**A numerical simulation for separation of two types of CTC with a novel spiral microchannel**

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**Abstract**

Microfluidic separation has recently absorbed considerable attention among scientists due to its distinct advantages over conventional separation methods, such as low cost and noninvasive performance. Microfluidic separation can be divided into two categories; active ones that use external force fields such as acoustic and magnetic; and passive ones that depend on the channel structure and fluid properties. Inertial microfluidics, one of the kinds of passive separation, is often favored because of its simplicity, low cost, and high throughput. Spiral ones have detached significant attention despite complicated concepts among the inertial microfluidics approach, such as straight channels with pillar array and expansion-contraction channels. This new means of cell separation are used in isolation of Circulating Tumor Cells (CTCs) that are cancer cells and originate from a primary tumor cell and travel to other body parts through the bloodstream. This article proposed a novel microchannel with which two types of CTC, including HL-60, and HEPG2, are human blood and lung cancer, respectively, are divided, with high efficiency and purity of 90% for HEPG2 and efficiency of 100% and purity of 83% For HL-60 separation. It is worth mentioning that the mean diameter of HL-60 is approximately 15μm, and that of HEPG2 is 18μm; therefore, this spiral channel can separate particles with a minimum size difference of 3μm.

**Keywords:** Passive separation, Spiral microchannel, CTCs, separation efficiency, separation purity

**شبیه­سازی عددی برای جداسازی دو نوع سلول سرطانی به کمک یک طرح نوآورانه ریزسیالی مارپیچ**

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# چكيده

امروزه استفاده از تراشه­های ریزسیالی به دلیل مزایای قابل توجه آن­ها در مقایسه با روش­های قدیمی جداسازی، توجه محققین زیادی را به خود جلب کرده است. از مزایای قابل توجه این روش جداسازی می­توان هزینه بسیار کم و غیرتهاجمی بودن آن را نام برد. به طور کلی جداسازی به کمک تراشه­های ریزسیالی به دو گروه روش فعال و غیرفعال تقسیم بندی می­شود؛ در روش فعال از یک نیروی خارجی مانند نیروی مغناطیسی و یا صوتی برای جداسازی ذرات استفاده می­شود، ولی جداسازی ذرات در تراشه­های غیرفعال وابسته به شکل کانال و ویژگی­های سیال می­باشد. جداسازی اینرسی که یک روش غیرفعال می­باشد، به دلیل سادگی در ساخت و استفاده، و هزینه بسیار پایین آن و همچنین نرخ جداسازی بالای این نوع تراشه­ها، در تحقیقات گسترده­ای مورد استفاده قرار گرفته است. در میان روش­های مختلفِ این نوع جداسازی، مانند کانال مستقیم با آرایه، کانال­های منبسط-منقبض شونده؛ تراشه­های اسپایرال با وجود ابهاماتی در اندیشه کلی آن­ها توجه زیادی را به خود جلب کرده­اند. این روش جدید جداسازی، در جداسازی سلول­های توموری گردش خون (CTCs) که سلول­های سرطانی هستند و از یک سلول تومور اولیه سرچشمه می­گیرند و از طریق جریان خون به سایر اعضای بدن سفر می­کنند، مورد استفاده قرار می­گیرند. در این مقاله یک میکروکانال جدید معرفی شده است که می­توان به کمک آن، دو نوع سلول سرطانی HEPG2 و HL-60 که سرطان خون و ریه انسان می­باشد را از خون لیز شده جداسازی کرد. بازده و خلوص جداسازی HEPG2بالاتر از 90% و برای جداسازی HL-60 بازده 100% و خلوص 83% می­باشد. شایان ذکر است که میانگین قطر سلول­هایHL-60 و HEPG2 به ترتیب ۱۵ و ۱۸ میکرومتر است بنابراین این کانال مارپیچ می­تواند ذرات با حداقل اختلاف اندازه ۳ میکرومتر را از هم جدا کند.

