Network simulation-based optimization of centrifugopneumatic blood plasma separation

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Automated and robust separation of 14 μl of plasma from 40 μl of whole blood at a purity of $99.81\% \pm 0.11\%$ within 43 s is demonstrated for the hematocrit range of 20%–60% in a centrifugal microfluidic polymer disk. At high rotational frequency, red blood cells (RBCs) within whole blood are concentrated in a radial outer RBC collection chamber. Simultaneously, plasma is concentrated in a radial inner pneumatic chamber, where a defined air volume is enclosed and compressed. Subsequent reduction of the rotational frequency to not lower than 25 Hz enables rapid transfer of supernatant plasma into a plasma collection chamber, with highly suppressed resuspension of red blood cells. Disk design and the rotational protocol are optimized to make the process fast, robust, and insusceptible for undesired cell resuspension. Numerical network simulation with lumped model elements is used to predict and optimize the fluidic characteristics. Lysis of the remaining red blood cells in the purified plasma, followed by measurement of the hemoglobin concentration, was used to determine plasma purity. Due to the pneumatic actuation, no surface treatment of the fluidic cartridge or any additional external means are required, offering the possibility for low-cost mass fabrication technologies, such as injection molding or thermoforming. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4979044>]

I. INTRODUCTION

Blood plasma is one of the most relevant biological samples for diagnostic purposes. Apart from electrolytes and dissolved gases, it contains more than 100 proteins that are relevant clinical analytes, so that a wide range of diseases can be diagnosed *via* biomarkers in the plasma (Anderson, 2002 and Ray *et al.*, 2011). When it comes to rapid testing at the point-of-care, miniaturized systems offer the possibility to perform entire laboratory workflows from the sample preparation to the detection of biomarkers, integrated in one microfluidic chip with a high degree of automation. Since optical detection of biomarkers requires pure plasma with a low concentration of red blood cells (RBCs), plasma separation is often the initial sample preparation step. On-chip plasma extraction methods include geometric filtering, dielectrophoresis, or they make use hydrodynamic effects.

Chen *et al.* presented dielectrophoresis-based plasma separation in a microfluidic channel, sandwiched by two metallized glass slides. Upon application of an AC voltage between the two electrodes, blood cells are polarized and attracted to the channel walls. As the propulsion of the blood sample by capillary force proceeds within the microfluidic channel, around 90% of the cells in the 150 μl sample volume are filtered out (Chen *et al.*, 2014). This process can be run

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at low voltages around 1 V and does not require a complex or bulky processing device. Still, the process duration to collect a significant amount of purified plasma is rather long.

Alternatively, blood cells can be filtered out geometrically either by fabrication of microchannels whose diameters are below the cell diameters (Szydzik *et al.*, 2015) or by implementation of filter membranes (Chen *et al.*, 2016 and Songjaroen *et al.*, 2012). While microchannels can be machined with high precision and relatively high effort in poly(dimethylsiloxane) (PDMS), filter membranes present a low-cost alternative with less homogenous filter structures. In both approaches, sample transport can be accomplished either by capillary action (Songjaroen *et al.*, 2012 and Szydzik *et al.*, 2015) or by the means of external pumps (Chen *et al.*, 2016). The latter one inherently involves the challenge to interface the pump to the chip with a certain risk of contamination when the sample is introduced. Purely capillary propulsion is generally relatively slow and leads to long processing times, which may be critical in point-of-care applications.

Hydrodynamic filter methods also require external pumps for fluid transport, but no additional filter membranes. Instead, the flow profile in microchannels is used to concentrate particles along defined streamlines, such that they can be sorted out in branching channels (Yang *et al.*, 2006). Nivedita *et al.* focused blood cells in spiraled microchannels along certain streamlines, where lift forces and Dean forces are balanced. At the end of the spiral, the channel splits into different chambers that either collect the cells or pure plasma. Since this effect of focusing particles relies on the fluid's Newtonian characteristics, blood samples need to be diluted by a factor of ~ 100 (Nivedita and Papautsky, 2013).

