A Microfluidic System for Continuous Magnetophoretic Separation of Suspended Cells Using Their Native Magnetic Properties

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ABSTRACT

This paper presents the characterization of a continuous magnetophoretic microseparator for separating white and red blood cells from whole blood by using a high gradient magnetic separation method and microfabrication technology. The magnetophoretic microseparator directly separates blood cells based on their native magnetic properties without the use of additives such as magnetic tagging or inducing materials. Experimental results show that the diamagnetic capture mode microseparator can continuously separate out 89.7 % of red blood cells and 72.7 % of white blood cells, and the three-stage cascade paramagnetic capture mode microseparator can continuously separate out 93.5 % of red blood cells and 97.4 % of white blood cells from whole blood by applying an external magnetic flux of 0.2 T using a permanent magnet.

Keywords: cell separator, diamagnetic capture mode, high gradient magnetic separation, magnetophoresis, paramagnetic capture mode

1 INTRODUCTION

Conventional magnetic separators [1-2] based on the high gradient magnetic separation (HGMS) method have been used for separating heavy metals, slightly magnetic radioactive particles, and for water purification. Additional research [3-4] has focused on magnetophoretic macroseparators using the HGMS method for separating biological cells because of its benefits, such as the capacity to produce a large separation force with simple device structures, low cost, ease to use, and the non-hydrolytic nature of magnetic fields. However, since the magnetic susceptibility of native biological cells usually is not large or specific enough to separate subpopulations, magnetic cell separation using magnetic beads has become the most common method used for separating biological cells.

On the other hand, much research [5-6], with a focus on the native magnetic properties of biological cells, has reported that deoxyhemoglobin red blood cells (RBCs) in whole blood are paramagnetic particles. According to these literatures, the relative magnetic susceptibility of the deoxyhemoglobin RBCs in plasma is about 3.9×10^{-6} (SI), which is much larger than that of other biological cells. Although the magnetic susceptibility of white blood cells (WBCs) are rarely reported, Takayasu et al. [7] reported that WBCs behave like diamagnetic particles in water. Therefore, based on the inherent magnetic properties of blood cells, some research [8-9] has focused on developing magnetophoretic macroseparators for blood cells. However, the conventional macro scale magnetophoretic separators, characterized by centimeter to millimeter scale dimensions, have the capability to generate relatively small magnetic flux gradients on biological cells. This fact, combined with the inherently small magnetic susceptibilities of blood cells, has led to limited success with macro scale systems. To overcome the low magnetic forces on biological cells and to take advantage of the geometrical scaling advantages of miniaturization, our group has previously proposed a high gradient magnetophoretic microseparator [10] fabricated by microfabrication technology. In our previous work, the theoretical model for calculating the magnetic forces produced by the magnetophoretic microseparator was derived and verified by comparison with finite element analysis using commercially available software, ANSYS.

This paper presents the design, fabrication, and characterization of a continuous magnetophoretic microseparator for separating blood cells based on their native magnetic properties. The magnetophoretic microseparator was designed for available to both diamagnetic capture mode (DMC) and paramagnetic capture mode (PMC) based on our previously developed general theoretical model [10] of the magnetophoretic microseparator. In the experimental results, quantitative measurements of the relative separation percentages of RBCs and WBCs flowing into each outlet are reported for both DMC and PMC modes and compared with theoretically estimated results.

2 DESIGN

Magnetophoretic microseparator consists of one inlet and three outlets, which were numbered #1 to #3 from left to right, and includes a ferromagnetic wire incorporated along the length of the microchannel, as shown in Fig. 1. A uniform external magnetic field, applied normal to the axis of a ferromagnetic wire, is deformed near the ferromagnetic wire, and generates a high gradient magnetic field. Therefore, blood cells flowing parallel to the ferromagnetic wire experience a magnetic force by the high gradient magnetic field created near the ferromagnetic wire. For the diamagnetic capture (DMC) mode magnetophoretic microseparator, an external magnetic field is applied

normal to the microchannel in the *x*-direction, as shown in Fig. 1(a). Then, the RBCs as paramagnetic particles are forced away from the ferromagnetic wire and the WBCs as diamagnetic particles are drawn closer. Thus, the RBCs are separated continuously into outlets #1 and #3, and the WBCs are separated continuously into outlet #2.

