Hydrodynamic Assists Magnetophoreses Rare Cancer cells Separation in Microchannel Simulation and Experimental Verifications

O Saeed¹², L Duru¹ and D Yulin¹

¹ School of Life Science, Department of Biomedical Engineering, Beijing Institute of Technology, Beijing 100081, China
² Faculty of science and technology, Department of Biomedical Physics, AL-Neelain University, Khartoum 1121, Sudan
E-mail: omerphy2007@bit.edu.cn

Abstract. A proposed microfluidic design has been fabricated and simulated using COMSOL Multiphysics software, based on two physical models included in this design. The device’s ability to create a narrow stream of the core sample by controlling the sheath flow rates Qs1 and Qs2 in both peripheral channels was investigated. The main target of this paper is to study the possibility of combing the hydrodynamic and magnetic techniques, in order to achieve a high rate of cancer cells separation from a cell mixture and/or buffer sample. The study has been conducted in two stages, firstly, the effects of the sheath flow rates (Qs1 and Qs2) on the sample stream focusing were studied, to find the proposed device effectiveness optimal conditions and its capability in cell focusing, and then the magnetic mechanism has been utilized to finalize the pre-labelled cells separation process.

1. Introduction

The use of single and pure cells in biological field represents a vital required demand for therapy, drug discovery and disease control and monitoring. As a result, cell separation, particularly of circulating tumour cells (CTCs) isolation and separation from a blood sample has gained high interest in the field of biology and biotechnology. Due to the fact that, the abundance of the CTCs in the blood can be as low as 1 in 10⁹ erythrocytes and 1 in 10⁷ leukocytes, and they somehow share characteristics and properties with leukocyte cells, their separation represents a challenges for researchers. The design of microfluidic platforms that have a high and precise ability in CTCs separation has been proven difficult. However, erythrocytes have their own unique physical, chemical and biological properties, which facilitate their separation and makes it easy to remove them from the blood[1][2].

Recently, several methods and mechanisms have been established for CTCs separation, they can generally be classified into two broadly approaches, firstly, biochemical methods, which were mainly achieved by merging the cell surface antigens express by the target cells, and intracellular markers fluorescent tagging to identify the CTCs. The CTCs identify as positive for cytokeratins (CK) fluorescent staining, while leukocytes were defined by staining for CD45 (receptor-linked protein tyrosine phosphatase).

The separation based on the second approach (biophysical methods) is totally based on the existence of differences of physical properties between CTCs and leukocytes cells e.g. size [3][4][5]. Deterministic lateral displacement (DLD) devices are also a part of this approach, which has been
utilized for separation based on cells and particles sizes [6][7]. Cell sorting techniques, as reviewed above and elsewhere [8][9], are limited in yield and purity, furthermore, the operation of these methods relies highly on the expertise of skilled personnel. Different fabricated microfluidic platforms have shown their high capability in the detection and separation of targeted cells from a mixture, which is highly demanded in biological and clinical research. Due to low costs, small sample volumes, fast processing times, large surface-to-volume and the ability to efficiently isolating single cell, and thanks to features available in microfluidic platforms[10], different methods based on microfluidic cell separation were established including dielectrophoresis [11], acoustophoresis[12] and hydrodynamics [13].

There are many microfluidic platforms that have been studied and utilized for CTCs separation based on biochemical methods [14][15]. Anti-epithelial cell adhesion molecule antibody (anti-EpCAM antibody), has been commonly used in studies for CTCs identification as U.S. FDA (Food and Drug Administration) approved Veridex Cellsearch® system (USA), considered as an example in utilizing this approach for clinical uses[16].

As shown in our previously published review paper[17], the different microfluidic methods that have been fabricated to separate cells including CTCs, suffers some limitations. Until now a few work has been done to overcome these limitation, by utilizing a microfluidic platforms that combine and merge two or more technique into one microfluidic platform. Here we are trying to overcome the one technique limitations, by utilizing a microfluidic device used to separate CTCs spiked in blood and/or buffer sample. Firstly, a hydrodynamic module used to concentrate and align the CTCs contained the sample in a narrow stream by controlling the two sheath flow rates \(Q_s\) in both peripheral channels. Secondly, exerting a magnetic force on the pre-labelled magnetic beads CTCs, generated by a permanent magnet located at specific distance from the main channel wall, enable the separation of pre-labelled CTCs from cell mixture and/or buffer sample. The study has been conducted in two stages, firstly the effect of both sample flow rate \(Q_i\) and the two sheath flow rates \(Q_{s1}\) and \(Q_{s2}\) on sample stream focusing were studied, to find the proposed device effectiveness optimal conditions and its capability, then the magnetic mechanism was utilized to finalize the separation proses of the pre-labelled cells with magnetic beads spiked in a cell mixture and/or buffer sample.

