CONTINUOUS RBC REMOVAL USING SPIRAL MICROCHANNEL WITH TRAPEZOID CROSS-SECTION

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ABSTRACT

Size-based inertial microfluidic sorters have been proposed to be an alternative blood cell separation technique, since they minimize the artificial alteration on cellular phenotypes by eliminating the long-term exposure of sensitive blood cells in nonphysiological conditions. However, the available separation resolution and the cell dispersion issue have been limiting the direct application of the technique to real-world samples. In this work, we demonstrated a novel design of spiral microchannel with higher separation resolution and its ability in isolating leukocytes from human blood with high accuracy for up to 2% hematocrit samples with negligible effect on activation profile of sorted cells.

KEYWORD

RBC removal, inertial microfluidics, spiral, trapezoid cross-section

INTRODUCTION

Red blood cells (RBC) or erythrocytes are the most abundant cell component in many biological fluids, including blood (where it makes up ~45% of the volume), bone marrow aspirate and peritoneal aspirate. Depletion of contaminating RBCs from those samples is often an indispensable sample preparation step before the application of any scientific, clinical and diagnostic tests [1], while avoiding artificial alteration on the phenotypes of sorted cells is an important criterion for all studies. This is especially important in the case of removing RBCs from human blood to isolate leukocytes, which play a key role in carrying out and mediating the immune response to various pathogens. The information extracted from the isolated leukocytes would be meaningful to facilitate disease prognosis only when the key features of leukocytes' original state are not masked by the sample preparation artifacts. However, several cases have been reported that the conventional methodologies for blood cell separation on the macroscale, including differential centrifugation and selective erythrocyte lysis, could result in altered imuno-phenotype [2, 3] or impaired viability [4, 5] of the isolated white blood cells (WBCs). Meanwhile, passive continuous microfluidic separation techniques utilizing the size-dependent hydrodynamic effects [6-8] have been considered as an alternative approach to bypass the issues associated with macroscale blood cell separation methods. In this work, we improved the separation resolution of curvilinear microchannel while maintaining the high-throughput feature by modifying the channel cross-section to be trapezoidal rather than rectangular, and demonstrated its ability for efficient RBC depletion from human blood sample with negligible effect on PMN immune-phenotype as compared to selective erythrocyte lysis method.

DESIGN PRINCIPLE

One major challenge of utilizing spiral microchannel in blood cell separation lies in the limited separation resolution and capacity of holding vast number of RBCs without affecting the separation efficiency. To accommodate the samples with higher hematocrit, we need to increase the spacing between equilibrium positions. Our approach is to modify the spiral microchannel cross-section into a trapezoid with higher channel depth on the outer channel wall (Figure 1A). The asymmetry of trapezoid cross-section alters the shape of velocity field and results in formation of strong Dean vortex cores skewed towards the outer wall with larger channel depth even at relatively low flow rates. Therefore, while in spiral with rectangular cross-section the interplay between inertial lift force and Dean drag force leads to the focusing of large particles close to the inner wall and the trapping of small particles at the core of Dean vortices located at the center of channel width, the modified velocity field of spiral with trapezoid cross-section leads to a greater shift for small particles towards the outer wall without affecting the focusing position of large particles, thus providing a greater difference in equilibrium positions between them, resulting in higher separation resolution (Figure 1B-E). The trapezoid cross-section also has an impact on the size- and flow-rate-dependence of particle focusing. In a rectangular cross-sectional spiral, particles with $a_p/D_h \geq 0.07$ (a_p and D_h indicates particle diameter and microchannel hydraulic diameter, respectively) initially focus near the inner channel wall at low Re_c (Channel Reynolds number), and then move towards the outer wall as Re_c increases. When Re_c is sufficiently high, Dean drag force dominates the particle behavior leading to defocusing of particles. Interestingly, while the particle behavior of trapezoid cross-section spiral displays a similar focusing-defocusing dependence on Re_c, an additional regime featured by the trapping of particles within the outer half of channel cross-section was observed when Re_c increased further (Figure 2). Moreover, the flow rate required to trap particles increases with particle size, making the isolation of particles within a specific size range feasible. Moreover, the trapping location is independent of the particle size and remains constant for all the particles tested in this work. The exact mechanism of particle trapping under high Re_c remains elusive.