Centrifugation is considered to be the gold standard for plasma separation. It uses the difference in density to separate the plasma from the cells. The artificial gravity generated by rotation enables fast sedimentation of RBCs that are either filtered out or concentrated at the bottom of a vessel prior to manual extraction of the supernatant plasma. The same artificial gravity is employed in centrifugal microfluidics as realized for nucleic acid analyses, immunoassays, and cell analyses (Cho *et al.*, 2007; Park *et al.*, 2012a, 2012b; Sundberg *et al.*, 2010; Furutani *et al.*, 2010; Amasia *et al.*, 2012; Noroozi *et al.*, 2011; Lee *et al.*, 2008, 2011; and Burger *et al.*, 2015). Thus, the implementation of blood plasma separation in centrifugal microfluidics is straight forward. Yet, transfer of purified plasma into spatially separated reaction vessels has often been omitted (Li *et al.*, 2010 and Grumann *et al.*, 2006). A major challenge for such a spatial separation of RBCs and plasma is the robust valving of plasma while preventing carryover of RBCs. Sedimentation alone can be used for applications that do not require highly purified plasma and/or include read-out at continuously high rotational frequencies so that cell resuspension is suppressed. Automated blood plasma separation with spatial separation of blood cells and plasma into separate reaction vessels, however, asks for more complex solutions.

One approach presented by Park *et al.* makes use of a normally closed ferrowax valve that is located radial inward of the shock interface, i.e., the interface between concentrated cells and purified plasma. Upon infrared laser irradiation, the wax is melted so that the purified plasma is released into the downstream fluidics. Implementation of a ferrowax valve allows for simple fluidic design which is achieved at the expense of higher complexity in fabrication and of the processing device (Park *et al.* 2012b).

Haeberle *et al.* presented a blood separation technique on a centrifugal microfluidic disk with continuous sedimentation in a quasi-isoradial channel. Within this narrow sedimentation channel, denser red blood cells are concentrated at the outer rim. At a rotational frequency of 40 Hz, a 5 μ l whole blood sample with a hematocrit in the normal physiological range (36%–52%) was processed within 20 s. While blood cells are concentrated in a first collection chamber, the plasma is extracted by overflow into a second collection chamber. In a second step, further transport of the purified plasma was realized by capillary action at low rotational frequency. This kind of liquid processing in two steps requires hydrophilic coating to enable frequency controlled plasma valving (Haeberle *et al.*, 2006).

Another approach uses a chamber for RBC sedimentation with an adjacent siphon for plasma transport. After sedimentation at a high rotational frequency is completed, the siphon is primed with purified plasma at a low rotational frequency by capillary action (Schembri *et al.*, 1995 and Nwankire *et al.*, 2014). In this way, Amasia *et al.*, processed 2 ml of whole blood

(HCT = 49%) within 320 s with a purity of 99.99%. Similar to the work of Haeberle *et al.*, frequency controlled valving by capillary action required hydrophilic coating of the siphon (Amasia and Madou, 2010).

Differently, Burger *et al.*, used a chamber for cell sedimentation with a valve for plasma drainage above the shock interface. This valve opens at high rotational frequencies above 50 Hz by centrifugo-pneumatic actuation so that the rotational frequency for sedimentation is limited to below 50 Hz. Hence, the speed of such a plasma separation process is always limited by the minimum rotational frequency to open the valve. With this method, a 5 μ l whole blood sample with a hematocrit in the physiological range was processed within 120 s (Burger *et al.*, 2013).

All of these centrifugal plasma separation techniques are either limited in rotational frequency, making the process longer, or require additional means such as ferrowax and lasers or use capillary forces such that hydrophilic coating and/or plasma treatment is indispensable for most polymer substrates.

