A novel microfluidic system using a reservoir and flow control system for single-cell release, migration, separation, and characterization

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***Abstract*—The microfluidic chambers have become the focus of many scientists and researchers because it ensures efficient control for many functions such as cell migration and separation. In addition, the microfluidic is interested in studying the behavior of fluids within microchannels. It is also interested in the technology of the manufacturing of these micro-channels. Single cell migration and separation are the key step in several applications such as lab-on-chip (measurement the electrical properties (i.e. Impedance, and capacitance) of a single-cell), biochemistry, and cell biology. In this study, a novel technique for single-cell migration and separation using a reservoir, flow control and dielectrophoresis (DEP) is purposed. Also, sensors for the capacitance measuring is implemented for cell characterization applications. The design of the proposed microfluidic chamber is simulated and tested using COMSOL Multiphysics 5.4. The results prove the ability of the proposed system for single-cell migration and separation using a reservoir and flow control system at a lower flow rate than previous work through a single sample.**

Keywords—Microfluidic, reservoir, single-cell, separation, characterization.

I. INTRODUCTION

Recently, many applications depend on a single-cell study, therefore single-cell migration is a necessity for many scientific areas. A lab on a chip is one of the most important applications that need to the single-cell migration. A lab on a chip is a system that integrates one or many functions of laboratories on a very small device using a single integrated circuit and microfluidics. Microfluidics concern the control, and the behavior of fluids that flow through micro-channels. It includes many functions transportation, mixing, and separation in order to enhance the efficiency and flexibility of a specific application as well as reducing sample and reagent volumes. Microfluidics serves many fields such as capillary, pressure driven, centrifugal, electro-kinetic, and acoustic. The proposed design concerns the microfluidics with flow control for single-cell migration and electro-kinetic for single-cell separation. Many of the previous works relied on the presence of an entrance that allows the release of single-cell but did not focus on how to achieve this property. The power of this study is solving the single-cell releasing problem using a reservoir. Yamada et al separated mixture of fluid and particles by the spreading streamlines according to their sizes [1]. Takagi et al. designed a microfluidic chamber (MC) using two inlets, one for the liquid that contains particles, and the other for a liquid without particles [2]. Vig and Kristensen enhanced pinched flow fractionation [3]. Lee et al. separated the particles by a contraction–expansion array. Also, the sheath was another solution was to pass single-cell [4-5]. Adams et al. improved the sorting using integrated acoustic-magnetic separation [6]. Lenshof A et al. designed a microfluidic chamber based on inlet and outlet of group particles [7]. Nam et al. designed a microfluidic chamber based on continuous sample release [8]. From this point, the proposed design is developed to solve two major problems: first problem; single-cell release, this is because many applications take into account the accuracy of measurements by measuring on a single-cell (i.e., cancer cell analysis [9-10], cellular heterogeneity [11], single-cell spectroscopy [12], and single-cell impedance measurement [13]). Second problem: the size of the sample volume. However, Previous work has been summarized into the following types: 1) continuous injection of a sample that includes single-cell at each step of time; which lead to consuming a large volume of sample to ensure the continuity of injection. In addition to the complex process to release single-cell at each step of time such as sheath [14]. 2) continuous injection of a sample with a random number of cells which leads to consuming a large volume of a sample, and low accuracy of measuring [15]. In this study; these two problems are solved as following: the technique and the geometry of the proposed design need to release tiny volume of a sample only once at the beginning of the experiment. In addition to allowing the self-continuity release of single-cell

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at each step of time depend on the flow rate of the inlet medium.

II. METHODS AND MATERILS

In this study, a whole microfluidic system for separation and characterization of biological particles is developed and implemented. The proposed system consists of three main phases as follows: first phase: single-cell releasing using a reservoir and flow control system. Second phase: cell separation using DEP. Third phase: microelectrodes for capacitance sensing application as shown in Fig.1. However, each phase will be briefly explained in the next sections.

