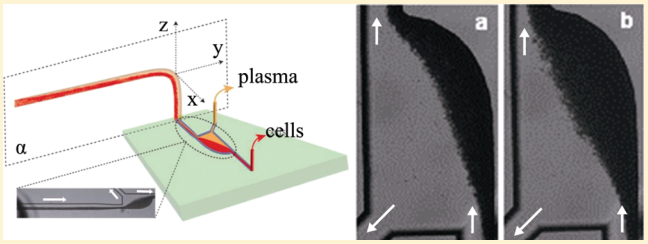
Gravitational Sedimentation Induced Blood Delamination for Continuous Plasma Separation on a Microfluidics Chip

Xian-Bo Zhang, Zeng-Qiang Wu, Kang Wang, Jie Zhu, Jing-Juan Xu,* Xing-Hua Xia,* and Hong-Yuan Chen

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China

Supporting Information

ABSTRACT: Continuous plasma separation will be greatly helpful for dynamic metabolite monitoring in kinetics research and drug development. In this work, we proposed a continuous on-chip plasma separation method based on the natural aggregating and sedimentation behavior of red blood cells at low shear rate. In this approach, a glass capillary was first used to realize quick and obvious delamination of blood cells from plasma. A novel "dual-elbow" connector was designed to change the direction of delamination. The blood was finally separated by laminar flow and bifurcation on the microchip. Results demonstrated that the present device can efficiently and stably separate plasma from blood in a continuous means, e.g., in a 4 h separation we did not observe clogging or a trend of clogging. In addition, the present approach can avoid the damage to cells which usually occurs in separation with high shear rate in a microchannel and possible contaminants to plasma. The proposed microchip device is robust, simple, and inexpensive for long time plasma separation with high plasma recovery and less sample consumption. The present work provides an effective tool for metabolite monitoring in pharmacokinetics research and drug development.



Blood composed of blood cells suspended in blood plasma performs essential but the most important functions within the body. Blood tests can provide valuable information for clinical diagnosis and drug development. Most of the biochemical blood tests are performed using blood plasma or serum, since blood cells and their constituent substances can falsify the results of measurements. Therefore, separation of plasma from whole blood (also named as plasmapheresis) is the prior step for further biochemical analysis.[1] Routine method of centrifugal separation for plasmapheresis commonly needs milliliters of blood and a labor-intensive handling process, which makes it difficult to conduct self-help and low-cost inspection of drug and other metabolites in blood. The sample pretreatment step becomes a bottleneck of the assay process. One classic example is blood glucose monitoring (BGM) in diabetes therapy.[2] In practical application, plasma BGM and whole blood BGM are usually used. The former is often adopted in hospital because of better accuracy. This method based on plasma separation by centrifugation requires more blood, safe operation, and bulk equipment and is not suitable for regular tests for patients. In order to meet the need of point of care (POC), portable BGM devices are recommended for self-monitoring. However, whole blood BGM level is obtained by these devices. The lysis of blood cells, anemia, and plethora leads to inaccuracy of blood glucose level outside of the cell. Recently, many researchers found that self-monitoring of blood glucose several times a day may lose some important information on the alteration of the blood glucose level especially at night and dawn, which may affect the therapy.[3] This issue may be solved by using a continuous glucose monitoring system (CGMS) which has been supplied by many corporations. In this case, the glucose level of extracellular fluid of subcutaneous tissue can be obtained every 5 min. Moreover, continuous BGM is more acceptable to the patient because of less pain of repeating puncturing.[4] The continuous dynamic monitoring mode is also recommended for the monitoring of other analytes in blood[5] and a reasonable choice for the study of pharmacology, toxicology, and pharmacokinetics in new drug development. Therefore, continuous plasma separation could be an important technique for the continuous dynamic monitoring mode.

