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Micromagnetophoreses based CTCs Detections in Simple Chip

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Abstract

Rare cells separation as in Circulating Tumor Cells (CTCs) from clinical blood sample has fetal importance in cancer monitoring and treatment. Here we study a method of CTCs separation from clinical sample or buffer sample by magnetic separation technique. Design and simulations of magnetic and drag forces in the microchannel for separation of magnetic particles and CTCs have been studied. Modeling software COMSOL Multiphysics 4.3a (COMSOL, Inc.) which uses finite element method used to design and simulation of the microchannels device for the characterization the device. The simulation was done in two stages first for magnetic field generated from the permanent magnet positioned at a specific distance from the microchannel wall and second the cell/particle tracing for cell separation verifications. The separation results with 93% purity and efficiency of the undesired particle was achieved 0.105m/s velocity and cancer cells size about 15 µm. Considering the results achieved, our proposed microchannel device is a good candidate for cancer cells detections in clinical uses.

Keywords: Cancer Cell, Computational Fluid Dynamics, Magnetophoresis, Microfluidics

1. Introduction

Biological cells separation and detections has been highly interested in recent years. There are many applications concerned for an easy and reliable way to separate samples into distinct different populations[1]. Microfluidic technology as in microfluidic lab on chip devices is one of the most important methods in the single cells separation. It can be achieved through different methods like microchannel system based on membrane[2,3], and membrane free microchannel systems[4,5]. The need of cancer cells among other cells to be selectively isolated from blood at earlier cancer stage has a high demand in cancer treatments and drug discovery analysis. Therefore, the cancer cells isolation and detection at earlier stage has gained great importance among the various fields of research[6,7].

As a result of the small sample required for analysis, the short required scan time and large surface to volume ratios, are available features in microfluidic systems[7,8], this technology act as a promising opportunities in field of single cell detection[6,9], as a result, various of different microfluidic structures have been manufactured and tested for separation of rare cells as well as (CTCs), fetal cells, and stem cells, where the differences in cell shape and size, are utilized for the separation[7–9], as reviewed in our previous published paper microfluidic device based cells separation and detections can be achieved using many different ways and mechanisms depending on the desired cells to be isolated[10]. Among these different criteria and mechanisms of the microfluidic device utilized for cells separations the magnetophorsis which is magnetic based separation represents an emerging research area

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in microfluidics field is utilizing magnetism to control particles and cells on the microfludic chip[7]. This is a very attractive and power full method as small, lightweight permanent magnets can easily be positioned on the microchip. Magnetic based methods have been utilized to be use in both particle and cells sorting , fluid mixing and cell lysis (breaking down of a cell wall)[8]. There are many well-known types of commercial magnetic particles and beads which can be coated with antibodies which will cause the particles to attach to specific cells[9]. Once the cell attached to a magnetic particle it can then be directed to a specific area on the microchannel[10], where it can be optically detected. Therefore, the magnetic cell separation totally dependent on the interaction between antigenslocated on the cell surface type and the type of antibodies that has been conjugated on magnetic particle surface, by utilizing external magnetic force it is easy permit manipulation and control even capturing the cancer cells labeled with specific magnetic. There are many different methods of magnetic cell and particle control that are used in microfluidics[7], Liu and Pang et al. stated a microfluidic device, it serves as the first device which has the ability to separate the low abundance cancer cells from among a mixture of Red Blood Cells (RBC) utilizing magnetic method[11], in this device a hexagonal film of nickel pillars was structured and patterned onto microfluidic channel botton to represent as a magnetic field gradient generator in order to control and trap the movement of the magnetic beads. Utilizing the magnetic cells separation method mechanism the spiked A549 cancer cells in RBCs mixture were captured and sorted effectively, the achieved capture rate was between 62% and 74%.

A method stated by Saliba and viovyetal[12], utilizing array of magnetic ink of bio-functionalized magnetic beads structured at the microfluidic channels bottom. The application of a magnetic field vertically leads to aggregation of magnetic particles in the top of the ink dot, applying this device in biomedical application by using a mixture of cells more than 94% recovery rate was achieved. In[13], with a 90% recovery rate had manufactured a device capable to separate lung cancer cells from patient blood sample by utilizing magnetic beads functionalized with a specific antibodies installed in a chamber bottom to recover and trap the cancer cells.