In the past few years, within the community dealing with centrifugal microfluidics a trend away from capillary valves towards centrifugo-pneumatic valves could be observed. This is due to the high pneumatic pressures that are generated by intended and well-defined air entrapment and compression. Typically, these pressures are orders of magnitudes higher than capillary pressures, which makes them particularly suitable for actuation of valves and switches realized by interplay of centrifugal and pneumatic pressures (Gorkin *et al.*, 2010; Godino *et al.*, 2013; Zehnle *et al.*, 2012; 2015; and Kinahan *et al.*, 2016a; 2016b). In this context, the basic principle of using pneumatic pressure to separate purified plasma from the sedimented cells by siphon valving has been demonstrated successfully (Godino *et al.*, 2013 and Kinahan *et al.* 2016a). Here, we present centrifugal microfluidic plasma separation using the pneumatic siphon valve, with optimized geometries and frequency protocol, robustness analysis and performance characteristics.

II. WORKING PRINCIPLE

The plasma separation working principle is depicted in Fig. 1. Initially, the whole blood sample is introduced into the reservoir. Upon centrifugation, three major phases are defined.

A. Loading

Initial ramp-up of the rotational frequency leads to transfer of the sample into the RBC collection chamber. When the fill level exceeds the constriction between the RBC collection chamber and pneumatic chamber, the air inside the pneumatic chamber is entrapped. Further loading of the pneumatic chamber with whole blood reduces the air volume in the chamber and increases the pressure of the enclosed air.

B. Sedimentation

At high rotational frequency, the air overpressure (=pneumatic pressure) in the pneumatic chamber is balanced by the centrifugal pressure in the inlet channel and the siphon, defined by elevated fill levels. Red blood cells sediment radially outward in the RBC collection chamber, while displacing the plasma into the pneumatic chamber.

C. Collection

The rotational frequency is reduced to below the threshold frequency. Centrifugal pressures are reduced accordingly. Consequently, the air volume in the pneumatic chamber expands and displaces the plasma into the siphon. Once the siphon is primed, the plasma volume in the pneumatic chamber located above the siphon inlet is transferred into the plasma collection chamber. As soon as the fill level in the pneumatic chamber falls below the inlet of the siphon, air is sucked into the siphon. Thus, no further liquid and no cells are transferred into the plasma collection chamber.

FIG. 1. Working principle of centrifugo-pneumatic plasma separation. (a) Whole blood sample is introduced into the reservoir. (b) Loading of sample and air compression in the pneumatic chamber. (c) Sedimentation of blood cells in the RBC collection chamber completed. (d) Plasma transfer through siphon into the plasma collection chamber completed.

III. SIMULATION AND FABRICATION

A network simulation with lumped model elements was set up in Saber 2004.06 (Synopsys, CA, USA) to predict the fluidic characteristics and optimize parameters, such as channel/chamber dimensions and the rotational protocol. The network simulation approach has already been introduced and discussed extensively in the literature (Zehnle *et al.*, 2015 and Schwarz *et al.*, 2016). In brief, the fluidic network is broken down into single elements, i.e., channels with radial and isoradial orientation, chambers and air vents. Each element includes a transfer function that relates the total pressure across the element Δp_{tot} to the volumetric flow rate q through the element. The total pressure Δp_{tot} across each element is the sum of the centrifugal (Δp_c), Euler (Δp_E), inertial (Δp_i), capillary (Δp_{cap}), and pneumatic (Δp_p) pressure, as well as pressure loss due to viscous dissipation (Δp_v) as given by Eqs. (1)–(6).