As a promising platform for developing the next generation of blood analysis technology, the microchip has received extensive attention in the recent years.[1,6] The basic idea of using the microchip platform is to shrink conventional laboratory analytical systems onto a centimeter sized chip. More importantly, microchip platform potentially support online continuous analytical functions. Currently, there are two main research branches in on-chip assay. One is developed for a disposable quick test based on a single sample.[7] Most of the previous work aims to instantaneously detect protein and DNA based on drops of blood. Commonly, the monitoring of

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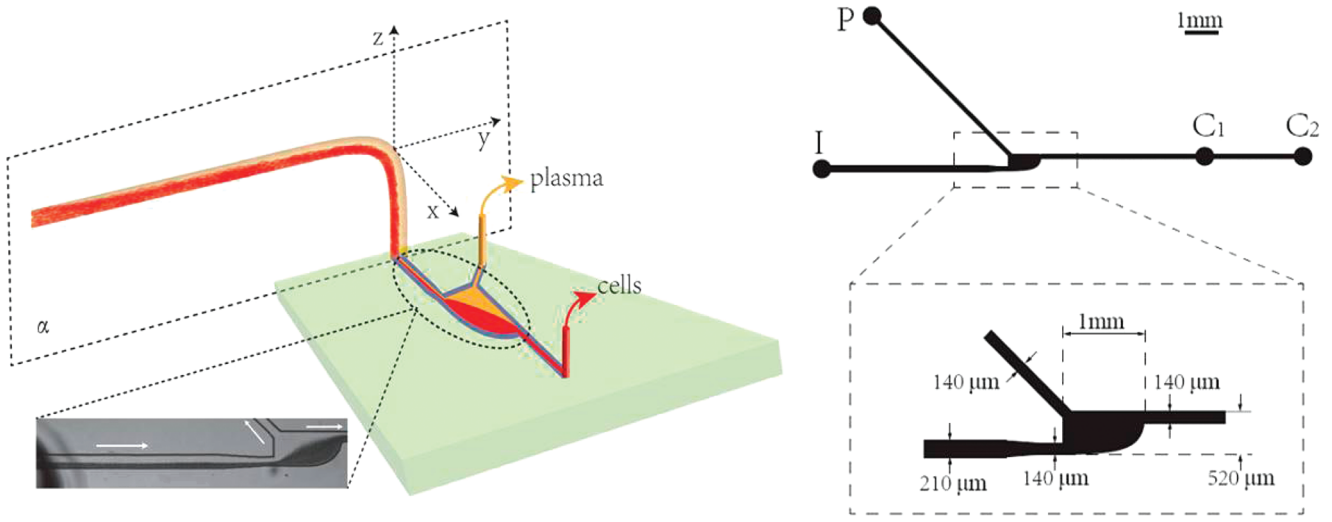

Figure 1. Principle of the plasmapheresis device (not to scale for schematic section). The zoomed-in parts are a photo image (left) and structure of the separation chamber (right).

many analytes also adopts this type of assay because of long sampling intervening time. The other is expected to be able to continuously monitor the analyte concentration for long time similar to CGMS. Up until now, less attention has been paid to this issue.[8] Zahn and his co-workers used a continuous plasma separation technique for inflammatory responses monitoring in cardiopulmonary bypass (CPB).[9] They first reported plasma separation on a PDMS chip based on the Zweifach-Fung effect.[9a] A 15% plasma sample was obtained from defibrinated sheep blood injected at 10 μL/h. Later they used a microfluidic chip integrated with a membrane filter to continuously separate plasma with a blood consumption of 80 μL/min for inflammatory response monitoring in CPB.[9b] For continuous plasma separation, clogging of the blood cells on chip is the most common problem unsolved. For practical application of continuous plasma separation, the stability of long-term continuous separation, sample consumption, plasma recovery, plasma quality, and manipulation facility should be well considered.

