A capillary dielectrophoretic chip for real-time blood cell separation from a drop of whole blood

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This study proposes a capillary dielectrophoretic chip to separate blood cells from a drop of whole blood (approximately 1 μ l) sample using negative dielectrophoretic force. The separating efficiency was evaluated by analyzing the image before and after dielectrophoretic force manipulation. Blood samples with various hematocrits (10%–60%) were tested with varied separating voltages and chip designs. In this study, a chip with 50 μ m gap design achieved a separation efficiency of approximately 90% within 30 s when the hematocrit was in the range of 10%–50%. Furthermore, glucose concentration was electrochemically measured by separating electrodes following manipulation. The current response increased significantly (8.8-fold) after blood cell separation, which was attributed not only to the blood cell separation but also to sample disturbance by the dielectrophoretic force. © 2013 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution 3.0 Unported License. [<http://dx.doi.org/10.1063/1.4802269>]

I. INTRODUCTION

Because blood analysis represents a dramatic development in point-of-care testing (POCT)^{1–3} and rapid testing products, whole blood instead of serum or plasma was used as the test sample. In most application scenarios, whole blood, in a proper sample, has the advantages of easy collection and operation. Lay users can collect whole blood samples by pricking their fingertips or alternative sites with a lancet. However, the blood cells (i.e., erythrocytes and white blood cells) in whole blood samples can interfere with the accuracy of test results. For example, in conventional blood glucose tests, glucose readings can be significantly reduced in proportion to the cell density of the said hematocrit (Hct) in the blood. The Hct range is suggested as 40%–54% for males and 37%–47% for females.⁴ However, Hct shows wide variations in people with anemia, dehydration, and hemodialysis. Whole blood with Hct of 10%–70% is typically found in clinical samples.⁴ The test bias of whole blood samples with the same plasma glucose but different Hct may be +50% or higher, a large challenge for conventional glucose test strip manufacturers.⁵ On the other hand, a blood sample collected from the fingertip can be as small as several microliters. Thus, it is difficult for a lay user to separate the plasma sample in a test. Consequently, a technique that can separate blood cells in tiny blood sample is desired for POCT and rapid test products to provide accurate results.^{6,7}

Blood cells can be separated by hydrodynamic^{8–18} and electrokinetic forces.^{19–25} Many disc-like microfluidic chips^{26–29} have been integrated with compact disk (CD) readers that can

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remove blood cells using centrifugal instruments. However, additional spinning instruments and a larger sample volume are required for these designs. In addition, some specific microfluidic channels have been proposed for cell separation based on their various flow characteristics in the channel. The sample is driven by an external pump to flow through the curve channels. The cells are retained at a specific area with a low flow field in the flow channel, thus, obtaining the plasma sample. However, these methods typically have a longer separation time, which is not suitable for *in situ* applications. Regarding the drawbacks of hydrodynamic technologies, electrokinetic techniques have demonstrated greater possibilities of meeting the requirements of *in situ* small-volume separation. There are numerous studies on electrophoresis (EP),³⁰ AC electro-osmosis (ACEO),^{31,32} dielectrophoresis (DEP),^{19,23–25,33,34} and AC electrothermal flow (ACETF)^{31,32,35–38} regarding bio-particle manipulation. One electrokinetic technology, DEP, has been widely used for manipulating, separating,^{33,39} focusing,⁴⁰ and concentrating⁴¹ cells/bacteria/DNA in microfluidic channels. However, the most of DEP with blood application have been developed continuous flow separation of cancer cell^{22,42} from dilute blood sample, few works with plasma separation²⁰ and blood cell separation.²³ In addition, Takashi's group²⁰ developed a chip to separate blood cells from a diluted whole blood sample using negative dielectrophoretic force. Although this chip device can isolate blood cells without an external pump, a sample with normal Hct cannot be directly applied.

This study developed a capillary dielectrophoretic chip for *in situ* blood cell separation with untreated whole blood using negative dielectrophoretic force. A drop of blood sample can automatically fill the flow channel using capillary force. And then, blood cells can be separated by the negative dielectrophoretic force generated by the electrodes within the flow channel. The separation efficiency can be evaluated by analyzing the images before and after DEP manipulation. Furthermore, a working electrode for the glucose sensor can be arranged between the separating electrodes for *in situ* glucose measurement following blood cell separation. The separating electrode can be used as the counter electrode for electrochemical detection. Blood cells on the electrode surface were rapidly removed following DEP manipulation. The response current should be high and independent of whole blood samples with differing hematocrits.