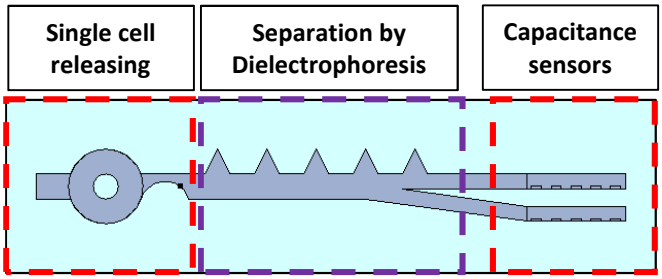

Fig. 1: the whole microfluidic system for separation and charactrization of biological particles.

A. Single-cell releasing

The proposed design includes a reservoir in a donut shape for sample release as shown in Fig.2. The reservoir has one inlet (free medium is injected with a specific flow rate to allow the particles to exit the reservoir) and one outlet with a specific shape (narrow) for single particle release. The second inlet within the channel to maintain the particles move in the middle of the channel for the next stage (separation). However, Fig.2 shows the dimension of inlet and outlet channels which are relative to the application and the size of the proposed particles. Also, the flow rate of the inlet medium at the two inlets is relative to the application. In this study, the flow rate of inlets is 200 µm/s. This rate is preferred for achieving suitable particles' velocity for the separation phase. The conclusion of the releasing process is that a tiny volume of sample is placed in the reservoir, then the free medium is injected through inlet 1 with a specific rate. The particles start to exit from the reservoir cell by cell due to the narrow way out. The particles forced to flow in the middle of the channel using the injection of the free medium through the inlet 2.

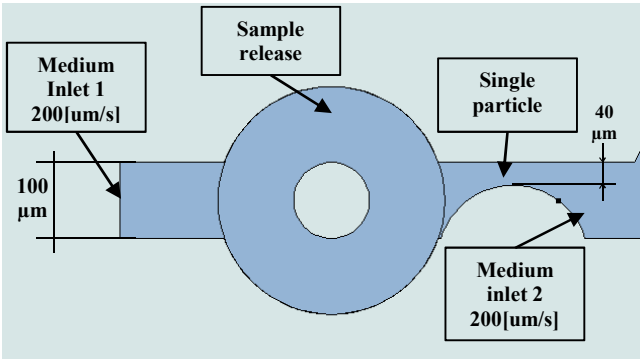

Fig. 2. First phase: single-cell releasing using a reservoir.

B. Separation using DEP and microfluidic

The proposed system for particles separation is developed as follows: 1) a sawtooth microelectrode for applying di-electrophoresis force on particles. 2) Inlet and outlet channels for inlet and separate particles at a specific position according to the movement of particles under the effect of DEP forces and fluid flow forces. However, the particles undergo two forces: dielectrophoretic force and the drag force due to the flow of medium as following [16]:

$$F_t = F_{DEP} - F_m$$

$$F_{DEP} = 2\pi\varepsilon_m r^3 \, Re(K_{cm}) \nabla |E|^2$$

Where ε_m Is the permittivity of the medium, r is the radius of particles, E is the electric field, $Re(K_{cm})$ Is the real part of Clausius-Mossotti factor defined as follows:

$$K_{cm} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\,\varepsilon_m^*}, \qquad \varepsilon^* = \varepsilon - j\frac{\sigma}{\omega}$$

Where ε_p Is the permittivity of the particle, σ is the conductivity, ω is the angular frequency of the electric field, j the imaginary unit.

$$F_m = f\,v, \qquad f = 6\pi\gamma r$$

Where v is the speed of the cells relative to the medium, γ is the viscosity.

The developed microelectrodes and channels for separation are shown in Fig.3. However, it includes 5 microelectrodes for DEP forces, one inlet, and two outlet channels for separation of different type of particles. The creeping flow study, electric current and particle tracing for fluid flow modules are used to simulate the DEP forces and fluidic forces. Where ± 10 volts is applied to the electrodes. The flow rate is 200µm/s at the inlets of free medium and the pressure is (0 pa) at the outlets as shown in Fig.2.

