SORTING OF BLOOD IN SPIRAL MICROCHANNELS

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ABSTRACT

In this work, we demonstrate continuous sorting of blood in a simple passive microfluidic device. Our approach takes advantage of the principles of inertial microfluidics and Dean drag forces in spiral microchannels. We report successful isolation of plasma and separation of blood cells with high throughput (1-2mL/min) and high separation efficiency (>90%). The developed approach caters to the need for blood cell sorting devices that can be integrated with an on-chip analysis system and rapidly provide separated sample with high purity.

KEYWORDS: Inertial microfluidics, cell-sorting, blood, dilution, spiral microchannels

INTRODUCTION

Each component of blood provides significant information for both diagnostics and therapeutics. The conventional tools for cell-sorting need expensive equipment and trained professionals, and are not compatible for on-chip and point-of-care devices for blood analysis. Inertial microfluidics offers a potential solution to both cost and labor intensive nature of blood analysis, along with a reagent-free method of separation and diagnosis.

In this work, we optimized the spiral inertial microfluidic devices we developed previously [3] and targeted two applications critical to blood analysis. The first was the extraction of cell-free plasma from blood, while the second was the sorting of RBCs and WBCs. Both of these applications are commonly used in clinical blood analyses and are generally performed by centrifugation.

DEVICE PRINCIPLE

Our device utilizes the balance of hydrodynamic forces acting on cells within laminar flow, and rotational Dean drag due to spiral microchannel geometry to focus the cells in streams near the inner channel wall (Fig.1b) [3,4]. In straight rectangular channels, the shear induced lift force and the wall induced lift force act on neutrally buoyant particles, focusing the particles in four positions along the channel periphery depending on their size.

In a spiral rectangular microchannel, the counter-rotating Dean vortices, induced due to the curvature, disrupt the balance of the inertial forces at the four focusing positions and the particles refocus in a single stream near the inner channel wall, with the largest particle focusing closest to the inner channel wall (Fig.1b). The single focusing position makes the size- based sorting and extraction of multiple cell-lines easier. The net Dean Force (F_D) is given by

$$F_D = 3\pi\mu U_{Dean} a_p \quad (1)$$

where U_{Dean} is the average Dean velocity, a_p is the diameter of the particle and μ is the viscosity of the fluid. The ratio of the net lift force and the dean force is highly dependent on the particle size (a_p^3) and this dependence can be used for size-based cell-sorting with appropriate outlet system to extract the sorted cells. While the lift forces are dependent on the Newtonian nature of the fluid, the Dean vortices can form in viscous fluids too. This differential effect of blood rheology

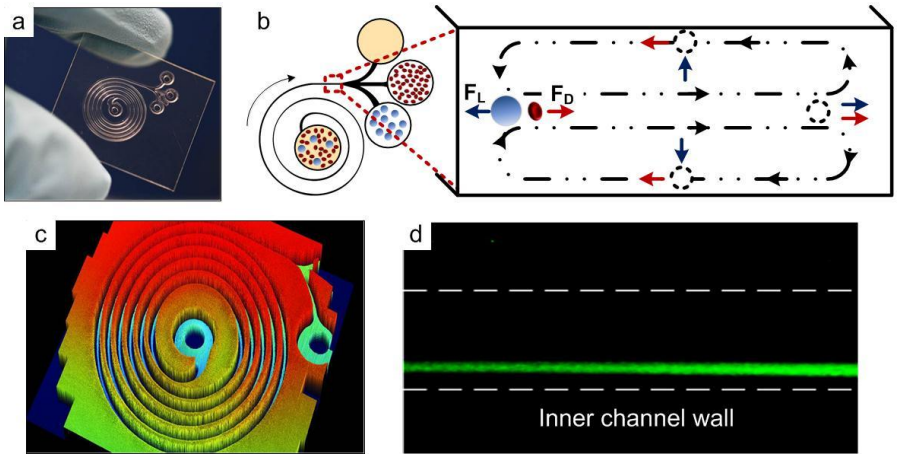

Figure 1. *(a) Image of a PMMA inertial device. (b) Schematic describing the focusing of blood-cells due to the balance of inertial lift forces (F_L) and Dean drag force (F_D). (c) Optical profile scan of the PMMA device to confirm the height of the channel across the whole device. (d) Fluorescent image of the focused 20μm particles near the inner channel wall close to the outlet.*

impacts sorting efficiency and throughput in spiral microchannels. Therefore, we first evaluated the optimal dilution for sorting blood cells.