Recently, on-chip plasmapheresis has been realized using filtration units including membranes, microdams, and micropillars.[10] These filtration-based plasma separation chips are especially suitable for immediate and disposable samples. However, these devices cannot provide continuous and dynamic plasma separation. Although some improvements including cross-flow filtration,[9b,11] great sample dilution,[12] and pulsed sample injection methods have been proposed,[13] clogging phenomenon is unavoidable over time. It should be noted that the decrease of the harvest velocity of plasma due to clogging would lead to inaccuracy of downstream on-chip manipulations such as mixing, dilution, and metering. Cell lysis, the other critical problem which is often suffered,[10b] leads to contamination from cellular components. An alternative strategy to fulfill the on-chip plasmapheresis is based on blood delamination. In this case, passive and active methods are usually adopted to achieve blood delamination. In the former case, plasmapheresis is driven by pump or capillary force without any external forces, and thus blood cells are separated hydrodynamically using various hydrodynamic effects including the plasma skimming effect,[7a,b,14] Zweifach-Fung effect,[9a,14e,15] and pinch flow effect.[14e] Narrow channels and fast flow rates are commonly needed to generate asymmetrical velocity profile and shear gradient for enhancing the hydrodynamic effects.[14b,c] As a result, a high percentage of cell damage, the problem of clogging, and lower recovery of plasma harvest were often found in their experiments. Another hydrodynamic effect based on cell−wall interaction is an inertial continuous separation which is often used for highly diluted blood fractionation.[16] The active delamination is driven by extra fields including centrifugation,[17] acoustophoresis,[18] magnetophoresis,[19] and dielectrophoresis.[20] Of these methods, on-chip centrifugation can achieve the highest separation efficiency, but it cannot perform separation continuously. Laurell et al. separated plasma from human blood on a chip integrated with an acoustic standing wave device. The wave nodes concentrate blood cells in the middle of the microchannel and form depletion of their concentration in the periphery of the channel.[18] However, long time performance of this acoustophoresis generates heat and may influence blood and other analytical units integrated on chips.[21] In addition, materials for the device must be chosen carefully to transmit the acoustic power to the fluid properly.[18]

Gravitation is a natural field because it exists everywhere in common circumstances. Making use of gravity to realize plasmapheresis will greatly simplify the chip system and make the on-chip blood test a step closer to real application. Blood is a complex non-Newtonian fluid, exhibiting special properties different from conventional fluids. A notable property of blood is that the erythrocytes aggregate at low shear rates, which accelerates the gravitational sedimentation of blood and thus forms a vertical delamination.[22] The aggregation-accelerated gravitational sedimentation in a small-sized glass capillary can cause an obvious blood delamination within a few minutes. For example, plasma separation has been obtained by combining cross-flow filtration and a gravitational sedimentation effect on an interchannel microstructure at a slow injection flow rate of 1 μL/min.[23] In another report, a self-powered microfluidics blood analysis system based on sedimentation was developed and used to realize instantaneous detection of protein in plasma from a single drop of blood.[7c] Although the chip-based plasmapheresis systems making use of blood delamination effect are suitable for disposable application purposes, their

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long time performance may cause clogging of the chip with narrow channels.

Herein, we report a continuous on-chip plasma separation method based on gravitational sedimentation induced blood delamination. An illustration of the separation device is shown in Figure 1. In this approach, delamination of the blood within a glass capillary before entering the chip is directly used as the separation step. Then, the delaminated blood flows through a "dual-elbow" structure by which the vertical blood delamination is converted to a left-to-right delamination structure. The blood delamination performed in the capillary and a relatively wide microchannel on the chip not only provides a decent plasma output flow but also greatly reduces the cell damage caused by high shear stress in a narrow microchannel. In the present approach, possible contamination of the plasma caused by cell damage can be effectively avoided due to the laminar flow property in microchannels and the left-to-right delamination flow structure remains very well. In addition, a broader chamber with a side bifurcation is designed for splitting flow of the delamination, which makes easier collection of plasma with higher efficiency. The quality of plasma was validated by quantitative analysis of free hemoglobin in separated plasma. The potential of downstream application was validated by the detection of theophylline in plasma separated from spiked blood on the chip. The present device can perform blood separation continuously and is promising to be an effective tool for metabolite monitoring in clinical therapy, pharmacokinetics research, and drug development, which requires long time plasma separation.

EXPERIMENTAL SECTION

Materials and Reagents. Refer to the Supporting Information.