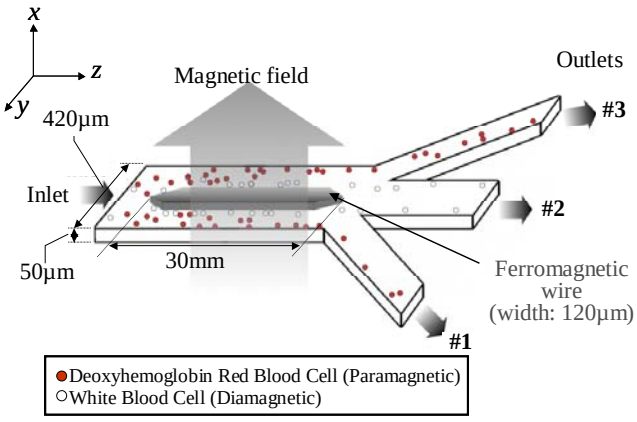
For the diamagnetic capture (DMC) mode magnetophoretic microseparator, an external magnetic field is applied normal to the microchannel in the *y*-direction, as shown in Fig. 1(b). Then, the RBCs are drawn closer to the ferromagnetic wire and the WBCs are forced away. Therefore, the RBCs are separated continuously into outlet #2, and the WBCs are separated continuously into outlets #1 and #3.

3 MICROFABRICATION PROCESS

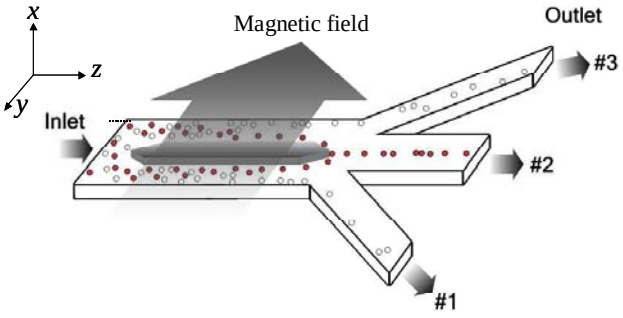
For the first fabrication step, the bottom glass substrate (Borofloat™ glass of 0.7 mm thick) was etched 50 µm in depth using 25% HF solution. Next, a Ti/Cu/Cr seed layer, for nickel electroplating, was evaporated onto the bottom glass substrate, as shown in Fig. 2(a). The ferromagnetic wire was fabricated by nickel electroplating, as shown in Fig. 2(b). After removing the seed layer, the glass chip of the magnetophoretic microseparator was completed by glass-to-glass thermal bonding at 685ºC for 3.5 hrs (Fig. 2(c)).

The integrated microfluidic interface (IMI) fabricated by stereolithography was used to realize the microfluidic interconnect [11]. Nitrile rubber o-rings were used for sealing the microfluidic interconnects. An ultraviolet (UV) adhesive was dropped into the openings for adhesive bonding on the IMI, and capillary forces pulled the adhesive into the gaps between the IMI and the glass chip. The UV adhesive was then cured by placing it under a UV light for 30 minutes, completing the fabrication of the magnetophoretic microseparator, as shown in Fig. 2(d). Finally, to reduce the adhering of blood cells to the microchannel wall, polyethylene glycol surfactant was coated onto the surface of the microchannel. Figure 3 show the fabricated magnetophoretic microseparator and a close-up of the separation point with the three outlets.

As mentioned above, the present microseparator was designed for both the DMC and PMC modes. Therefore, the microchannel should be located at the edge of glass chip for the PMC mode. As a result, the three outlet channels are bended away from the edge of the glass chip with careful consideration for fluidic resistance of the three outlet channels, as shown in Fig. 3.



