High-throughput Separation of White Blood Cells from Whole Blood using Inertial Microfluidics

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Abstract—White blood cells (WBCs) constitute only about 0.1% of human blood cells, yet contain rich information about the immune status of the body, thus separation of WBCs from the whole blood is an indispensable and critical sample preparation step in many scientific, clinical and diagnostic applications. In this work, we developed a continuous and high-throughput microfluidic WBC separation platform utilizing the differential inertial focusing of particles in serpentine microchannels. First, separation performance of the proposed method is characterized and evaluated using polystyrene beads in the serpentine channel. The purity of 10- μm polystyrene beads is increased from 0.1% to 80.3% after two cascaded processes, with an average enrichment ratio of 28 times. Next, we investigated focusing and separation properties of Jurkat cells spiked in the blood to mimic the presence of WBCs in whole blood. Finally, separation of WBCs from human whole blood was conducted and separation purity of WBCs was measured by the flow cytometry. The results show that the purity of WBCs can be increased to 48% after two consecutive processes, with an average enrichment ratio of 10 times. Meanwhile, a parallelized inertial microfluidic device was designed to provide a high processing flow rate of 288 ml/h for the diluted ($\times 1/20$) whole blood. The proposed microfluidic device can potentially work as an upstream component for blood sample preparation and analysis in the integrated microfluidic systems.

Index Terms—Inertial microfluidics, particle focusing and separation, blood cell separation, white blood cells, leukocytes, serpentine microchannel

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I. INTRODUCTION

BLOOD contains massive information about the functioning and status of the body. Separation of blood sample is a requisite and critical step in many research applications, and clinical diagnoses and therapeutics [1-5]. Current methods for blood cell separation require complex and expensive equipment. Centrifugation, the “gold standard” and the most common method for blood cell separation, is labor-, energy- and time-intensive and relies on well-trained operators [6, 7]. Another common approach is to expose whole blood to the hypotonic solution to lyse red blood cells (RBCs), which are more sensitive to osmotic shock than nucleated white blood cells (WBCs) [8]. In this method, centrifugation is still required to remove the lysed RBC debris from WBCs. Moreover, exposure to a high acceleration or osmotic shock will probably influence the immunophenotype [9] and viability [10, 11] of the cells.

Microfluidics has been emerging as a powerful technology and holds the potential for high resolution and high efficient separation of blood cells in microchannels. In microfluidic devices, active external (electric, acoustic and magnetic) force fields were applied to facilitate the fractionation of blood cells. Han and Frazier [12] presented lateral-driven continuous dielectrophoretic (DEP) microseparators to separate RBCs and WBCs from diluted ($\times 1/6$) whole blood at a flow rate of 50 $\mu\text{l}/\text{min}$. The separation efficiency of WBCs for the divergent and convergent type DEP microseparators was 92.1% and 76.9%, respectively. Meanwhile, free flow acoustophoresis (FFA) was proposed and developed to separate polystyrene particles and blood cells [13]. This method has been further developed to fractionate WBCs subpopulations (lymphocytes, monocytes, and granulocytes) [14]. For lymphocytes and granulocytes, flow cytometry data reveal high purity (95.2 % and 98.5 %, respectively) and high recovery (86.5 % and 68.4 %, respectively). However, despite a high recovery (83.1%), a relatively low purity (25.2%) was obtained for monocytes. In addition, employing intrinsic differences in magnetic property between WBCs and RBCs can also enable the separation of WBCs and RBCs. Han and Frazier [15] developed continuous cascade paramagnetic capture (PMC) mode microfluidic separators for high efficient separation of RBCs and WBCs based on the native magnetic properties. The PMC microseparator continuously separated 93.5% of RBCs and 97.4% of WBCs from whole blood at a volumetric flow

rate of 5 $\mu\text{l/h}$. Moreover, Iliescu et al. [16] reported a microfluidic device for magnetophoretic capture of RBCs from blood under continuous-flow condition. The ferromagnetic wires generate a gradient of magnetic field which is perpendicular to the flowing direction and enlarges the magnetic force to capture RBCs on the bottom of the microfluidic channel, while the rest of the blood flows through and is collected at the outlet. Experimental results show that 95% of RBCs were trapped in the device.