Design and Fabrication of Device. Simple photolithography and wet chemical etching technique were used to fabricate microfluidics glass chips (refer to the Supporting Information). As shown in Figure 1, the microchannel structure for plasmaphoresis has four connectors: connector I is the inlet for blood sample; connector P is the outlet for collecting the extracted plasma, and the other two outlets (C_1, C_2) are for collecting the concentrated cells. A plasma collection channel of 6 mm in length is placed on the corner of the separation chamber with an angle of 45°. The inlet channel before the separation chamber is 5.7 mm long. All these channels and the separation chamber have a depth of 50 μm. In order to adjust the back pressure, either of the outlets C_1 and/or C_2 for collecting the concentrated cells can be easily closed by an end-sealed capillary tube. An L-shaped glass capillary was manufactured by heating a 10 cm straight glass tube with an alcohol burner with one end of ~2 cm and another end of ~8 cm long. The shorter end was inserted into the inlet hole on the PDMS slice of the chip with a required steering angle. The L-shaped glass capillary and the microchannel before the separation chamber hence form a "dual-elbow" structure. A silicone tube (0.7 mm i.d., 8 cm long) was used for connecting the syringe pump and the glass capillary. A homemade clamp was used to control the flow rate of the plasma collection. Another capillary tube was inserted into the outlet hole to collect the concentrated cells. The assembled microdevices were flushed with 2% (w/v) aqueous solution of bovine serum albumin (BSA) for 10 min to passivate the channel walls for preventing cell adhesion. The feeding flow rate of solutions was controlled through a syringe pump (Lange Scientific, Baoding, China). A photograph of the experimental setup is shown in Graph S1 in the Supporting Information.

Sample Preparation. Whole human blood was collected by venipuncture from healthy consenting male volunteers into blood collection bag containing sodium citrate anticoagulant (donated by Nanjing Red Cross Blood Center, Jiangsu, China). The volume ratio of blood to anticoagulant was 5:1. The blood samples were diluted by PBS. Hematocrit (HCT) was measured by the centrifugal method according to the standard clinical procedure. Blood samples were used within 3 days of collection.

Imaging and Image Analysis. A DMIRE2 inverted fluorescence microscope (Leica, Germany) equipped with a DP71 CCD (Olympus, Japan) was used for microimaging (bright-field) and movie recording. Image-Pro Plus (IPP) software was employed for image analysis.

Analytical Procedures. The blood sample in the syringe pump was stirred magnetically every 2 min to avoid aggregation and settling of erythrocytes to mimic running blood. The nonseparation mode means that the plasma layer is not collected from the separation chamber and the blood sample flowing out from the cell outlet was directly collected in the experiments during studying the effect of individual separation parameters. The delamination of plasma and cell components was observed on a microscope.

To evaluate the influence of different steering angles on delamination efficiency, blood samples were injected into the device with different steering angles by a syringe pump. Blood samples of three different concentrations were used to study the effect of sample dilution on plasma separation efficiency. The effect of feed flow rate on plasma separation was also studied with a wide flow rate ranging from 10 to 100 μL/min. Under optimized conditions, the blood sample (8% HCT) was separated and collected. Different feed flow rates and plasma collection rates were monitored and their effects on the overall plasmaphoresis were compared. After the on-chip blood separation, the number of blood cells remaining in the plasma was counted using a Neubauer hemocytometer under the microscope. The concentration of free hemoglobin in plasma samples was also measured. For comparison, a series of control experiments were carried out with plasma samples obtained using centrifugation. A blood sample of the same concentration was centrifuged at 2500 rpm (relatively acceleration 1886g) for 10 min to get the supernatant for the free hemoglobin test. The free hemoglobin of collected plasma supernatant was detected by a free hemoglobin test kit on a UV-3600 spectrophotometer (Shimadzu, Kyoto, Japan). Theophylline spiked blood was separated and detected by dual ultraviolet wavelength spectrophotometry (refer to the Supporting Information).

RESULTS AND DISCUSSION

Transport Properties of Blood in Glass Capillary and Microchannel. In whole blood, about 95% of the blood cells are erythrocytes. Erythrocyte has a biconcave disk shape with a greater thickness at its outer ring (diameter, 7.7 ± 0.7 μm; central and peripheral thicknesses, 1.4 ± 0.5 μm and 2.8 ± 0.5 μm).[24] The mass density of a red cell is approximately 1.13 g/mL, higher than that of plasma (about 1.03 g/mL). This slight difference in mass density leads to a gravitational settling of individual red cells in plasma. However, because the density difference is small, the sedimentation rate of an individual red cell in plasma is only about 0.13 μm/min.[25] At shear rates below 50 s^{-1}, red cells can reversibly clump which is known as