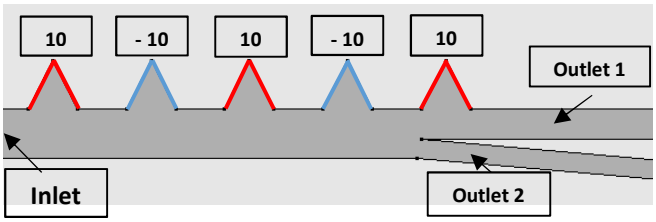

Fig. 3. Second phase: separtion of particles using DEP and microfluidic forces.

C. Capacitance sensor application

A microelectrode for capacitance sensing of particles is proposed as an application for measuring stage of a single-cell. There are many applications of integrated capacitance sensors (i.e. Fingerprint sensing [17], particle position sensing [18], physical property (humidity) sensing [19], and particles identification [20]. Using the electrostatics and stationary source sweep studies the capacitance of the particles is estimated.

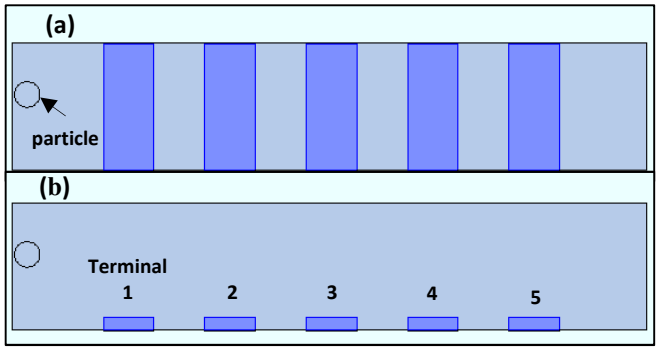

Fig. 4. Third phase: the microelectrode for capacitance sensing of a particle.

Fig.4 shows the top and the side view of the proposed microelectrode for capacitance sensing. The proposed design includes 5 electrodes that are defined as terminal from 1 to 5 as shown in Fig. 4. However, by firing one electrode with 1v and grounded others, the Maxwell capacitance at each electrode is measured.

III. RESULTS AND DISCUSIONS

In this section, the detailed results were presented within three categories in order to develop a microfluidic channel for injecting single-cell and manipulating it for separation and characterization using capacitance sensing as an application. However, the three categories are defined as follows: 1) the fluid flow conditions, 2) the output results of the dielectrophoretic microelectrode, and 3) the output of capacitance sensing microelectrode.

A. The fluid flow conditions

The inlet/ outlet flow rates are defined especially for the proposed design as described previously. Fig.5 (a) shows the distribution of the fluid flow pressure over the whole system. However, the rainbow legend bar includes the red color that represents the maximum values of the fluid pressure and the blue color represents the minimum value. However, the highest value of pressure has occurred at the inlet and the lowest at the outlet. This is logical in order to the fluid moves from the inlet to the outlet until the separation and characterization processes are interrupted. Fig.5 (b) shows the velocity distribution of fluid flow. The maximum velocity is achieved at the nozzle due to the path is narrowed at this nozzle. Therefore, the particles in the medium undergo the first force which is the drag force.

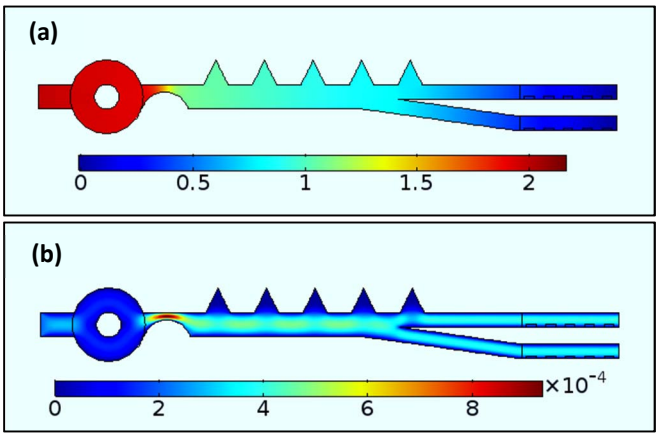

Fig. 5. a) the pressure distribution, and b) in the whole microfluidic system.