$$\Delta p_c = \frac{1}{2} \rho \omega^2 (r_2^2 - r_1^2), \quad (1)$$

$$\Delta p_E = \rho L \dot{\omega} r, \quad (2)$$

$$\Delta p_i = -\frac{\rho L}{A} \dot{q}, \quad (3)$$

$$\Delta p_{cap} = 4 \frac{\sigma}{a} \cos \theta, \quad (4)$$

$$\Delta p_p = p_0 \left(\frac{1}{1 - \Delta V/V_0} - 1 \right), \quad (5)$$

$$\Delta p_v = -28.4 \mu \frac{L}{a^4} q. \quad (6)$$

In Eqs. (1)–(6), ρ is the liquid density, μ is the dynamic liquid viscosity, ω is the angular rotational speed, r_1 and r_2 are the radial inner and outer limit positions of liquid in the corresponding element, L is the channel length filled with liquid, r is the radial position for channels with isoradial orientation, A is the cross-sectional area, σ is the surface tension and θ is the contact angle of the processed liquid, a is the edge length of the channels with squared cross section, p_0 is the ambient pressure, V_0 is the initial air volume in the pneumatic chamber, and ΔV is the change of said air volume.

The plasma separation module is designed to cover the hematocrit range of 20%–60%. Whole blood underlies strong viscosity variations depending on the hematocrit with shear thinning behavior, i.e., viscosity decreases as the shear rate increases. To account for this effect, the viscosity was implemented as a function of the wall shear rate, $\dot{\gamma}_w$, using a power law model according to

$$\mu(\dot{\gamma}_w) = K \dot{\gamma}_w^{n-1} + \mu_0, \quad (7)$$

where K and n denote the consistency and the flow index, respectively, and μ_0 represents a constant Newtonian viscosity offset. These parameters were determined empirically based on viscosity measurements using a Physica MCR 101 rheometer (Anton Paar GmbH, Austria). Further details and numerical values on the network simulation are given in the [supplementary material](#).

SolidWorks 2011 (Concord, MA, USA) CAD software was used to design the microfluidic plasma separation structure according to Fig. 2. For this specific approach, all chambers were chosen to be 1.5 mm deep, and the inlet channel and the siphon were designed with $250 \mu\text{m} \times 250 \mu\text{m}$ squared cross-sections.

Disks were fabricated *via* CNC micromilling in 4 mm thick Poly(methyl methacrylate) (PMMA) substrates. After milling, the disks were rinsed with de-ionized (DI) water, dried with pressurized nitrogen, and sealed with a pressure sensitive adhesive (# 900 320, HJ Bioanalytik, Germany) using lamination rolls.

The RBC collection chamber was designed as wide as possible to geometrically minimize the cell sedimentation distance, while still allowing the plasma to rise into the pneumatic chamber. Similarly, the pneumatic chamber was also designed as wide as possible with the slope of the radial outer flanks steep enough to allow for the sedimented blood cell slurry to slide into the RBC collection chamber (Kim *et al.*, 2013).

With a fixed sample volume of $40 \mu\text{l}$, the volume of the RBC collection chamber determines the maximum hematocrit that can be processed. We designed the RBC collection chamber to hold $25.9 \mu\text{l}$ thus covering 64.75% of the total sample volume with a buffer of 4.75% above the maximum shock interface, which reduced the risk of resuspending red blood cells due to Euler forces during frequency changes at high hematocrit levels, as reported in the literature (Burger *et al.*, 2013 and Amasia and Madou, 2010). Yet, siphon priming by pneumatic actuation allows for continuous operation at high rotational frequencies—also during siphon priming and plasma collection.

In this work, the rotational frequency is limited to 75 Hz. The radial outer position of the siphon is chosen to be 45 mm allowing for cascading of further microfluidic structures on a centrifugal platform.

IV. EXPERIMENTS

Characterization of the plasma separation module was carried out with human EDTA whole blood collected from a healthy donor. The hematocrit was measured via photometric measurement of the hemoglobin concentration with an EKF Hemo Control (EKF-diagnostic GmbH, Germany). Subsequently, the whole blood samples were stored at $+4^\circ\text{C}$ for 3 h to sediment the blood cells before supernatant plasma was extracted or added to obtain a hematocrit

FIG. 2. Projected view on the plasma separation module including dimensions. Radial positions are marked by dashed lines, where $r_{\text{reservoir}}$ and r_{pc} denote the radial outer positions of the reservoir and the pneumatic chamber, respectively, and r_{crest} denotes the radial position of the siphon crest. Air vents that are drilled through the substrate are indicated by ground symbols.