Meanwhile, fractionation of blood cells by employing intrinsic hydrodynamic force or cell-cell interactions without external force fields was also reported. Cross flow filtration which filtrates small particles from large ones by an array of side branches has been developed for blood cell separation [17-20]. Yamada and Seki [17] proposed a hydrodynamic filtration in a microchannel with multiple side branch channels to concentrate WBCs from ($\times 1/10$) diluted blood samples at a flow rate of 20 $\mu\text{l/min}$. The ratio of WBCs to RBCs was increased from 0.13% to 3.71% after two rounds of filtration. In addition to side branch, the surface membrane was integrated within a crossflow filtration scheme in a microfluidic chip to sort WBCs from whole blood [19]. The recovery rate of WBCs was 27.4% and purity was 93.5% with a sample-throughput of 1 ml/h.

WBC margination, which is recognized as an outcome of the cell deformability and shape differences between RBCs and WBCs, is also used for blood separation [8, 21]. Jain et al. [8] developed a biomimetic postcapillary expansion technique using repeated triangular expansions/contractions to extract nucleated cells at a rate of 18 $\mu\text{l/h}$ on the unprocessed whole blood. The recovery rate of 94% and purity of 5% were obtained. By patterning slanted ridges on the top of the straight channel, Kim et al. [21] developed a microfluidic platform to achieve deterministic separation of WBCs from whole blood, achieving high-throughput separation of cells (150 $\mu\text{l/min}$ for undiluted whole blood). The ratio of WBCs to RBCs was 110:1000 in the WBCs reservoir after multistage ($n=4$) device operation, with a recovery rate of 80%.

Recently, inertial microfluidic technology which manipulates microparticles by the finite inertial effects of fluid and particles has attracted significant attention due to the advantages of simple structure, high throughput, and precise manipulation [22-26]. Wu et al. [27] presented a novel inertial platform for continuous sheathless particle and blood cell separation in straight microchannels containing expansion-contraction microstructures. The processing rate for the device with 72 parallel channels for diluted ($\times 1/400$) blood was 10.8 ml/min, with separation purity and efficiency as high as $\sim 90\%$. Spiral microchannels, which induce Dean flow to modify inertial focusing and facilitate cell separation, were proposed and applied for blood cell separation. Nivedita and Papautsky [28] demonstrated the continuous separation of RBCs and WBCs from a diluted ($\times 1/500$) sample of blood in a spiral microchannel at a flow rate of 1.8 ml/min, equivalent to the flow rate of 3.6 $\mu\text{l/min}$ for undiluted whole blood. Moreover, Wu et al. [29] presented a novel design of a spiral inertial microfluidic device with a trapezoidal cross-section for high efficient WBCs separation. For 0.5% hematocrit blood sample, $\sim 98.4\%$ of total WBCs (99.4% granulocytes and 92.4% mononuclear leukocytes) were recovered at a throughput of

$\sim 900 \mu\text{l/min}$, equivalent to 10 $\mu\text{l/min}$ for whole blood (at 45% hematocrit). However, the throughput of these inertial microfluidic devices is still on the order of $\sim 10 \mu\text{l/min}$ for the undiluted blood, which is not desirable for large volume sample preparation.

In the present work, we developed a continuous and high-throughput inertial microfluidic device with symmetric serpentine microchannels to further improve the throughput. The separation principle is based on the differential inertial focusing of particles in a microchannel. First, we characterized and evaluated the separation performance of the proposed methods using polystyrene beads. The purity of 10- μm polystyrene beads was increased from 0.1% to 80.3% after two processes, with an average purity enrichment ratio of 28 times. Next, Jurkat cells, a human T lymphocyte cell line, were spiked in the blood sample to mimic the presence of WBCs in whole blood, and its focusing and separation performance in the whole blood was investigated in the proposed microchannel. Finally, separation of WBCs from diluted whole blood was conducted in a cascaded manner, and flow cytometry was used to characterize the separation purity of WBCs. A parallelized inertial microfluidic device was designed and fabricated to provide a high processing flow rate of 288 ml/h for the diluted ($\times 1/20$) whole blood. We envision that the parallelized serpentine channels could be a useful alternative to conventional centrifugation for high efficient and high-throughput RBC depletion and WBC purification.