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rouleaus formation. When the shear rate increases above 50 s^{-1}, the shear field will disrupt cell aggregation and cause red cell deformation. Red cell aggregation greatly increases the settling rate since the settling rate of particles depends on the square of the particle size. For a fluid at stasis or a low flow rate, the terminal velocity is given by eq 1, which shows that the terminal velocity increases with the square of the particle size.[26]

$$U = \frac{2}{9}\frac{a^2}{\nu}\left(\frac{\rho}{\rho_{fl}} - 1\right)g \quad (1)$$

where, U, a, ν, and g are the terminal velocity, the particle radius, a kinetic viscosity, and acceleration of gravity, respectively. ρ and ρ_{fl} are the density of particle and fluid, respectively. Aggregation of erythrocytes will increase the particle size and thus accelerates the delamination of the cells and plasma. At low flow rate, delamination results in a two-phase flow with cells at the bottom layer and plasma at the upper layer.

In the glass capillary used in the present work, a flow rate of 20 μL/min gives a pseudoshear rate (lineal velocity/hydrodynamic diameter) of 0.11 s^{-1},[27] thus meets the conditions of aggregation and settlement of erythrocytes. However, the aggregation generally increases the risk of clogging in the microchannel for many on-chip plasmapheresis methods. The key strategy is to keep the delamination unperturbed in the glass capillary and microchannel. Therefore, a novel steering structure was designed, and cells and plasma can be successfully separated by laminar flow in a chamber. A schematic illustration of the principle of plasma separation is shown in Figure 1. A photo image of plasma separation is shown on the bottom-left corner. A bifurcation is designed to magnify the delamination for observation and separation.

In our experiments with different blood concentrations and flow rates, blood behaves as laminar flow. We calculated the maximal *Re* under the experimental conditions according to the equation of Reynolds number (*Re*). At a maximum flow rate of 100 μL/min, the average linear velocity of blood is 0.53 mm/s in glass capillary and 73 mm/s in microchannel. The corresponding value of *Re* is 1.2 and 13, which are lower than 2000. This means the fluid behaves as laminar flow. In accordance, the blood can realize and keep delamination before separation. We had modified the microchannel with polyvinyl alcohol to compare the separation of different surface energy (data not shown) and did not find any significant alteration of the separation results. The separation of plasma and cells layers can be realized as long as the flow shows laminar character. Thus, the present device is not specific to PDMS-glass.

In addition, continuous plasmapheresis was performed on the device for 4 h. We did not observe clogging or a trend of clogging. The delamination and laminar character of flow behaved well in the whole process. The current device can effectively avoid the clogging problem in the microchannel. High pressure is not needed in plasma separation, thus it is robust enough for long time use.

Conversion of the Blood Delamination Direction. We studied the influence of steering angles on separation. As shown in Figure 1, the plane of the glass capillary is denoted as plane α. A Cartesian coordinate system can be established with the y and z coordinates in the plane α. Rotation of the α plane along the z axial results in different steering angles. The flow direction of blood in the microchannel is along the x axis. The flow direction of blood in the glass capillary is along the y axis. The steering angle is defined as the one between the plane α and the flow direction of blood in the straight microchannel, i.e., Figure 1 (left) shows a steering angle of 90°, and Figure 2 show

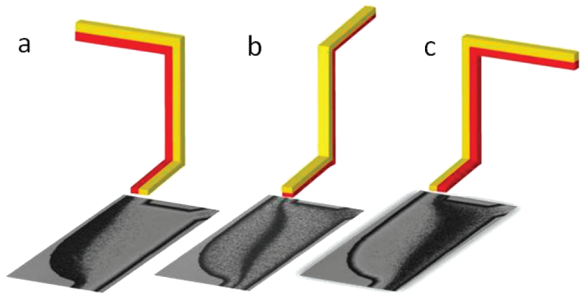

Figure 2. Illustrations and photo images of lamination on the microchip device with different steering angles for 8% HCT blood sample injected at 20 μL/min. Steering angle of 90° (a), 0° (b), and −90° (c).

steering angles of 90° (a), 0 (b), and −90° (c). With the steering angles of 90° (a) and −90° (c), the delamination in the microchannel is along the horizontal direction. For a steering angle of 0° (b), the delamination is along the z-axis, which means the direction of delamination does not change as compared to the one in a glass capillary. The delamination in the $x-y$ plane is of benefit for chip fabrication and separation manipulation.

