Low-cost, disposable microfluidics device for blood plasma extraction using continuously alternating paramagnetic and diamagnetic capture modes

Pilkee Kim,¹ Eng Hui Ong,¹ King Ho Holden Li,^{1,a)} Yong-Jin Yoon,^{1,b)}
Sum Huan Gary Ng,² and Khuntontong Puttachat²

¹*School of Mechanical and Aerospace Engineering, Nanyang Technological University,
50 Nanyang Ave., Singapore 639798*

²*Singapore Institute of Manufacturing Technology (SIMTech), 71 Nanyang Dr.,
Singapore 638075*

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Blood plasma contains biomarkers and substances that indicate the physiological state of an organism, and it can be used to diagnose various diseases or body condition. To improve the accuracy of diagnostic test, it is required to obtain the high purity of blood plasma. This paper presents a low-cost, disposable microfluidics device for blood plasma extraction using magnetophoretic behaviors of blood cells. This device uses alternating magnetophoretic capture modes to trap and separate paramagnetic and diamagnetic cells away from blood plasma. The device system is composed of two parts, a disposable microfluidics chip and a non-disposable (reusable) magnetic field source. Such modularized device helps the structure of the disposable part dramatically simplified, which is beneficial for low-cost mass production. A series of numerical simulation and parametric study have been performed to describe the mechanism of blood cell separation in the microchannel, and the results are discussed. Furthermore, experimental feasibility test has been carried out in order to demonstrate the blood plasma extraction process of the proposed device. In this experiment, pure blood plasma has been successfully extracted with yield of 21.933% from 75 μ l 1:10 dilution of deoxygenated blood.
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I. INTRODUCTION

Human blood is composed of red and white blood cells and platelets suspended in blood plasma. The blood plasma that occupies 55% volume in blood contains various biomarkers and substances (dissolved proteins, clotting factors, glucose, electrolytes, hormones, enzymes, waste product, etc.) in blood fluid. By quantifying the biomarkers in blood plasma, general physiological condition of an organism can be evaluated,¹ and a variety of diseases such as paraproteinemias can be diagnosed as well.² However, there are a number of blood cells, which prevent an accurate diagnostic test, in human blood; $4\text{--}6 \times 10^6$ red blood cells (RBCs)/ μ l, $0.4\text{--}3 \times 10^4$ white blood cells (WBCs)/ μ l, and $1.5\text{--}4 \times 10^5$ platelets/ μ l.^{3–5} Accordingly, the extraction of high purity of blood plasma is essential prior to a diagnostic test. One of the most conventional methods to extract blood plasma from whole blood is to use centrifugation equipment, which requires large volume of blood sample, high cost, and labor-intensive and time-consuming processes. During the past decades, Lab-On-Chip (LOC) or microfluidics device has been considered as an alternative approach to overcome the drawbacks of the conventional method. The main advantages of LOC devices are smaller volume requirement of blood sample, lower cost, faster reaction, and higher throughput.

^{a)}Electronic mail: HoldenLi@ntu.edu.sg. Tel.: (65) 6790 6398.

^{b)}Electronic mail: YongjinY@ntu.edu.sg. Tel.: (65) 6790 5033.

Many different types of microfluidics devices and approaches have been proposed in order to efficiently separate blood cells and blood plasma in blood.^{5–23} One of representative separation methods is to use filtration method. Crowley and Pizziconi⁹ demonstrated a microfluidics device which separated blood plasma from whole blood sample using cross filtration method. Similar device was also proposed by VanDelinder and Groisman.¹⁰ This device caused a little hemolysis of RBC which contaminated the extracted blood plasma. On the other hand, Shim *et al.*¹¹ developed a microfluidics device based on a conventional type filtration method, which used various sizes of silica beads as a filter. The silica beads were packed inside the microchannel. The bead-packed microchannel induced capillary action force which allowed blood plasma to move through the microchannel faster than blood cells. This device was able to separate blood plasma from non-diluted blood without hemolysis of blood cells.

Furthermore, hydrodynamic force has also been used in separation of blood cells and blood plasma. Nivedita and Papautsky¹² proposed Archimedean spiral device which utilized hydrodynamic force coupled with Dean force to separate blood cells from blood plasma in diluted blood with dilution factor of >100X. Other hydrodynamic forces such as Zweifach–Fung bifurcating effect have been used to extract blood plasma.^{8,13–15} Shamsi *et al.*¹⁵ designed and fabricated a microfluidics device with a main channel and a series of daughter channels for blood plasma separation using bifurcating effect and plasma skimming.

Another method to separate blood cells and blood plasma is to use dielectrophoretic behaviors of blood cells. The blood cells in the blood tend to be polarized when placed in non-homogenous electric field, and dielectrophoretic force attracts or repels the cells along the electric field line. Nakashima *et al.*¹⁶ presented a microfluidics device which could separate and extract blood plasma from several microliters of blood without external mechanical driving sources. The device consisted of main and side channels with two electrodes. Han and Frazier¹⁷ used electric field to separate RBCs and WBCs. In their study, microfluidics devices were designed with two different types of electrode comb patterns, convergent type and divergent type.

