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Blood Cell Tracing in a Microchannel by Using Dielectrophoresis Force

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ABSTRACT

The conventional techniques of platelet separation are by using a centrifugation and acoustic. However, by using these techniques, the platelets become active due to the relatively high-speed centrifugal force and large amount of a sample usage. Therefore, to overcome this problem, an electric separation technique is preferred as it relies more on the dielectric properties of particles, that allow for highly selective and sensitive analysis. One of them is dielectrophoresis (DEP), which is the force resulting from an interaction between bipolar particles in a non-uniform electric field that can be used to separate neutral particles. The DEP force allows the separation of platelets from red blood cells due to dielectric properties of the particles and medium as it requires small quantities of samples, lower reagents usage, and low-cost equipment. In this study, H-type microchannel with two inlets and outlets was developed by using COMSOL© software to observe the cell distribution at blood concentrations; C_A=0.01, 0.015, 0.02, 0.025 and 0.03 mol/m^3, with feed velocities at the inlet B; v_B=600, 700, 800, 900 and 1000 μm/s and at voltages charged; V=-30V to 30V that applied at the five electrodes. The simulation results show the optimization of blood cell concentration and velocity were achieved at the outlets at C_A=0.01 mol/m^3 and v_B=800 μm/s, respectively. Moreover, the optimum voltage for platelet cell separation was at V=10V as it shows the highest DEP force; F_{DEP}=-3.19×10^{13} N/m as compared to the other tested voltages.

Keywords: Blood cell; dielectrophoresis; particle tracing; microchannel; COMSOL©

INTRODUCTION

Blood is the most important biological liquid for performing a basic function to transporting nutrients and oxygen to the tissues and organs, as well to control pH and temperature inside the human body. It also provides an efficient transit system for transport of immune cells in strengthening the human body's defence mechanism against the foreign microbes and wound healing. The main components of blood cells are red blood cells, white blood cells, plasma and platelets. Since the numbers of red blood cells are relatively high, the separation of blood cell components become an issue. Thus, the separation on a microfluidic-based blood has gained attention and growth developed as it shows a high progress over the last few years. The concept of the inertial particles migration in the microchannel has been demonstrated (Choi et al. 2010; Othman et al. 2013; Liu et al. 2013; Yao et al. 2015; Othman et al. 2015 and Othman & Takei 2015).

In a blood cell, platelet is the smallest type of cell, which is about 2-4 μm in a diameter that plays an important role in haemostasis and thrombosis processes, which is process of stopping the bleeding in human body (Colman et al. 2006). Once bleeding occurs, platelets will be aggregated in the wound area to initiate formation of thrombus until the bleeding stopped. Besides, patients who undergoes general surgery, organ transplants and trauma treatments often require platelet transfusion (Triulzi & Grifith 1998). Likewise, it applied to the patients with platelet dysfunction or thrombocytopenia, which has low platelets count in their body.

Among the most common diseases associated with platelet cell dysfunction is Von Willebrand's that has abnormal genes as a protein that plays an important role in blood clotting (Huebsch & Harker 1981). When the protein is at the low level or unable to function normally, the platelets tend to stick to the blood vessel's wall while the injury occurs. Besides, Autoimmune Thrombocytopenia (AITP) disease which has a low platelet count that was attacked by antibodies produced by the human immune system (Lanzkowsky 2011). The platelet cells are also needed to detect antibody cells that invade platelet cells to treat the patients who are affected by this disease. Thus, it is very important to treat the patient by separating the platelet cells from blood cells.

The traditional techniques of platelet separation are centrifugation and acoustic. Yet, using these techniques, the platelets become active due to the relatively high-speed centrifugal force and large amount of a sample usage (Yousuff et al. 2017). Therefore, to overcome this problem, an electric separation technique is preferred as it

relies more on the dielectric properties of particles, which allow for highly selective and sensitive analysis (Gascoyne & Vykoukal 2002). The electrical separation technique consists of two types of forces that used to separate particles; electrophoresis (EP) and dielectrophoresis (DEP) forces.