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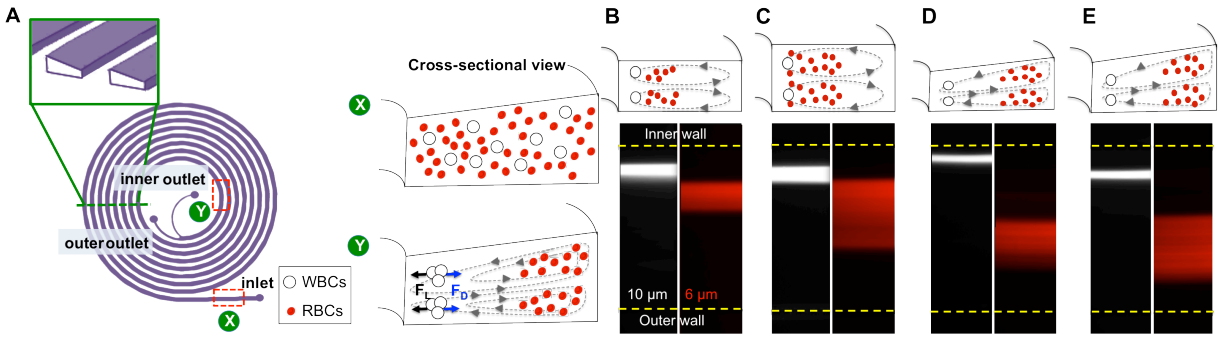

Figure 1 *Design principle of spiral channel with trapezoid cross-section. (**A**) Schematic (not to scale) of spiral channel with trapezoid cross-section illustrating the operation principle. Schematic (not to scale) and average composite fluorescent images indicating the inertial focusing of 10 μm (white) and 6 μm (red) beads in (**B**) spiral channel with rectangular cross-section of 500 μm × 90 μm (W×H) under optimal flow rate: 1 ml/min (Dean number, $De = 4.31$); (**C**) spiral channel with rectangular cross-section of 500 μm × 120 μm under optimal flow rate: 2 ml/min ($De = 8.63$); (**D**) spiral channel with trapezoid cross-section of 500 μm width, 70 μm (inner) and 100 μm (outer) depth under optimal flow rate: 0.8 ml/min ($De = 4.22$).(**E**) spiral channel with trapezoid cross-section of 500 μm width, 90 μm (inner) and 120 μm (outer) depth under optimal flow rate: 0.8 ml/min ($De = 4.32$).*

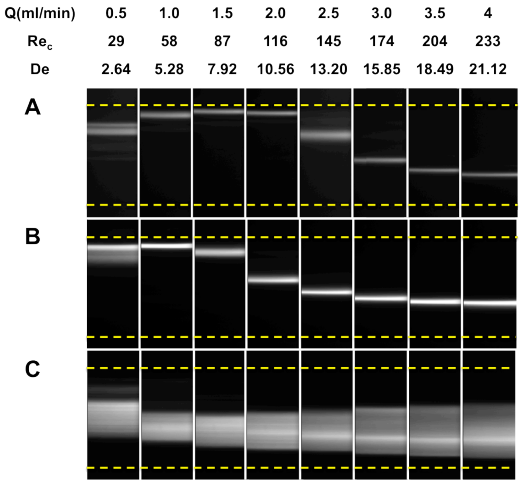

Figure 2 *Top-down view images demonstrating the focusing behavior of fluorescent particles as a function of flow rate (Q) inside spiral channel with trapezoid cross-section of 500 μm width, 70 μm (inner) and 100 μm (outer) depth. (**A**) 15.5 μm particles; (**B**) 10 μm particles; (**C**) 6 μm particles. Yellow lines indicate the position of channel walls, while the inner channel walls were shown on the top side of the images.*

DEVICE PERFORMANCE ON HUMAN BLOOD SAMPLE

The optimized PDMS device for RBC removal developed consists of a 1-inlet, 2-outlet spiral microchannel with trapezoid cross-section of 500 μm width (485.00 μm ± 2.31 μm), 70 μm (inner wall, 72.84 μm± 1.16 μm) and 100 μm (outer wall, 102.65 μm ± 3.55 μm) depth. Near the outlet region, the 485 μm wide channel was split into two outlet channels with a channel width ratio of 3 : 7 (inner : outer), while their channel lengths were adjusted to be equal with each other. We defined the inner outlet to be the WBC outlet with RBC-depleted sample and the outer outlet to be the RBC waste. The optimal flow rate was experimentally determined to be 0.8 mL/min ($Re_c = 46.52; De = 4.22$). PMNs and MNLs isolated via centrifugation using Moly-Poly Resolving Medium (MP-RM) were injected through our device separately to determine their equilibrium positions inside the channel (Figure 3A). The optimal performance was achieved for 0.5% hematocrit blood sample with ~95% RBC removal and 98.4% of total WBC recovery (99.4% PMN recovery and 92.4% MNL recovery) after a single pass (Figure 3B). Further increase in input sample hematocrit would cause a decrease in both RBC removal and MNLs recovery but the total WBC recovery and PMN recovery remained relatively stable. Up to 1.5% hematocrit, the device can still achieve 86.8% RBC removal and 96.2% of total WBC recovery. A 2-stage process, where the output sample from WBC outlet of the 1st run was used as the input of 2nd run without any dilution, can be fashioned to achieve high RBC removal while maintaining good WBC recovery for 1%~1.5% hematocrit sample (Figure 3C, D). In addition, given the high sensitivity of white blood cells to external stimuli, we compared the effect of different RBC removal techniques on the expression level of cell surface marker, CD18, which is a classical activation marker for PMNs. As shown in Figure 3E, both spiral process and centrifugation using MP-RM had negligible effect on PMN activation, whereas the RBC lysis method increased the percentage of activated PMNs significantly, questioning the validity of information extracted from WBCs isolated via selective erythrocyte lysis method.