Recently, a few studies have presented incorporation of magnetism into microfluidics devices for blood plasma and blood cell separation.^{18–23} Generally, deoxygenated RBCs in the blood tend to be attracted by the magnetic field due to their paramagnetic property, whereas WBCs tend to be repelled due to their diamagnetic property. Based on such magnetophoretic behaviors of the cells, Qu *et al.*²² designed a microfluidics device to separate RBCs away from whole blood. The magnetic field was applied across the microchannel with thin nickel wire in the middle to create high gradient magnetic field (HGMF). Iliescu *et al.*²³ created a microfluidics device with ferromagnetic beads layer under the microchannel to trap RBCs when the magnetic field is applied across the channel.

In this study, a low-cost, disposable microfluidics device for magnetophoretic extraction of blood plasma has been designed and fabricated. The device comprises a disposable microfluidics chip and non-disposable magnetic field source, respectively, on its upper and lower sides. The disposable part of the device is in a very simple structure and beneficial for low-cost mass production, because the magnetic field source is a separate part rather than an embedded part in a microfluidics chip. In particular, for the magnetic field source, ferromagnetic metal elements are periodically arranged on a permanent magnet in order to impose non-uniform magnetic field in the channel; thereby, paramagnetic and diamagnetic capture modes for blood cells are alternated along the channel. When blood flows through the microchannel, the paramagnetic and diamagnetic cells in the blood are continuously trapped on the bottom of the channel in an alternating manner by magnetophoretic attractive and repulsive forces, and therefore the pure blood plasma can be readily obtained at the end of the channel. A series of theoretical simulation for a single magnetizable cell, deoxygenated RBC or WBC, is performed in order to demonstrate the dynamic behaviors of a paramagnetic or diamagnetic cell in the proposed device and also describe the fundamental cell-capturing/trapping mechanism. As for the platelet, dynamic simulation study is not available due to unknown diamagnetic susceptibility, but instead the consistent effectiveness of the proposed capturing scheme is briefly addressed with a plausible reasoning. Furthermore, experimental feasibility test has been carried out with a lab-

scale device, and the blood cell counting results support that the proposed device can successfully extract pure blood plasma.

II. LOW-COST, DISPOSABLE DEVICE FOR BLOOD PLASMA EXTRACTION

Fig. 1 shows the schematics of (a) microfluidics device for blood plasma extraction and (b) its magnified cross section view. The microfluidics device for blood plasma extraction proposed in this study uses the magnetophoretic behaviors of blood cells to separate them away from blood plasma. The mechanism of this design is based on alternating paramagnetic and diamagnetic capture modes for blood cells. The design of this device consists of three parts. As shown in Fig. 1, the top, middle, and bottom parts of the device are the microfluidics chip, the ferromagnetic metal elements, and the permanent magnet, respectively. The microfluidics chip is designed to have only one inlet for blood sample input and one outlet for blood plasma output. The magnetic field is applied orthogonal to the chip or to the ground by the permanent magnet. Additionally, the ferromagnetic metal elements in form of metal mesh sheet are placed above the permanent magnet, creating alternating HGMF to induce magnetic force on blood cells.

Fig. 2 shows the magnetic field strength distributed in space above the right angle quadrilateral metal elements ($0.3 \times 0.9 \text{ mm}^2$ in cross-section area). Note here that the magnetic field strength has been evaluated using its analytical expression which will be provided later (through Eqs. (3)–(8) in Section III A). The detailed parameter values are listed in Table I. From Fig. 2, it is shown that the magnetic field strength is strongest on the surface of the metal elements and gradually decreasing along y-axis. On the other hand, the magnetic field strength between the metal elements is weakest on the channel bottom and increasing along y-axis. Under such magnetic field profile, the paramagnetic cells (e.g., deoxygenated RBCs) are likely to be attracted towards metal element regions where the magnetic field strength is getting stronger.

FIG. 1. Schematics of (a) proposed microfluidics device and (b) its enlarged cross section view A-A.

FIG. 2. Magnetic H-field strength above the right angle quadrilateral metal elements. The black thick lines represent the position of the metal elements in x-axis.

On the other hand, the diamagnetic cells (e.g., WBCs) are likely to be repelled away from the metal element regions, rather attracted toward the gap regions of the metal elements where the magnetic field strength is getting weaker. When passing through metal elements, the paramagnetic cells must experience attractive force downwards which makes them to sediment at a higher rate, while the cells passing through the empty gaps must experience repulsive force. The opposite behaviors of the diamagnetic cells can be readily deduced. Both types of cells with opposite magnetism are likely to be trapped at the bottom of the microchannel, separated away from the blood plasma, and the blood plasma can be readily collected at the outlet of the channel.