II. METHODS AND MATERIALS

A. Device Design and Fabrication.

The microchannel in the experiments is 15.2 mm long, comprised of 15 zigzag periods. The depth and width of the microchannel are 42 μm and 200 μm , respectively. The width and length of each U-turn are both 700 μm . At the end of the serpentine channel, a trifurcation outlet is implemented, and the two-sided symmetrical branches are merged for an easy fluid collection. In order to amplify the processing throughput, eight serpentine channels were parallelized along the planar direction in a single microfluidic device. All components were fabricated by the standard photolithography and PDMS soft lithography techniques [30].

B. Particle and Cell Preparation

Polystyrene particles internally stained with fluorescent dyes were obtained from Thermo Fisher Scientific. Particles with diameters of 3 μm (Product No. R0300, CV5%) and 10 μm (Product No. G1000, CV5%) were respectively dispersed in deionized (DI) water and the particle weight ratio is about 0.05%. Tween 20 (Sigma-Aldrich, Product No. P9416) with was added in the DI water as a surfactant at a weight ratio of 0.1% to avoid particle aggregation. For the separation test, a mixture of polystyrene beads was prepared by mixing 3- μm and 10- μm particles to a final concentration of 4×10^7 counts/ml and 4×10^4 counts/ml in the DI water, respectively.

For cell experiments, Jurkat cells, an immortalized line of human T lymphocytes, were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (ThermoFisher Scientific) containing 10% fetal bovine serum (Bovogen Biologicals) and 1% L-glutamine (ThermoFisher Scientific)

within a humidified 5% CO₂, 37 °C incubator (Thermo Scientific) using the standard protocols. Jurkat cells, which grow in the culture medium, were centrifuged at 450×g for 5 min. After aspirating the supernatant, the cells pellet was resuspended gently in a pre-warmed CellTracker Green CMFDA solution (Product NO. C2925, CellTracker) and incubated for 30 min. Finally, the stained cells were centrifuged at 450×g for 5 min, diluted in the phosphate-buffered saline (PBS) to the desired concentration of $\sim 1 \times 10^5$ cells/ml, or spiked in whole blood to mimic the presence of WBCs. The cells were stained to facilitate observation of cell trajectory in the serpentine microchannel and counting of recovered cells. Whole blood was collected from human volunteers into lithium heparin tubes (Greiner Bio-One). Blood sample was diluted 20 times with PBS before being infused into the microfluidic device.

C. Experimental Setup and Method

The microfluidic devices were located on the stage of an inverted fluorescence microscope (CKX4, Olympus). A syringe pump (Legato 100, KD Scientific) was used to control the flow rate and infuse particle suspension and blood sample into the microfluidic devices. An high-speed CCD camera (Optimos, Q-imaging) mounted on the microscope recorded the trajectories of particles and cells, and the captured images were then post-processed and analyzed by a commercial image analysis software (Q-Capture Pro 7, Q-imaging). The experimental setup is shown in Fig. 1(a). The concentrations of polystyrene beads and blood cells were measured using a hemocytometer. The purity of polystyrene beads mixture collected from outlets after two cascaded processes was calculated based on three measurements. An LSR II flow cytometer (BD Biosciences) measured the Forward scatter (FSC) and side scatter (SSC) of blood samples before and after the process. FlowJo software (Tree Star) was used to analyze the data and evaluate the ratio of blood cell subpopulations.

III. THEORY AND MECHANISM

A. Inertial Migration

Particle inertial migration in straight channels is induced by the two inertial effects: the shear gradient lift force F_{LS} and the wall lift force F_{LW} . The shear gradient lift force F_{LS} originates from the parabolic fluid velocity profile and finite particle size, directing toward the channel wall. And the wall lift force F_{LW} arises when particles are near channel wall, always pointing to the channel centerline. The particle focusing positions are the locations where both inertial lift forces counteract each other. If the particle size is far smaller than the channel size, the net inertial lift force F_L can be expressed as following [22, 31]:

$$F_L = \frac{\rho_f U_m^2 a^4}{D_h^2} f_L(Re, z) \quad (1)$$

$$Re = \frac{\rho_f U_m D_h}{\mu} \quad (2)$$

where a is particle diameter, and D_h is the hydraulic diameter of the channel. ρ_f , μ and U_m are the fluid density, dynamic viscosity, and maximum velocity, respectively. $f_L(Re, z)$ is the dimensionless coefficient of the inertial lift force, which is a

function of the particle cross-sectional position z and channel Reynolds number Re [22].