of 60% or 20%, respectively. The disk was spun with a brushless EC motor (Maxon, Switzerland) that was controlled *via* a personal computer with a custom-made software, and synchronized with a stroboscope and a camera that grabs one real-time image per revolution. After completion of the plasma separation process, the plasma was retrieved from the disk. The purity was determined *via* remaining concentration of hemoglobin. First, lysis of the remaining red blood cells in the purified plasma was performed by mixing $6 \mu\text{l}$ of purified plasma with $1.2 \mu\text{l}$ of triton x-100 solution (0.1%) (Dow Chemical) and $40.8 \mu\text{l}$ phosphate buffered saline (PBS) solution at 60°C for 10 min under continuous shaking. Then, the lysate was mixed with $36.0 \mu\text{l}$ of aqueous thiocyanate solution (1:200) and $36.0 \mu\text{l}$ of PBS. Finally, light extinction by the lysate between 400–700 nm was measured with a spectrometer. In the absorption spectrum, a peak at 576 nm is characteristic for the hemoglobin concentration. In order to obtain background independent results, the difference of absorption between 563 nm and 576 nm was evaluated for both the calibration curve with 0%, 0.1%, 0.33%, and 0.5% HCT and the samples retrieved from the automated on-disk plasma extraction.

V. RESULTS AND DISCUSSION

Recorded real-time images were used to evaluate fill levels and hence determine liquid volumes. Fig. 3 shows an image sequence of the plasma separation process performed with the 60% hematocrit samples.

Initially, $40 \mu\text{l}$ of the whole blood are introduced into the reservoir. As the rotational frequency increases in the loading phase, the blood is transferred into the RBC collection chamber first (Fig. 3(a)) and then into the pneumatic chamber (Fig. 3(b)). In this phase, the liquid must not surpass the siphon crest, as indicated by the safety range in Fig. 3(b). During the sedimentation phase, the blood cells concentrate in the RBC collection chamber and the plasma in the pneumatic chamber (Fig. 3(c)). Reduction of the rotational frequency results in air expansion in the pneumatic chamber so that the siphon is filled with purified plasma. At the end of the

FIG. 3. Image sequence of the plasma separation process. Dashed lines are used to indicate fill levels. (a) The whole blood sample is transferred from the inlet reservoir to the RBC collection chamber. (b) Loading into the RBC collection chamber and pneumatic chamber complete, while frequency still ramps up. Fill levels in the siphon and in the pneumatic chamber are given by I_1 and I_2 , respectively. The safety range indicates that the fill level in the siphon does not reach the siphon crest, thus premature siphon priming is prevented. (c) Sedimentation into the RBC collection chamber complete. (d) Plasma transfer from the pneumatic chamber into the plasma collection chamber complete. Reproduced with permission from Zehnle *et al.*, 16th International Conference on Miniaturized Systems for Chemistry and Life Sciences (2012), pp. 869–871. Copyright 2012 The Chemical and Biological Microsystems Society (CBMS).

collection phase, the entire purified plasma is transferred from the pneumatic chamber to the plasma collection chamber (Fig. 3(d)). The corresponding spin protocol is plotted in Fig. 4 together with the simulated and experimental fill levels.

As predicted by network simulation, the whole blood in the pneumatic chamber reaches a certain fill level that remains constant until it decreases again and approaches zero in the collection phase. Differently, but also predicted by simulation, the fill level in the siphon shows a distinct peak in the loading phase. In Fig. 3 and in Fig. 4, the safety range indicates the distance between the peak fill level during loading and the siphon crest. When the rotational frequency reaches its maximum at 75 Hz, the fill level in the siphon approaches the equilibrium level. In the collection phase, the fill level piles up to maximum, indicating complete siphon priming before it drops to zero, meaning that the plasma transfer has been completed.