(a) Diamagnetic capture mode



(b) Paramagnetic capture mode

Figure 1: Illustrations of the magnetophoretic microseparator having one inlet and three outlets.

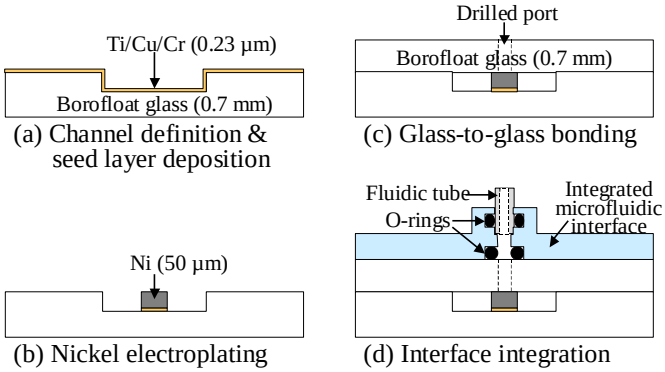


Figure 2: Microfabrication process.

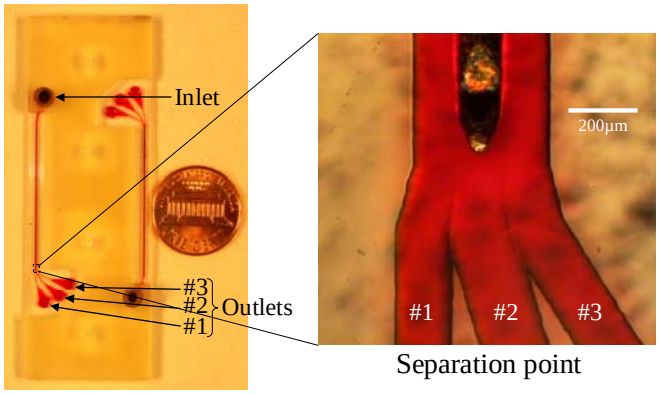


Figure 3: Fabricated magnetophoretic microseparator.

4 EXPERIMENTAL RESULTS

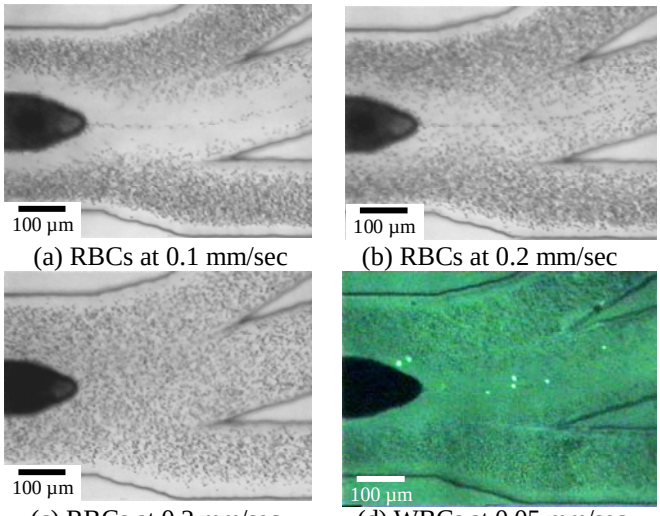
4.1 Diamagnetic Capture Mode

Instrument setup for the magnetophoretic microseparator consists of a permanent magnet to create the external magnetic flux of 0.2 T, and one syringe pump used to drive the fluid. To prepare blood sample, bovine whole blood was diluted to a ratio of 1:10 using a 3 mM isotonic sodium hydrosulfite solution. Figures 4(a) and 4(b) display images of RBCs flowing at average velocities of 0.1 mm/sec and 0.2 mm/sec through the microchannel of the DMC microseparator with an external magnetic flux of 0.2 T using a permanent magnet. Figure 4(c) shows an image of RBCs flowing at average velocity of 0.2 mm/sec without the external magnetic flux. The images demonstrate that RBCs are forced away from the wire with the application of an external magnetic field. The measured relative percentage of RBCs at each outlet (Fig. 5(a)) shows that the DMC microseparator separates out 89.7% of RBCs from whole blood at a 0.1 mm/sec average flow velocity.