RESULTS AND DISCUSSION

Devices for this work were fabricated by roll-by-roll embossing of PMMA [5] or standard PDMS soft lithography. For PMMA devices, ports were connected with UV-cured epoxy (Loctite). A ferrule and tubing were bonded to ports to prevent leakage. PMMA devices were found to have quite uniform height (±3 µm) across the entire device (Fig.1c), and their performance was generally identical to that of PDMS devices. Due to their uniformity and higher robustness, the PMMA devices replaced the PDMS devices, which were used in the pilot experiments. The devices used for plasma and cell-separation consisted of a 4-loop Archimedean spiral with three outlets and channel dimensions of 250µm×75µm. The devices used for sorting RBCs and WBCs consisted of a 6-loop Archimedean spiral with four outlets and channel dimensions of 500µm×100µm.

Blood is a non-Newtonian fluid and its viscosity is highly dependent on hematocrit, plasma protein concentration, platelet count, and leukocyte count [1]. Plasma concentration also affects cell-to-cell interactions in blood [2]. Considering that inertial microfluidic devices rely on hydrodynamic forces acting in a Newtonian, Poiseuille flow, separating blood components presents a challenge. Sample dilution can reduce these non-Newtonian effects. To investigate the effects of blood dilution on sorting efficiency and determine the optimum dilution needed to achieve both high throughput and high efficiency of blood-cell sorting in spiral microchannels, whole blood from a human-male donor was diluted 10-700× with 0.9% saline. At each dilution, sorting efficiency was assessed, at flow rates in the 0.1-3mL/min range, by collecting and staining the blood cells with Wright-Giemsa stain (Fig.2b).

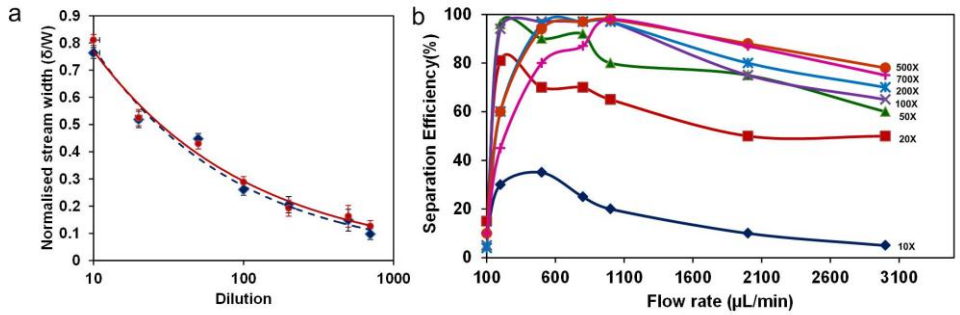

Figure 2. *(a) The log-plot shows the trend of focusing in both the devices at the flow rate of 1mL/min (δ is the width of the focused stream of cells and W is the width of the channel). The cells focus in a tighter stream as the dilution is increased. (b) Plot illustrating the effect of blood dilution on sorting efficiency of the plasma and blood-cells.*

Our results show that the optimal flow rate for sorting is dependent on sample dilution and thus controls the throughput of the device (Fig.2a). The width of the focused stream of cells was measured at each dilution to determine the precision of focusing. At lower dilution, the sheer concentration of cells overwhelmed the focusing positions, thereby disrupting the adjacent focusing positions and affecting the efficiency of separation. Since, Dean Vortices act strongly even in viscous fluids, obtaining cell-free plasma was possible at lower dilution. But, for sorting blood-cells, we needed the inertial lift forces to be strong enough to control the focusing the positions of the cells depending on their diameter. Therefore, it was necessary to use a higher dilution sample for better efficiency (Fig.2b).

