LABEL-FREE LATERAL MAGNETO-DIELECTROPHORETIC MICROSEPARATION METHOD FOR SEPARATING NUCLEATED CELLS FROM PERIPHERAL BLOOD

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ABSTRACT

We present a label-free continuous lateral magneto-dielectrophoretic (MAP-DEP) microseparator for highly efficient enrichment of circulating nucleated cells from peripheral blood, based on native magnetic and dielectric properties of blood cells. A nickel ferromagnetic wire array inlaid on bottom of the microchannel creates lateral magnetophoretic (MAP) force acting on red blood cells (RBCs). A planar interdigitated electrode array patterned on top generates lateral dielectrophoretic (DEP) force acting on white blood cells (WBCs). The lateral MAP and DEP forces are opposite direction each other on the whole area of the microchannel, thereby improving the separation efficiency. Experimental results showed that 96.7% WBCs and 3.3% RBCs were collected from an outlet, which means that the present lateral MAP-DEP microseparator can continuously enrich nucleated cells from peripheral blood in a high separation efficiency.

KEYWORDS: Blood, Cell Separation, Label-Free, Magneto-Dielectrophoresis

INTRODUCTION

The lateral MAP and DEP microseparators [1, 2] based on native magnetic and dielectric properties of blood cells were hard to totally separate RBCs and WBCs from peripheral blood because WBCs have been separated by diffusion from a high-density stream of RBCs, although they were forced in the same direction with MAP / DEP forces acting on RBCs. Thus, the lateral MAP and DEP microseparators required long time to separate WBCs from peripheral blood and their separation efficiency was moderate. To overwhelm the limitations and obtain high enrichment efficiency, we propose a continuous lateral MAP-DEP microseparator, which simultaneously generates opposite directional lateral MAP and DEP forces, acting on RBCs and WBCs, respectively.

THEORY

The lateral MAP-DEP microseparator consists of sample and buffer inlets and two outlets (Fig. 1A). Additionally, it includes a ferromagnetic wire array inlaid on the bottom of the microchannel and a planar interdigitated electrode array patterned on the top (Fig. 1B), that they are formed in symmetry to each other with respect to the direction of flow. When blood cells injected through the sample inlet pass over the ferromagnetic wire array at angle and the interdigitated electrode array at another angle, RBCs and WBCs are forced towards the bottom of the microchannel and forced laterally in the opposite direction to each other (Fig. 1A). RBCs that settle at the bottom are primarily affected by the MAP force and are moved to one sidewall of the microchannel, thereby flowing into outlet #1. In contrast, WBCs settling at the bottom are affected primarily by the DEP force and are driven to the other sidewall of the microchannel, thereby flowing into outlet #2. Although the MAP force acting on RBCs and the DEP force acting on WBCs interfere somewhat because the directions of their lateral forces are opposite to each other, RBCs and WBCs are driven primarily by the MAP and DEP forces, respectively. Consequently, because the MAP and DEP forces are generated evenly over the whole area of the microchannel, they can be used for separating RBCs and WBCs in the present lateral MAP-DEP microseparator, with the inlaid ferromagnetic wire array and planar interdigitated electrode array; it potentially has greater separation efficiency than the previously reported lateral MAP [1] and DEP microseparators [2].

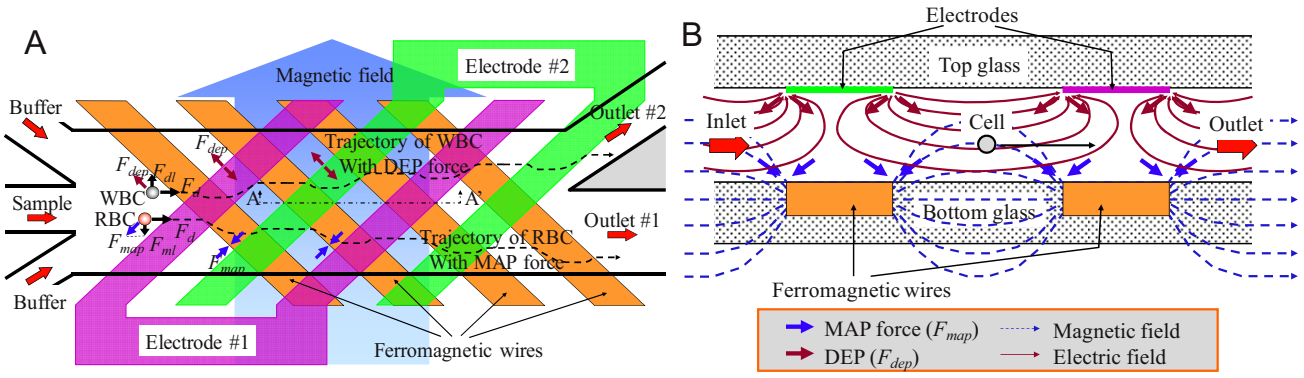

Figure 1: Schematic of the lateral displacement and MAP and DEP forces on blood cells passing through the microchannel. (A) Top view of the lateral MAP-DEP microseparator and (B) cross-sectional view along the A-A' cross section in Fig. 1(A)

