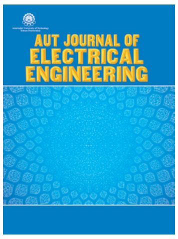

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Parametric Investigation of Separating RBCs from Platelets using Dielectrophoresis

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ABSTRACT: This paper discusses a simulation of the continuous separation of blood cells using a non-uniform electric field. Numerous factors influencing the separation of RBCs and platelets are addressed and examined in this numerical analysis. The simulation utilizes the equations of continuity, Navier-Stokes, and Newton's second law to understand the behavior of blood cells in the non-uniform electric field and to separate them based on their dielectric properties. The DEP force is modeled using Newton's second law equation, and its influence on the separation of RBCs and platelets is examined. The simulation was conducted using the COMSOL Multiphysics software, which employs a 2D FEM algorithm to investigate the cases. Various microchannel serpentine geometries were studied, and electrodes embedded along the microchannels applied a non-uniform electric field on the particles. The simulation results revealed that the separation of blood cells can be achieved using Dielectrophoresis based on their dielectric properties. The results of the simulation show that the separation of platelets from red blood cells can be achieved efficiently using the DEP mechanism. It was found that the separation efficiency is affected by the geometry of the channel, the voltage applied, the frequency of the electric field, and the velocity of the inlet stream. By optimizing these parameters, high separation efficiency can be achieved. And it was found that better separation occurs in the triangular, rectangular (where the height is less than the width), and square geometries in a higher voltage range.

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1- Introduction

Microfluidics is a rapidly growing interdisciplinary field that deals with the manipulation and analysis of fluids at the microscale. At these small-length scales, fluids exhibit a range of behaviors that differ significantly from those observed at larger scales. For instance, the effects of surface tension become more pronounced, as do energy dissipation and fluidic resistance. These factors play a critical role in determining the behavior of fluids in microfluidic systems, and understanding how they interact is key to developing new applications. Numerous applications can be made use of microfluidic devices, including e-skin applications [1], monodisperse double emulsion production [2], particle focusing [3], particle separation [4,5], and cell separation [6–8].

The medical industry has made significant progress in recent years with the development of new diagnostic and therapeutic techniques. One of the key challenges in this field is the capacity to distinguish between different types and sizes of biological cells. This is essential for a range of medical processes, including diagnostic, and therapeutic. Cell separation and sorting play a crucial role in cell biology research and various diagnostic and therapeutic procedures. In recent times, there has been a growing interest in techniques that do not rely on the use of biochemical labels [9]. Overall, the integration of microfluidics with the medical industry has opened up new possibilities for cell separation and analysis and is expected to play a significant role in future developments in this field.

The cultivation and separation of blood cells, which afterwards might be employed in medical treatments, is one of the key uses of laboratory microchips in stem cell therapy, cancer treatments, the chemical industry, diagnostic applications, and other areas [10,11].

There are various methods for sorting and isolation microparticles, which are typically classified as active or passive methods based on their function. Active methods rely on external forces, such as electric or magnetic fields, to manipulate the particles and separate them according to size, shape, or other characteristics. Examples of active methods include Dielectrophoresis [12,13], magnetophoretic [14,15], electrophoresis [16], and acoustic separation [17,18]. These methods require an external energy source and are often highly precise and controllable. Passive methods, on the other hand, rely on the physical properties of the particles, such as size, density, and shape, to sort them. Examples

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of passive methods include filtration [19], sedimentation [20], and Cell deflection which is carried out in a variety of ways, including using steeped obstacles [21], deterministic lateral displacement [22], and inertial focusing [23]. These methods do not require an external energy source. Parameters including power, efficiency, performance precision, energy consumption, method controllability, and other factors are frequently used to assess an approach's quality.

Although Dielectrophoresis has been a topic of discussion since the early 1900s, it was in 1951 that Martin Paul conducted a comprehensive study on it and gave it its name [24,25]. In essence, Dielectrophoresis is the force that acts on a dielectric particle when it is exposed to an electric field that is non-uniform and typically varies with time. The response of the particles is influenced by their electrical properties as well as those of the fluid surrounding them. Depending on the characteristics of the electric field, depending on the direction of the high field, particles may either be attracted there (positive Dielectrophoresis) or driven away from them (negative Dielectrophoresis). Therefore, to ensure that particles are influenced by non-uniform electrical fields, the system must be designed accordingly.