II. THEORY

The phenomenon of dielectrophoresis describes a motion caused by the field-induced polarization of a dielectric particle in a non-uniform electric field.⁴³ The time average dielectrophoretic force (F_{DEP}) acting on a spherical particle is given by

$$F_{DEP} = 2\pi r^3 \epsilon_m \text{Re}(f_{CM}) \nabla E^2, \quad (1)$$

where r is the radius of the particle, ϵ_m is the permittivity of the suspending medium, E is the electric field, and $\text{Re}(f_{CM})$ is the real part of the Clausius-Mossotti (CM) factor given by

$$f_{CM} = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*}, \quad (2)$$

where ϵ_p^* and ϵ_m^* are the complex permittivity of the particle and the medium, respectively. $\epsilon^* = \epsilon - j\sigma/\omega$; in which ϵ is the permittivity, σ is the conductivity, ω is the angular frequency. The real part of the frequency dependent CM factor determines whether the F_{DEP} is positive or negative. When the particle is more polarizable than the medium ($\text{Re}(f_{CM}) > 0$), the particle will be attracted toward the strong electric field (positive DEP, pDEP). When the particle is less polarizable than the medium ($\text{Re}(f_{CM}) < 0$), it will be repelled away from the strong electric field (negative DEP, nDEP).

III. MATERIALS AND METHODS

A. Chip design and fabrication

The chip was constructed with electrode, spacing, and cover layers, as shown in Fig. 1(a). Two aligned finger electrodes formed on a glass substrate. Briefly, titanium (Ti; 40 nm) and

FIG. 1. (a) Schematic illustration of the chip assembly. Doublesided tape (height of $30\ \mu\text{m}$) was used to bond the hydrophilic membrane and electrode chip together. (b) Image of the capillary dielectrophoretic chip. (c) Result of blood cells separation in the chip. (d) Schematic illustration of the blood cell separation process on the chip.

gold (Au; 250 nm) layers were sequentially deposited on the glass slide ($76 \times 26\text{ mm}$) by an electron beam evaporator (E-beam VT1-10CE, ULVAC). Next, the electrode pattern was transferred onto the Au layer using a conventional photolithography technique. Following the Ti and Au etching steps, multiple electrode pairs were formed on the slide, and the electrode pairs ($14 \times 26\text{ mm}$) were individually incised with a diamond cutter. A double-sided tape ($30\ \mu\text{m}$ in thickness) with an opening ($5 \times 2\text{ mm}$) was used to bond a hydrophilic membrane (3M 9917) and the electrode chip together. The completed capillary dielectrophoretic chip is shown in Fig. 1(b). The gap between the electrode chip and the hydrophilic layer formed a flow channel. The blood sample flows into this channel automatically when the capillary force contributes to the hydrophilic surface, and then blood cells were separated by the negative dielectrophoretic force generated by the electrodes within the flow channel, as shown in Fig. 1(c). The operation scenario is shown in Fig. 1(d).

B. Numerical simulation

The dielectrophoretic force is sensitive to electrode design, based on the previous reports,⁴⁴ this study adopted COMSOL MULTIPHYSICS 3.5 (COMSOL, Inc., Burlington) to simulate the gradient of electric field square (E^2) and the electric field distribution in the electrode plane. A 2D simulation was performed at quasi-static conditions. The simulation parameters were given as DC mode. The conductivity of the solution was set to be 1.2 S m^{-1} in order to match the conditions of experiment. The potential of each electrode was set at $+20\text{ V}$ and -20 V as boundary conditions, and the other were set to be electrically insulated. More than 5×10^4 elements were used for the meshes to obtain the accurate results.

C. Blood cells separation

A whole-blood sample was withdrawn from a healthy volunteer with a BD Vacutainer tube and gently shook to mix with the anticoagulant. The sample was spanned to separate the blood cells as pellets, and the supernatant was collected separately. Whole-blood samples with various Hcts were prepared by mixing the pellets and supernatant at different volume ratios. Varying separation voltages (i.e., $10\text{--}25\text{ V}_{\text{p-p}}$) at 100 kHz were applied to two-finger electrodes prior to sample application. A drop of whole blood (approximately $1\ \mu\text{L}$) was dipped in the inlet of the flow channel and was allowed to fill the channel automatically. We used a conductivity meter