The delamination phenomenon in the microchannel is shown in Figure 2. The red and yellow layers represent the blood cells and plasma, respectively. Although a concentrated cells layer can be obtained with three different steering angles, efficient separation of cells and plasma is difficult on the device with a steering angle of 0° (Figure 2b). On the contrast, efficient separation and collection of cells and plasma can be easily achieved on the microchip device with the steering angles of ±90° (Figure 2a,c). It is observed that the volume fraction of the two layers does not change notably after the blood flows through the bifurcation structure. These phenomena imply that gravitational sedimentation plays a major role in the delamination process. The bifurcation structure used in our approach is different from the literature in size and function.[9a,15a] The width of the microchannel is larger than 140 μm, which can avoid clogging problems and cell damage. The main function of the bifurcation is to collect plasma from the split flow of the delamination with high efficiency due to the larger distance between the cell layer and outlet for plasma collection. The present bifurcation can make the separation of plasma and blood cells layers more easily and without disturbance. The Fåhraeus effect (the average concentration of red blood cells in human blood decreases as the inner diameter of a capillary for human blood flowing decreases), constriction and pinch flow are neglectable. It is worth noting that delamination can be achieved in our device with lower linear flow rate, while such phenomenon can only be observed at higher linear flow rates on the reported microdevices based on the constriction effect.[14b,e] Therefore, a steering angle of 90° was adopted to carry out plasma separation in the following experiments. In addition, the designed bifurcation structure makes collection of plasma at the collection corner much easier. All the results demonstrate that the designed steering structure makes the plasma separation easier without disturbing the laminar flow and delamination.

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Hematocrits Level and Blood Delamination. HCT is the volume percentage of blood cells in whole blood. The relationships among HCT, aggregation, and sedimentation rate have been experimentally verified by Fabry.[22] The author reported that larger RBC aggregates have faster sedimentation rates. The aggregate size increases with HCT when it is lower than 15%, while it decreases or even becomes amorphous at higher HCT because there are not enough macromolecules to connect RBCs for aggregation. Besides, the interaction between neighboring erythrocytes becomes significant when the HCT is larger than 15%, which will lower the red cell sedimentation rate as well.[22] On the present microchip device, the influence of the entrance hematocrit level on delamination was also studied at a feed rate of 25 μL/min, and the results are shown in Figure 3. It is clear that the separation efficiency of blood decreases

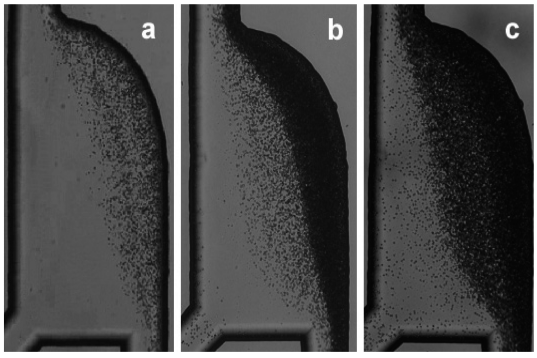

Figure 3. Delamination of different blood concentrations at a feed flow rate of 25 μL/min. Blood concentration (HCT): (a) 4%, (b) 8%, and (c) 14%.

with the increase of HCT. In the case of the nonseparation mode, a small amount of cells was found at the entrance of the plasma collection channel because of the continuous accumulation of remaining cells in the plasma layer at this corner by the vortex as reported previously.[28] According to this result, the sample concentration of 8% by volume is reasonable to achieve excellent separation efficiency on the current microchip device.

Because of the typical non-Newtonian fluid property of blood, its fluidness decreases with the increase of cell concentration. To keep the continuous flow in separating a higher concentration blood (even whole blood) in the capillary and microchannel, a modified structure containing multiple cell outlets in the sedimentation channel is additionally needed to acquire enough separation efficiency. At the same time, macromolecules such as dextran and glutin can be used to enhance the aggregation of erythrocytes for higher HCT samples. However, the recovery and quality of plasma may be lower than the present device. It requires an understanding of balancing the consideration of a high plasma recovery, low blood sample consumption, and the actual requirement of concentration in real assays.