In order to provide uniform flow in microchannels for cancer cell separation utilizing a hybrid Dielectrophoresis configuration, Kang et al. [26] proposed a rectangular device. The study presented in this article provides evidence that by using this technique and in combination with electroosmotic flow, biological cells with diameters varying from a few to tens of micrometers can be continuously separated into separate wells. By modifying the voltage outputs of the electrodes, target cells of a particular size can be isolated with ease.

In order to avoid using electrodes, Lapizco et al. [27] developed an insulator-based DEP (iDEP) and incorporated circular arrays to control the low and high field strengths for the efficient sorting of bacteria according to type. The initial use of iDEP for the concurrent separation and concentration of live and dead bacteria demonstrates its capability as a promising pre-processing approach for bacterial analysis.

Shafie et al. [28] recommended adding a second wall with a 20 mm thick barrier in addition to the channel's primary wall. Through the active separation technique of Dielectrophoresis, the separation of platelet cells from other blood cells is simulated in this article in a continuous stream. The impact of diverse voltages and frequencies on the electric field gradient and device efficiency was explored through numerical analysis and corroborated by experimental findings. The microchannel geometry used in the study was based on the work of Piacentini et al. [29] Various factors that affect the separation of blood cells, including frequency, voltage, and flow velocity, were examined.

Bisceglia et al. [30] developed a microfluidic system to trap and analyze whole blood cells using parallel electrodes and positive Dielectrophoresis force. They also computed the imaginary part of the Clausius Mossotti coefficient for blood cells and found that it agreed well with experimental results.

Park et al. [31] employed a numerical model with Dielectrophoresis field-flow fractionation to sort white blood cells, red blood cells, and platelets solvent based on their size in blood utilizing potential at low voltage. The study investigated the effects of flow velocity in channel intake, frequency, and voltage applied to the electrodes, and particle size on the quality of blood cell separation using a medium fluid to transport blood cells to the electrodes. The displacement and residence time of the cells were estimated to determine how many separated blood cells. Different outlet designs were proposed to improve the separation of blood cells. As the quantity of inlet cells and flow rates increased, the recovery rate decreased, and particle-particle interactions had a significant impact on sorting.

Ishak Ertugrul et al. [32] designed and analyzed a microfluidic chip using the COMSOL Multiphysics software. The Dielectrophoretic (DEP) force technique was used to separate platelets and RBCs in the blood flowing through the channel, and the DEP force feature was given importance in the channel design. The voltage applied to the channel and the blood inlet velocity were found to affect the fluidic velocity and pressure, and the separation of platelets from RBCs depended on input data. Parham et al. [33] offered the development of a dielectrophoretic microfluidic system for manipulating microparticles such as polystyrene microbeads and Saccharomyces cerevisiae cells. The device was fabricated through a simple and precise process and was tested to determine the effects of different parameters on particle concentration and live/dead cell separation. The results showed that the device could efficiently and continuously perform with high throughput, achieving an efficiency of approximately 100% under optimum conditions. The device has four triangular electrodes for continuous focusing and separation of particles.

In this article, the separation process of platelet cells from red blood cells in continuous streams using the Dielectrophoresis mechanism is studied. A simulation approach is adopted to investigate the effect of various parameters such as geometry, voltage, frequency, and inlet velocity on the separation process. Examining diverse literature sources, simulations were conducted to investigate the impact of various electrode structures and shapes, different voltage settings, and distinct chip exit configurations on particle manipulation and separation [29,31,34,35]. As far as our knowledge extends, no prior studies have systematically explored parametric investigations for various geometries. The objective of this study has been to conduct a parametric exploration of different geometries for the separation of red blood cells (RBCs) and platelets, as there is currently a lack of research in this domain. The focus has been on elucidating the parametric differences in the geometries and their impact on the separation efficiency of these blood components. The objective is to enhance the efficiency of separating red blood cells (RBCs) and platelets using different geometries, including (a) triangular shape [36], (b) rectangular shape where the height is less than the width [37], (c) square shape [37], (d) rectangular shape where the height is greater than the width [37], (e) circular shape [37], and (f) omega-shaped configuration. The simulation model takes into account the electrical and fluid dynamics of the system to predict the behavior of cells in a microfluidic channel. Prior to manufacture and the experimental analysis of the setup, the parameter study can act as a low-cost test and provide information on how each parameter affects the separation outcome.