The three essential parts of the proposed device, microfluidics chip, metal elements, and permanent magnet, can be fabricated easily and separately. The microfluidics chip is disposable, while the metal elements and permanent magnet can be reused. Unlike the earlier devices for

TABLE I. Constant parameters used throughout the simulations.^{25,28}

μ_0 (H/m)	$4\pi \times 10^{-7}$
M_{es}	1.3687×10^6
$H_{ex,y}$ (A/m)	329 928
w (mm)	0.15
t_b (mm)	0.1
h_c (mm)	0.075
X_f	-7.7×10^{-6}
ρ_f (kg/m ³)	1000
η (kg/m s)	0.001
WBC	
ρ (kg/m ³)	1070
R (μ m)	5
V (μ m ³)	524
X	-9.2×10^{-6}
RBC	
ρ (kg/m ³)	1100
R (μ m)	3.84
V (μ m ³)	88.4
X	-3.9×10^{-6}

blood cell separation,^{17–20} where the metals or magnets were integrated into the microfluidics chip, the present device is modularized with three independent and separate parts, which is beneficial to the reducing of manufacturing time and cost.

III. THEORETICAL INVESTIGATION OF BLOOD CELL BEHAVIORS UNDER MAGNETIC FIELD

A. Mathematical modeling

From microscopic point of view, the whole blood can be largely divided into biological micro-cells and blood plasma. The blood plasma tends to behave as a Newtonian fluid similar to water.²⁴ On the other hand, the complicated rheological characteristics of blood originate mostly from the mechanical and electromagnetic behaviors of the biological cells and the interaction effects between multiple cells.²⁴ The theoretical investigation of such microscopic aspects of numerous cells in the blood requires very involved modeling (such as microstructure based model or particle dynamics model) and simulation, which are not within the scope of the present study. For simplicity of analysis, this study considers only a single magnetizable micro-cell in a Newtonian blood plasma flowing along a microchannel under an applied magnetic field. This simplification would be efficient and useful for understanding the fundamental magnetophoretic behaviors of the blood cells in the proposed device.

A mathematical model to simulate the dynamic motion of a single blood cell in a microchannel under a non-uniform magnetic field has been well established by Furlani's theoretical studies.^{25,26} In this study, a series of numerical simulation is performed based on Furlani's model²⁵ in order to investigate the motion of a single deoxygenated RBC or WBC in the present device. However, it should be noted that the direction of the gravitational force in the present system is different from that in Furlani's model, which leads to significant difference in blood cell motion. In this section, the mathematical derivation steps are briefly summarized.

When blood flows through the microchannel, it is assumed that a blood cell experiences four fundamental forces, i.e., magnetic force, gravitational force, buoyancy force, and drag force. Thus, the force balance equation for a floating blood cell in blood can be expressed as^{25,26}

$$\sum \vec{F} = \vec{F}_m + \vec{F}_g + \vec{F}_b + \vec{F}_d = \rho V \frac{d\vec{v}}{dt}, \quad (1)$$

where $\vec{F}_m$, $\vec{F}_g$, $\vec{F}_b$, and $\vec{F}_d$ are the vector forms of magnetic force, gravitational force, buoyancy force, and drag force acting on the blood cell, respectively, and ρ , V , and $\vec{v}$ are the density, volume, and velocity vector of the blood cell, respectively. Besides, the blood cell is treated as a perfect sphere, and its Brownian motion is not taken into account.

The magnetic force acting on a magnetic particle (a blood cell in this case) is given in the form²⁷

$$\vec{F}_m = F_{m,x}\hat{i} + F_{m,y}\hat{j} = \mu_0 V (X - X_f)(\vec{H} \cdot \nabla) \vec{H}, \quad (2)$$

where $F_{m,x}$ and $F_{m,y}$ are the x - or y -directional components of the magnetic force, respectively, $\hat{i}$ and $\hat{j}$ are the unit vectors in the x and y directions, respectively, μ_0 is the permeability of space, X is the magnetic susceptibility of the blood cell, X_f is the magnetic susceptibility of the blood plasma, and $\vec{H}$ is the magnetic field strength. In Eq. (2), $\vec{H}$ is the combination of magnetic field strengths generated by the permanent magnet and the magnetized metal elements ($\vec{H}_{ex}$ and $\vec{H}_a$, respectively), which can be expressed as^{25,26}

$$\vec{H} = \vec{H}_{ex} + \vec{H}_a = H_{a,x}\hat{i} + (H_{a,y} + H_{ex,y})\hat{j}, \quad (3)$$

where the x or y after a comma in the subscript denotes the x - or y -directional component of magnetic field strength, and $\hat{i}$ and $\hat{j}$ are the unit vectors in the x and y directions, respectively.

In Eq. (3), $H_{ex,x}$ is set to zero with the assumption of infinite dimensional permanent magnet, and the variation in $H_{ex,y}$ is assumed to be negligible.