2. Theories

2.1. Hydrodynamic focusing

In the symmetrical hydrodynamic focusing as shown in Figure 1 below where \(Q_{s1}\) and \(Q_{s2}\) are equal, the sample stream width can be focused, constrained and centered in the middle of the microchannel, using the two equal sheaths flow rates from the peripheral channel inlets.

\[
\frac{w_f}{w_0} = \frac{v_0 Q_i}{\bar{v}_f (Q_i+2(Q_s))}
\]

Figure 1. Hydrodynamic sample stream focusing mechanism

In the same manner this focusing can be determined regardless of the sample stream width location within the channel, by using an asymmetric hydrodynamic focusing, where the sheath flow rates \(Q_s\) and \(Q_{s2}\) are unequally injected. Here we only consume symmetrical focusing conditions. The width of hydro-dynamically focused stream \((w_f)\), relates to the inlet flow rates \((Q_i)\) and the two side channels flow rates \((Q_s)\) as follow [18]
Where \( w_0 \) Outlet channel width, \( \bar{v}_f \) and \( \bar{v}_0 \) are focused stream and outlet channel average flow velocities, respectively.

In order to determine the focused stream width, both \( \bar{v}_f \) and \( \bar{v}_0 \) velocities, in equation (1) should be calculate as stated below

As viscous forces are predominant and the Reynolds number value is low in microfluidics, therefore solving the equation for a fully devolved flow in the outlet channel is straight forward. When applying wall no-slip boundary conditions consideration, the solution can be expressed as [19].

\[
\frac{u(y,z)}{4h^2} = \left( -\frac{dp}{dx} \right) \sum_{n=0}^{\infty} (-1)^n \times \left\{ 1 - \frac{\cosh(\frac{(2n+1)\pi y}{h})}{\cosh(\frac{(2n+1)\pi w_0}{2h})} \right\} \frac{\cosh(\frac{(2n+1)\pi y}{h})}{(2n+1)^3} 
\]

Where \( \mu \) The fluid dynamic viscosity, \( -w_0/2 \leq y \leq w_0/2 \) and \( -h/2 \leq z \leq h/2 \) are the width and height of the outlet channel respectively.

Equation (2) is a rectangular channel with a Poiseuille velocity profile. To find \( \bar{v}_f(y) \) the average velocity of the fluid, we can integrate equation (2) along \( z \)- direction using the previous boundary conditions

\[
\bar{v}_f = \frac{1}{\pi^2 w_f} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^3} \times \frac{\sinh(\frac{(2n+1)\pi w_f}{2h})}{\cosh(\frac{(2n+1)\pi w_0}{2h})} 
\]

Using equation (3) both \( \bar{v}_f \) and \( \bar{v}_0 \) velocities for the outlet and focused stream can be determined as

\[
\bar{v}_f = 1 - \frac{192h}{\pi^2 w_f} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^3} \times \frac{\sinh(\frac{(2n+1)\pi w_f}{2h})}{\cosh(\frac{(2n+1)\pi w_0}{2h})} 
\]

\[
\bar{v}_0 = 1 - \frac{192h}{\pi^2 w_0} \sum_{n=0}^{\infty} \frac{\tanh(\frac{(2n+1)\pi w_0}{2h})}{(2n+1)^3} 
\]

Equation (1) has been analytically solved for different \( (Q_s/Q_i) \) ratio values, particularly, 0 to 20, for a microfluidic channel with aspect ratio \( (\varepsilon = h/w_0) \) equal to 0.5.

2.2 Magnetic separation

A simple model was studied to separate magnetic particles and magnetically labelled cells from sample mixture.

A computational model using COMSOL Multiphysics software was devolved to find the magnetic field produced by the rectangular magnet located at pre-specified distance from the channel wall.