**کليدواژه­ها:** جداسازی غیرفعال، میکروکانال مارپیچ، تومور گردشی، بازده جداسازی، خلوص جداسازی

**Introduction**

Each blood component has vital information about the health of bodies that can be used for diagnosis and treatment; therefore, Enrichment and isolation of blood cells is often the first step in blood analysis. For instance, by separating RBC, diseases such as Anemia and Thalassemia can be diagnosed, and dividing WBC can be used in distinguish immunodeficiency and infection. Moreover, separation of CTC has been vital for the past two decades because of its extensive benefit for treatment monitoring and drug response investigation. Nowadays, microfluidic chips have attracted researcher's attention for separating different components of blood due to their massive influx of merits compared to the conventional methods; for instance, high throughput, low manufacturing cost, and fewer blood sample requests in these systems, in the hope of being widely applied not only in different medical issues but also in various aspects such as diagnostics, therapeutic and cell biology[1].

As mentioned previously, size difference plays a critical role in cell separation by spiral microchannel; it can be said that the size of CTCs is between 14μm and 25μm, which is generally bigger than other blood components that the size of WBC is about 14μm, and RBC is about 8μm. Although spiral microchannel can be used to separate these cells from blood according to their size, separating particles with high throughput and, at the same time, high separation efficiency is challenging; because these cells are so rare in the blood sample. For better understanding, it must be said that the number of CTCs is in order of 1-30 in 1 milliliter of blood, and the number of WBCs is in order of and the number of RBCs is about in 1 milliliter of blood sample[2].

Scientists try to use spiral microchannels with higher Reynolds numbers compared with other passive ones to take advantage of both inertia and fluid viscosity for separation; in these microchannels, the Reynolds number is between Stokes and turbulent regimes. Moreover, the existence of secondary flow contributes to two vortices next to the walls that result in better separation; these two vortices are created because of momentum mismatch in the center of the channel and near-wall zone at the same time meet the mass conservation law. As can be seen In Figure 1. in a straight channel without secondary flow, neutrally buoyant particles with and similar properties focus in four equilibriums position within the straight microchannel but in the spiral channel, because of additional force due to secondary flow, these four equilibrium positions are unified; which results in better particle focusing and consequently increases the separation efficiency[3].

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| Figure 1: (a) equilibrium positions within the cross-section of microchannel where no dean drag force exists. (b) equilibrium position within the spiral microchannel, four equilibrium positions are unified due to the dean vortices[3]. |

Excessive research tried to use a spiral channel with different configurations to separate particles; for example, Ho W et al., 2017 used a 7-loops spiral channel with a rectangular cross-section that had height and a low aspect ratio of ; it was successful in separating three different sizes of particles with high efficiency of and a flow rate of . A remarkable point in that research was the ability to collect closely-spaced particle's streams[4]. Joen et al. in 2020; took advantage of a double spiral microchannel with a S-shaped transition with a rectangular cross-section that had height and width to separate WBCs from RBCs with an efficiency of 97% for RBCs and 95% for WBCs in the golden flow rate [5] .

Since 2010, the separation of two types of CTCs from blood has absorbed pronounced attention among scientists; in 2012, Sun et al. with a double spiral microchannel with 12 loops, obtained a separation efficiency of 96.77% to separate Hela and MCF7 from blood[6]. Shen et al. 2017 separated MCF7 and Hela by creating additional secondary flow in a spiral channel with micro-obstacles. The efficiency of 97.5% for MCF7 and 92.3% for Hela was obtained in that research at a flow rate of [7]. Abdulla et al. used a cascade channel that consisted of two spiral channels with five loops and a zigzag channel to separate two kinds of CTCs from blood, A549, which is about and MCF7that is about , it’s separation efficiency was about 80.75% for A549 and 73.75 for MCF7[8]. Shiriny et al. in 2021, designed a novel spiral channel with only a 0.75 loop for continuous-flow separation of two types of CTC from blood; they reached an efficiency of 100% at a wide range of Reynolds numbers of 30-120[9].