As depicted in Fig. 1, a number of magnetic metal elements are arranged side by side along the microchannel. The magnetic field strength generated by those metal elements can be calculated by superposition principle. Following the derivation steps reported in Refs. 25 and 26, the x - and y -directional components of the total magnetic field caused by the magnetized metal elements can be obtained in the following forms:

$$H_{a,x}(x, y) = \sum_{n=0}^{N-1} \frac{M_e}{4\pi} \left\{ \ln \left[\frac{(x - nz + w)^2 + (y - h)^2}{(x - nz + w)^2 + (y + h)^2} \right] - \ln \left[\frac{(x - nz - w)^2 + (y - h)^2}{(x - nz - w)^2 + (y + h)^2} \right] \right\}, \quad (4)$$

$$H_{a,y}(x, y) = \sum_{n=0}^{N-1} \frac{M_e}{2\pi} \left\{ \tan^{-1} \left[\frac{2h(x - nz + w)}{(x - nz + w)^2 + y^2 - h^2} \right] - \tan^{-1} \left[\frac{2h(x - nz - w)}{(x - nz - w)^2 + y^2 - h^2} \right] \right\}, \quad (5)$$

$$z = 2w(1 + r), \quad (6)$$

where N is the number of the magnetic elements, z is the distance between the centers of two nearby magnetic elements, r is the width ratio of an empty gap to a metal element, h and w are the half height and half width of the magnetic element, respectively, and M_e is the magnetization of the magnetic element. In general, the magnetization of magnetic materials exhibits non-linear behavior such as saturation phenomenon under strong external magnetic field. Particularly, the magnetization of the magnetic material with large susceptibility only depends on its shape, and the unsaturated and saturated magnetization is then given by²⁷

$$\text{Unsaturated } M_e = \frac{H_{ex}}{N_d} \text{ for } H_{ex} < N_d M_{es}, \quad (7)$$

$$\text{Saturated } M_e = M_{es} \text{ for } H_{ex} \geq N_d M_{es}, \quad (8)$$

where N_d and M_{es} are the demagnetization factor and saturated magnetization of the metal element, respectively. In Eqs. (7) and (8), the demagnetization factor only depends on the aspect ratio p of the rectangular element, i.e., $p = h/w$ (refer to the Appendix for the detailed expression of the demagnetization factor).

The gravitational force is the downward force acting on blood cell in the y direction due to gravity. The gravitational force can be expressed as follows:

$$F_{g,y} = -\rho V g, \quad (9)$$

where g is the gravitational acceleration, 9.81 m/s^2 .

On the other hand, the buoyancy force is the upward force acting on the blood cell by blood plasma. The buoyancy force can be expressed as follows:

$$F_{b,y} = \rho_f V g, \quad (10)$$

where ρ_f is the density of blood plasma.

For low Reynolds number and low velocity flow on a small spherical particle, Stokes' drag (rather than Newton's drag) should be used. The Stokes' drag force is given as

$$\vec{F}_d = -6\pi\eta R(\vec{v} - \vec{v}_f), \quad (11)$$

where η is the dynamic viscosity of the blood plasma, R is the Stokes' radius of the blood cell, and $\vec{v}_f$ is the velocity of the blood plasma. For fully developed internal laminar flow in the microchannel with no slip wall condition and constant average flow velocity, the velocity profile of the blood plasma can be written as

$$v_{f,x} = \frac{3}{2} u_{f,m} \left(1 - \left(\frac{y - h - t_b - h_c}{h_c} \right)^2 \right), \quad (12)$$

$$v_{f,y} = 0, \quad (13)$$

where $u_{f,m}$ is the average velocity of the blood plasma, t_b is the thickness of the base of the microfluidics chip, and h_c is the half height of the microchannel.

By substituting four force components derived by Eqs. (2)–(13) into Eq. (1), the equation of motion of the blood cell is obtained as follows:

$$\rho V \frac{\partial^2 x}{\partial t^2} = F_{m,x} - 6\pi\eta R \frac{\partial x}{\partial t} + 6\pi\eta R \frac{3}{2} u_{f,m} \left(1 - \left(\frac{y - h - t_b - h_c}{h_c} \right)^2 \right), \quad (14)$$

$$\rho V \frac{\partial^2 y}{\partial t^2} = F_{m,y} - \rho V g + \rho_f V g - 6\pi\eta R \frac{\partial y}{\partial t}. \quad (15)$$

B. Numerical simulation

Based on the mathematical model derived in Eqs. (14) and (15), numerical simulations have been performed, and the associated results are presented and discussed in this section. The parameters used in the simulation study are listed in Table I.

Fig. 3 shows the trajectories of a single (a) RBC and (b) WBC in the microchannel, obtained with different initial positions of the cells. The cell trajectories have been computed by the direct numerical integration of Eqs. (14) and (15). At the inlet of the channel, a single blood cell is released from several different vertical positions between 0.4 mm and 0.25 mm, with a horizontal initial velocity. The initial velocity of the cell is same as that of the blood fluid at the given initial location. In Fig. 3, the vertical axis is the distance away from the bottom of the microchannel, i.e., $y^* = y - h - t_b$. In Fig. 3, it is clearly observed that the RBC and WBC are captured via the paramagnetic and diamagnetic capture modes, respectively. As shown in Fig. 3(a), the RBC tends to move downward due to magnetic attraction, passing

FIG. 3. (a) RBC trajectories with $u_{f,m} = 0.05$ mm/s and (b) WBC trajectories with $u_{f,m} = 0.1$ mm/s in microchannel when $p = 3$ and $r = 1$. The black thick lines represent the position of the metal elements in x-axis.

through the metal elements; whereas it tends to move upward due to magnetic repulsion, passing through the empty gaps between the metal elements. These tendencies are getting more significant as it approaches nearer to the bottom surface, because of higher gradient magnetic field. The RBC is finally trapped on the region above the metal elements. On contrary, the WBC tends to move upward above the metal element regions (or downward above the other regions), and eventually it is trapped on the channel bottom above the empty gaps.