2- Governing Equations

In this simulation, a non-uniform electric field has been used for the continuous separation of blood cells. One of the situations when a homogeneous spherical particle is suspended in a medium and subjected to an uneven electric field, Dielectrophoresis can be seen. In this scenario, eq.1 [38] can be used to explain the dielectrophoretic force (DEP force) operating on a particle with a radius of a. To solve the fluid flow within the microfluidic channel, the continuity equation (eq.5) and Navier-Stokes equations (eq.6) are utilized. These equations are used to describe the conservation of mass and momentum in the fluid, respectively. The continuity equation relates the rate of change of the fluid density to the velocity field, while the Navier-Stokes equations describe the relationship between the pressure, velocity, and viscosity of the fluid. The solution of these equations helps to understand how the blood cells behave under the influence of the non-uniform electric field and how they can be separated based on their dielectric properties. Overall, the use of these governing equations plays a crucial role in the simulation of the continuous separation of blood cells using a non-uniform electric field.

$$F_{DEP} = 2\pi\epsilon_m a^3 Re\left[K_e\right]\nabla E_{rms}^2 \tag{1}$$

$$K_e = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \tag{2}$$

$$\epsilon_p^* = \epsilon_p - j\,\frac{\sigma_p}{\omega} \tag{3}$$

$$\epsilon_m^* = \epsilon_m - j\,\frac{\sigma_m}{\omega} \tag{4}$$

$$\nabla\vec{V} = 0 \tag{5}$$

$$\rho\frac{D\vec{V}}{Dt} = -\nabla p + \rho\vec{g} + \mu\nabla^2\vec{V} \tag{6}$$

The complex permittivity coefficients of the particle and suspension medium are indicated in the descriptive equation for the dielectrophoretic force (eq. 1) by the symbols ϵ_m and ϵ_p, respectively. The electrical conductivity coefficients for the suspension medium and particle are σ_m and σ_p, respectively. The real component of the Clausius-Mossotti factor is referred to as $Re\left[K_e\right]$. ∇E_{rms}^2 is the gradient of the square of the electric-field intensity, f (Hz) is the frequency of the applied electric field, and ω=2πf is the angular frequency in radians. The real part of the Clausius-Mossotti factor plays a key role in determining the direction and magnitude of the dielectrophoretic force experienced by a particle in a non-uniform electric field. The factor's real part decides whether the force is positive or negative, depending on the relative polarizabilities of the particle and the surrounding medium. If the particle is more polarizable than its surroundings, the force is positive, moving the particle toward higher electric field intensity. In contrast, if the medium is more polarizable than the particle, the force is negative, pulling the particle towards lower electric field intensity. This understanding can aid researchers in designing and optimizing microfluidic systems that utilize Dielectrophoresis for particle separation and manipulation based on their dielectric properties. In this study, the real part values of the Clausius-Mossotti factor for Red Blood Cells (RBC) and Platelets (PL) at a frequency of 100,000 Hz are determined to be -0.1359 and -0.0884, respectively.

3- Numerical Method

COMSOL Multiphysics has been used to create a 2D FEM algorithm to investigate the cases. Three physics equations—electric currents, creeping flow, and particle tracking in fluid flow—were employed in this simulation to mimic the model. It is assumed that the fluid is both incompressible and non-turbulent. The Navier-Stokes equation does not include the internal component because the fluid flow is steady and the Reynolds number, which is the ratio of inertial forces to viscous forces, is relatively small (Re<<1). The GMRES solver is advanced using the Jacobi iteration approach, while the non-linear solver uses the Newton method.

Fig. 1 illustrates the various microchannel geometries that were investigated, which consist of (a) triangular shape, (b) rectangular shape where the height is less than the width, (c) square shape, (d) rectangular shape where the height is greater than the width, (e) circular shape, and (f) omega-shaped configuration. The microchannel geometry used in the present simulation is characterized by having two inlets and two outlets, along with specified lengths. Electrodes embedded along the microchannels apply a non-uniform electric field on the particles as they move through the channel. The electrodes are positioned near the top of the microchannel, with positive electrodes placed along the wave crests and negative electrodes placed along the troughs. This placement is consistent across all of the microchannel geometries studied. As illustrated in Fig. 1, blood cells are introduced into the microchannel through Inlet 1, or the upper inlet, while buffer solvent is introduced through Inlet 2, or the lower inlet. The width of the microchannel, as well as the inlet and outlet, is 500 μm. The microchannel has a unit length of 2000 μm, which is repeated to match the number of wave