B. The output results of dielectrophoretic microelectrode

The applied signal for generating dielectrophoretic force is presented in Fig.6 (a). However, the red color and the blue color represent the maximum and minimum values of the applied electric potential (±10 volt). Which mean that a sequence of signals with 180° phase shift is applied. Fig.6 (b) shows the strength of the produced electric field norm. the highest values have occurred at the areas that are in between the microelectrode.

C. the output results of capacitance sensing microelectrode

the capacitance measuring is achieved by firing one electrode and grounded the others. Then, the capacitance is sensed at the same electrodes. Fig.7 presents the directions and distribution of the generated electric field norm due to the firing of one electrode for capacitance measuring.

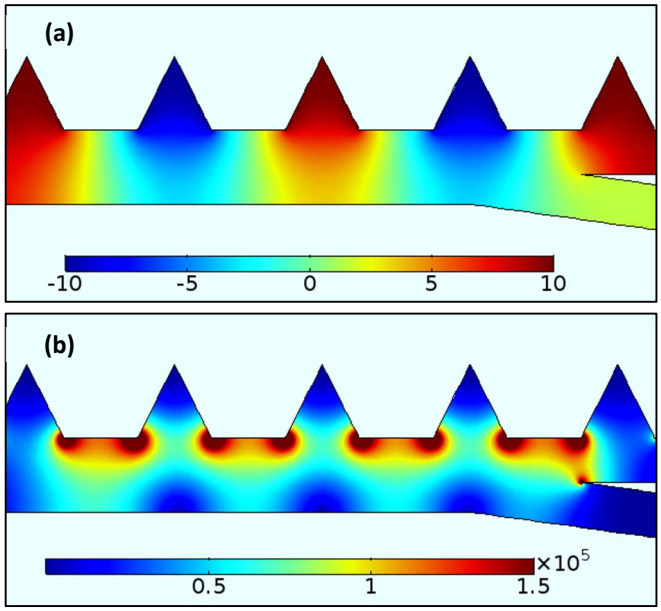

Fig. 6. a) The electric potential of the applied signal ±10 Volt, b) The distribution of the electric field norm over the developed microelectrode.

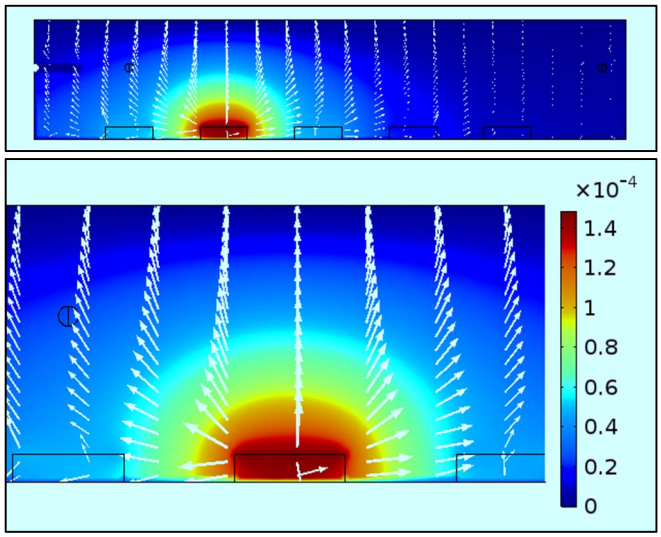

Fig. 7. the side plane of the capacitance sensing microelectrode and the focused of the fired electrodes.

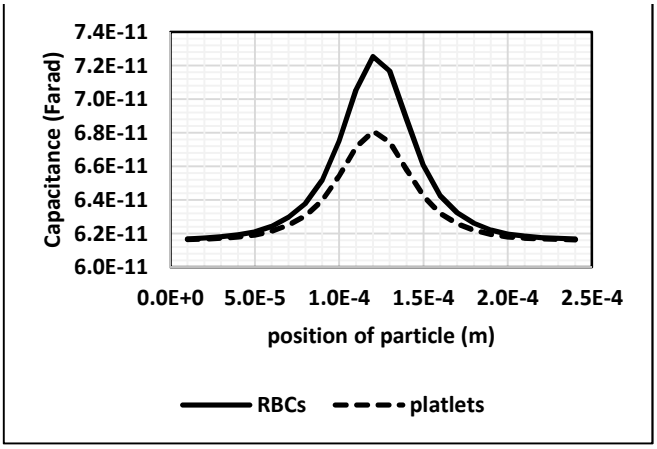

Fig.8: the estimated capacitance of RBCs and platelets.