The alternating trap of the RBC and WBC along the channel can be easily understood by investigating the variation in the magnetic force exerted on the blood cells. Fig. 4 shows the variations in (a) horizontal and (b) vertical magnetic forces acting on the RBC and WBC, calculated with a constant vertical position ($y^* = 30 \mu\text{m}$) along the microchannel. The horizontal magnetic forces acting on the blood cells (denoted in Fig. 4(a) by the solid and dotted lines for the RBC and WBC, respectively) force the RBC and WBC to move towards the centers of the metal elements and the empty gaps, respectively. Note that the solid and dotted arrows in Fig. 4 indicate the expected movement of the RBC and WBC, respectively. On the other hand, the vertical magnetic forces (shown in Fig. 4(b)) help the sedimentation processes of the RBC on the metal elements and the WBC on the empty gaps. The alternative upward and downward magnetic forces in the vertical direction cause the fluctuating motion of the blood cells shown in Fig. 3. These horizontal and vertical magnetic forces underlie the continuous trapping of the RBC and WBC by alternating paramagnetic and diamagnetic capture modes.

Fig. 5 shows the settling distance for the RBC and WBC with respect to the vertical initial height of the cells when the magnetic field is present and absent. In this figure, it is seen that the RBC under the magnetic field settles down in a shorter distance, when compared with the case without the magnetic field, while the WBC settles in a longer distance. Such tendencies originate from stronger magnetic attraction of the RBC in vertical direction than magnetic repulsion or stronger repulsion of the WBC than attraction, as shown in Fig. 4(b). Furthermore, the settling distance of the RBC is always longer than that of the WBC, since the mass of the RBC (and also gravitational force) is generally smaller. From the above observation, it can be deduced that the settling distance of the RBC seems to be an important factor in determining

FIG. 4. x-component (a) and y-component (b) magnetic forces acting on RBCs and WBCs. The black thick lines represent the position of the metal elements in x-axis.

FIG. 5. Settling distance of the RBC and WBC with respect to the vertical initial position when $p = 3$, $r = 1$, and $u_{f,m} = 0.1$ mm/s.

the total length of the microchannel, and the use of the magnetic field is beneficial to the miniaturization of the device. Additionally, the step-wise increase of the settling distance can be understood in that the settling distance of a cell increases with the initial vertical position but discontinuously when the cell-trapping region is shifted, as illustrated in Fig. 3, due to the nature of alternating cell capture modes.

Fig. 6 shows the settling distance of the blood cells with respect to the aspect ratio p of the metal element. The blood cell is released from the top of microchannel at the inlet. Similar to Fig. 5, the RBC (or the WBC) under the magnetic field always settles down in a shorter (or longer) distance. The WBC in general exhibits significantly shorter settling distance than the RBC. It can be seen that the settling distance of the RBC decreases while the WBC increases as the p increases, which attributes to the strengthening of magnetic field. As the aspect ratio has been defined as the ratio of height to width in this study, the larger aspect ratio, the taller metal element. Generally, ferromagnetic materials are magnetized more strongly in a longer axis, so that a taller metal element generates stronger magnetic field strength in the vertical direction. The magnetic field intensity increases with the aspect ratio, thereby the cell-trapping region can be shifted with the discontinuous trend shown in Fig. 6. This increasing

FIG. 6. Settling distances of the RBC and WBC with respect to the aspect ratio p when $r = 1$ and $u_{f,m} = 0.1$ mm/s.

magnetization with p follows Eq. (7). However, for $p > 3$, the settling distances of both the RBC and WBC are no longer increasing but become constants with the constant magnetic field owing to the saturated magnetization M_{es} of the metal elements given in Eq. (8). Therefore, it can be concluded that the saturation point (when $p \cong 3$ in Fig. 6) is an optimal condition for the aspect ratio p to maximize the blood plasma extraction performance with a small size of a device.

Fig. 7 shows the settling distances of the RBC and WBC with respect to r with a fixed w and p of metal elements. As the width of the metal element is fixed at $300\ \mu\text{m}$ in this study, the variation in the parameter r means the change in the ratio of metalized area against non-metalized area, and also the relocation of metal elements. In Fig. 7, the saw-like discontinuous shift of the cell-trapping region is caused by the variation in the placement of metal elements and the associated magnetic field distribution with the increasing parameter r . General tendencies in settling distances of the RBC and WBC are similar to Figs. 5 and 6. Interestingly, the blood cell capturing performance of the present device is getting worse as the density of metal elements in a confined area is too much low or high, owing to the break of proper alternating capture modes. As shown in Fig. 7, the deviation in the settling distance between the cases with and without magnetic field is more remarkable in the region of medium r , when compared with small or large r . Accordingly, there is also an optimal condition for the parameter r , with which alternating capture modes would be actively operating, and thus the device performance is possibly enlarged.