(Radiometer analytical company, CDM230) to measure the blood plasma conductivity. Because whole-blood samples are high conductivity of solutions (σ_m approximately 1.2 S m^{-1}), the $\text{Re}(f_{CM})$ of blood cells is approximately -0.5 at a frequency of 100 kHz based on previous studies.^{19,20} Thus, negative dielectrophoretic force was generated to repel blood cells away from high electrical field when the sample contacted the symmetrical electrode. The separation phenomenon of each condition was recorded with a CCD camera (Olympus, MIC-D) operating at 30 frames per second. Images at various separation times for each experiment were analyzed with IMAGEJ software. The separation efficiency was defined as

$$\text{Separation efficiency} = \frac{A_i - A_r}{A_i} \times 100\%, \quad (3)$$

where A_i is the area of the defined separation zone, and A_r is the area covered by the residual blood cells following DEP manipulation. A_r was calculated by summarizing each red spotted area in the separation zone.

D. Preparation of an *in situ* glucose sensor

A working electrode ($3.3 \times 0.05 \text{ mm}$) was arranged within the separation zone for an *in situ* glucose sensor. A glucose oxidase (GOx, EC 1.1.3.4, 181.6 U mg^{-1}) reagent ($3 \mu\text{l}$) that was prepared with a 10 mM phosphate buffer ($\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$, $\text{pH } 7.35$) was dipped on the electrode surface. The reagent was spread over the electrode surface by a spin coater (1000 rpm , 20 s). Thereafter, another potassium ferricyanide reagent (50 mM) that was dissolved with the same phosphate buffer was applied and incubated for 1 min at 60°C . Thereafter, the spacer and cover layers were sequentially constructed on the chip. All chemicals and enzymes were purchased from Sigma-Aldrich. The blood sample was introduced from the inlet to fill the channel and stopped at the end of the flow channel. The electrode area was defined by the electrode width and flow length. The glucose measurement was conducted immediately following 30 s separation using an electrochemical analyzer (CH Instrument, CHI 660 C). The detection voltage across the working and counter electrode was established as 0.6 V for 10 s , and the current response at the 3rd second following the pulse was acquired for analysis.

IV. RESULTS AND DISCUSSION

A. Numerical simulation in the various electrode configurations

Figs. 2(a) and 2(b) show the simulation results of various electrode configurations. Gradient of E^2 with symmetrical electrodes of the same width (i.e., $100 \mu\text{m}$) but different gaps (i.e., $50 \mu\text{m}$ and $100 \mu\text{m}$) were compared to obtain the optimal design. We study these two dimensions ($50 \mu\text{m}$ and $100 \mu\text{m}$ gaps) in the study to consider fabrication limitation of commercial production. The arrows indicate the electric field gradient, which agreed with the direction of the negative DEP force. Blood cells in the described electric field gradient were expected to move in the directions indicated by the arrows. A longer arrow length indicates a stronger negative DEP force. A high electrode field gradient presented at the separation zone and a low electrode field gradient presented in the other zones. Because the electric field gradient determines the negative DEP force, a high gradient should provide a larger negative DEP force to accelerate blood cell separation. In the separation zone, blood cells are first repelled along the electrode gaps because of the larger electric field gradient. The residual cells within the electrode gaps move to the center of the gap and leave the separation zone in up and down directions. The blood cells at the edge of the separation zone are repelled in the direction of the arrow. Finally, the blood cells within the separation zone are removed. Figs. 2(c) and 2(e) show that the gradient of E^2 and the electric field in the cross-sectional lines with different electrode gaps in the middle of the symmetrical electrode were acquired and plotted against an x-axis distance. The $50 \mu\text{m}$ gap configuration showed a lower electric field gradient and greater electric field strength in the zone of the four electrodes at the cross-section. Therefore, it can avoid blood

FIG. 2. (a) and (b) Gradient of E^2 and negative field gradient for the negative DEP force in the horizontal plane for the symmetrical electrode (left: the electrode gap is 100 μm ; right: the electrode gap is 50 μm). The surfaces indicate the gradient of E^2 , and the arrows indicate the negative field gradients of the negative DEP force. (c) The magnitudes of the gradient of E^2 profile in the horizontal positions ($y = 50 \mu\text{m}$) with various electrode gaps. (d) The magnitudes of the gradient of E^2 profile in vertical positions with various electrode gaps. (e) The magnitudes of the electric field profile in the horizontal positions ($y = 50 \mu\text{m}$) with various electrode gaps. (f) The magnitudes of the electric field profile in vertical positions with various electrode gaps.