We successfully demonstrated separation of plasma at the flow rate of ~1mL/min (Fig.3) and separation of blood cells at the flow rate of ~2mL/min (Fig.4). From these results, it was evident that for plasma and cell-separation, >90% separation was possible at 50× dilution, but for sorting RBCs and WBCs, at least 500× dilution was required for >90% separation efficiency. These results indicate that although the Dean vortices are strong enough to regulate the flow

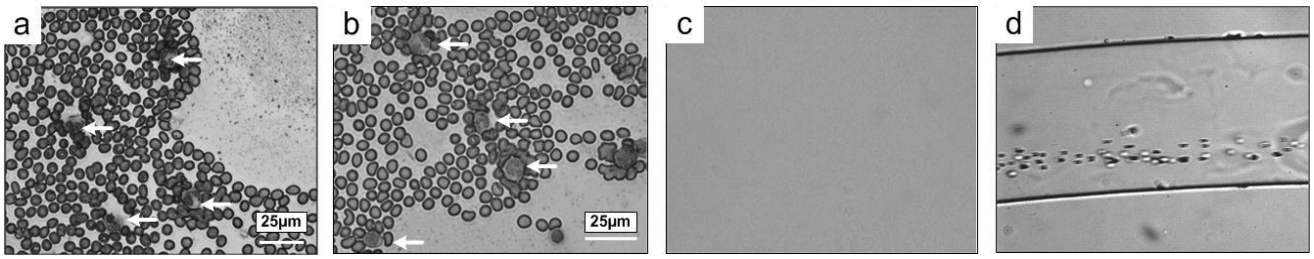

Figure 3. *(a) Bright field image of the stained blood cells at the input. The arrows indicate the WBCs in the sample. (b) Sample collected at the first and second outlets (RBCs, WBCs and platelets are present). (c) Sample collected at the third outlet (no cells present, only plasma). (d) Bright field image of the cells focused closer to the inner channel wall at 50× dilution and 0.8mL/min flow rate in the first device (250µm×75µm).*

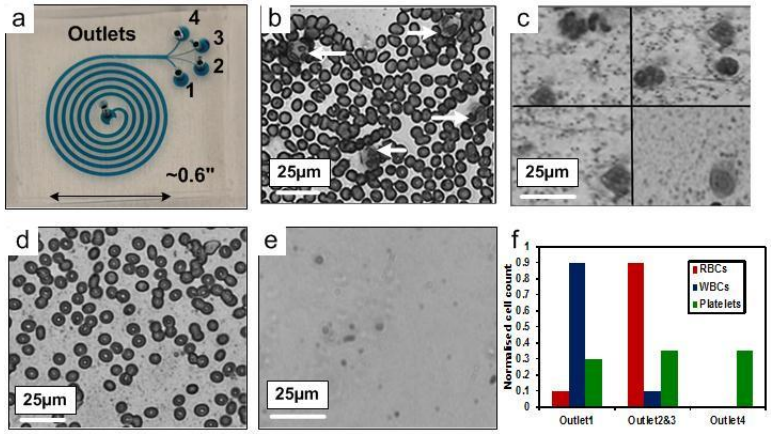

Figure 4*. (a) Image of the second device used for sorting RBCs and WBCs at 500× dilution and 2mL/min flow rate. (b) stained cells at the inlet.(WBCs are indicated by arrows). (c)Stained cells collected at outlet1(mostly WBCs are present). (d)Stained RBCs collected at outlet2&3. (e) Stained platelets collected at outlet4. (f)Results of hemocytometer count.*

of blood cells towards the inner channel wall to cause plasma and blood cell separation, strong inertial lift forces are required to specifically separate the RBCs ad WBCs. This also requires blood dilution and flow rate to be large enough where the forces can act effectively on the cells without the cell-to-cell interaction and viscosity of plasma interfering in the process.

We were able to achieve complete cell-free plasma, although it was difficult to sort out platelets from blood cells (Fig.4c-e). Since, platelets do not satisfy the condition, $a_p/D_h>0.07$, the inertial lift forces were not strong enough to focus them in a single stream and the platelets rotated along the dean vortices, eluting in all the four outlets. The hemocytometer results show a >90% sorting efficiency for separation of WBCs from RBCs. This is a higher rate of sample purity as there is no contamination due to sedimentation based lysing that is usually observed in centrifugation methods of separation.

CONCLUSIONS

This work not only demonstrates the use of spiral microchannels for sorting blood, but also the effect of rheology of blood on their sorting ability. These devices address the tradeoff between efficiency and throughput which has been faced by earlier passive microfluidic devices based on deterministic lateral displacement and pinched flow fractionation. This sorting technique is label-free and does not require specialized steps or instrumentation. The devices are small and efficient, and can be easily integrated with on-chip sample preparation systems paving way for point-of-care blood analysis.

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