The simulation results for the RBC and WBC (discussed by Figs. 3–7) could provide physical insights to understand the paramagnetic and diamagnetic cell dynamics in the proposed device and also roughly predict the relations between the system parameters and the device performance. Besides the RBC and WBC, there is another blood cell, the platelet, to be addressed in this study. The simulation on the dynamic behaviors of a platelet could not be performed inevitably, due to unknown diamagnetic susceptibility that makes it difficult to determine the relative magnetism in human blood. Nevertheless, according to the experimental results of Ref. 5, it is readily expected that the absolute diamagnetic susceptibility of the platelet is larger than that of the RBC, but its magnetophoretic effect is smaller than that of the WBC. In other words, the absolute susceptibility of the platelet can be assumed to be located in a certain range where the alternating magnetic capture mode of the proposed device still applies to the platelet. Thus, it is concluded that a single platelet after released from the inlet tends to move toward the channel bottom of the present device, and eventually trapped, whatever its relative magnetism is.

Additionally, it should be noted that there are certain interaction effects between multiple cells, e.g., the aggregation of cells and the magnetic interaction between magnetized cells under

FIG. 7. Settling distances of the RBC and WBC with respect to the parameter r when $p = 3$ and $u_{f,m} = 0.1\ \text{mm/s}$.

an applied magnetic field. Although not treated in this study, such interaction effects between multiple cells may not break the fundamental paramagnetic and diamagnetic modes in the proposed device. But, it is believed that further experimental and theoretical studies are needed to shed more light on the complex behaviors of numerous blood cells and also to accurately predict the microphoretic cell-capturing performance of the device.

IV. EXPERIMENTAL FEASIBILITY TEST

A. Fabrication

In this study, a lab-scale device for blood plasma extraction has been fabricated, and its feasibility has been evaluated. Fig. 8 shows (a) the fabricated microfluidics chip and (b) metal elements in the form of metal mesh sheet. The material used for the microfluidics chip is PMMA. The PMMA chip has a dimension of $25\text{ mm} \times 70\text{ mm} \times 1\text{ mm}$. The microchannel with length 379 mm, width 1 mm, and depth 0.15 mm was milled onto the PMMA chip in serpentine manner. Two holes were drilled at the two ends of the microchannel as inlet and outlet. The microchannel side of the chip was then covered with 100 micron polyethylene terephthalate (PET) film. The PMMA tube has been connected right on the inlet of the microchannel in the vertical direction (same as gravitational direction). On the other hand, the syringe pump is connected to the outlet to generate negative pressure that allows the blood to flow through the microchannel. A metal mesh sheet with alternative straight metal lines and straight empty gap line patterns is used to mimic metal elements in the present device, as described in Fig. 1. The metal sheet shown in Fig. 8(b) is made out of mild steel and has a thickness of 0.3 mm. The metal line has a width of 0.3 mm, and the empty gap line has a width of 0.45 mm. The 0.45 mm empty gap was removed by laser cutting. The permanent magnet used in the experiment is a neodymium iron boron (NdFeB) block magnet. The magnet has a dimension of $50\text{ mm} \times 100\text{ mm} \times 25\text{ mm}$. It is rated with surface magnetic strength of 0.4146 T.

FIG. 8. Overview of (a) PMMA microfluidics chip and (b) mild steel metal mesh sheet.

B. Blood cell counting

The number of the blood cells in blood sample has been measured before and after blood plasma extraction process. Disposable plastic hemocytometer was used for blood cell counting. The hemocytometer (Fig. 9(a)) contains a pair of sample loading point, counting chamber with grid line. As illustrated in Fig. 9(b), the grid line consists of nine $1\text{ mm} \times 1\text{ mm}$ squares (indicated by red). The squares are then subdivided into $0.25\text{ mm} \times 0.25\text{ mm}$ (indicated by blue), $0.2\text{ mm} \times 0.25\text{ mm}$ (green), $0.2\text{ mm} \times 0.2\text{ mm}$ (yellow), and $0.05\text{ mm} \times 0.05\text{ mm}$ (black) squares. The depth of the grid is 0.1 mm .