The EP force is an electrostatic coulomb power that consist of particle surface charge interaction and electric current field. But, the EP force is not suitable for biological particles that are neutral since the EP force does not have a net charge. On the other hand, DEP is a force that resulting from interaction between bipolar particles and non-uniform electric field, that can be used to separate neutral particles (Li et al. 2014 & Oshii et al. 2010). Nowadays, the application of DEP force includes separation of bacteria from nano particles, live yeast cells from dead yeast cell, white blood cells from blood cells and etc. Therefore, the objective of this study is to apply the DEP force to observe the blood cell tracing in a microchannel with the effects of an inlet velocity, blood's concentration and voltage in order to achieve the optimum platelet separation.

THEORY

DEP force is applied based on the theory that particles such as synthetic beads, biological cells, proteins and DNA can be manipulated by forces exerted by non-uniform electric fields (Pohl 1978). This concept was first studied by Herbert A. Pohl in the early 1950s. Meanwhile, polarization is the ability of material to produce charge at the interface or the material's ability to react in electric field. The polarization has three basic mechanisms either by an electronic, atom or orientation polarization.

The interface polarization is an additional mechanism caused by the accumulation of charge between two different dielectric properties in the frequency range of 10 kHz to 100 MHz. The DEP force can be calculated by using Eq. (1) where ε_m is medium permittivity, r is a particle radius, Re[K(ω)] is a Clausius-Mossotti Factor and ∇E is an electric field gradient. It shows the DEP force is depending on various parameters such as particle size and conductivity of particles and medium, electric field gradient and Clausius-Mossotti (CM) factor.

$$\left|F_{DEP}\right| = 2\pi\varepsilon_m r^3 Re\left[K(\omega)\right]\nabla E^2 \qquad (1)$$

The CM factor plays an important role on DEP force in representing the dielectric properties of the particles and medium in the DEP area. As the particles dissolved in the medium and exposed in the electric field, the charge in the particles and medium will be reallocated at the medium and particle interface. If the particle is more polarized than the medium (Re[K(ω)] > 0), then the particle is pushed towards electric field and the movement is called a positive DEP (p-DEP). On the other hand, if the medium is more polarized than the particle (Re[K(ω)] < 0), then the particle moves away from the electric field and it is called negative DEP (n-DEP) (Gascoyne & Jody Vykoukal 2002). In order to solve Eq. (1), the CM term can be calculated by using Eq. (2) where $\bar{\varepsilon}$ is complex permittivity, ε is permittivity, σis particle conductivity, ω is angular frequency and j is imaginary unit. The p and m subscripts show particles and medium, respectively. The Eq. (2) shows the term of CM factor is liable on the complexity of particle and medium, as well as frequency of the external electric field.

$$K_{CM} = \frac{\bar{\varepsilon}_p - \bar{\varepsilon}_m}{\bar{\varepsilon}_p - 2\bar{\varepsilon}_m}, \quad \bar{\varepsilon} = \varepsilon - j\frac{\sigma}{\omega} \qquad (2)$$

METHODOLOGY

In this study, AutoCAD® and COMSOL® software academic version 5.2 are used to develop and design the model of H-type microchannel in order to observe the blood cell distribution. The main element in designing the H-type microchannel is to offer a new approach in separating particle from fluid without any membrane filter. It also provides a broad surface interface to improve the separation efficiency. It can be achieved by designing the main channel of microchannel with both height and width of 40 µm. This is because the diameters of each red blood cell and platelets are about 8 µm and 4 µm, respectively. With this particle size condition, 40 µm dimension of main channel can make the flow in the separation region (Reynolds number <0.05), so that both streams are not mixed up together. This microchannel has two inlets; A and B, two outlets; C and D, and five electrical electrodes as shown in Figure 1.

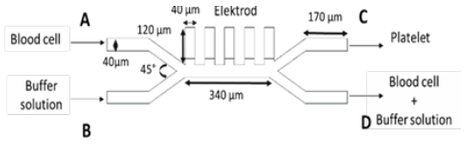
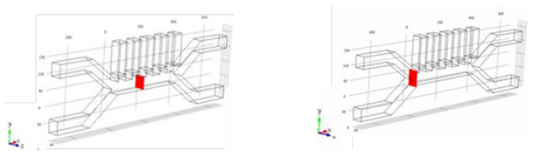


FIGURE 1. Model configuration of H-type microchannel

Inlet A was used as the feed of blood cell while inlet B was used as the feed of buffer solution (PBS). The electrode was used to charge the DEP force to the blood cell in order to provide external force for them to move in the microchannel fluid. The length of the straight microchannel from inlets to outlets is 340 µm with the diameters of inlet and outlet are 40 µm. The length of the electrical electrode distance is 40 µm and the metal electrode used is a platinum.