Research trends incline to separate two types of cancer cells; in this regard, the present work focuses on a novel design that is capable of separating HL-60 and HEPG2.

**Governing equations**

The immense difference between spiral microchannels and conventional ones is that in traditional microchannels, the flow remains almost within the Stokes flow region that Reynolds number is insufficient, but in the spiral microchannel, Reynolds number is between stokes and turbulent regimes; therefore, inertia contributes to the microfluidics phenomena. In other words, the existing influence of both inertia and fluid viscosity in the spiral channel is a key point that contributes to better cell separation[10].

The process of investigating the location of particles is divided into two parts. At first, laminar flow equations, including the Navier-Stokes equations (1) and the continuity equation (2), were solved to obtain the velocity profile; after that, particles were added to the microchannel, and by applying lift forces and drag forces, the equilibrium positions of particles have been tracked. It is worth mentioning that laminar flow and particle tracing have been assumed to be steady-state flow and time-dependent, respectively. In these equations, is the density of the fluid, is flow velocity, is the pressure, is the time, µ is the dynamic viscosity of the fluid, and are the velocity components in directions respectively.

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|  | (1) |
|  | (2) |

In general, it can be said that particles in a flow experience shear and normal stresses that are applied on their surface, which contribute to a parallel and perpendicular force to the main flow direction named drag force and lift force, respectively. Four lateral forces operate on particles; the first one is the Magnus force (3), which results from the particle's rotation. The second force is the Saffman force (4), which is created by slip-shear and direct particles toward a position where the relative velocity magnitude is greater. The third force is wall-induced lift force (5), as can be seen in Figure 2; this force stems from different pressure magnitudes in the center of the channel and near the wall; in other words, when particles reach the area near the channel's wall, because of more significant pressure that acts on particles on that place, it tends to move toward the center of the channel. The last force is the shear gradient lift force (6); as shown in Figure 3. the velocity gradient creates shear stress, and due to the higher velocity gradient near the walls, shear stress is also higher in that area compared with the area in the center of the channel. Shear-induced lift is due to this difference in shear stress, and its direction is toward the wall as there is more shear stress next to the wall compared with the center of the channel[11].

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|  | (3) |
|  | (4) |
|  | (5) |
|  | (6) |



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| Figure 2: Schematic illustration of wall-induced lift force [11] |

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| Figure 3: Schematic illustration of shear-induced lift force [11] |

It should be noted that Magnus and Saffman's forces are negligible compared to others. Furthermore, the effect of the wall-induced lift force and shear-induced lift force was calculated via the net inertia lift force equation; Evidently, this force depends on the channel Reynolds number and the particles' normalized position, as shown in equation (7). In this equation is a non-dimensional lift coefficient that depends on the Reynolds number and the normalized position of particles , that x is the position of particles and is half of the channel's height, is the particle's radius, and  is the mean flow velocity.

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|  | (7) |

It is to be noted that the Forces discussed earlier act on a particle within bounded channel flows. There is an extra force in a spiral microchannel because of a momentum mismatch in the center and near the wall of a curved channel and the law of conservation of mass, two dean vortices formed to help particles reach their equilibrium position more quickly and reduce the channel length footprint. The secondary flow is defined by a non-dimensional number that is shown in equation (8). In this equation and are the channel's hydraulic diameter and curvature's radius, respectively. Stokes' law also gives the magnitude of the Dean drag force in equation (9), in which is the velocity that depends on which can be calculated by equation (8).

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|  | (8) |
|  | (9) |
|  | (10) |

Although, with a higher Dean number, particles reach their equilibrium position sooner; if this number exceeds a specific number, the result will be opposite from what has been expected, and particles will remain in vortices and never reach their equilibrium position. Therefore, finding a golden flow rate is essential to use the positive impact of dean drag force on particle separation[12].