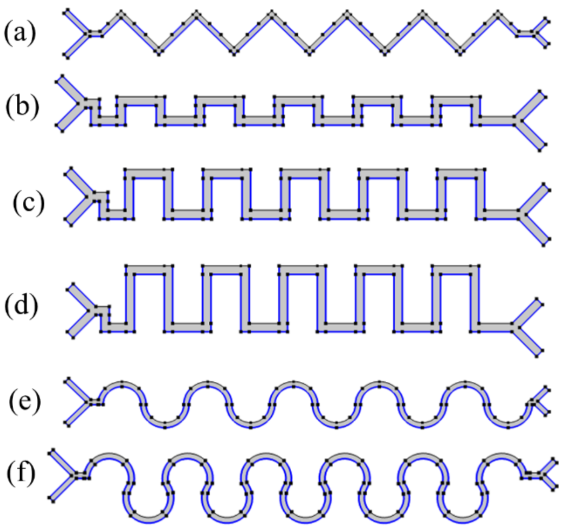

Fig. 1. schematic view of microchannels: (a) triangular shape, (b) rectangular shape where the height is less than the width, (c) square shape, (d) rectangular shape where the height is greater than the width, (e) circular shape, and (f) omega-shaped configuration, where the black lines show the location of electrodes.

Table 1. Simulation properties

Fluid properties	Symbol	fluid	Red blood cells	platelets
Radius (μm)	r	-	5	1.8
Membrane thickness (μm)	d	-	9	8
Relative permeability	ε_r	80	59	50
Relative membrane permeability	ε_{mem}	-	444	6
Electric conductivity (mS/m)	σ	55	31	25
Membrane electric conductivity (mS/m)	σ_{mem}	-	0.001	0.001
Density (Kg/m^3)	ρ	1000	-	-
Fluid viscosity (Kg/m.S)	μ	0.001	-	-

periods. Upon reaching the end of the microchannel where the two outlets are located, the platelets, after separation, are directed towards the upper outlet or Outlet 1, whereas the red blood cells are directed towards the lower outlet or Outlet 2. Table 1 provides the electrical and mechanical properties of both the particles and fluids used in the study. Specifically, pure water at a temperature of 20 degrees Celsius was utilized as the fluid medium in the investigation.

4- Result and Discussion

Fig. 2 displays a randomly selected section used to evaluate the mesh independence of the model. The results demonstrated that the solution obtained from a case containing approximately 2000 elements was mesh-independent, as depicted in Fig. 3.

Fig. 4 illustrates the distribution of electric potential within the triangular, circular, and square geometries. It is