This research paper stated the use of magnetophoresis forces to control particles on the microchannel. This project aims to separate and sort magnetic particles in a flow and ultimately to separate and identify magnetically tagged breast cancer cells from background healthy cells and buffer media. The mechanism depend on the conjugation of the micro-beads with anti-epithelial cell adhesive molecule antibodies (anti-EPCAM) which bind to epithelial cancer cells, to utilize the way towards detecting cancer cells from a blood sample was investigated.

2. Material and Method

2.1 Theory

Four main forces act on a magnetic bead suspended in a liquid medium, are the magnetic force F_{mag}, the viscous drag force F_D, the gravity force F_g and the F_{buo} buoyancy force, as schematically explained in Figure 1 below

The most dominant are drag and magnetic forces while the other forces such as gravity force can be neglected, in this model we just consider the both dominant forces, from the schematic above figure we get

$$\vec{F}_{mag} = -\vec{F}_d \quad (1)$$

The magnetic beads are moving in a fluid flow, and thus the exerted drag force (F_D) given as

$$F_D = 6\pi r\eta v \quad (2)$$

Where:

η = fluid viscosity, r = radius of the particle, and v = the fluid velocity.

The magnetic force ($\overline{F}_{mag}$) of the magnetic beads due to the applied magnetic field can be expressed by the following equation:

$$F_{mag} = \frac{4\pi r_b{}^3 \Delta\chi}{(3\mu_0)(B.\Delta)\mathbf{B}} \quad (3)$$

Where:

B =the magnetic field from the magnet, $\mu 0$ = the permeability of thespace, Dx= magnetic susceptibility of the bead and that of the fluid (Dx= 1.1704) and r_b= radius of the bead magnetic core (0.5 μm).

In our model we have described the movement of the magnetic beads in both x and y directions in order to a chive that we have to find the magnetic force in both

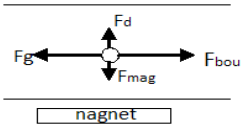

Figure 1. Four main forces act on a magnetic bead suspended in a liquid medium.

directions that affect the magnetic beads movement in the microchannel. The magnetic forces in x and y direction on the magnetic beads is given by

$$F_{mag,\,x} = \frac{4\pi r_p^{\,3}\Delta\chi}{3\mu_0} H_x \frac{\partial H_x(x,y)}{\partial x} \quad (4A)$$

$$F_{mag,\,y} = \frac{4\pi r_p^{\,3}\Delta\chi}{3\mu_0} H_y \frac{\partial H_y(x,y)}{\partial y} \quad (4B)$$

The governing equations for the flow of magnetic beads, i.e., cell, super paramagnetic bead, or cell bead complex, based on a Lagrangian particle tracking method and Newton's law

$$\mathbf{m_p}\frac{\mathbf{d}}{\mathbf{dt}}\mathbf{v_p} = \mathbf{F_{mag}} + \mathbf{F_{drag}} \quad (5)$$

Here, m_p and v_p are the mass and velocity of the particle.

2.2 Design of the Microchannel

The microfluidic device in this study consists of one straight channel with dimensions (700 µm x 30mm) (width × length),the height of the microchannel is 60 µm. Neodymium (NdFeB) earth rare magnet was positioned laterally on the side of the straight channel with a lateral distance about (1mm). Polydimethylsiloxane (PDMS) device was finished using standard soft lithography techniques described in the literature[14]. At the end a microfluidic device consists of two inlets (sample and buffer) to provide a confining flow, with a single flow channel, and two outlets for collection was achieved as shown in Figure 2(a). The sample and buffer were pumped through the two inlets at constant flow rates by separate tow syringe pumps (Dual syringe infusion/withdrawal pump, Cole-Parmer, Vernon Hills, IL, USA). The relative widths of sample and buffer flows within the channel are determined by the relative flow rates of each. Grade N52 NdFeB magnets was positioned laterally at specific distance from the channel wall in order to generate an external magnetic field that continually deflects the flow path of the magnetic particles and the cells pre-coated with super-magnetic beads only. Cells that have enough number of magnetic micro beads becomes deflected during the flow and moves laterally to the flow direction, following this process leading to the cells separation lastly at the desired end as schematically shown in Figure 2 (b).