The width of the electrode should be equal to the width of main channel of the microchannel. The height of electrode is 120 µm, where the electric current field can reach down to a base microchannel. The electrodes are connected at the straight channel part as shows in Figure 1 in order to analysis a particle movement under the DEP force. And, for the purpose of the discussion of the analysis, two areas are

defined in the cross-section, viz.: center area and mixing point as shows in Figure 2.



a b

FIGURE 2. (a) Cross sectional view for center area and (b) cross-sectional view for mixing point

Figure 2 (a) shows the position of the cross-sectional view in the microchannel at location of 160 µm in the *x*-axis that is positioned between the 2nd and 3rd electrode. This cross-sectional position was used to observe the pattern of cell distribution at the center of the microchannel flow. Meanwhile, Figure 2 (b) shows the microchannel cross sectional view positioned at 0 µm in the *x*-axis. This position is the region where blood and PBS have a contact surface before mixed up in the straight microchannel. The position was set up in order to observe interaction of blood cell and PBS flows.

The simulation was carried out by separately injecting the blood cells and PBS through inlets A and B using micro-pumps. In the first simulation, the blood cells distribution was observed at various PBS velocity at the inlet B; v_B=600, 700, 800, 900 and 1000 µm/s with the blood cell velocity at the inlet A, v_A was constant at 130 µm/s. Then, the simulation was repeated at various blood cell concentration at the inlet A; C_A=0.01, 0.015, 0.02, 0.025 and 0.03 mol/m^3, with the concentration of PBS solution at the inlet B was constant at 0.01 mol/m^3. Lastly, the effect of the DEP force on the cells distribution was observed at several voltage charged on the five electrodes; V=-30V to 30V.

In COMSOL simulation, three modules are used; an electrical current, creeping flow, particle tracing in a fluid flow and transport of species. The electric current module was used to supply the non-uniform electric current fields. The creeping flow was used to simulate fluid flow at very low Reynolds numbers where the inertia term in the Navier-Stokes equation can be ignored. Besides, the model of particle tracing in a fluid flow and transport of species are applied in order to observe a movement of particles in the microchannel. The boundary conditions were specified in this simulation which are feed velocity at the inlet A, pressure and flow rate of blood feed. The velocity at inlet A was fixed at 130 µm/s to observe the effect of velocity distribution at inlet B on the movement of particles in microchannel, while pressure and inlet flow B were set up by default.

Based on the simulation, red blood cells will move further from the electrode as the DEP force reject large cells and red blood cells from the separation region. The cells which move away from the electrode will passage into the buffer solution, concentrated on the one side of the main channel and exit through the outlet D. However, the platelet cells cannot be pushed out strongly as the platelet has lower Clausius-Mossotti Factor than the red blood cells (Pohl 1978). Thus, the platelet cells have been continuously flow in the laminar flow regime throughout the microchannel and flow out through the outlet C.

RESULT AND DISCUSSION

Figure 3 shows the velocity distribution profile in the microchannel where the velocity of PBS solution at the inlet B, v_B was varied at v_B=600 µm/s (Figure 3a), 700 µm/s (Figure 3b), 800 µm/s (Figure 3c), 900 µm/s (Figure 3d) and 1000 µm/s (Figure 3e), while the velocity at the inlet A, v_A was constant at 130 µm/s. The color contour represents the mixture velocity distribution inside the microchannel where the blue color indicates the lowest velocity of 100 µm/s, while the red color indicates the highest velocity of 1000 µm/s. From the simulation results, the variances in the velocity distribution pattern was observed at two position; front view which is the overall view along the microchannel and the selected cross-section area at the center of microchannel; 160 µm in the *x*-axis (refer Figure 2a)

The results show as the velocity at the inlet B increased, the mixture velocity achieved at the outlets area was increased. For example, the velocity distribution at v_B=1000 µm/s in Figure 3(e) was slightly higher at the inclined and horizontal plane from the transverse plane of the outlets C and D as compared to the other velocities. It was occurred due to pressure difference that yield the decrease on the cell velocity distribution. However, as the velocity at the inlet B increased, the pressure also increased and produced higher inertia lift force to move and transport the cells in the microchannel.