**Figure 2.** The four main forces act on a magnetic bead suspended in a liquid medium

Figure 2 above shows the main forces that acts the magnetic particle flowed in a liquid media, under effect of magnetic field, where \( \vec{F}_d, \vec{F}_{mag}, \vec{F}_g \) and \( \vec{F}_{bou} \) are the drag, magnetic, gravity and the bouncy forces respectively. Here we just consider the magnetic and fluidic forces which are the most dominant forces.

The generated magnetic force on the magnetic particle can be given by [20]:

\[
\vec{F}_{mag} = \frac{\mu_0 M \cdot A}{2\pi r^2} \vec{r}
\]
\[
\mathbf{F}_{\text{mag}} = -\frac{4\pi r_p^3 \Delta x}{3\mu_0} (\mathbf{B} \cdot \Delta \mathbf{B})
\]

(6)

Where

\( \mathbf{B} \) is the magnetic field, \( \mu_0 \) is the magnetic permeability of free space, \( r_p \) magnetic particle radius and \( \Delta x \) is the bead, fluid magnetic susceptibility difference.

Here in our configuration, the \( \mathbf{y} \) directions of the magnetic force, which leads to the deflection of magnetic particle and so the pre-labelled cells during their path was calculated as follow

\[
\mathbf{F}_{\text{mag}, \mathbf{y}} = \frac{4\pi r_p^3 \Delta x}{3\mu_0} \mathbf{B}_y \frac{\partial B_z(xy)}{\partial y}
\]

(7)

2.3 The calculation of MNPs trajectory

The trajectories of the magnetic particles within the microfluidic device under magnetic force effects were achieved using force balance principle.

As mentioned previously, it was considered that the gravity and bouncy forces on the magnetic particles are quite small and can be neglected, comparing to the magnetic and viscous drag forces.

Using Stokes’ approximation for the drag on a sphere, the hydrodynamic drag force \( \mathbf{F}_{\text{d}} \) can be expressed as

\[
\mathbf{F}_{\text{d}} = 6\pi \eta r_p (\mathbf{v} - \mathbf{v}_p)
\]

(8)

Where

\( \eta, \mathbf{v} \) and \( \mathbf{v}_p \) are the fluid viscosity, fluid and particle velocities respectively. The governed equation of the magnetic particle, given by

\[
\frac{\mathbf{v}_p}{\text{d}t} = \frac{\mathbf{F}_{\text{mag}, \mathbf{y}}}{6\pi \eta r_p}
\]

(9)

Equation (9) can be numerically solved; hence the magnetic particles trajectories can be determined.

3. Methodology

In our device, the hydrodynamic focusing has the ability to focus, align the particle and to orient cells into a narrow stream line located in the microchannel centre. Using this technique the particle manipulation can be achieved at a high throughput rate. As mentioned previously, the pre-labelled CTCs were firstly focused to a single narrow streamline using symmetrical hydrodynamic focusing technique, then in the second stage, the external magnetic applied field would alter and deflect the magnetic beads labelled CTCs from the focused particle streamline, thus the targeted cells could be separated with high efficiency from mixture of non-targeted cells sample or buffer.

The above equations were utilized for magnetic bead focusing and tracing in 2D microfluidic channels, as shown schematically in figure 3 (A) below. The designed microchannel’s width (w) and length (L), used here in this analysis were 200 \( \mu \)m and 30 mm in \( y \) and \( x \) direction respectively.

Firstly, in our study, the different flow rates, that affect the hydrodynamic focusing of the particles were investigated. Different flow rates ratios value of \( Q_s/Q_i \) were conducted, and the corresponding focused stream width was calculated analytically and numerically simulated used the above equations, furthermore, the focused stream widths, were experimentally measured.

In this study, the employed microchannel has three inlets and three outlets. The hydrodynamic focusing experiments were worked out using deionized water and deionized water-immersed 5 \( \mu \)m polystyrene beads as sheath and sample fluid respectively, followed into the microchannel using two different syringe pumps (LongerPump\textsuperscript{b}, TS-1B, China), in order to control the sheaths and sample flow rates precisely. Optical microscopy (OLYMPUS-IX7, Japan), was used to acquire the experimental hydrodynamic focusing effect images and analysis.
Figure 3. (A) Schematic shows the magnetic beads tracing. (B) The fabricated micro-device. (C) Anti-EpCAM coated beads and breast cancer cell line (MCF7) cells