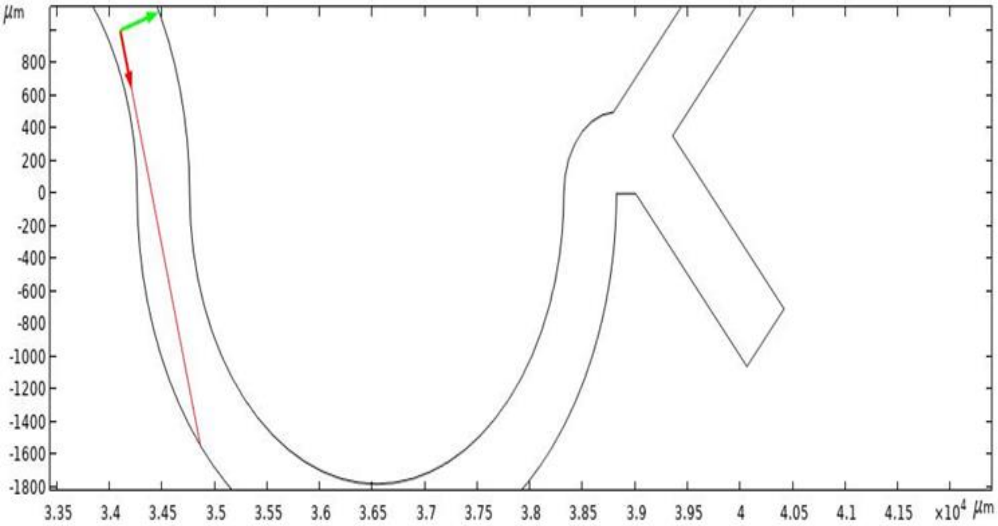

Fig. 2. Random line for mesh independency study

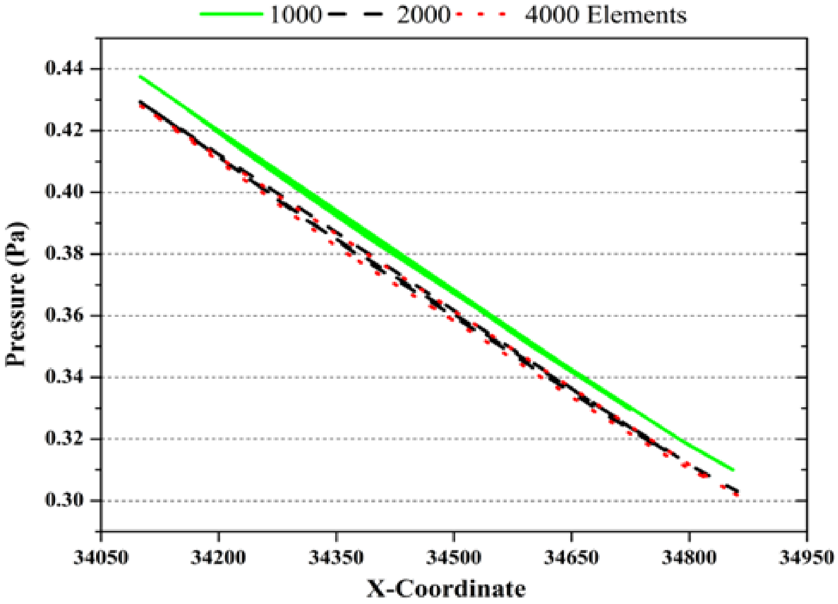

Fig. 3. Mesh independency study results for 3 different element counts

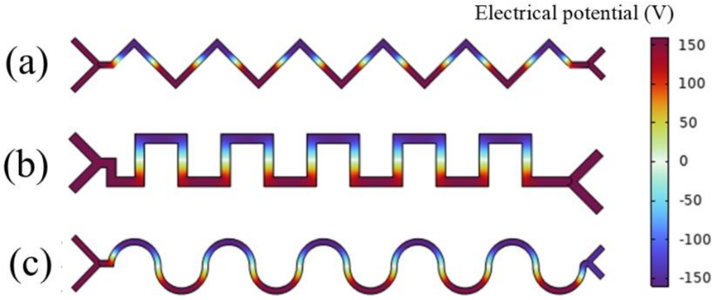

Fig. 4. Distribution of electric potential contours at 3 different geometries: (a) triangular shape, (b) square shape, and (c) circular shape

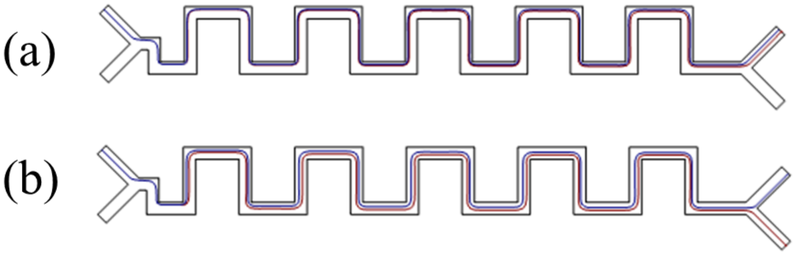

Fig. 5. Particle traces of red blood cells (RBC) and the platelets in square shape where (a) V=100 v, v=200 (μm)/s , and f=10000 Hz (b) V=200 v , v=200 (μm)/s , and f=10000 Hz

worth noting that this distribution remains consistent across all other voltages and geometries, with only the corresponding values being affected.

Particle traces are shown in Fig. 5, where the path of red blood cells (RBC) is represented by the red line and the path of platelets is shown in blue. As is evident, separation did not occur in scenario (a), whereas it was successfully achieved in scenario (b).

Dielectrophoresis (DEP) is a powerful technique for manipulating and separating particles in microfluidic devices based on their polarizability differences. One of the most important parameters in DEP separation is the applied voltage. The effect of voltage on DEP separation can be understood through the concept of the dielectrophoretic force (DEP force), which is the force acting on a particle due to its interaction with an electric field gradient. The strength of DEP force is proportional to the square of the electric field strength, which is in turn proportional to the applied voltage. Therefore, increasing the voltage leads to an increase in the strength of the DEP force, which can result in more efficient particle separation. However, there is a limit to the voltage that can be applied, and exceeding this limit can lead to undesirable effects such as particle aggregation or even damage to the particles. The dielectric breakdown of human RBCs is reported to range between 4 - 5 kV/cm [39], and platelet activation under voltages as high as 8.5 kV/cm [40] has been reported to be viable for clinical use; hence, the product of the proposed devices' operation can be considered viable.