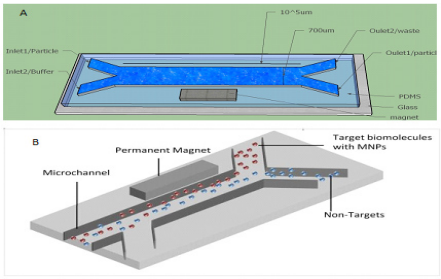

Figure 2. **(a)** Schematic representation of the microfluidic device dimentions. **(b)** The working mechanism of the device the different solutions used in inlets 1 and the black dots represent MPs across the channels and the expected behavior.

2.3 Cell Culturing and Labeling

MCF-7 cells line,purchased from American Type Culture Collection (ATCC), the culture media was Eagle's Minimum Essential Medium (EMEM, ATCC) supplemented with 0.01 mg ml−1 bovine insulin (Sigma-Aldrich) and 10% fetal bovine serum (FBS, Gemini Bio Products). Trypsin-EDTA (Invitrogen) use to harvest the cells, the harvested cells were suspended and diluted in culture media. Haemocytometer and microscope were used to count the cells and to determine the concentration. The cell suspension spiked into buffer with specific concentrations. Anti-EpCA Mantibody purchased from Abcam. Streptavidin conjugated superparamagnetic beads with 1 µm diameter were obtained from Sigma Aldrich. We saturated the beads (20 µL, 10 mg mL−1) with enough amounts of antibody (10 µL, 0.2 mg mL−1) in Phosphate Buffer Saline (PBS) at room temperature for 1 hour, Figure 3 and re-suspending in PBS to form the anti- EpCAM beads.

3. Results

3.1 Magnetic Field and Fluid Flow Simulation

A 2- Dimensional geometry model (2D) utilizing the COMSOL Multiphysics 4.3a (COMSOL, Inc.) modeling software was used to study the magnetic field intensities in different domains, inside the microchannel domain

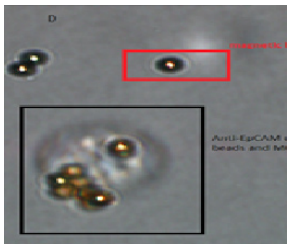

Figure 3. Mixture of anti-EpCAM coated beads and MCF7 cellsSchematic of magnetic bead coated with anti-EpCAM streptavidin biotin attached.

and the microchannel surrounding domain and to study the effect of the magnetic field generated on the CTCs paths. The conditions of simulation model were set for incompressible liquid flow with stationary state, the inlets fluid velocity were determined, a zero pressure boundary condition was set to the outlets boundaries for the fluid flow, in our solutions we consider the (0, 0) for the model at the center of the magnet.

As shown in Figure 4(a), the simulation results of the magnetic field, T, as function of distance (5 mm, 4 mm, 2 mm, and at the surface of the magnet) vertically from the permanent magnet shows that the high magnetic field intensities positions of the configuration are in the edge of the permanent magnet. Figure 4(b) shows the surface plot of the magnetic field intensity which illustrates the magnet position with respect to the microchannel.

3.2 MCF7 Cells Separation

A Neodymium (NdFeB) earth rare magnet was located laterally to the microchanel, will generate an external magnetic flux of about 250 mT in the surface as shown in Figure 4(a), leads to external magnetic force generated in y direction of the microchannel. MCF7 cells which were pre-coated with magnetic beads will get deflected along y direction, as shown schematically in Figure 2.

We practically studied the deflection and separation of the MCF-7 through the observation of the track changes of the cell micro-magnetic beads complex using a microscope. During the flow of the cells and buffer samples, the flowed cells from both outlets collected in 1.5 ml after the observation during the whole of the flow time using fluorescent microscope, as shown in Figure 5 (a), (b) which shows the captured images of the flow during the experiment time, from this we can conclude that the separation of 1 μm magnetic beads in the microfluidic channel is going well. The simulated trajectories of the beads Figure 5(c), (d) is in well agreement with the experimental one for both with and without effect of the magnetic field produced by (NdFeB) magnet.