Influence of Feed Flow Rate on Delamination. As has been mentioned above, the shear force will counterbalance the gravitational sedimentation of cells and may damage the cell as well. In this section, the influence of feed rate on the cell separation was studied. These experiments were carried out with the same feed hematocrit of 8% sample but varying the flow rate from 10 to 60 μL/min. As shown in Figure 4, the feed flow rate considerably altered the plasma layer (cell-depleted

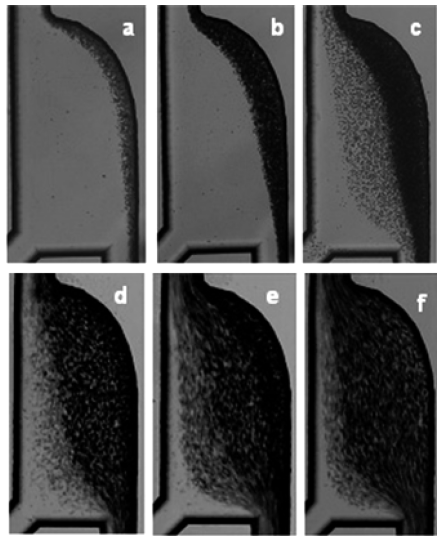

Figure 4. Influence of feed flow rate (μL/min) on the delamination. Feed flow rate: (a) 10, (b) 15, (c) 30, (d) 40, (e) 50, and (f) 60 μL/min.

zone) in the cell-plasma separation chamber. Higher feed flow rates resulted in a thinner plasma layer. Since the time for erythrocytes settlement decreases with the increase of linear velocity, the extent of erythrocytes aggregation decreases as the shear rate increases. The results confirm that gravitational sedimentation plays a major role in the separation process. However, for a feed flow rate lower than 5 μL/min, the delamination would be unstable on the current device. These results demonstrate that lower feed flow rates enable us to obtain distinct delamination. At higher flow rates (e.g., flow rate larger than 60 μL/min), the Fåhraeus effect and pinch flow effect become prominent, which results in more sample consumption and lower separation efficiency. The higher flow rates also increase the risk of damaging the cells through greater shear forces and occurrence of turbulence at the connection interface between glass capillary and microchannel. We did not find pronounced change of the volume ratio of plasma to cell layers from the straight microchannel to the separation chamber, confirming continuous laminar flow remains in the microchannel. Therefore, the designed separation chamber can facilitate the plasma collection but does not affect blood delamination. As shown in Figure 4b, the feed flow rate of 15 μL/min is adoptable for its excellent performance of delamination. Since the separation can be monitored continuously, we can change the separation parameters and observe their effects in real-time, enabling online feedback.

Separation Efficiency. The influence of feed flow rate on the separation purity efficiency at a plasma collection velocity of 10 μL/min was studied. The separation purity efficiency is defined as

$$E_p = 1 - \frac{C_p}{C_f} \tag{3}$$

where C_p and C_f are the cell numbers per milliliter at the plasma collection outlet and at the feed inlet, respectively. The dependence of separation efficiency on the feed flow rate is shown in Figure 5. It is clear that the percentage of cells removed from the plasma decreased with the feed flow rate. The separation purity efficiency decreases slowly within the feed flow rate of 30 μL/min and then it decreases rapidly from

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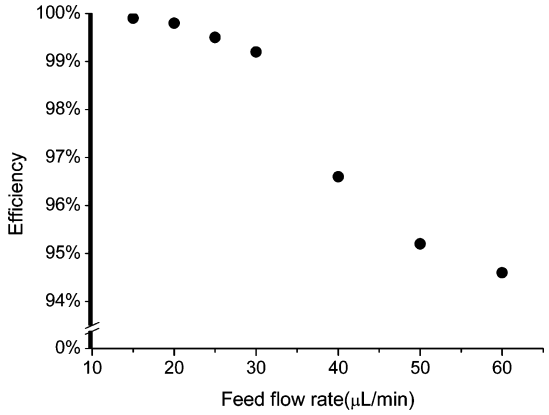

Figure 5. Separation efficiency as a function of feed flow rate at 10 μL/min of plasma collection rate.