cell retention in the zone and reduce the number of blood cells in the adjacent zone moving toward the zone. Figs. 2(d) and 2(f) show that the gradient of E^2 and the electric field at the cross-section in the zone between the four electrodes were acquired and plotted against a y-axis distance. The results indicated that the 50 μm gap configuration had greater electric field gradient and strength in the zone of the four electrodes at the cross-section. Therefore, it can accelerate the movements of the blood cells away from the zone. If the electrode gap is decreased to less than 50 μm , the electric field strength in the zone with the four electrodes is similar to that of the zone with two electrodes. Although this increases the electric field strength and gradient in the zone with four electrodes, it increases the difficulty of chip fabrication. According to the simulation results, the configuration with the 50 μm electrode gap had the greatest electric field gradient surrounding the electrode edges and gaps. The blood cells within these electrode configurations were expected to have effectively removed in the separation zone.

B. Real-time blood cell separation

Based on the simulation results, we prepared two separation chips with electrode gaps of 50 and 100 μm , separately. The same separation voltage (20 $\text{V}_{\text{p-p}}$, 100 kHz) was applied to the

symmetrical electrodes before a whole-blood sample (40% hematocrit) was introduced. The sample introduction and blood cell separation activities were video recorded, and the images at time intervals were analyzed. The time interval is 10 s. Fig. 3(a) shows the sequential images captured 0, 10, 20, and 30 s following sample introduction. At 0 s, the cell densities in the separation zone were less than in the other area, suggesting that the blood cells were repelled immediately after the cells flowed into the separation channel. The separation voltage was maintained for continuous separation during the separation period. The blood cells at the electrode edges were first repelled because of the high electric field gradient. The blood cells gradually moved along the centers of the gaps and finally exited the separation zone. These figures demonstrate that a 50 μm gap chip can separate blood cells faster than one with a 100 μm gap, because of blood cell removal area of 50 μm gap chip is better than 100 μm gap chip at the same time. The density of the separation zone in each image was calculated as an index to quantify the separation efficiency (see Fig. 3(b)). This shows that the separation efficiency increased in proportion to the separation time, finally reaching different plateaus. Numerous blood cells still resided in the separation zone of the 100 μm gap chip, even when the separation time was extended by several minutes. This phenomenon suggests that a minimal applied voltage is required to repel the residual blood cells. If the separation voltage cannot be as high as 20 $V_{\text{p-p}}$, chips with smaller gaps should be used to achieve better separation efficiency.

A chip with a 50 μm gap design was used to investigate the separation efficiency at 10, 15, 20, and 25 $V_{\text{p-p}}$ separation voltages. Fig. 4 shows the relationships between the separation efficiency and various applied voltages at a frequency of 100 kHz with a 40% Hct blood sample. The DEP force was proportional to the voltage squared; therefore, increase in the voltage can enhance the nDEP force acting on the blood cell to accelerate the removal of blood cells resulted in better separation efficiency. The separation efficiency reached approximately 80%

FIG. 3. (a) Sequential images of the blood cell separation at times of 0, 10, 20, and 30 s following sample introduction. (b) The relationships between separation efficiency and separating time for various electrode gaps when an AC voltage of 20 $V_{\text{p-p}}$ at 100 kHz was applied.

FIG. 4. The relationships between separation efficiency and the separating time for various applied voltages at the frequency of 100 kHz for the whole blood (40% hematocrit).

within 25 s with 15 V_{p-p}, and a 90% separation efficiency was achieved within 20 s using 25 V_{p-p}. Higher separation efficiency was achievable by increasing the separation voltage. However, the highest applied voltage was limited by the blood sample conductivity and electrode reliability. Because sometimes the air bubbles were generated and the electrodes were damaged when a separating voltage of 25 V_{p-p} at 100 kHz was applied in our experiment.