FIG. 4. Spin protocol (blue) and fill levels (black) in the siphon (I_1) and in the pneumatic chamber (I_2) resulting from simulation and experiments with whole blood of 60% hematocrit. Experiments were performed in triplicates. Error bars denote one standard deviation. Reproduced with permission from Zehnle *et al.*, 16th International Conference on Miniaturized Systems for Chemistry and Life Sciences (2012), pp. 869–871. Copyright 2012 The Chemical and Biological Microsystems Society (CBMS).

The 20% hematocrit samples were processed to confirm that premature siphon priming is prevented also for samples with such low viscosity, as predicted by network simulation (see the [supplementary material](#)).

Plasma separation processes using capillary siphons, as discussed in the introduction, revealed that the duration of siphon priming is the limiting factor for fast processing. Low capillary pressure heads require time spans for priming in the range of 30 s. Due to pneumatic actuation and continuously high rotational frequencies in the hereby presented method, the duration of the entire plasma collection phase was 2.0 s with a standard deviation of 0.2 s (simulation: 1.7 s) for a deceleration rate of 16 Hz s^{-1} , and 1.5 s with a standard deviation of 0.3 s (simulation: 1.1 s) for a deceleration rate of 32 Hz s^{-1} .

Fig. 5 and Table I illustrate and summarize all criteria for the spin protocol to ensure robust operation, i.e., the rotational frequency does not enter the shaded area in Fig. 5. For a given inlet channel resistance, the initial deceleration rate must be high enough to prevent premature siphon priming. Once the sample volume is loaded into the pneumatic chamber, the rotational frequency $f_{\text{Sedimentation}}$ must not drop below the threshold frequency, $f_{\text{Threshold}}$, during sedimentation. Subsequent deceleration below $f_{\text{Threshold}}$ triggers siphon priming and initiates the plasma collection phase. During this phase, the rotational frequency, $f_{\text{Collection}}$, must be low enough to prevent disruption of the liquid stream. This was observed for $f_{\text{Collection}} > 30 \text{ Hz}$ and may be due to either meniscus instability or evolution of gas due to the low pressure within the siphon and thereby reduced solubility of dissolved gases (CO_2 , O_2). High gas evolution leads to gas bubbles that clog the siphon channel and results in malfunction. Despite this, $f_{\text{Collection}}$ must be chosen as high as possible to prevent resuspension of blood cells due to Euler forces, while the rotational frequency is being reduced. In this work, the plasma separation module is operated so that the centrifugal force that sediments the blood cells is at any time at least two orders of magnitude higher than the Euler force.

For a spin protocol with $f_{\text{Sedimentation}} = 75 \text{ Hz}$, a duration of 40 s for loading and sedimentation, and a deceleration rate of 32 Hz s^{-1} for plasma collection with $f_{\text{Collection}} = 25 \text{ Hz}$, $14.0 \mu\text{l}$ of plasma with a standard deviation of $0.6 \mu\text{l}$ were extracted. The purity of extracted plasma was defined as $P = 1 - \text{HCT}$, where HCT is the remaining hematocrit in the plasma, and amounted to 99.81% with a standard deviation of 0.11%. Similarly, three samples were processed with a deceleration rate of 16 Hz s^{-1} during the collection phase thereby extracting $14.0 \mu\text{l}$ of plasma (standard deviation: $0.5 \mu\text{l}$) with a purity of 99.84% (standard deviation:

FIG. 5. Schematic of an optimized spin protocol for robust operation preventing undesired effects that occur in the critical, shaded frequency bands: (a) Undesired siphon priming due to insufficient air compression in the pneumatic chamber. (b) Cell resuspension by Euler forces. (c) No siphon priming above the threshold frequency. (d) Disruption of liquid transfer through siphon due to air bubble entrapment. Reproduced with permission from Zehnle *et al.*, 16th International Conference on Miniaturized Systems for Chemistry and Life Sciences (2012), pp. 869–871. Copyright 2012 The Chemical and Biological Microsystems Society (CBMS).