Additionally, the microchannel was simulated numerically, using COMSOL Multi-Physics software; two dimensional simulations were carried out. Laminar flow, transport diluted species (tds), and particle tracing module (fpt) were the physics involved in this simulation. The sample channel flow rate was adjusted at 10 μl/min, while different sheath flow rates values were chosen according to the selected sample/sheath flow rate ratio values. The Full width at half maximum (FWHM) of the concentration cross-sectional plots was calculated and considered as the focused stream width $w_f$ values, for the different conditions and adjusted sample/sheath flow rate ratios. Hydrodynamic focusing helps in cell or particle alignment and focusing in a narrow single sample stream, after assuring of the hydrodynamic process works well, the magnetic separation process takes place.

To determine the magnetic field distribution in the configuration above, a 2 D model was constricted and simulated using Finite Element Method (FEM) package COMSOL. The simulation model includes the polydimethylsiloxane (PDMS) channel, the permanent magnet and the flow media, as shown in figure 3. In order to limit the simulation domain, outer circle was used. A 5 mm cubic shape and 49600 A.m saturation magnetization NdFeB magnet was used. This magnet was placed at 2 mm away from the channel wall. Magnetic field strength $H_y$ in both channel walls (at $y = 0$ and $H$) was computed, so as the magnetic field gradient in y direction $\frac{dH_y(xy)}{dy}$ throughout the microchannel width has been estimated to be 592.813 A/m.

We fabricated this device in order to separate the CTCs cells from buffer/sample based on magnetic method after aligning the cell sample into single narrow stream using hydrodynamic effect, super-magnetic beads coated with Anti-EpCAM antibodies were used to label MCF7 cells (taken here as a CTCs model), the MCF-7 cell line was purchased from American Type Culture Collection (ATCC). MCF7 were cultured in Eagle's Minimum Essential Medium (EMEM) with 0.01 mg ml$^{-1}$ bovine insulin (Sigma-Aldrich) and 10% fetal bovine serum (FBS, Gemini Bio Products) as supplements. Trypsin-EDTA (Invitrogen) was used for MCF-7 cells harvesting, and then cells were re-suspended in media. Haemocytometer with microscope were used for cells counting, the average of three calculations used here as cells number.

To conjugate the magnetic beads into the desired antibody, a mixture of 10 μL from Anti-EpCAM antibody (Abcam ) added to 20 μL from streptavidin modified magnetic bead (1 μm in diameter) for one hour at 27°C, then the complex was washed three times with PBS and column magnet, lastly anti-EpCAM beads were suspended in PBS buffer. The mixture of Anti-EpCAM coated beads and breast cancer cell line (MCF7 cells) showed a very high binding of the beads to the cells surface as shown in Figure 3(C).
4. Results and Discussion

4.1 Hydrodynamic focusing

As the aspect ratio in microchannel, is low, the velocity cross the micro channel width has a parabolic profile, using analytical solution of equation (3), gives the average flow velocity, which has been studied for different aspect ratio \( \varepsilon \) values. The results for the fully developed flow in the microchannel, given below in figure 4. As we can see from the graph, the average velocity field profile shape is totally based on the aspect ratio of the channel. Here the velocity field profiles for 0.1, 0.2, 0.5, 1 and 1.5 aspect ratios were calculated analytically using equation (3). One can clearly observe the transient behaviours of the velocity profile, between parabolic and plug-like profile during increasing and decreasing the aspect ratio respectively.

![Velocity Profile](image)

**Figure 4.** Velocity profiles for 0.1, 0.2, 0.5, 1.0 and 1.5 aspect ratio (\( \varepsilon \)) microchannel.

Firstly, as we mentioned previously, we studied the hydrodynamic focusing of the core sample stream under different conditions. The 0 to 20 values (\( Q_s = 10-200 \) \( \mu \)l/min) of the flow rate ratios (\( Q_s/Q_i \)) has been studied, for the 0.5 aspect ratio microchannel. Particularly, by solving the pre-stated equations analytically, additionally, we compared the results given by analytical solution with that from the numerical and experimental one.