According to the change in the position or the type of particle, the value of capacitance is changed. Fig.8 shows two important issues which are: 1) the closer the particle is to the sensor, the greater the capacitance value. As shown in Fig.8, the maximum value of capacitance for both type of particles is achieved at the middle of the electrode whose capacitance is measured. 2) the type of the tested particle affects the maximum value of capacitance. As shown in Fig.8, the RBCs have a higher max value of capacitance than platelets. Therefore, the proposed sensor able to differentiate between different type of particles.

Fig.9 shows the particle trajectories in different stages, where, the RBCs are displayed in red and the platelets in blue. Fig.9 (a) the sample release. (b) the injection of the medium at inlet 1 for single-cell release in the channel. (c) the injection of the medium at inlet 2 in order to force the cells to move in the middle of the channel. (D) the effect of the applying of dielectrophoretic force. (e) the separation of particles according to its movement after applying dielectrophoretic force. (f) The cells are directed to electrodes for the measurement phase.

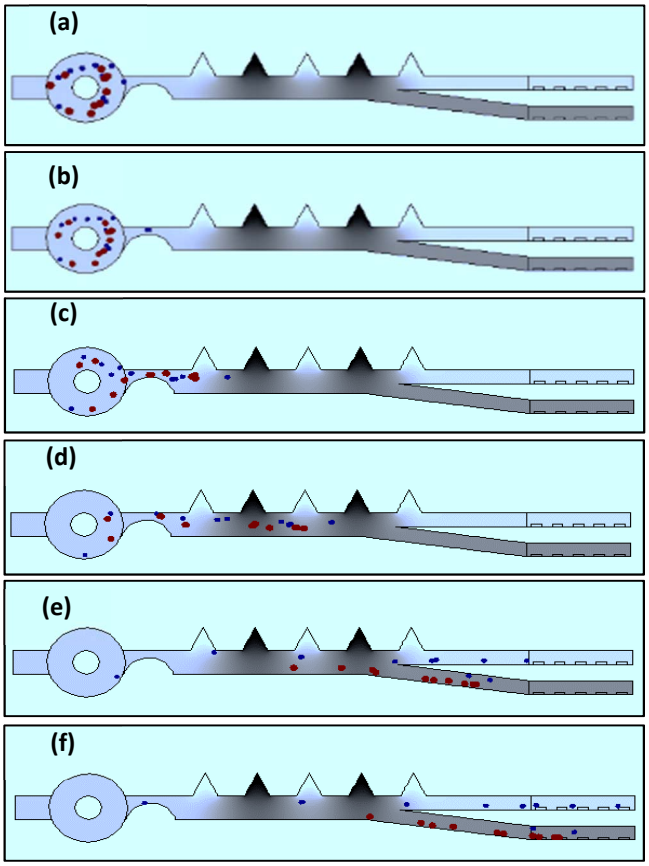

Fig. 9. Particle Trajectories. The RBCs are displayed in red and the platelets in blue.

IV. Conclusion

A novel technique for releasing single-cell using a reservoir is designed and implemented. A developed array of microelectrode for producing dielectrophoretic force in case of separating different types of cells is designed and implemented. A capacitance sensing microelectrode as measuring application is implemented and discussed. A model of a whole microfluidic system (sample release, microfluidic channels, separation technique (DEP), and capacitance sensing) is developed and implemented. The results show that the proposed novel technique complete the following tasks efficiently: permits single-cell releasing with only one sample drop using the reservoir, separation of different type of cells using DEP microelectrodes, and distinguish between the two types of cells using the capacitance sensors. All these completed tasks lead to reducing sample consumption, and high accuracy of characterization of particles unlike the previous methods.

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