In addition, the viscosity and density of fluid also play an important role in order to observe the cell movement in the channel flow. As the viscosity increased, the velocity distribution decreased. In fact, the viscosity of PBS solution was much lower compared to the blood cell and the inlet velocity B was higher than inlet velocity A, thus the mixture velocity distribution at the inlet B was much higher than at the inlet A.

Figure 4 shows the concentration distribution in the microchannel at various initial concentration of a blood cell at the inlet A; C_A=0.01 mol/m^3 (Figure 4a), 0.015 mol/m^3 (Figure 4b), 0.02 mol/m^3 (Figure 4c), 0.025 mol/m^3 (Figure 4d) and 0.03 mol/m^3 (Figure 4e), while the concentration of PBS solution at the inlet B was constant at 0.01 mol/m^3 as the dense concentration of the PBS solution will alter the biochemical nature of the blood cells (Di et al. 2016). The contour color bar represents the cells concentration in the microchannel where the red color indicates the highest concentration of 0.03 mol/m^3, while the blue color indicates the lowest concentration of 0.01 mol/m^3.

It shows as the concentration of the blood cell at the inlet A increased, the flow pattern of the cell concentration distribution increased especially in the inclined plane at near

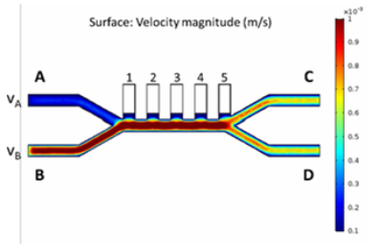
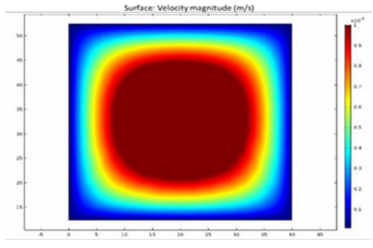
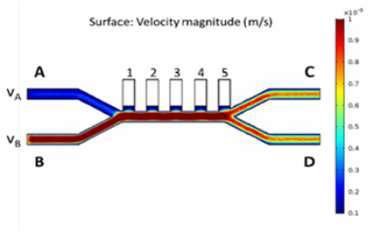
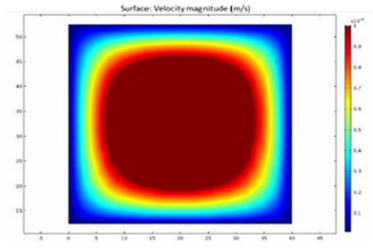
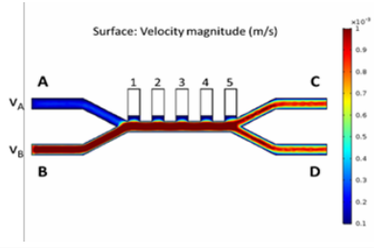
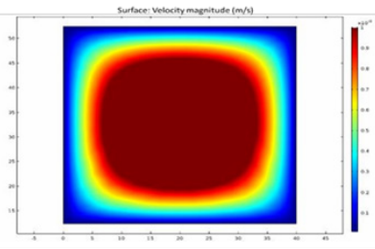
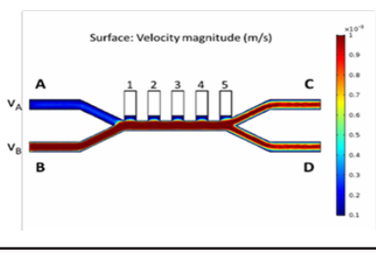
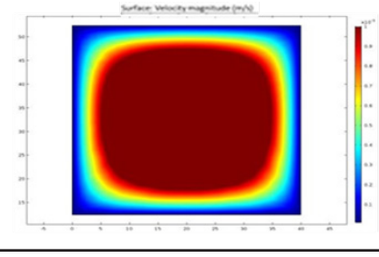
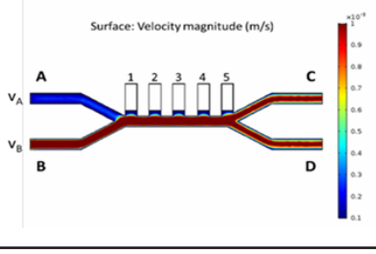
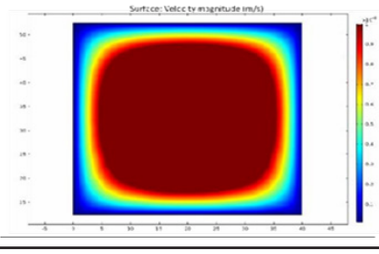
Parameter	Front view	Cross-sectional view
(a) v_A = 130 µm/s, v_B = 600 µm/s		
(b) v_A = 130 µm/s, v_B = 700 µm/s		
(c) v_A = 130 µm/s, v_B = 800 µm/s		
(d) v_{Av} = 130 µm/s, v_B = 900 µm/s		
(e) v_A = 130 µm/s, v_B = 1000 µm/s		