To improve reliability of experiment related to WBC, fresh bovine blood was used within 12 hrs after it is obtained from a cow. The WBC-rich blood sample was prepared through the centrifuged WBCs, the bovine whole blood, and the 3 mM isotonic sodium hydrosulfite solution is mixed to a ratio of 1:1:10. Figure 4(d) shows an image of fluorescence dyed WBCs flowing at average velocity of 0.05 mm/sec with the external magnetic flux. The measured relative percentage of WBCs at each outlet (Fig. 5(b)) shows that the DMC microseparator separates out 72.7% of WBCs from whole blood at a 0.05 mm/sec average flow velocity.

4.2 Paramagnetic Capture Mode

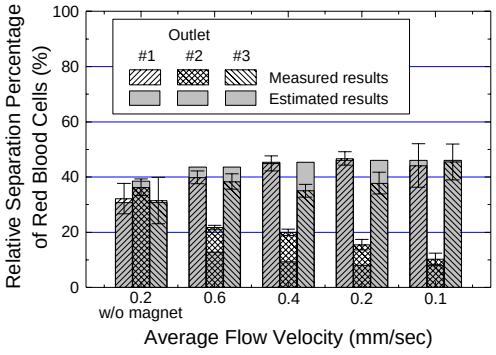
Figures 6(a) and 6(b) display images of RBCs flowing at average velocities of 0.1 mm/sec and 0.2 mm/sec through the microchannel of the PMC microseparator with a 0.2 T external magnetic flux. Figure 6(c) shows an image of RBCs flowing at average velocity of 0.2 mm/sec without the external magnetic flux. The images demonstrate that RBCs are drawn closer to the wire with the application of an external magnetic field. The measured relative percentage of RBCs at each outlet (Fig. 7(a)) on three-stage cascade PMC microseparator shows that 93.5 % of RBCs is separated out from whole blood at a 0.1 mm/sec average flow velocity. Figure 6(d) shows an image of fluorescence dyed WBCs flowing at average velocity of 0.05 mm/sec with the external magnetic flux. The measured relative percentage of WBCs at each outlet (Fig. 7(b)) shows that the three-stage cascade PMC microseparator separates out 97.4 % of WBCs from whole blood at a 0.05 mm/sec average flow velocity.



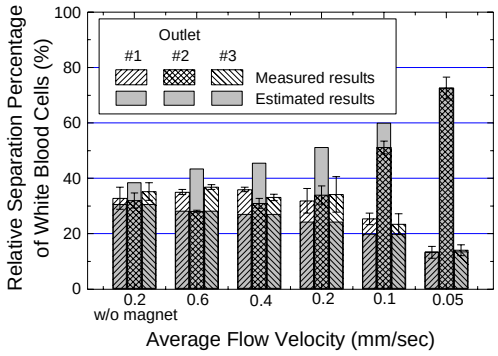
(a) RBCs at 0.1 mm/sec (b) RBCs at 0.2 mm/sec

(c) RBCs at 0.2 mm/sec without magnetic field (d) WBCs at 0.05 mm/sec

Figure 4: RBCs and WBCs passing through the microchannel of the DMC microseparator for various average flow velocities with and without an applied magnetic field.