Figure 5(A) below, shows examples of the calculated focused stream widths values for image sequences at (2,4,6,12,16, and 20) (\( Q_s/Q_i \)) flow rate ratio values respectively, and the method used in \( w_f \) calculations as FWHM of the cross-sectional concentration plots for COMSOL MULTIPHYSIC simulations figure 5(B). Additionally, the ratio of the collected particles during 100 seconds from both peripheral outlets after hydrodynamic focusing and the desired outlet after magnetic separation to the total number of the particle in the inlet (transmission probability), was calculated as shown in figure 7(A,B), in the simulation and practical process we used 30 and \( 10^4 \) particles in buffer solution respectively, using Dyna beads magnetic properties as stated by H Shirzadfar (2015)[21], the determination of the focused stream width \( w_f \), was determined experimentally by utilizing deionized and deionized water-black ink mixture solution (1:5) as the two side and sample inlets respectively. Microscope integrated with a CCD camera (OLYMPUS- IX71), was used for flow images acquiring in figure 5(C), the images were analysed by ImageJ software, in order to get the light intensity of the cross-sectional plots. Again the FWHM of the light intensity cross-sectional plot cross the microchannel width was calculated.

Finally, using equation (1), the focused stream width \( w_f \) is computed analytically using MATLAB for the same different flow rate ratios values, the results has been tabulated in table 1 below. The results of the focused stream width obtained, using the three methods has been summarized in figure 6, we can conclude that, all the three methods are perfectly matched with each other in focused width estimations and calculation. Furthermore, it clearly showed that the focused stream width is correlate strongly (linearly decrease) up to certain flow rate ratio value. As the flow rate ratio increases, the changes in the focused stream width no longer occurred or a little change happened, since non-linear correlation has been observed in case of high flow rate ratios values.
Figure 5. (A) Focused stream width images sequences from Comsol Multiphysics simulation. (B) Plotted curve example of the sample stream concentration along the width and the FWHM calculation method. (C) The selected experimental images (1, 2, 3 and 4) were obtained when $\frac{Q_x}{Q_i}$ values are 2, 4, 6 and 20 respectively.

Table 1. Analytically calculated $w_f$ results.

| $\frac{Q_x}{Q_i}$ | 1   | 2   | 4   | 6   | 8   | 10  | 12  | 14  | 16  | 18  | 20  |
|-------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| $w_f (\mu m)$     | 50.494 | 29.8553 | 16.5316 | 11.4349 | 8.7411 | 7.0743 | 5.9418 | 5.1218 | 4.5006 | 4.0139 | 3.6222 |

Figure 6. The results of the focused stream width obtained, using the three methods.
Particle trajectories for both focusing and magnetic simulations for time saving, was simulated only in the main channel domain. This domain has the same dimension parameters of the channel (200 um and 30 mm) high and length respectively. Figure 7(A) below showed the main device working characterization concept. For hydrodynamic focusing our desire is to statistically evaluate the particles that have been collected in outlet B (middle) with respect to the total particle from the inlet (B), while after magnetic separation the same analysis will be done for the particle collection from outlet (A)

The numerical particle focusing which has been conducted using comsol multiphysics results, has been analysed and used to calculate the Transmission Probability.

![Image](image_url)

**Figure 7.** (A) schematically graph used for characterizations. (B) Transmission Probability after hydrodynamic focusing and magnetic separation at $Q_s = 4$. (C) Transmission Probability experimental results. (D) Magnetic beads trajectories simulation at the inlet, middle (magnet zone) and outlet.

Which gives the ratio of the collected to the total particle number per time, as in figure 7 (B) (black line) we can figure out how efficient hydrodynamic focusing is, since within 82 to 85 second the particle reach the exit (B) (middle one). Additionally, Transmission Probability value reached 1 indicating 100% of particles that entering the channel exit out through the desired outlet (B)(middle), hence, 100% focusing efficiency achieved. We also verified these numerical results with an experimental one which resulted in 96 % focusing efficiency, by using known particle concentrations samples and counting the collected from the middle outlet using hemocytometer device. These differences can be returned to imprecision on the collected particles counting and concentration determination or particle stacking in the microchannel walls.

4.2 Magnetic separation

After assuring the hydrodynamic process, as used here to facilitate the cells separation by cell focusing and aligning in a narrow stream works well, the magnetic separation of the pre-magnetic beads labelled cells will take place, firstly a 2 dimensional simulation on COMSOL MULTIPHYSICS, has been constricted to study the magnetic field produced by the magnetic configuration used, beside studying magnetic force that acts on the magnetic beads and the trajectories have been investigated. X and Y components of the magnetic field gradient distribution, have been plotted at three different distances (1000, 1500 2100 um) from the magnet. In addition, we have calculated the y component of the magnetic force that acting on the magnetic particle trajectories based on equation (7) along the horizontal line at the middle of the microchannel (2100um from the magnet), the results shown in figure 8. It’s clearly that the Y – component of the Magnetic field gradient has negative values,
indicating the attractive force that deflects the magnetic beads during their path toward the desire outlet.