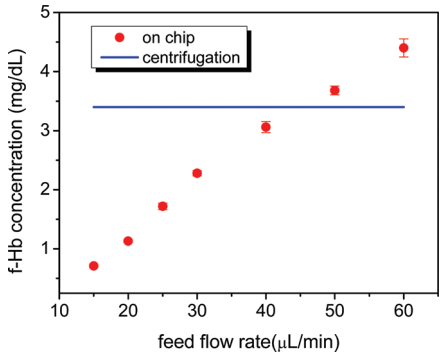

Figure 6. Plot of free hemoglobin concentration in plasma as a function of feed flow rate (red solid circles). The blue line shows the mean concentration of free hemoglobin (3.4 μg/dL) from the centrifuged plasma.

99% to 94.5% with the feed flow rate from 30 to 60 μL/min. This phenomenon could be due to the fact that higher feed flow rate results in shorter time for sedimentation and more obvious turbulence at the connector.

At a certain feed flow rate of sample, the separation efficiency will be influenced by the plasma collection rate as well. As shown in Figure S1 in the Supporting Information, the phenomenon of delamination is distinct at a feed flow rate of 15 μL/min when the plasma collection rate is 0. With the increase of plasma collection rate from 3 to 10 μL/min, the cell layer expands accordingly. However, the separation efficiency still remains 99% (the first point in Figure 5) if we use a bifurcation structure which can keep the plasma harvest away from the turbulence of separation. In this case, the recovery of plasma, which is defined as the volume ratio of plasma and whole blood, reaches 66% at the collection rate of 10 μL/min. For the video of separation at a collection rate of 5 μL/min, refer to Movie S1 in the Supporting Information.

Separation Quality. Concentration of free hemoglobin appearing in the harvested plasma is another important parameter to evaluate the quality of acquired plasma, since free hemoglobin reflects the damaging of red cells during the whole separation process. In our measurements, the free hemoglobin concentration in plasma was determined by a free hemoglobin test kit and used to evaluate the shearing damage to cells on the present separation device. In the separation experiments, blood sample (8%) was injected at different feed flow rates while the plasma collection rate was kept at 10 μL/min. As shown in Figure 6, the concentration of free hemoglobin increases almost linearly with the feed flow rate. It clearly shows that the damage of cells at the feed flow rate lower than 40 μL/min is less than that from the centrifugation method (the mean concentration of free hemoglobin of centrifuged plasma is 3.4 μg/dL, shown as solid line in Figure 6. The result is also much better than those obtained based on narrow constriction.[14b] The high quality of the collected plasma obtained on the present separation device can be attributed to the fact that the delamination process from gravitational sedimentation experiences less shearing damage to cells than the separation conditions that rely on higher flow rates and narrow channels.[14c] In addition, laminar performance in the microchannel can keep the plasma layer away from contamination by the cells layer.

Validation for Downstream Application. Here, we used theophylline as an internal standard to validate the feasibility for downstream application by our separation method. Theophylline is a small molecular drug that calls for drug dose determining and suitable usage for an individual. Theophylline in plasma separated by a centrifuge and our device, respectively, was detected to demonstrate the efficient separation on the present device. These two separation methods did not show a significant difference (Figure S2 in the Supporting Information).

CONCLUSIONS

In summary, we have developed a continuous, on-chip plasma separation method. The proposed microchip device is robust, simple, and inexpensive for long time continuous flow plasma separation. In this approach, aggregation and enhanced gravitational settlement behavior of erythrocytes, novel design of the connector for orientation changing, and laminar flow character of the microchannel are used to realize high-performance plasmapheresis. Results demonstrated that the present device can efficiently and stably separate plasma from blood in a continuous mode. Without a detection method that can be finished quickly, the continuous plasma separation can be used to research the metabolism kinetics of the analyte. To realize instantaneous feedback of monitoring, a quick enough detection mean is also necessary. It has been reported that a fluorescent polarization immunoassay (FPIA) can realize quick analysis in 65 s on a chip.[29] FPIA does not require several processes such as washing and reflowing and immobilizing of antibodies or antigens in the channel and can be integrated on a chip in the future. Therefore, the present microchip device is expected to be an effective tool for metabolite monitoring in pharmacokinetics research and drug development.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: xujj@nju.edu.cn (J.-J.X.); xhxia@nju.edu.cn (X.-H.X.).

Notes

The authors declare no competing financial interest.

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