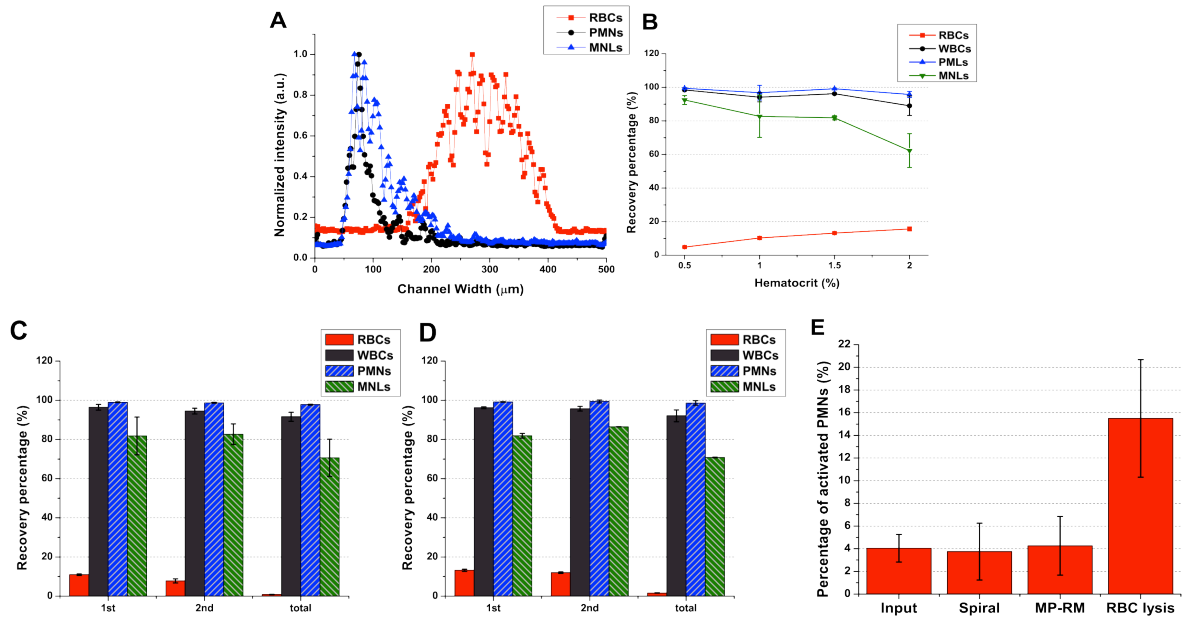

Figure 3 *Characterization of blood cells in spiral channel with trapezoid cross-section. (**A**) Normalized intensity line scan indicating the distribution of polymorphonuclear leukocytes (PMLs), mononuclear leukocytes (MNLs) and RBCs (0.1% hematocrit) across channel width at 0.8 ml/min. (**B**) Single-pass recovery percentage of total WBCs, PMNs, MNLs and RBCs at different hematocrit. Recovery percentage of 1% hematocrit (**C**) and 1.5% hematocrit (**D**) input sample after processed by trapezoid cross-sectional spiral in a 2-stage cascade manner. (**E**) Comparison of PML activation by spiral and other RBC removal techniques, such as density centrifugation (MP-RM) and RBC lysis, based on FACS analysis of CD66b+ CD18 + cells. Error bars represent standard deviation of results from three tests.*

CONCLUSIONS

In this work, we developed a novel high throughput RBC removal technique using trapezoid cross-sectional spiral, which provides higher resolution separation as compared to rectangular cross-section with similar dimensions and showed no effect in activation profile of isolated PMNs. While many other size-based separation methods, especially those with membranes or pillars, have limited operation times and low throughput due to the clogging issues, our device works in a clogging-free continuous mode. Besides, compared to other types of continuous cell separation methods, such as "deterministic lateral displacement" [7] and pinched flow fractionation [8] techniques, our spiral microchannel functions at high operation flow rate (~mL/min) with large channel dimension accommodating the abundant RBCs (up to ~2% hematocrit), and thus possesses high throughput and is amenable to process blood samples. We envision that the novel trapezoid cross-sectional spiral microchannel developed can be used as a generic, high-throughput method for removing erythrocytes and enriching target cells from other biological fluids, such as in the harvesting mesenchymal stem cells (MSCs) from bone marrow aspirates.

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