The effect of voltage on DEP separation can also be dependent on the geometry of the microchannel in which the separation is taking place. As shown in Fig. 6, in triangular geometries, no separation occurs at voltages below 100 V, which means the low voltage failed to produce sufficient FDEP for blood cell sorting (Fig. 7a), and above 260 V, RBCs tend to stick to the channel walls (Fig. 7 b). However, separation occurs between voltages of 100 V to 260 V (Fig. 7 c). Raising the electric voltage resulted in an elevation of

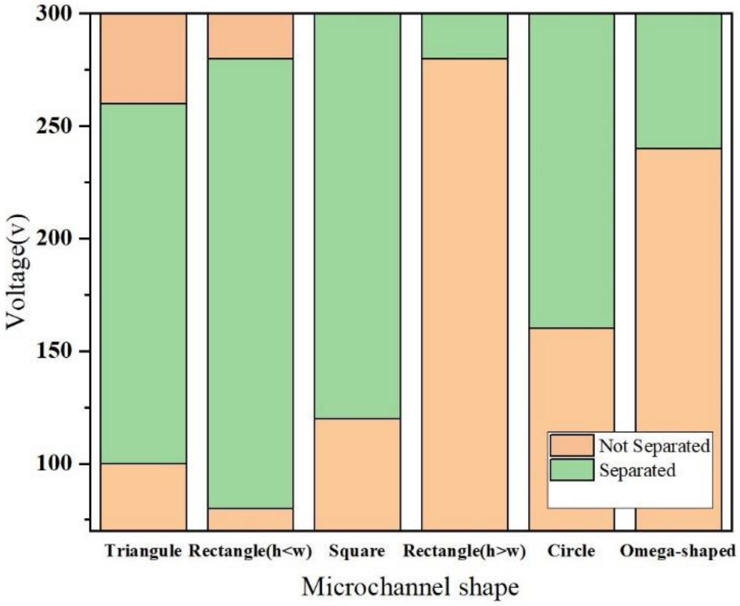

Fig. 6. The effect of voltage on separation in different geometries where v=200 (μm)/s and f=10000 Hz

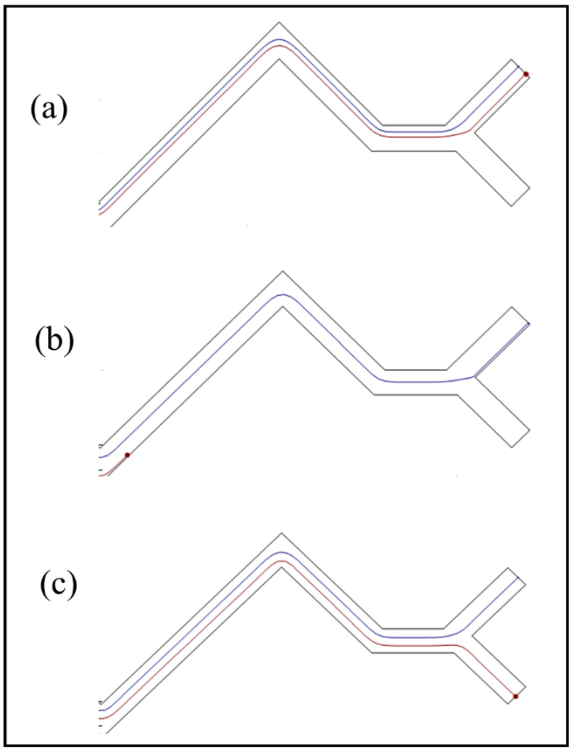

Fig. 7. Schematic view of (a) not separated cells where V=100 v , (b) cells attached to the channel wall where V=200 v , and (c) separated cells where V=280 v in triangular geometry and v=200 (μm)/s , and f=10000 Hz