FIGURE 3. Velocity distribution in the microchannel at the front and cross-sectional view

to the outlets C and D. As well, the cell concentration at the outlet horizontal of plane C is less than the outlet D. Also, from the front view, it shows as the concentration of the blood cells increased, the increment on the concentration also occurred at the area of liquid electrode, inclined plane of the inlet A, and inclined plane of the outlet C and D. This is because the viscosity and density of the blood cells are higher than PBS solution and therefore, the cell more concentrated due to an aggregation of blood cells because of the high inertia in the microchannel.

Figure 5 shows the electrical field distribution and DEP force in the microchannel at various voltage charged at the electrodes; V=±30V. The positive voltages of V=10V, 15V, 20V, 25V and 30V were charged at electrodes 1, 3 and 5, meanwhile the negative voltages of V=-10V, -15V, -20V, -25V and -30V were charged at electrodes 2 and 4. The voltages of V=±10V are selected as the lowest voltages as the separation between platelet cells and blood cells can be separated at V=±7V. The red color represents the highest voltage of 30V, while the blue color represents the lowest voltage of -30V. The electrical field distribution was observed at two positions; at the front view and cross-sectional view at the center of microchannel. The different voltages charged at the electrodes resulted in reducing of

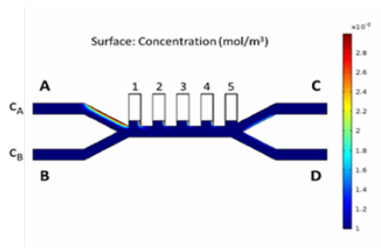
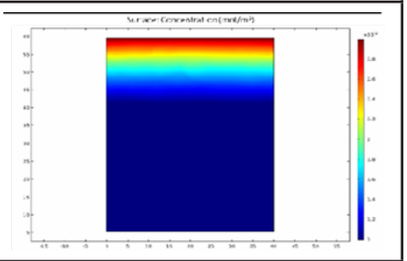
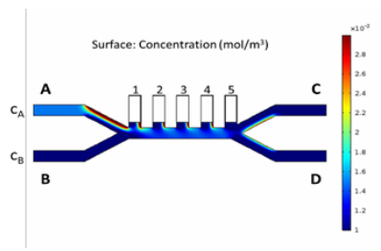
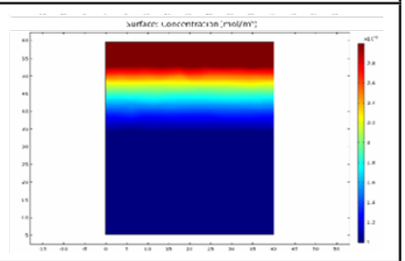
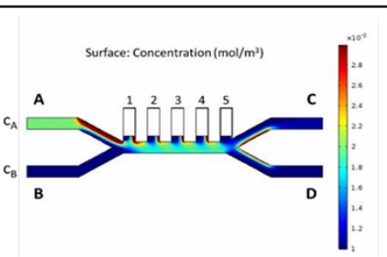
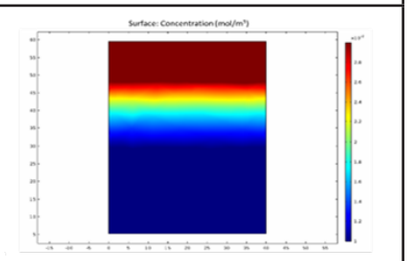
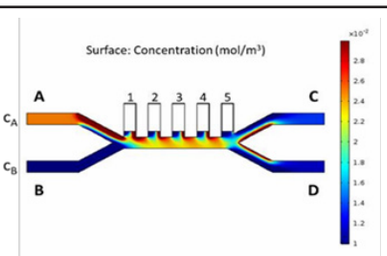
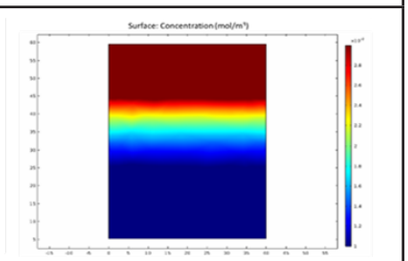