In addition to the manipulation of the dielectrophoretic force, separation efficiency was further evaluated with whole-blood samples at various Hcts (i.e., 10%, 20%, 30%, 40%, 50%, and 60%). Fig. 5 shows the relationships between separation efficiency and the various hematocrit values when a separating voltage of 20 V_{p-p} at 100 kHz was applied. For whole-blood sample with low hematocrit (i.e., 10%), the blood cells were rapidly repelled, and the separation index reached 95% within 5 s. It took 10 s to completely remove the blood cells from the separation zone. The separation efficiency was approximately 90% within 30 s when the hematocrit was in the range of 10%–50%. Conversely, more than 30 s were required to repel the blood cells with a separating efficiency of 67% for high hematocrit (60%) sample. These results indicate that blood cells were repelled from the separation zone, but were stacked in the other area. More blood cells were guided to leave the separation zone along the same gaps when the hematocrit of the whole-blood sample was increased. Even blood cells bear the same DEP force, and separation efficiency is reduced in reverse proportion to hematocrit. In addition, we also observed pearl chaining phenomenon in the separation zone due to high concentration of red blood cells in whole blood sample, and the number of pearl chaining were increased with the increase in the hematocrit. Pearl chaining of red blood cells were formed along the AC field line due to particle polarization based on the previous studies.⁴⁵ Although pearl chaining of red blood cells were formed within seconds in the separation zone, which soon were dispersed and separated into the low electric field zone due to nDEP domination.

C. *In situ* glucose measurement following blood cell separation

A whole-blood sample (40% hematocrit) was introduced in the channel for the *in situ* glucose measurement. A working electrode was arranged in the separation zone, and a reagent layer was coated on the electrode surface for the blood glucose measurement. Because of blood cell interference, a higher response current was expected for the whole blood sample following

FIG. 5. The relationships between separation efficiency and the separating times for various hematocrit values when an AC voltage of 20 V_{p-p} at 100 kHz was applied.

blood cell separation. The blood cells homogeneously covered the electrode surface following sample introduction (see Fig. 6(a)). Next, a glucose measurement was performed immediately after 30 s incubation. In contrast, the separating voltage was applied to the separation electrodes for 30 s following sample introduction. Fig. 6(b) shows that most of the blood cells were repelled from the working electrode by a negative DEP force. Thereafter, the response current was measured without blood cell interference. The response currents were acquired and plotted, as shown in Fig. 6(c). The currents with and without blood cell separation were

FIG. 6. (a) The blood cells homogeneously covered the electrode surface following sample introduction. (b) Separation of the blood cells from whole blood when an AC voltage of 20 V_{p-p} at 100 kHz was applied for 30 s. The dashed line represents the working electrode. (c) Comparison of the current responses of the blood glucose from whole blood at the 3rd second when a DC voltage of 0.6 V was applied.

$3.70 \pm 0.36 \mu\text{A}$ and $0.42 \pm 0.06 \mu\text{A}$, respectively. The blood cells significantly affected the response current by acting as diffusion barriers, reducing the effective electrode area. This phenomenon is in agreement with the hematocrit interference of commercial electrochemical strips. However, it indicated a nearly 8.8-fold response in the experiment, which was higher than the approximately 1-fold increase found in commercial strips. Because of commercial strips detected the same blood glucose in whole blood and blood plasma, the response current of blood plasma was higher than the response current of whole blood approximately 1-fold (data not shown). Some additional effects caused by DEP separation were considered to explain this result. ACETF induces the fluid flow because of the joule heating of the fluid produces a temperature gradient in the conductivity solution,⁴⁶ which must be manipulated in the high conductivity solution ($>0.1 \text{ S/m}$) and high frequency ($\sim\text{MHz}$).^{32,35–38,47} Too high temperature can cause the blood cells breach and produce the phenomenon of hemolysis. Therefore, we were operated in the low-frequency (100 kHz) and the appropriate voltage to reduce the electrothermal effect and achieve micro mixed effect. Electrothermal fluid flow can be generated in high conductivity of blood sample, the dissolved reagent be mixed well with the sample, and the tiny sample volume can be heated using an electrothermal force during the separation. These two effects should enhance the catalytic reaction of the glucose oxidase and glucose prior to electrochemical measurement.

V. CONCLUSION

A capillary dielectrophoretic chip was developed for real-time blood cell separation in a tiny sample. For whole-blood samples, the separation efficiency can reach 90% in 30 s following application of the separation voltage. The separation efficiency is proportional to the gradient of the electrical field, which can be enhanced by increasing the separation voltage or by reducing the electrode gaps. In addition, significantly different responses before and after blood cell separation suggest that electrothermal fluid flow can be generated during separation in a tiny sample volumes. Both the mixing and heating effects caused by the electrothermal flow enhanced the catalytic reaction, resulting in a higher current response. An additional advantage of dielectrophoretic separation is easy integrates with electrochemical detection. The proposed chip enables miniaturization, tiny sample volume, and high separation efficiency in the application of POCT devices, particularly tests based on electrochemical detection.

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