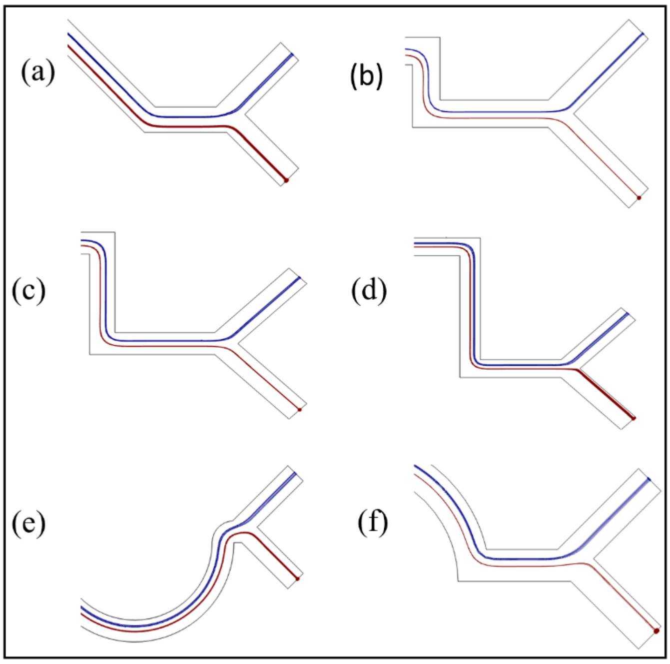

Fig. 8. Schematic view of separated cells at the end of microchannels: (a) triangle where V=200 v, (b) rectangle(h<w) where V=200 v, (c) square where V=200 v, (d) rectangle(h>w) where and v=280 (μm)/s, and f=10000 Hz , (e) circle where V=200 v, and (f) omega-shaped where V=280 v and v=200 (μm)/s , and f=10000 Hz

the electric field and FDEP within the cells. This heightened FDEP induced a vertical deflection of both red blood cells (RBCs) and platelets away from each other. These findings suggest that an electric voltage ranging from 100 to 260 V could be suitable for the envisioned design of a triangular microfluidic device to achieve an effective separation of RBCs and platelets. In rectangular geometries where the height is less than the width, no separation occurs below 80 V and above 280 V, while in square geometries, no separation occurs below 120 V. In rectangular geometries where the height is greater than the width, an increase in channel height has a negative impact on separation efficiency as it reduces the electric field gradient and therefore the strength of the DEP force. This can also be observed in circular and omega-shaped channels, where no separation occurs below 160 V and 240 V, respectively. Fig. 8 provides a visual representation of the isolated cells of red blood cells (RBCs) and platelets as they are observed at the exits of various microchannels. This image serves as a crucial illustration to demonstrate the specific locations where RBCs and platelets have been successfully separated from each other.

In conclusion, the applied voltage plays a critical role in DEP separation, and the optimal voltage for separation can depend on the geometry of the microchannel. Careful consideration of these factors can lead to more efficient and effective DEP separation of particles.

Fig. 9 illustrates the effect of speed on separation for a voltage of 200 volts and a frequency of 10,000 Hz. In a triangular geometry, separation occurs at speeds greater than 150 micrometers per second. In a rectangular geometry where the height is less than the width, separation occurs at speeds greater than 175 micrometers per second. In a square geometry, separation occurs at speeds greater than 150 micrometers per second. However, in geometries such as rectangular, where the height is greater than the width, circular, and an omega shape, no separation occurs. This suggests that the effect of voltage on separation is more significant compared to the effect of speed.

In conclusion, understanding the relationship between voltage, frequency, and speed in Dielectrophoresis is essential in optimizing the separation of particles in different geometries.

Fig. 10 illustrates the impact of frequency on Dielectrophoresis separation under constant voltage (160 volts) and speed (200 micrometers per second). In triangular and rectangular geometries with height less than width, as well as in square and circular geometries, separation occurs in the frequency range of 10000 to 500000 Hz. However, in rectangular geometry with height greater than width and in omega-shaped geometry, no separation is observed, indicating that voltage has a stronger impact than frequency on separation efficiency in these geometries.