TABLE I. Summarized undesired effects A-D according to Fig. 5 to be prevented for robust operation. The critical parameters can be tuned by varying the inlet channel resistance (affecting the critical acceleration during loading), the gas volume in the pneumatic chamber, or the radial position of the siphon crest (both affecting $f_{\text{Threshold}}$).

Undesired effect	Critical parameter		Condition
A	Premature siphon priming	Acceleration during loading	$\geq 27 \text{ Hz s}^{-1}$
A	Premature siphon priming	$f_{\text{Threshold}}$	$> 53 \text{ Hz}$
B	Cell resuspension by Euler force	$f_{\text{Collection}}$	As high as possible
C	No siphon priming during collection	$f_{\text{Threshold}}$	$< 53 \text{ Hz}$
D	Disruption of liquid stream in siphon	$f_{\text{Collection}}$	$< 30 \text{ Hz}$

0.03%). For comparison to other microfluidic plasma separation techniques, a benchmarking is summarized in Table II. It shows that the characteristics of centrifugo-pneumatic plasma separation (i.e., throughput and purity) are competitive to the state of the art.

In contrast to other microfluidic plasma separation techniques, centrifugo-pneumatic plasma separation uses fluidic channels with diameters that are orders of magnitudes larger than the cell diameters and that can be fabricated by common injection molding or thermoforming. Furthermore, its pneumatic actuation principle circumvents the use of surface effects, making it fast and more predictable at the moderate expense of additional real estate on the disk for the compressed gas volume. Since the speed of cell sedimentation does not depend on the chamber depth, similar performances may be expected for a deeper or shallower separation structure with up- or down-scaled sample volume, respectively (Kim *et al.*, 2013).

VI. CONCLUSION AND OUTLOOK

We developed a fast and robust microfluidic processing chain for separation of plasma from whole blood samples covering the entire physiologically relevant hematocrit range. Simplicity in design and operation and scalability of processed volumes as well as the ability to optimize fluidic operation by network simulation make it a universal sample preparation process chain that can easily be combined with downstream analysis, e.g., immunoassays or the determination of parameters in clinical chemistry. The monolithic integration into polymer materials does not require any surface treatment and allows for low-cost fabrication in mass production. Fluidic downstream elements can be integrated radially outward or inward by cascading an inward pumping unit operation to the plasma separation. Furthermore, it may be used for the separation of other kinds of dispersions, such as bead solutions. By addition of density gradient medium and further outlet channels, its functionality can be extended to buffy coat extraction, thereby enabling downstream analysis of white blood cells and rare cells. Recently, several

TABLE II. Benchmarking of existing microfluidic plasma separation techniques.

References	Separation mechanism	Purity (%)	Throughput of sample	Devices required
Nivedita <i>et al.</i> (2013)	Focussing of blood cells in a spiraled channel by balancing of Dean and lift forces	100	1 ml min ⁻¹ of diluted (100×) whole blood	Syringe pump
Chen <i>et al.</i> (2016)	Geometric filtering with a polycarbonate membrane bonded to the microfluidic chip	92.5 ^a	20 $\mu\text{l min}^{-1}$	Syringe pump
Szydzik <i>et al.</i> (2015)	Dielectrophoresis, sample transport by capillary action	100	15 μl in 15 min	AC voltage source
Haeberle <i>et al.</i> (2006)	Centrifugation, plasma separation by decanting	99.89	5 μl in 20 s	Centrifuge
Centrifugo-pneumatic blood plasma separation		99.81	40 μl in 43 s	Centrifuge

^aDefined as percentage of RBCs that were filtered out (=separation efficiency).

centrifugal microfluidic disks for automated pathogen detection have been published which require blood plasma as a sample material (Noroozi *et al.*, 2011; Czilwik *et al.*, 2015; and Strohmeier *et al.* 2015). In the future, these and other systems can monolithically be complemented with the presented plasma separation module for fully integrated sample-to-result systems.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for the network model of the plasma separation, the implementation of the hematocrit dependent viscosity in simulation, and further simulation results.

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