Before counting blood cells on the hemocytometer, phosphate buffered saline (PBS) is dyed using 0.4% Trypan Blue solution with ratio of 1:20. The dyed PBS is then used to dilute the sample and also dye the blood cells, so that it is easier to visualize and count the blood cells under the microscope. $10\text{ }\mu\text{l}$ of the dyed diluted sample is used for counting blood cells on hemocytometer. The sample is loaded into the counting chamber via sample loading point. After the counting chamber is covered with the sample via capillary action, the hemocytometer is placed under a microscope, and the number of blood cells on the central square is counted with the aid of the grid line. And then, the density of blood cells in the blood can be calculated using the counted number of blood cells, as follows:

$$\text{Blood cells density} \left(\frac{\#}{\mu\text{l}} \right) = \frac{\text{Number of blood cells} \times \text{Dilution factor}}{\text{Volume of central square}}. \quad (16)$$

C. Blood plasma extraction test

Fig. 10 shows the picture of the experimental set-up used in this study. The metal mesh sheet is placed on the top of the NdFeB magnet, and the PMMA chip is adhered on the top of the metal mesh sheet. The whole set of the device is then placed on a movable platform which is under a microscope. The microscope is connected to a computer for picture taking. The outlet of the PMMA chip is connected to a syringe pump.

The blood used is human whole blood with EDTA anticoagulant sourced from Innovative Research. The whole blood is first diluted with PBS to dilution ratio of 1:10. The number of blood cells in the diluted blood has been counted first to obtain the initial density of blood

FIG. 9. Overview of (a) the hemocytometer and (b) the grid line in its counting chamber.

FIG. 10. Device experiment testing setup.

cells. $75\ \mu\text{l}$ of diluted blood sample is then loaded into the inlet. Note that the blood sample has been sufficiently mixed by a vial vibrator before loaded in the PMMA inlet tube. The syringe pump is then switched on with withdrawing rate of $0.01\ \text{ml/min}$. When the blood flow reaches the end of the microchannel, the blood sample is immediately collected from the outlet. The number of blood cells in the output blood sample is then counted again to evaluate the purity of blood plasma. The parameters used to quantify the output blood plasma sample are purity and yield, both of which can be expressed by

$$\text{Purity} (\%) = \left(1 - \frac{\text{Final cells density}}{\text{Initial cells density}} \right) \times 100 \%, \quad (17)$$

$$\text{Yield} (\%) = \frac{\text{Output sample volume}}{\text{Input sample volume}} \times 100 \%. \quad (18)$$

Fig. 11 shows the pictures of the blood plasma extraction process for the 1:10 diluted blood sample using the present device, which are obtained at (a) 1 min 20 s and (b) 4 min 34 s after the blood sample has been injected at the inlet of the microchannel. For comparison purpose, the process without magnetic field is also presented in Fig. 11(c). The blood sample reaches the

FIG. 11. Blood cells separation process for the diluted sample at (a) 1 min 20 s and (b) 4 min 34 s. (c) The process without magnetic field is also given for comparison.

4th row of channel counting from the inlet channel row at 1 min 20 s (Fig. 11(a)). At this moment, it is observed that the color of the blood sample starts to become notably pale. This lighter blood color indicates the decrease of blood cell density in the blood sample due to the trapping of blood cells through the passing region in the microchannel. The blood sample reaches the outlet of the channel at 4 min 34 s (Fig. 11(b)). As can be seen in the enlarged pictures in Fig. 12, the last two rows (6th and 7th rows) of the microchannel are wholly occupied by transparent blood sample, in which blood cells have been entirely removed and only pure blood plasma remains. Therefore, in this case, the pure blood plasma of $16.45\ \mu\text{l}$ can be extracted with a yield of 21.933% from the last two row of the channel. After directly counting the number of blood cells (using the method described in Section IV B), it has been confirmed that there are not any blood cells in the transparent output sample but pure blood plasma with purity of 100%. The whole process takes about 5 min from loading input sample to output sample extraction. On the other hand, for the process without magnetic field (Fig. 11(c)), the color of the blood sample at the outlet becomes slightly paler than that of the injected blood at the inlet. The blood cell counting result has indicated that the purity of the output blood sample is 52.17%. For the case without magnetic field, the sedimentation of blood cell due to the gravitational force might proceed but it is too slow to obtain pure blood plasma in a confined length of the microchannel.

From the abovementioned observations, it can be concluded that the magnetophoretic trapping of the blood cell enhances the performance of blood plasma process. However, the performance may depend on various factors such as the viscosity of the blood sample and the dilution rate. Fig. 13 shows the pictures of the blood plasma extraction process for non-diluted blood, taken at the centers of second to seventh rows of the microchannel. The non-diluted blood obviously contains larger number of blood cells than the diluted blood used in Fig. 12, and also its viscosity must be higher. For the non-diluted sample, the whole process until the sample reaches the outlet has taken 8 min 40 s, which is about 4 min longer than that for the diluted sample. Such longer process time might be due to a higher viscosity of the non-diluted sample, which suffers from larger drag force in the channel. Furthermore, as shown in Fig. 13, the colors of the non-diluted blood sample are almost same throughout the microchannel and not visually distinguishable. By blood cell counting, the purity of the non-diluted sample at the outlet has been measured as only 6.875%. There may be several reasons for such poorer performance for the non-diluted sample. The highly dense populated blood cells tend to interact to each other more often in non-diluted blood. The interaction between the blood cells may prevent the trapping of the blood cell, leading to lower sedimentation efficiency. Besides, the

FIG. 12. Microscope views for the diluted blood taken at the center points of six (2nd to 7th) rows of the microchannel. The numbers on the top right hand corner of sub pictures represent the number of microchannel row counted from the inlet row.