In Figure 6 bellow the flow pattern of the cell-beads complex inside the channel in the presence. Figure 6(a) or

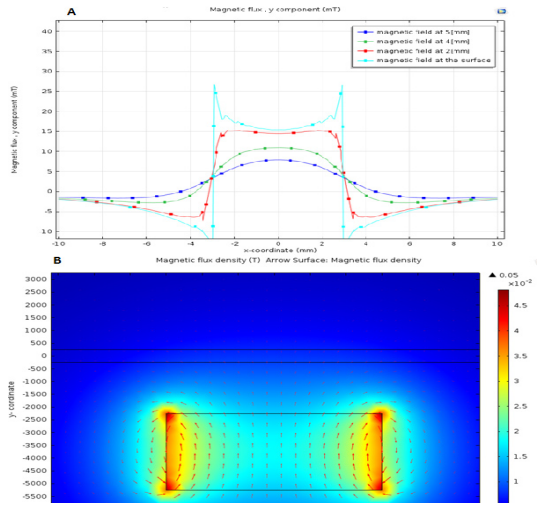

Figure 4. **(a)** The simulation results of the magnetic field intensity mT (A) as function of distance (5mm, 4 mm, 2 mm, and at the surface). **(b)** The surface plot of the magnetic intensity.

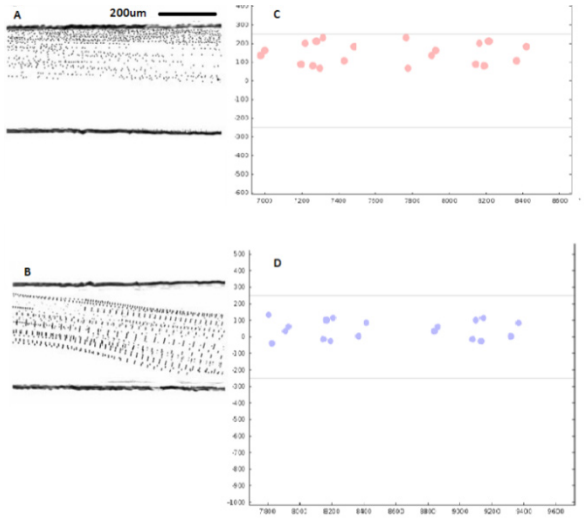

Figure 5. Images of the flow of 1 μm magnetic beads inside the channel,in case of without. **(a)** or with. **(b)** The magnetic field applied using a neodymium magnet .the simulation of the μm magnetic beads trajectories in the channel without. **(c)** or with. **(d)** the magnetic field applied using.

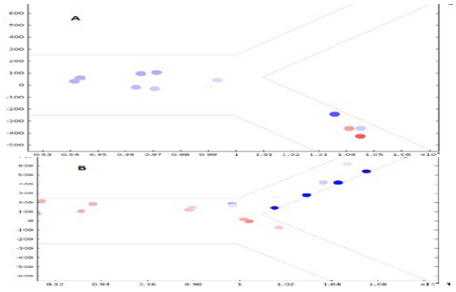

Figure 6. The trajectories of the complex at the end of the channel (exits). **(a)** With the magnetic force. **(b)** without the magnetic force.

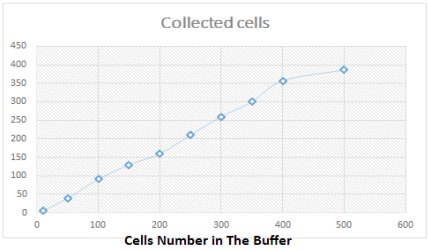

Figure 7. Experimental colected cell vs spiks one.

absence Figure 6(b) applied magnetic field using (NdFeB) magnet it clears that the magnetic beads as well as MCF7 cell–beads complex are well separated from their paths laterally under the effect of the magnetic force produced by the permanent magnet, in addition we are calculated the lateral displacement of the beads in y –direction during their path in the channel

Experimentally we observed the capability of the device in the separation till to 93% of the spiked MCF-7 cancer cells at a flow rate 40μL/min in buffer sample with a flow rate 100 μL/min, as shown in Figure 6.

4. Conclusion

This paper describes the mechanism and practical results of a simple microfluidic design capable to isolate selectively cancer cells. Utilizing magnetic cell sorting method, we fabricated and designed simple costless chip for separation of CTCs from buffer solution. We computationally simulated the external static magnetic intensity using COMSOL Multiphysics 4.3a (COMSOL, Inc.) software. The designed micro-device with lateral positioned permanent magnet isolates the CTCs into the lateral-exit of the flow in the micro-channel. The micro-device was observed experimentally to isolate about 93% of (MCF-7) in buffer solution at a flow rate of 40/100μL/min with respect to the whole buffer solution. Since the described designed micro-device protocols are very simple and costless, and regarding the results achieved, our proposed microchannel device is a good candidate for cancer cells detections in clinical uses.

5. Acknowledgment

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