EXPERIMENTAL

The instrument setup for the lateral MAP-DEP microseparator included two permanent magnets to generate an external magnetic flux of 0.3 T and used a 2-MHz sinusoidal voltage of 4 Vp-p to create the DEP force acting on blood cells. We used two syringe pumps, which provide controlled flow of the sample and buffer through the microchannel. The syringes were connected to the inlets through capillary tubing to push the diluted blood sample and buffer into the microchannel. A deoxyhemoglobin blood sample was prepared from human whole blood, diluted to a ratio of 1:5, using 3 mM isotonic sodium hydrosulfite solution.

The lateral MAP-DEP microseparator consisted of a microchannel, defined by the SU-8 photoresist, between two glass slides 1.1 mm thick. To form grooves for the ferromagnetic wires, the bottom glass was etched at a depth of 30 μm. Then, a permalloy ($Ni_{0.8}Fe_{0.2}$) film 40 μm thick was electroplated onto the bottom glass, and the ferromagnetic wire array was formed and inlaid on the bottom glass using a chemical-mechanical nickel polishing technique. Next, SU-8 2050 photoresist was spun and patterned to define the microchannel 30 μm thick. The interdigitated electrodes were made of Cr/Au (200 Å/ 2000 Å) evaporated and patterned on the top glass. The top glass was then bonded to the bottom glass using ultraviolet (UV) adhesive. A plastic microfluidic interface, fabricated by stereolithography, was assembled on the glass chip by UV adhesive bonding. Finally, to reduce adherence of the blood cells to the microchannel walls, the surface of the microchannel was coated with a surfactant. Figure 2 shows the fabricated lateral MAP-DEP microseparator on two permanent magnets and an enlarged view of the separation point with the two outlets.

RESULTS AND DISCUSSION

Without both the lateral MAP and DEP forces, RBCs and WBCs passing through the microchannel are uniformly distributed and flowed into the same direction of flow (Fig. 3A). When both the lateral MAP and DEP forces are applied in simultaneous, RBCs are mainly driven by the lateral MAP force caused by the ferromagnetic wires and flowed into outlet #1. In contrast, WBCs are mainly forced by the lateral DEP force generated by the interdigitated electrodes and laterally driven to the opposite direction that RBCs migrate. As a result, WBCs are continuously separated into outlet #2 (Fig. 3B). The experimental results show that percentages of RBCs and WBCs collected from outlet #2 were 3.3% and 96.7%, respectively (Fig. 4). Consequently, the lateral MAP-DEP microseparator facilitates the enrichment of nucleated cells from peripheral blood in applications such as a genomic sample preparation and diagnosis of blood borne disease.

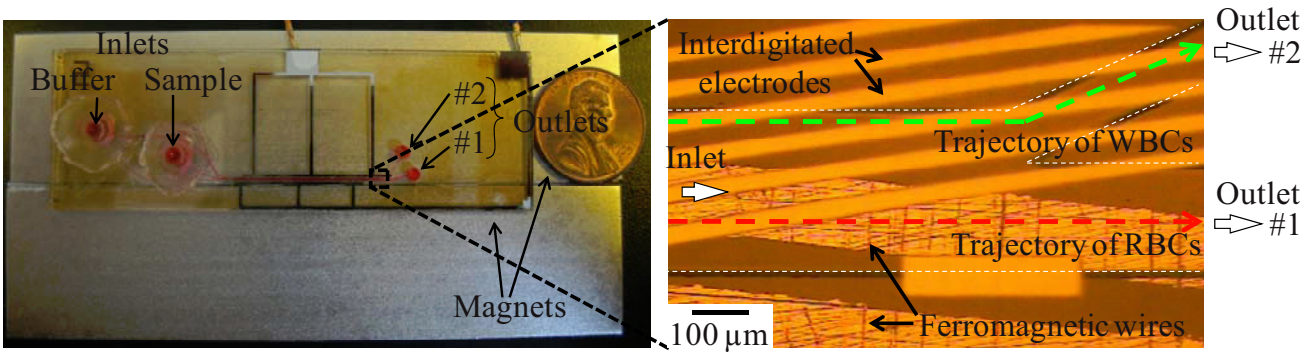

Figure 2: Photographs of the fabricated lateral MAP-DEP microseparator placed on a permanent magnet and enlarged view of the separation point with the two outlet channels