Fig. 1. (a) A diagram of experimental setup. The microfluidic chip is placed on an inverted microscope which is mounted with a digital camera. A syringe pump is used to control and infuse particle/cell suspension into the microfluidic chip at a specific flow rate, and the trajectories of particles/cells are recorded by the digital CCD camera and analyzed by the Q-capture Prof 7 software. (b) Inertial focusing and separation of microparticles in the symmetric serpentine microchannel. (c) The inertial equilibrium position of 3- μ m and 10- μ m polystyrene beads under different flow Reynolds numbers in the serpentine microchannel. (d) Separation of 3- μ m (red) and 10- μ m (green) polystyrene beads by differential equilibrium positions.

B. Secondary Flow

In a curving channel, fluid momentum mismatch in the center and near-wall region within the curvature causes a pressure gradient along the radial direction, which subsequently induces a secondary flow [22]. The fluid elements near the channel centerline flow outwards due to a higher momentum than those near the wall, and push the relatively stagnant fluid elements near the wall inwards along the circumference, thus producing two counter-rotating streams, also called Dean vortex [22]. The strength of the secondary flow U_D is approximated as [32, 33]:

$$U_D = 1.8 \times 10^{-4} De^{1.63} \quad (3)$$

where the Dean number De is a function of the Reynolds number Re , the radius of the channel curvature R , and the hydraulic diameter of the channel D_h [34]:

$$De = \sqrt{\frac{D_h}{2R}} Re \quad (4)$$

For a particle moving at a speed of U_p , the Dean drag force F_D on the particle can be calculated using Stokes' drag law [35]:

$$F_D = 3\pi\mu a(U_D - U_p) \quad (5)$$

The additional lateral forces from the secondary flow induced by the channel curvature could assist and modify inertial focusing process and positions [36]. The particle focusing pattern in the microchannel (i.e. the position, number, and bandwidth of the particle positions) can be analyzed by comparing the relative strength of the secondary flow drag and the inertial lift force [22]. If the secondary flow in the serpentine channel is too weak to offset the inertial lift force, the focusing pattern is still dominated by the inertial lift force, and particles are normally ordered along the two sidewalls [37]. If the secondary flow is strong enough, it will modify the particle focusing positions. In such case, a single stream can be obtained in a symmetric serpentine channel [38]. Benefitting from the property that the relative strength of these forces is highly dependent on particle size, particle separation by size in the serpentine channel can be obtained by the overlap of the two different regions.

IV. RESULTS AND DISCUSSION

A. Inertial Focusing of Polystyrene Beads

Blood cells consist of three main classes: (i) leukocytes or WBCs, spherical cells 8-12 μm in diameter, and concentration is $\sim(5-10)\times 10^6$ counts/ml; (ii) erythrocytes or RBCs, discoid, anuclear cells ~ 8 μm in diameter and ~ 2.5 μm in thickness, and concentration is $\sim 5\times 10^9$ counts/ml; and (iii) platelets, discoid particles 1-3 μm in diameter, and concentration is $(2-5)\times 10^8$ counts/ml [18, 39, 40]. The significant size difference of WBCs with other blood cells makes the size-based separation of WBCs possible.

As a first step, the rigid spherical polystyrene beads with similar size to relevant blood cells were used to characterize the inertial focusing behavior (e.g., inertial equilibrium positions and distribution) in the symmetrical serpentine microchannel. Initially, particles are focused as two streaks along the sidewalls. Increasing Reynolds numbers from 10 to 200 (corresponding to flow rate of 50-1000 $\mu\text{l}/\text{min}$), particles migrate towards the channel centerline, and above a certain Re threshold, the large (10- μm) particles focusing streams merge as a single beam along channel centerline. In contrast, small particles (3- μm) still occupy the two sidewalls for a wider Re region, Fig. 1(c). Even above a threshold, no central focusing is observed but the defocusing emerges. Defocusing can be explained by the stirring effects of secondary flow on the small particles [38]. By employing the overlap of the central focusing status of large (10- μm) particles and the two-sided focusing status of small particles (3- μm), continuous and efficient separation of binary particles in the serpentine microchannel can be achieved, Fig. 1(b and d), which is the principle of WBCs separation in this work.