According to equations (7-9), The parameters that influence the magnitude of lift force and dean drag forces depend on the flow rate, geometry, and particle size. Since, turns to pull particles in the secondary flow in competition with lift forces; the balance between their magnitude is essential to focus particles in their equilibrium position. This point implies that particles with different sizes occupy different lateral positions within the microchannel cross-section. Furthermore, that is particle size and is channel hydraulic diameter should be bigger than 0.1 that the balance of the forces explained above fixes particles in a single equilibrium position. These forces and their direction are illustrated in Figure 4.

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| Figure 4: forces and their direction that apply to particles |

It is essential to govern the length of the spiral microchannel to determine the forces that affect the particles and put them in their equilibrium position. The channel length that is necessary for particles to focus at their equilibrium position completely is given in equation (11) in which is the average fluid velocity, is particle's lateral migration velocity that can be seen in equation (12) and is the migration length of particles.

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|  | (11) |
|  | (12) |

**Microchannel design**

Figure 5 illustrates the spiral microchannel that is designed to separate two types of CTC (HL-60 and HEPG2) from lysed blood samples. This design consists of two parts. The first part is a one and half-loop spiral geometry with two inlets; the first inlet is for a lysed blood sample, and the second one is for a sheath sample. When particles pass through this part of the spiral channel, particles with a mean diameter of focus on their equilibrium positions near the inner wall and collect in outlet No.3; Particles with mean diameters of and move near the outer wall and are directed to the second part of the channel. Then, after passing the particles from a 0.75-loop spiral channel, namely the second part, particles accumulate next to the outer wall and exit from outlet No. 5, and the particles with are concentrated next to the inner wall and exit from outlet No.4.

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| Figure 5: design of spiral microchannel to separate two types of CTC (HL-60 and HEPG2) from a lysed blood sample |

The cross-sections of the first and second part and their vortices are shown in Figure 6 and Figure *7*, respectively; in selecting their dimensions, two points are considered: one- in the first part, the ratio of particle diameter to hydraulic diameter of the channel must be greater than 0.1 for targeted cells that their diameter is and for the rest of the particles it should be smaller than 0.1, and also in the second part, the ratio of particle diameter to hydraulic channel diameter for particle should be greater than 0.1 and for the smaller particle ( It should be smaller than 0.1, in addition, two-the height should be the same in both cross-sections so that the chip can be made by a soft lithography method.

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| Figure 6: The cross-sections of the first part and its velocity vector |
| Figure 7: The cross-sections of the second part and its velocity vector |

Furthermore, the length of each part has been calculated according to equation (11), and it should be noted that pressure drop plays a critical role in this design if the pressure drop in outlet No.3 is more significant than the pressure drop in the second part of channel all of the particles enter the second part of the channel; otherwise, all particles come out of the third outlet. Therefore, pressure drop has been calculated, and its respective extension added to the end of outlet No3. as it is evident in Figure 8, the pressure at the initial position of the third outlet is equal to the pressure at the initial position of the second part of the chip and are equal to .

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| Figure 8: pressure in designed microchannel |

**Results**

In any numerical study, validation is required to ensure the simulation is performed. Therefore simulation is validated against experimental work reported by Hou et al.[13]. To this end, as shown in Figure 9a, Hou's geometry is reconstructed and reached its result by simulation with COMSOL Multiphysics 5.6 software. As shown in Figure 9b, particle trajectories with diameters of 15 and 6 micrometers are close to their experimental result in the inlet and outlet of the microchannel. Furthermore, in Figure 9c, it is evident that in the dean cycle NO.1 equilibrium position of are close in the present study and Hou et al experimental result.

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| Figure 9: (a) Hou's geometry and the geometry that is reconstructed. (b) particle trajectories with diameters of 15 and 6 micrometers in their experimental result and present study. (c) equilibrium positions of in the present study and Hou et al. experimental result |

The second step that seems to be required in simulation research is to show that the solution is independent of the number of mesh elements. We use uniform hexahedral mesh in our simulation and find the best number of mesh needed in the channel's height and width. According to the number of meshes in the channel's height and width that is shown in Figure 10, the velocity profile in a specific position in the microchannel cross-section with seven mesh in height and 14 meshes in width of the channel is similar to 9 and 18 mesh in height and width of channel respectively; therefore, we use H\_7/W\_14 mesh element to reduce the cost of simulation.