(a) RBCs

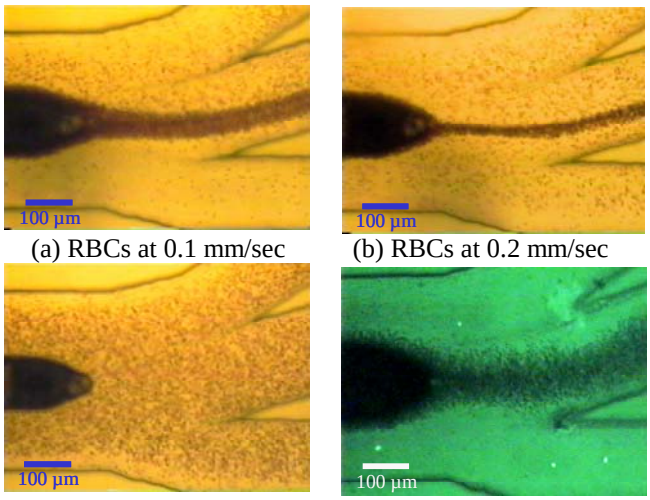


(b) WBCs

Figure 5: Measured and estimated relative separation percentage of RBCs and WBCs at each outlet of the DMC microseparator for various average flow velocities.

5 CONCLUSIONS

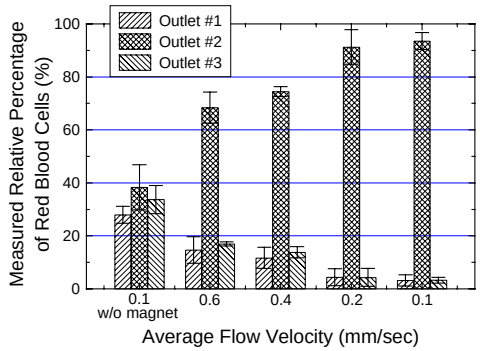
By using the HGMS method, a continuous magnetophoretic microseparator for both DMC and PMC modes was designed and successfully demonstrated for directly separating red and white blood cells from whole blood based on their native magnetic properties. Previously developed theoretical model was used to design of magnetophoretic microseparator. The magnetophoretic microseparator was fabricated using microfabrication technology, enabling the integration of micro-scale magnetic flux concentrators in an aqueous microenvironment, providing strong magnetic forces, and fast separations. For the experimental setup, a permanent magnet was used to create an external magnetic flux of 0.2 T. Experimental results showed that the DMC microseparator separated out 89.7 % of the RBCs from outlets #1 and #3 at 0.1 mm/sec flow velocity. By monitoring WBCs probed with a fluorescence dye, it was observed that 72.7 % of WBCs were separated into outlet #2 at 0.05 mm/sec flow velocity. The three-stage cascade PMC microseparator separated out 93.5 % of the RBCs from outlet #2 at 0.1 mm/sec average flow velocity. By monitoring WBCs probed with a fluorescence dye, it was observed that 97.4 % of WBCs were separated into outlets #1 and #3 at 0.05 mm/sec average flow velocity. Consequently, the magnetophoretic microseparator both DMC and PMC extracted highly concentrated WBCs from whole blood.



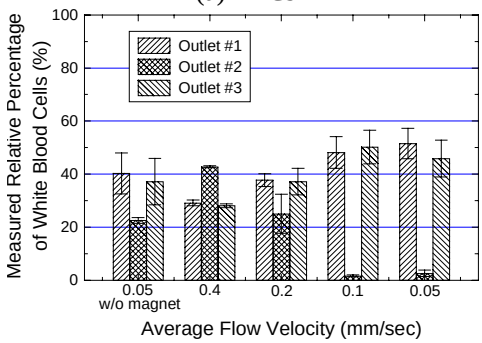
(a) RBCs at 0.1 mm/sec (b) RBCs at 0.2 mm/sec

(c) RBCs at 0.2 mm/sec without magnetic field (d) WBCs at 0.05 mm/sec

Figure 6: RBCs and WBCs passing through the microchannel of the PMC microseparator for various average flow velocities with and without an applied magnetic field.



(a) RBCs



(b) WBCs

Figure 7: Measured relative separation percentage of RBCs and WBCs at each outlet of the three-stage cascade PMC microseparator for various average flow velocities.

ACKNOWLEDGMENT

This work was supported by the National Institutes of Health (NIH) / National Institute for Environmental Health Sciences (NIEHS) under Grant ES10846.

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