Additionally, the correlation between the magnetic field gradient and the corresponding generated force acts on the bead illustrated in figure 8 (B, C), shown direct relation as stated in equation (7). 

Transmission Probability, as calculated for hydrodynamic focusing, also calculated her after magnetic separation as in figure 7(B) red line for outlet (A), as well as experimentally verified in figure 7(C), the simulations and experimental results shown a little bit different 92% and 95% respectively, which can be justified to the same reasons mentioned above, in addition to magnetic particles trapping near to the magnet zone in the microchannel.
To simulate magnetic pre-labelled cells movement in COMSOL MULTIPHYSICS, firstly, we have to calculate the magnetic bead- cell complex magnetic susceptibility, which is totally different from that of the magnetic bead alone. Here we considered the combinations of the bead to the cell has no effect on the overall drag force, as the diameter of the bead considerably has negligible effect on the complex volume. Using the given bead data sheet information[22], volume, mass and ion content mass was calculated as 11.489 µ3, 16.083x10−15kg and 1.930x10−15kg respectively, the same parameters have been used for cell as calculated by[23]. The magnetic bead susceptibility and calculated cell mass with and without protein have been used to calculate the complex susceptibility as (0.087) using the following expression:

$$x_c = x_p \times \text{icont}$$

Where $x_c$, $x_p$ and icont are the complex, bead susceptibility and ion content respectively.

For practical magnetic cell separation tests, we prepared 500 µL white blood cells (WBCs) ($2\times10^3$ cells), in phosphate buffer saline (PBS) after 0.1 % bovine serum albumin (BSA) adding, spiked with 100 MCF-7 cells. Cells were injected into the microchannel through sample inlet at fixed 10 µL/min flow rate value and a solution of PBS contained 0.1% BSA was used as the two sheath fluids through the two sheaths inlets.

The microfluidic device characteristics were quantitatively estimated, using fluorescent microscope images as shown in figure 9, in which EpCAM, CD45, and DAPI are used to characterize MCF-7 from WBCs. Figure 9(A,B) shows the fluorescent images of the sample mixture before the separation and the fluorescent images of the collected cells sample.

A parameterized study for different spiked MCF7 cell number was carried out; the corresponding isolated number from the collected sample has been calculated. Also the purity was observed under fluorescent microscope, by counting the total number of the isolated MCF-7 cell and the contaminated...
WBCs founded in the collected sample, as 98 and 5 cells respectively, as in figure 8(A,B,C) below, using the flowing expression:

\[ \text{Purity} = \frac{C_{\text{iso}}}{C_{\text{tot}}} \]  

(11)

Where:

- \( C_{\text{iso}} \) And \( C_{\text{tot}} \) are the isolated and total numbers of MCF-7 respectively.

The MCF7 Cell separation purity using our device was 92.65 %. Additionally, the recovery rate for different spiked number of MCF7 (10-500) cells in 500 µl PBS a 0.1 % BSA buffer solution was calculated as shown in figure 8 (D).

![Microfluidic device Characterisations](image)

**Figure 9.** Microfluidic device Characterisations (A, B): fluorescent images of the sample mixture before the separation and the fluorescent images of the collected cells sample. (C): purity. (D): Recovery rate of the separated cells.