B. Inertial Separation of Polystyrene Beads

In whole blood, the concentration ratio of WBCs to RBCs can be as low as 1:1000, thus making efficient separation and purification of WBCs from whole blood a technical challenge. In order to characterize the separation performance of the

symmetric serpentine channel, we prepared polystyrene beads mixture (3- μm and 10- μm) with a similar concentration ratio and infused the particle mixtures into the microchannel at the flow rate of 600 $\mu\text{l}/\text{min}$ ($\text{Re}=120$). The concentrations of 3- μm and 10- μm particles are 4×10^7 counts/ml and 4×10^4 counts/ml, respectively. After the first process through the serpentine channel, the concentration of 10- μm particles from the central collection increased to 1.4×10^5 counts/ml, whereas the concentration of 3- μm particles decreased to 1.19×10^6 counts/ml, Fig. 2(b). The optical images in Fig. 2(a) clearly show significant depletion of 3- μm particles and enrichment of 10- μm particles. Therefore, the purity of 10- μm particles is enhanced from 0.1% to 10.59% after a process, resulting in an enrichment ratio of 100 times, Fig. 2(c).

Fig. 2. Separation performance of the proposed microchannel for 3- μm and 10- μm polystyrene beads. (a) Brightfield images of particle mixture at (i) inlet, and (ii) central outlet collection from the first process and (iii) central outlet collection from the second process. The input sample for the second process is the central outlet collection from the first process. (b) The concentration of particle mixture before and after each process. (c) The percentage of 3- μm and 10- μm polystyrene beads before and after each process. The portion of 10- μm polystyrene beads increased significantly from 0.102% to 80.31% after two cascaded processes.

After a second process using the central collection from the first one, the concentration of 10- μm particles is further raised to 4.35×10^5 counts/ml, with the purity improved to 80.3%, Fig. 2(b, c). Whereas, the concentration of small particles is decreased to 1.075×10^5 counts/ml, occupying only 19.7% of the total number of particles at the central outlet. In summary, the purity of 10- μm particles is increased from 0.1% to 80.3% after two cascaded processes, with an average purity enrichment ratio of about 28 in each circle. Meanwhile, the separation efficiency (or recovery ratio, defined as the number ratio of collected target particles to the input target particles) of 10- μm particles is more than 95% in each process.

C. Separation of Jurkat Cells Spiked In Blood Samples

Jurkat cells, which are an immortalized line of human T lymphocytes, were used to investigate the inertial focusing behavior of cells in the symmetric serpentine microchannel. Jurkat cells were cultured and stained using CellTracker Green CMFDA to allow visualization of cell trajectory, Fig. 3(a). The diameter of Jurkat cells is 14.5 ± 2.2 μm ($n=167$). Jurkat cell suspensions were infused into the symmetric serpentine

channel at flow rates from 200 $\mu\text{L}/\text{min}$ to 900 $\mu\text{L}/\text{min}$. Their focusing positions at the end of the serpentine channel were captured, Fig. 3b. We can see that the focusing phenomenon of Jurkat cells is similar to that of the rigid polystyrene beads observed above: the two-sided focusing streaks migrated toward the channel centerline with an increment of flow rate and finally merged as a single streak at the centerline. This can be further validated by the fluorescent profile, Fig. 3(c).

Fig. 3. Inertial focusing of Jurkat cells in the symmetrical serpentine microchannel. (a) Brightfield (top) and fluorescent (bottom) images of the stained Jurkat cells. (b) Inertial focusing positions of Jurkat cells at flow rates ranging from 200 $\mu\text{L}/\text{min}$ to 900 $\mu\text{L}/\text{min}$. (c) Fluorescent profiles of cell focusing stream at the end of the serpentine microchannel.