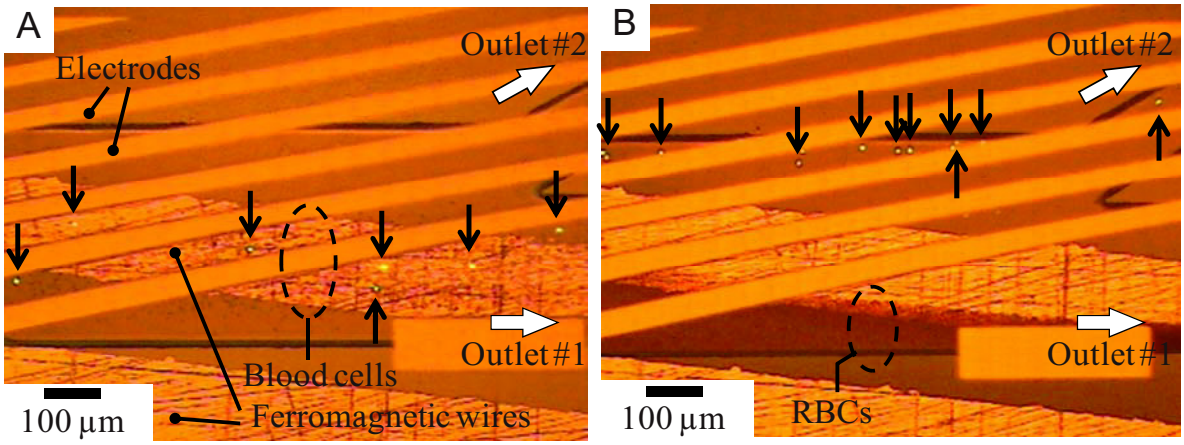

Figure 3: RBCs and fluorescent-dyed WBCs passing through the microchannel of the lateral MAP-DEP microseparator at a volumetric flow rate of 8 μl/h (A) without both the lateral MAP and DEP forces, and (B) with both the lateral MAP and DEP forces. The arrows (↑) indicate fluorescent-dyed WBCs.

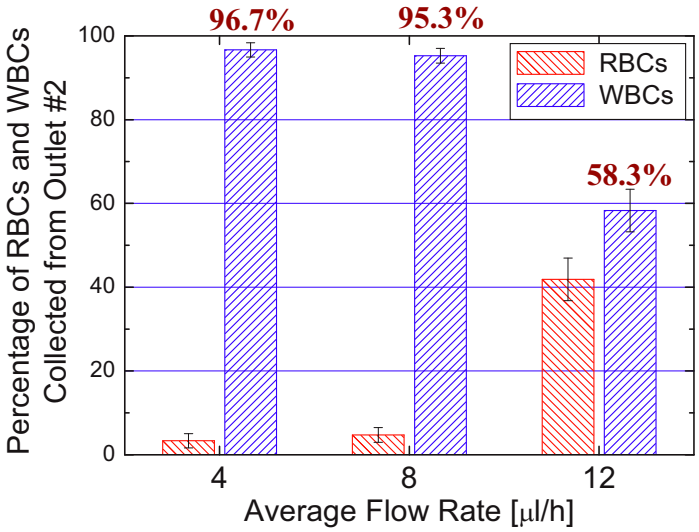

Figure 4: Percentages of RBCs and WBCs collected from outlet #2.

CONCLUSION

In this study, we designed, developed, and characterized a lateral MAP-DEP microseparator for the highly efficient enrichment of nucleated cells from peripheral blood, based on the native magnetic and dielectric properties of blood cells. Using a ferromagnetic wire array inlaid in the bottom of the microchannel and placed at an angle to the direction of flow, a lateral MAP force acting on blood cells was generated over the whole area of the microchannel. Using the planar interdigitated electrode array patterned on the top of the microchannel and placed at another angle to the direction of flow, a lateral DEP force acting on blood cells was generated simultaneously with the lateral MAP force. Using the ferromagnetic wire array and the interdigitated electrode array formed symmetrically to each other with respect to the direction of flow, the lateral MAP and DEP forces were generated in opposite directions to each other. The experimental results showed that due to the opposite direction of the lateral MAP force and the lateral DEP force, the percentages of WBCs and RBCs collected from outlet #2 at a flow rate of 4 μl/h were 96.7% and 3.3%, respectively. Thus, the lateral MAP-DEP microseparator could achieve highly efficient enrichment of nucleated cells from peripheral blood.

In contrast to MACS and FACS, which require magnetic and/or fluorescent tagging materials, the present lateral MAP-DEP microseparator uses only the native magnetic and dielectric properties of blood cells, and therefore eliminates the need for laborious sample preparation procedures before and after enrichment, is easy to use, and has reduced cost. Furthermore, the label-free enrichment technique allows the present microseparator to be readily incorporated with fully automated on-chip genetic preparation systems. Consequently, the future development of a high-precision lateral MAP-DEP microseparator may facilitate the enrichment of rare nucleated cells (*e.g.*, circulating tumor cells, regulatory T-cells, and peripheral dendritic cells) from peripheral blood in applications such as early diagnosis of cancer, genetic sample preparation, and blood-borne disease detection.

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