5. **Conclusions**

In this paper, we described the theoretical principles behind both hydrodynamic and magnetic phenomena utilized in microfluidic channel, along with experimental verifications of these theories. From the results given here, it obvious that compared to our previous paper which used only magnetic based cell[24], using hydrodynamic as an assistance tool has good impacts on the overall separation process enhancing. As our results for hydrodynamic focusing shows the efficiency of the hydrodynamic technique in particle as well as in cell focusing, as we get almost 100% transmission probability , indicating that almost all the particles have been focused and exited the microchannel at the desired middle outlet. And the overall particle loss was almost zero, which is clearly shown in figure 7. Additionally, we have clarified the high possibility given by hydrodynamic techniques, in focused stream width controlling, as it’s a vital issue in cell separating and sorting for flow-cytometer devices fabrication. The effects of the sheath flow rates (Qs1 and Qs2), on the sample stream focusing have been studied, that to get the optimal conditions for the proposed device effectiveness and its capability in cell focusing. In the microchannel geometry used here, we found the optimal flow rate ratio, which gives the best fitted cells size (MCF7 Here, Dia 15.6 um) as being 6, at this flow rate ratios, the corresponding focused stream width was 16.5316 µm, considerably in the same order of the cell size. At this end, the cells are aligned and sorted in a single narrow beam. Here the most obvious benefit is avoiding the cell accumulation and aggregation in specific region throughout the microchannel. The numerical simulations of the magnetic field gradient and the related force, gave fully consistent results with the analytical expression. **Transmission Probability concept,** has been calculated after each steps in our devise, additionally this concept used for the characterization of the fabricated device. Our combined hydrodynamic- magnetic microdevice, has shown it’s highly ability
in magnetic cell separation (MCF7 cells). As experimentally, we achieved more than a 96% recovery rate of MCF7 cells separation from a white blood cells mixture. Our fabricated device is based on easy and cheap manufacturing process and operations procedure, which are the main requirements for clinical application instruments. Combining two or more techniques into microfluidic platforms, are promising solution for the limitations microfluidic chip cell separation.

6. Acknowledgements

This work in the school of life science, Beijing institute of technology and financially was funded by the National Key Scientific Instrument and Equipment Development Project of China (No. 2012YQ040140).

7. References

[1] E. W. K. Young and D. J. Beebe, “Fundamentals of microfluidic cell culture in controlled microenvironments,” Chem. Soc. Rev., vol. 39, no. 3, pp. 1036–1048, Mar. 2010.
[2] P. R. C. Gascoyne and J. Vyckoukal, “Particle separation by dielectrophoresis,” Electrophoresis, vol. 23, no. 13. NIH Public Access, pp. 1973–1983, Jul-2002.
[3] C. W. Shields Iv, C. D. Reyes, and G. P. López, “From chip-in-a-lab to lab-on-a-chip: towards a single handheld electronic system for multiple application-specific lab-on-a-chip (ASLOC),” vol. 15, 2014.
[4] S. J. Tan, L. Yobas, G. Y. H. Lee, C. N. Ong, and C. T. Lim, “Microdevice for the isolation and enumeration of cancer cells from blood,” Biomed. Microdevices, vol. 11, no. 4, pp. 883–892, Aug. 2009.
[5] C. Wyatt Shields IV, C. D. Reyes, G. P. López, D. Guo, C. Shen, W. P. Wong, K. Halvorsen, O. C. Farokhzad, G. S. Teo, J. A. Phillips, D. M. Dorfman, R. Karnik, J. M. Karp, L. Malaquin, J. L. Viovy, H. A. Wakelee, S. X. Wang, L. V. Sequist, R. J. Lee, K. J. Isselbacher, S. Maheswaran, D. A. Haber, M. Toner, A. Bardia, L. V. Sequist, D. N. Louis, S. Maheswaran, R. Kapur, D. A. Haber, and M. Toner, “Microfluidic cell sorting: a review of the advances in the separation of cells from debulking to rare cell isolation,” Lab Chip, vol. 15, no. 5, pp. 1230–1249, Feb. 2015.
[6] Siyang Zheng, Raylene Yung, Yu-Chong Tai, and H. Kasdan, “Deterministic lateral displacement mems device for continuous blood cell separation,” in 18th IEEE International Conference on Micro Electro Mechanical Systems, 2005. MEMS 2005., pp. 851–854.
[7] S. L. Feng, A. M. Skelley, A. G. Anwer, G. Liu, and D. W. Inglis, “Maximizing Particle Concentration in Deterministic Lateral Displacement Arrays.”
[8] A. Armakolas, Z. Pantaleakou, A. Nezos, A. Tsouma, M. Skondra, P. Lembessis, N. Pissimisis, and M. Koutsilieris, “Detection of the circulating tumor cells in cancer patients,” Futur. Oncol., vol. 6, no. 12, pp. 1849–1856, Dec. 2010.
[9] V. Zieglschmid, C. Hollmann, and O. Böcher, “DETECTION OF DISSEMINATED TUMOR CELLS IN PERIPHERAL BLOOD,” Crit. Rev. Clin. Lab. Sci., vol. 42, no. 2, pp. 155–196, Jan. 2005.
[10] R. N. Zare and S. Kim, “Microfluidic Platforms for Single-Cell Analysis,” Annu. Rev. Biomed. Eng., vol. 12, no. 1, pp. 187–201, Jul. 2010.
[11] H. Zhu, X. Lin, Y. Su, H. Dong, and J. Wu, “Screen-printed microfluidic dielectrophoresis chip for cell separation,” Biosens. Bioelectron., vol. 63, pp. 371–378, 2015.
[12] F. Petersson, L. Åberg, A.-M. Śwärd-Nilsson, and T. Laurell, “Free Flow Acoustophoresis: Microfluidic-Based Mode of Particle and Cell Separation,” Anal. Chem., vol. 79, no. 14, pp. 5117–5123, Jul. 2007.
[13] M. Kersaudy-Kerhoas, R. Dhariwal, M. P. Y. Desmulliez, and L. Jouvet, “Hydrodynamic blood plasma separation in microfluidic channels,” Microfluid. Nanofluidics, vol. 8, no. 1, pp. 105–114, Jan. 2010.
[14] Y. Gao, Y. Zhu, Z. Zhang, C. Zhang, X. Huang, and Z. Yuan, “Clinical significance of pancreatic circulating tumor cells using combined negative enrichment and immunostaining-fluorescence in situ hybridization,” *J. Exp. Clin. Cancer Res.*, vol. 35, no. 1, p. 66, Dec. 2016.