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| Figure 10: velocity profile for showing the mesh independency |

The size difference between CTC and WBC is used to separate them from others by microfluidic chips. Furthermore, there is a difference between CTC's size for different types of cancer; This size difference has created a new approach in the design of microfluidic chips that can simultaneously isolate different cancer cells.

The simulated model is three-dimensional, with two rectangular cross-sections with different widths, depicted in Figure 6 and Figure *7*. as it can be seen in Figure 11 in the first cross-section, HEPG2 as the biggest particle that is about , are focused near the inner wall because of the balance of inertial lift force and Dean drag force while HL-60 and WBCs migrate along with the Dean vortices, exiting the first cross-section and enters the next cross-section. In the next part, HL-60, as the biggest cell, can be collected from the inner outlet No.4 and WBC from the outer outlet No.5. WBC and HL60 trajectory can be seen in Figure 12.

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| Figure 11: HEPG, HL60, and WBC trajectories in the first part of the channel |
| Figure 12: HL60 and WBC trajectories in the second part of the channel |

Since flow rate has a significant effect on particle separation, it is necessary to investigate the efficiency and purity of particle separation at different flow rates to achieve the golden chip flow rate in which we have the highest efficiency and purity. It is worth mentioning that efficiency means the ratio of cell numbers in the expected outlet to the numbers of that cell in all the outlets, which is shown in equation (13), and purity means the ratio of target cells to total cells in the desired outlet that is shown in equation (14).

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|  | (13) |
|  | (14) |

The efficiency and purity of HEPG cell that is and should be collected from outlet No.3 are shown in Figure 13 and 14; as can be seen in the flow rate of the efficiency and purity are higer than 90%. And for more and fewer flow rates, the efficiency and purification are significantly reduced.

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| Figure 13: purity for separation of HEPG cell |

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| Figure 14: efficiency for separation of HEPG cell |

The efficiency and purity of the HL-60 cell, which is 15𝜇𝑚 and must be collected from outlet No.4, are shown in Figures 15 and 16; as seen in the golden flow rate, the efficiency and purity are 100% and 83%, respectively.

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| Figure 15: purity for separation of HL-60 cell |
| Figure 16: efficiency for separation of HEPG cell |

The efficiency and purity of WBC that should be collected from outlet No.5 are shown in Figure 17Figure 18; as can be seen in the golden flow rate, the efficiency and purity are 90% and 100%, respectively.

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| Figure 17: purity for separation of WBC |
| Figure 18: efficiency for separation of WBC |

As it can be seen in Figure 14-18, when the flow rate is less than 310 μL/min so as to this fact that dean number and secondary flow are not that substantial to bring cells toward their equilibrium positions, the efficiency and purity of these cells are small; Therefore, weak dean number is responsible for decreasing the efficiency and purity of cells. Furthermore, as the flow rate has increased, the secondary flow becomes too powerful for cells to be trapped in vortices; as a result, the efficiency and purity become less and less.

**Conclusion**

In conclusion, an inertial microchannel has been designed and investigated numerically in this paper to separate two types of CTC, HL-60 and HEPG2, that are human blood cancer and human lung cancer, respectively, from lysed blood samples; according to what has been reported in the flow rate of HEPG2 with a mean diameter of can be isolated with efficiency and purity higher than 90%. Furthermore, in this flow rate, HL-60, with a mean diameter of is separated with an efficiency of 100% and purity of 83%. And finally, WBC isolation with this microchannel has an efficiency of 90% and purity of 100%.

To sum up, it can be said that as a future direction, it is suggested that this design can be improved to work with a whole blood sample and offers high throughput while keeping suitable efficiency and purity.

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