To mimic the whole blood and further evaluate the inertial focusing and separation of Jurkat cells in the presence of blood cells, the Jurkat cells were spiked into the blood in a number ratio of about 0.1% to mimic the behavior of WBCs in the whole blood. The brightfield and fluorescent images, which were for the better observation of RBCs and the stained Jurkat cells respectively, were composited to verify their distinguished focusing positions, Fig. 4(a). The RBCs (the dark streaks) are obviously focused along two sidewalls, whereas the Jurkat cells (the white streak) are concentrated tightly along the channel centerline. We also investigated the effects of flow rates on their focusing status, Fig. 4(b). The optimal flow rate was around 600 $\mu\text{L}/\text{min}$, when the flow rate was too low (400 $\mu\text{L}/\text{min}$), Jurkat cells could not focus tightly along the centerline,

and some exited from the up and bottom outlets. However, if the flow rate was too high (800 $\mu\text{L}/\text{min}$), the RBCs defocused and occupied a wide region of channel width due to the mixing effects of the secondary flow. In this case, large amount of RBCs will follow Jurkat cells, entering the central outlet.

Fig. 4. (a) Inertial focusing and separation of Jurkat cells spiked in the diluted ($\times 1/20$) whole blood. (b) Inertial focusing positions and distribution of stained Jurkat cells and blood cells at flow rates of (i) 400 $\mu\text{L}/\text{min}$, (ii) 600 $\mu\text{L}/\text{min}$, and (iii) 800 $\mu\text{L}/\text{min}$. (c) The optical images of Jurkat cells spiked in the blood under a hemocytometer before and after the process by the inertial microfluidic device. The dark points are blood cells, and the bright points are the stained Jurkat cells. The ratio of spiked Jurkat cells is increased by 11 times, from 0.1% to 1.1%.

At an optimal flow rate, the RBCs can be depleted and Jurkat cells can be concentrated significantly at the central outlet, Fig. 4(c). The ratio of spiked Jurkat cells in the blood is enhanced from 0.1% to 1.1% after a single process, with an enrichment ratio of 11 times. It is noted that this value is significantly lower than that of polystyrene beads, for which the enrichment ratio for the average enrichment ratio is 28 times. This is due to the fact that intensive cell-cell interactions in the blood sample deteriorate the cells focusing and separation performance. Although the blood sample was diluted $\times 1/20$ using PBS, the concentration of cells was still in the order of 10^8 counts/ml, which was one order of magnitude higher than that of polystyrene beads sample.

D. Separation of WBCs From The Whole Blood

From the above characterization of separation performance using polystyrene beads mixture and Jurkat cells spiked in blood, we found that the purity of target cells is unsatisfactory after single process, so a cascaded procedure that further processes the targeted central collection from the previous one is needed, Fig. 5(a). Meanwhile, the dilution of the whole blood sample is required, which will reduce the net flow rate, therefore a parallelization design to amplify the whole throughput is desired. Here, we designed and fabricated a parallelized device with eight parallel serpentine channels, Fig. 5(b). The flow rate of diluted ($\times 1/20$) whole blood infused into the device is 288 ml/h, equivalent to 14.4 ml/h for the undiluted blood. The throughput can be further enlarged by stacking several layers of microchannels along the height direction.

The input blood sample and central collections from two consecutive processes were tested by a flow cytometry, Fig. 5(c). The separation purity of WBCs increased to about 48%

after two processes, with an average enrichment ratio of about 10 times, which is sufficient for many clinical assays [8]. We observed that the separation purity of WBCs after each process was lower than that of polystyrene beads separation. In addition to the intensive cell-cell interaction as discussed above, a wide size distribution of WBCs, ranging from $\sim 8 \mu\text{m}$ in diameter (small T and B lymphocytes) to $\sim 11 \mu\text{m}$ (small granulocytes like neutrophils and eosinophils) and $\sim 14 \mu\text{m}$ and above for the largest subpopulations (large T and B lymphocytes, monocytes, and larger granulocytes like basophils) [14], may also contribute to the degradation of separation performance.

Fig. 5. (a) A schematic of cascaded separation and purification of white blood cells (WBCs) from whole blood. (b) A picture of the working inertial microfluidic device with eight parallel serpentine microchannels and optical images of the input blood sample and collections in the tubes after two cascaded processes. (c) Flow cytometry data indicate the percentage of white blood cells (WBCs), red blood cells (RBCs) and platelets (PLTs) numbers in the (i) whole blood sample, (ii) central collection after the first process, and (iii) central collection after the second process.