[15] W. Sheng, O. O. Ogunwobi, T. Chen, J. Zhang, T. J. George, C. Liu, and Z. H. Fan, “Capture, release and culture of circulating tumor cells from pancreatic cancer patients using an enhanced mixing chip.,” *Lab Chip*, vol. 14, no. 1, pp. 89–98, Jan. 2014.

[16] A. Truini, A. Alama, M. G. Dal Bello, S. Coco, I. Vanni, E. Rijavec, C. Genova, G. Barletta, F. Biello, and F. Grossi, “Clinical Applications of Circulating Tumor Cells in Lung Cancer Patients by CellSearch System.,” *Front. Oncol.*, vol. 4, p. 242, 2014.

[17] O. O. Saeed, R. Li, and Y. Deng, “Microfluidic Approaches for Cancer Cell Separation: Review,” *J. Biomed. Sci. Eng.*, vol. 07, no. 12, pp. 1005–1018, Oct. 2014.

[18] C. Kunstmann-Olsen, B. D. James Hoyland, and B. Horst-Günter Rubahn, “Syddansk Universitet Influence of geometry on hydrodynamic focusing and long-range fluid behavior in PDMS microfluidic chips Influence of geometry on hydrodynamic focusing and long-range fluid behavior in PDMS microfluidic chips.”

[19] G.-B. Lee, C.-C. Chang, S.-B. Huang, and R.-J. Yang, “The hydrodynamic focusing effect inside rectangular microchannels,” *J. Micromechanics Microengineering*, vol. 16, no. 5, pp. 1024–1032, May 2006.

[20] P. Chen, Y.-Y. Huang, K. Hoshino, and J. X. J. Zhang, “Microscale Magnetic Field Modulation for Enhanced Capture and Distribution of Rare Circulating Tumor Cells,” *Sci. Rep.*, vol. 5, no. 1, p. 8745, Aug. 2015.

[21] H. Shirzadfar, M. Nadi, D. Kourtiche, S. Yamada, and P. Shahabi, “Characterization of a needle-type giant magnetoresistance sensor for detection of Escherichia coli’s magnetic marker,” *Int. J. Smart Sens. Intell. Syst.*, vol. 8, no. 1, pp. 220–234, 2015.

[22] “Magnetic Handling of Your Target 12.”

[23] C. Fumarola, S. La Monica, R. R. Alfieri, E. Borra, and G. G. Guidotti, “Cell size reduction induced by inhibition of the mTOR/S6K-signaling pathway protects Jurkat cells from apoptosis,” *Cell Death Differ.*, vol. 12, no. 10, pp. 1344–1357, Oct. 2005.

[24] O. Saeed, R. Li, and D. Yulin, “Micromagnetophoreses based CTCs Detections in Simple Chip.”