FIG. 13. Microscope views for the non-diluted blood taken at six (2nd to 7th) rows of the microchannel. The numbers on the top right hand corner of sub pictures represent the number of microchannel row counted from the inlet row.

blood cells that have already been trapped might form a sort of thick biological layer on the channel bottom, making a new channel surface with weak magnetic field; thereby, new blood cells can hardly be trapped. In this case, a longer process with an extended microchannel would be needed to extract pure blood plasma.

V. CONCLUSIONS

In this study, a magnetophoretic approach to extract pure blood plasma was presented. The microfluidics device was based on alternating paramagnetic and diamagnetic capture modes, respectively, which trap blood cells on the bottom of the microchannel. In this device, a ferromagnetic mesh was used on a permanent magnet to realize high gradient magnetic field with alternating positive and negative gradient along the microchannel. Unlike the previous designs reported in Refs. 19, 20, 22, and 23, where magnetic field source part was integrated into the microfluidics chip, the design proposed in this study was composed of two separate parts of the microfluidics chip and the magnetic source. Therefore, in the present device, the microfluidics chip can be readily disposable, while the magnetic field source including the metal mesh sheet and the permanent magnet is non-disposable and re-usable. The modularized parts are beneficial to simplifying the structure of the microfluidics chip; thus, advantageous in faster and simpler manufacturing process and lower cost. Particularly, the PMMA microfluidics chip used in this study did not contain any complicated pattern, so that its mass production could be readily realized via low cost and high throughput hot embossing method.

To describe the blood plasma extraction mechanism of the present device, a series of theoretical simulation has been performed. The simulation results for the trajectories of the blood cells showed that the deoxygenated RBC and WBC could be captured and trapped alternately along the bottom of the microchannel. The simulation also demonstrated that the RBC under magnetic field always settled down in a shorter distance, while the WBC settled down in a longer distance, when compared with the results under no magnetic field. Since the settling distance of the RBC was generally much larger than that of the WBC, the blood plasma extraction process depended mainly on the RBC rather than the WBC. Furthermore, numerical parametric studies on the aspect ratio and density of the ferromagnetic elements showed the existence of certain optimal parameters to maximize the blood plasma extraction. Based on the present simulation results of the RBC and WBC and an existing experimental report,⁵ it could be supposed that the absolute diamagnetic susceptibility of the platelet is located in the range where the alternating magnetic capture mode of the proposed device still applies to the platelet. This implies that a single platelet would be trapped on the channel bottom of the present device,

whatever its relative magnetism is. But, the detailed magnetophoretic behaviors of the platelet in this type of device may still be an open issue to be uncovered.

To confirm the feasibility of the proposed device for the blood plasma extraction, an experiment study has been performed using a lab-scale device. The purity of the extracted blood plasma has been evaluated by directly counting the number of remaining blood cells. When 1:10 diluted blood sample (75 μ l) was injected into the microchannel at average rate of 0.01 ml/min, the proposed device successfully separated blood cells from blood plasma, and it was able to produce the blood plasma with purity of 100% and yield of 21.933% in 5 min. On the other hand, only blood plasma of low purity (measured by 52.17%) was obtained without help of the magnetophoretic trapping of blood cells. The abovementioned experimental comparison study supported the effective functionality of the proposed device for blood plasma extraction. This study theoretically and experimentally presented the magnetophoretic mechanism and feasibility of a low-cost, disposable device for blood plasma extraction using alternating paramagnetic and diamagnetic capture mode. However, further theoretical and experimental studies should be needed in order to completely reveal the relation between the device performance and the design factors such as the material and shape of the magnetic element and the properties of blood sample.

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APPENDIX: DEMAGNETIZATION FACTOR OF MAGNETIC ELEMENT IN EQS. (7) AND (8)

Demagnetization of the magnetic element, N_d , in Eqs. (7) and (8) (Ref. 25)

$$N_d = \frac{4}{\pi} \frac{[E(k) - k'^2 K(k)][E(k') - k^2 K(k')]}{k'^2}, \quad (\text{A1})$$

with

$$k' = \sqrt{1 - k^2}, \quad (\text{A2})$$

$$p = \frac{E(k') - k^2 K(k')}{E(k) - k'^2 K(k)}, \quad (\text{A3})$$

$$E(k) = \int_0^{\frac{\pi}{2}} \sqrt{1 - k^2 \sin^2(\varnothing)} d\varnothing, \quad (\text{A4})$$

$$K(k) = \int_0^{\frac{\pi}{2}} \frac{1}{\sqrt{1 - k^2 \sin^2(\varnothing)}} d\varnothing, \quad (\text{A5})$$

where p is the aspect ratio of metal element, i.e., $p = h/w$, $K(k)$ and $E(k)$ are complete elliptic integral of first and second kinds, respectively. Note that the quantity k is obtained by solving Eqs. (A2) and (A3) with a given value of p .

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