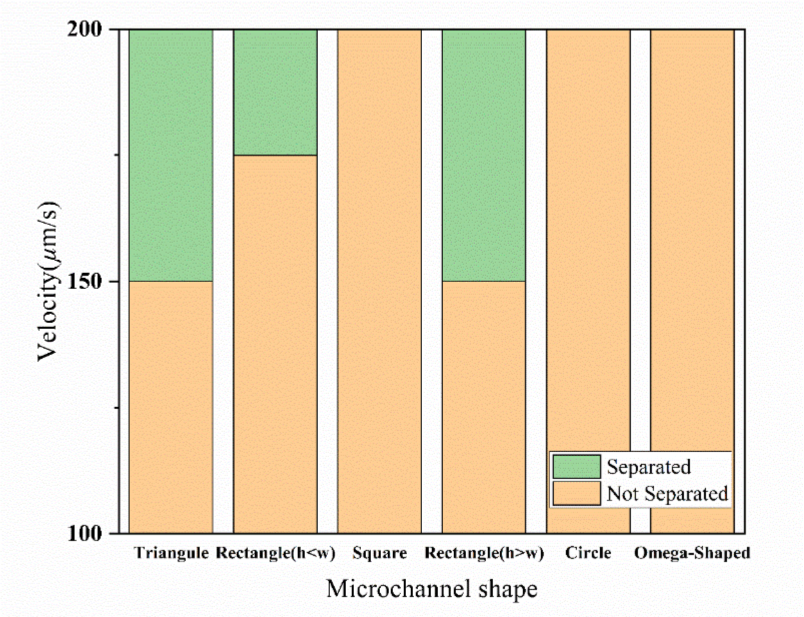

Fig. 9. The effect of velocity on separation in different geometries where V=200 v and f=10000 Hz

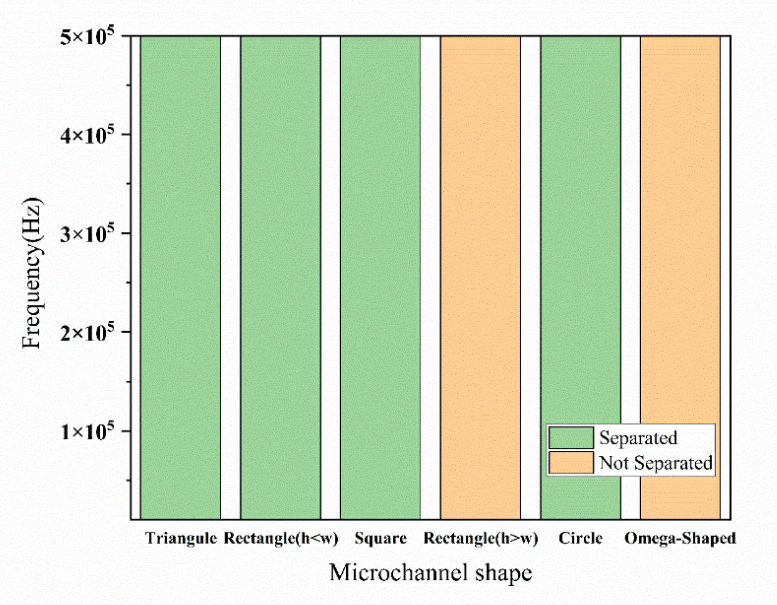

Fig. 10. The effect of frequency on separation in different geometries where v=200 (μm)/s and V=160 v

It is worth noting that the optimal frequency for Dielectrophoresis separation depends on various factors, including the size and shape of the particles or cells to be separated, the electrical properties of the suspending medium and the electrodes, and the applied voltage. Therefore, it is essential to carefully select the frequency and other experimental parameters to achieve the best separation efficiency in a specific system.

5- Conclusion

This paper describes an investigation of the separation of blood cells using Dielectrophoresis (DEP) in microfluidic devices. The simulation was performed using COMSOL Multiphysics software and involved three physics equations: electric currents, creeping flow, and particle tracking in fluid flow. The microchannels had various geometries, including triangular, rectangular, square, circular, and omega-shaped configurations. Electrodes embedded along the microchannels applied a non-uniform electric field on the particles as they moved through the channel, allowing for the separation of platelets and red blood cells. The study found that the voltage applied played a crucial role in DEP separation, and the geometry of the microchannel could also impact the efficiency of separation. The results showed that successful separation was achieved in rectangular microchannels with a height less than the width, square microchannels, and omega-shaped microchannels, among others, at specific voltages. The study also found that the geometry of the microchannel could affect the separation efficiency and that an increase in channel height had a negative impact on separation efficiency in some geometries. The study provides important insights into the use of DEP separation in microfluidic devices for blood cell separation.

To enhance our understanding of microchannel separation efficiency, it is recommended future research- focus on conducting a comprehensive parametric study. This study should investigate the impact of various parameters, including voltage, speed, frequency, and microchannel dimensions on separation efficiency across all geometries. Additionally, laboratory work will be necessary to conduct this study effectively. By systematically varying these parameters and analyzing the resulting data, researchers will be able to determine the optimal conditions for achieving high separation efficiency.

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