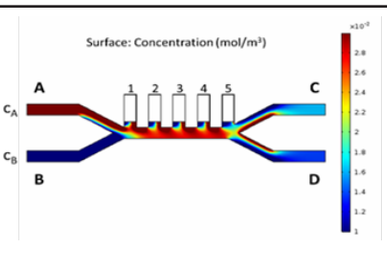
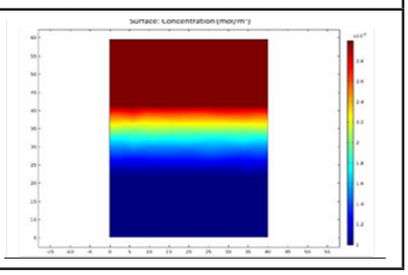
Parameter	Front view	Cross-sectional view
(a) C_A = 0.010 mol/m^3, C_B = 0.010 mol/m^3		
(b) C_A = 0.015 mol/m^3, C_B = 0.010 mol/m^3		
(c) C_A = 0.020 mol/m^3, C_B = 0.010 mol/m^3		
(d) C_A = 0.025 mol/m^3, C_B = 0.010 mol/m^3		
(e) C_A = 0.030 mol/m^3, C_B = 0.010 mol/m^3		

FIGURE 4. Concentration distribution in the microchannel at the front and cross-sectional view

the voltage distribution pattern as the distance between the electrodes become further. This is due to the voltage inside the microchannel flow will be lower than the voltage specified on the electrode.

By charging the voltage to the electrodes, the DEP force inside the microchannel was calculated. First, the CM factor of the platelet cells and blood cells were determined by setting up the frequency. In this study, the frequency was set up at 100 kHz. Based on the Gimsa et al. 1991, CM factor of platelet cells at 100 kHz was -0.5 (Gimsa et al 1991). After obtaining the CM factor of the platelet cell, the magnitude of the electric field gradient was determined. Based on the maximum value of the electric field gradient magnitude at various voltages, the DEP force was calculated from the Eq. (1).

As well, the simulation result in this work was compared with the previous experimental work done by Oshii (Oshii et al, 2010). Figure 6 shows the comparison result between the experiment and simulation study on the effect of the electric field intensity; E=1-25 V/mm on the DEP force.

It shows almost similar relationships and trend pattern where the DEP force is decreased as the electrical intensity charged on the electrodes was increased. At the lower electrical intensity, E<15 V/mm, it shows the DEP force