The proposed microfluidic device holds several advantages. First, there is no clogging issue in the proposed method compared to the filtration based methods [17, 19]. Second, compared with the reported inertial microfluidic systems, which process highly diluted blood sample ($\times 1/400$ - $\times 1/200$) [27, 28], the proposed microfluidic device can handle much less ($\times 1/20$) diluted blood sample. Third, the proposed methods can provide a high throughput. The equivalent throughput of the proposed device for the undiluted blood is $240 \mu\text{L}/\text{min}$, which is 30 times that of DEP microseparators [12], 48 times that of paramagnetic capture (PMC) mode microseparators [15], 14-1000 times that of cross flow filtrations [17-19], 1.6-800 times that of margination-based techniques [8, 21], and 9-66 times that of the state-of-art inertial microfluidic devices [27-29].

Table 1 summarizes the WBCs separation performance of different microfluidic methods. It should be noted that the shear

stress may affect the functionality and viability of WBCs in the separation process. In this work, WBCs are focusing at the center of microchannel where shear rate is minimum, with an order same as that in Kim's work [21], where WBCs are focused along one sidewall of the microchannel with the shear rate at $1.3 \times 10^4 \text{ s}^{-1}$. In their work, the shear rate did not impair the integrity and function of WBCs. Besides, the duration of WBCs in the microfluidic device is very short, only about 15 ms. Therefore, the effect of shear rate on the functionality and viability on WBCs is believed negligible. This can be further supported by our previous work using the same device for separation of neuron and glial cells [41].

Table 1 Comparison of different microfluidic methods on WBCs separation.

Methods	Sample	Flow rate	Purity	Efficiency	Refs
Dielectrophoresis	(1/6) diluted	$50 \mu\text{L}/\text{min}$	N/A	92.1%, 76.9%	[12]
Paramagnetic capture	Undiluted	$5 \mu\text{L}/\text{h}$	N/A	97.4%	[15]
Cross flow filtration	(1/10) diluted	$20 \mu\text{L}/\text{min}$	3.71%	N/A	[17]
	Undiluted	$1 \text{ mL}/\text{h}$	93.5%	27.4%	[19]
	(1/20) diluted	$40 \mu\text{L}/\text{min}$	$\sim 95\%$	NA	[20]
WBC margination	Undiluted	$18 \mu\text{L}/\text{h}$	5%	94%	[8]
	Undiluted	$150 \mu\text{L}/\text{min}$	$\sim 10\%$	80%	[21]
Inertial microfluidics	(1/400) diluted	$10.8 \text{ mL}/\text{min}$	91%	89.7%	[27]
	(1/500) diluted	$1.8 \text{ mL}/\text{min}$	N/A	95%	[28]
	(1/9) diluted	$900 \mu\text{L}/\text{min}$	N/A	98.4%	[29]
	(1/20) diluted	$288 \text{ mL}/\text{h}$	48.1%	$>90\%$	This work

In this work, we manually collected the central collection after the first process and infused into the same device to conduct the second round. It could also be carried out by connecting the central outlet of the first device with the input of the second one in a cascaded way to avoid human intervention. In this case, a sheath flow or a delicate outlet design is required to adjust the flow rate and stream division in each serpentine microchannel, ensuring every channel working at the optimal status.

V. CONCLUSIONS

In this work, we developed a continuous and high-throughput microfluidic platform for WBCs separation utilizing the symmetric serpentine microchannel, which provides differential inertial focusing of WBCs and RBCs along the lateral direction. Inertial focusing and separation of polystyrene beads and mammalian cells in the serpentine channel were conducted to characterize separation performance of the channel. The purity of $10\text{-}\mu\text{m}$ polystyrene beads was increased from 0.1% to 80.3% from after two processes, with the average purity enrichment ratio of 28 times. The purity of WBCs from the whole blood was increased to 48% after two consecutive processes, with an average enrichment ratio of 10 times. Also, we designed and fabricated a parallelized inertial microfluidic device, which can provide a high processing flow rate of $288 \text{ mL}/\text{h}$ for the diluted ($\times 1/20$) whole blood, equivalent to $14.4 \text{ mL}/\text{h}$ for the undiluted whole blood. The proposed

inertial microfluidic device can be applied to the high volume blood sample preparation and analysis in the upstream of an integrated microfluidic system.

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