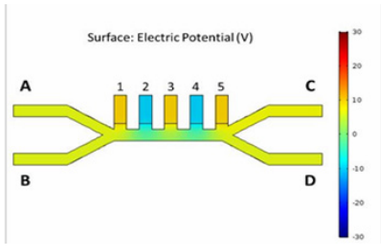
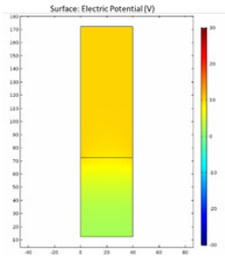
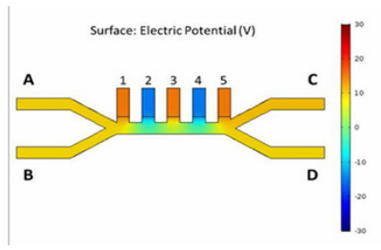
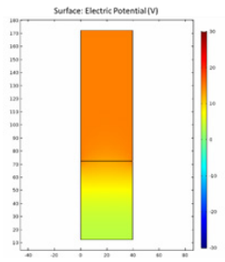
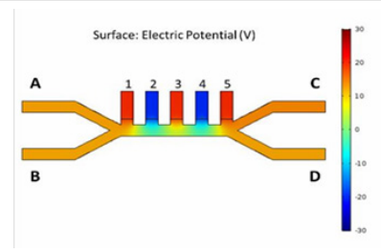
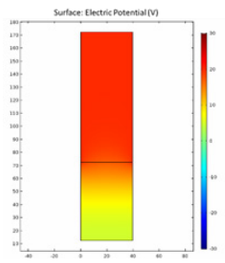
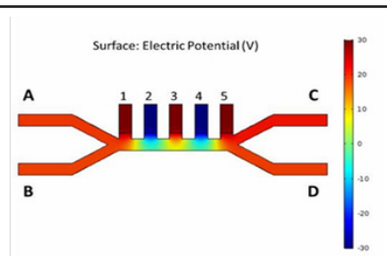
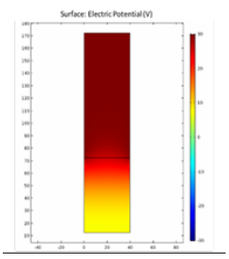
Parameter	Front view	Cross-sectional view
(a) electrode 1,3,5 = 10 V, 2,4 = -10V		
(b) electrode 1,3,5 = 15 V, 2,4 = -15V		
(c) electrode 1,3,5 = 20 V, 2,4 = -20V		
(d) electrode 1,3,5 = 30 V, 2,4 = -30V		

FIGURE 5. An electrical field distribution inside the microchannel at various voltage that charges at the 5 electrodes; V=-30V to 30V

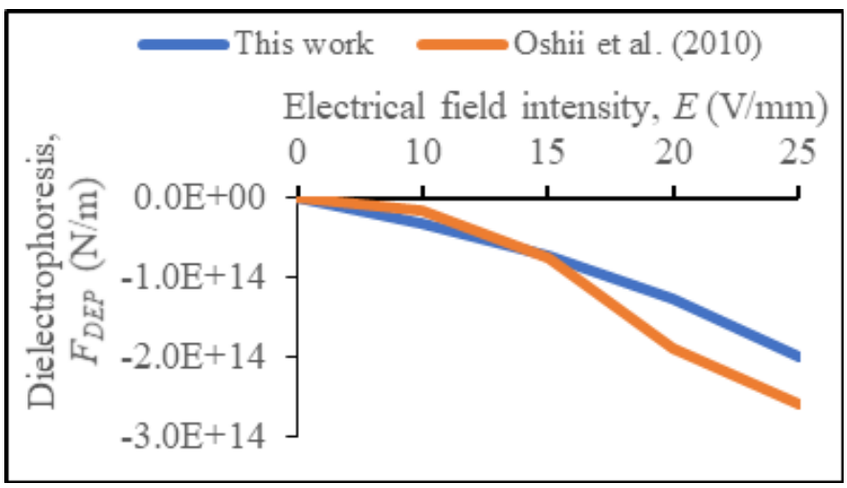


FIGURE 6. Comparison result on the effect of the electric field intensity, *E* on the DEP force

decreased gradually, however as the *E* is increasing, the DEP force shown sudden declined due to the square factor in the Eq. (1).

CONCLUSION

The H-type microchannel was developed to observe the effects of inlet velocity, blood cell concentration and voltage charged on the blood cell distribution. The results show the optimum concentration of the blood cells for better mixing process was achieved at C_A =0.01 mol/m^3 and v_B=800 μm/s as the lower concentration tend to reduce any mixing between a medium and the blood cell. The optimum voltage for platelet cell separation was achieved at V=10V as it shows the highest DEP force, F_{DEP}=-3.19×10^{13} N/m as compared to the others.

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DECLARATION OF COMPETING INTEREST

None.

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