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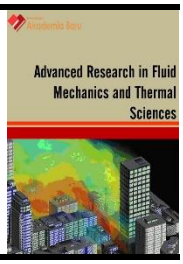

Efficient Magnetic Microbeads Trapping using Lab-on-Chip Magnetic Separator

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ABSTRACT

Lab-on-Chip (LoC) magnetic separation is a simple and effective method in separating bioparticles labelled with magnetic microbeads in microfluidics flow condition. In this work, trapping efficiency of magnetic microbeads using LoC magnetic separator and a microfluidics channel with chamber design is determined. The polydimethylsiloxane (PDMS) microfluidics channel was designed with an inlet, an outlet and a circular trapping chamber at the center. Standard soft lithography technique was used to replicate the PDMS microfluidics channel from the SU-8 mould. In a continuous hydrodynamics flow of 1.0 μL/min, trapping efficiency of 99.5 % and 94.9 % for 4.5 μm and 2.5 μm magnetic microbeads respectively was achieved. Flow analysis using COMSOL Multiphysics has been conducted in predicting the possible location of the magnetic beads trapping inside the microfluidics channel. The trapping is possible whenever the magnetic force is larger than the drag force experience by the magnetic microbead. The microfluidics channel with chamber design had facilitated low hydrodynamics drag force on the magnetic beads and resulted high efficiency trapping. Therefore, the development of this LoC magnetic separator may be promising to be utilized for biological studies and point-of-care testing (POCT) applications.

Keywords:
Lab-on-Chip (LoC), magnetic separator, microfluidics, magnetic microbeads, trapping efficiency

1. Introduction

The medical transformation resulted in world health care spending trend to change drastically by the year of 2025. The spending by patient for illnesses treatment will be substituted by the cost for prediction, diagnosis, and monitoring diseases [1]. Clinical diagnostics device invention for prediction, diagnosis, and monitoring diseases are expected to provide resourceful health condition information. In the near future, this ingenious device is able to be produced at cheaper price and will emerge for the end user market. Point-of-care testing (POCT) is part of reorganizing the clinical diagnostics testing where more illnesses can be predicted in the doctor's office or at home. Some of the benefits of POCT are less time and reduce cost for physician meeting with faster diagnostic results and

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treatment decision [2]. This devices are essential to the world population particularly the developing and poor nations due to limited access of sophisticated and expensive laboratory equipment. In recent years, research and development of Lab-on-chip (LoC) for next generation POCT medical devices for clinical diagnostics and bioparticles separation have increased significantly. Lab-on-chip (LoC) device integrates magnetic Microelectromechanical System (MEMS) and microfluidics channel in order to separate the intended biological cells of interest. The development of LoC magnetic separator has greatly contributed to new and greater opportunities in LoC research [3]. LoC magnetic separator exploited magnetic force in isolating or separating certain biological cells population which have been labelled with functionalized magnetic beads. The advantages of using the LoC magnetic separator are magnetic field is easy and simple to generate. In addition magnetic field is contactless process which results in no damage to the biological cells. Furthermore, the large surface-to-volume ratio and commercially available bio-functionalized magnetic beads of different sizes are also some of the LoC magnetic separator advantages.

Magnetic system of permanent or electromagnet is usually employed as part of LoC magnetic separator. The magnetic field characteristics including its direction, strength and gradient are important in ensuring the effective magnetic particles trapping [3]. The three main configurations of LoC magnetic separator are external macro-sized magnetic system, integration of microelectromagnet system and the combination of both system [4]. In a hybrid system of external macro-sized and micro-sized magnetic system, a low gradient magnetic field is always an issue. A low gradient magnetic system will generate low magnetic force on a magnetic microbead. Therefore, an efficient magnetic microbeads trapping will not be achieved with high drag force in the microfluidics continuous flow. Development of high gradient magnetic separation (HGMS) system as LoC magnetic separator enabling efficient trapping of magnetic microbeads. The reason for that are due to a strong localized magnetic force generation on the magnetic microbeads possibly be achieved [4]. In the previous studies, the integration of internal or external high gradient magnetic structure have successfully trapped magnetic micro- or nanobeads in the microfluidics continuous flow [5-9]. In spite of the high magnetic gradient generated from the system, inability in switching on and off the magnetic field, complicated fabrication processes and clogging tendency in the microchannel are some of the drawbacks associated with the current design of HGMS LoC magnetic separator. Therefore, in overcoming all the limitations, a new novel design of HGMS LoC magnetic separator is indispensable.

In this work, trapping efficiency of magnetic microbeads of nominal diameter 2.5 μm and 4.5 μm is investigated using a LoC magnetic separator. The novel design of the LoC magnetic separator consisted of an electromagnet system with V-shaped nickel ferrite ($Ni_{80}Fe_{20}$) core and a microfluidics channel which circular trapping chamber. The continuous microchannel flow is set between 1.0 μL/min to 60 μL/min using a microsyringe pump. The trapping efficiency is done by counting the magnetic microbeads using hemocytometer. The highest trapping efficiency will reflect the optimum performance of the LoC magnetic separator at the specific microchannel volumetric flow rate.

2. Methodology

2.1 Theoretical Concept of the LoC Magnetic Separator

The trapping and separation of the biological cells labelled with magnetic microbeads depend on the interaction of magnetic force generated by the magnet system and drag force from the hydrodynamic microfluidics flow. Trapping is enabled in microfluidics continuous flow whenever magnetic force is greater in comparison to the drag force experience by the magnetic beads. In trapping biological cells labelled with magnetic microbeads within a microchannel fluid volume, an

inhomogeneous high magnetic field and its gradient are required. The concept and design of the high gradient LoC magnetic device used in this work has been described in the prior works [10, 11]. For a magnetic microbead of volume, $V = \left(\frac{4}{3}\right)\pi R^3$, with difference in magnetic susceptibility $\Delta\chi$, ($\chi_p$for particle and χ_{pm} for the fluid buffer medium), magnetic constant, μ_o of $4\pi \times 10^{-7}$and strength and gradient of the magnetic flux density, B, the magnetic force is theoretically computed using Eq. (1).

$$\vec{F}_m = \frac{V\Delta\chi}{\mu_o}\left(\vec{B}\cdot\vec{\nabla}\right)\vec{B} = \frac{V\Delta\chi}{2\mu_o}\vec{\nabla}\left(\vec{B}\cdot\vec{B}\right) \quad (1)$$

As stated in Eq. (1), in order to have a high magnetic force on a magnetic microbead, a high value of the magnetic gradient is required. The high magnetic gradient is possible to be achieved with design and positioning configurations of the magnetic system.

A magnetic microbead of radius, R experiences hydrodynamics drag force in the microfluidics channel flow of *x*-direction. According to Stoke`s theorem, the drag force, F_d is expressed as in Eq. (2).

$$\vec{F}_d = 6\pi\eta R\left(v_p - v_{medium}\right) \quad (2)$$

where v_p is the particles velocity, v_{medium} is the fluid velocity and η is the fluid viscosity. In order to successfully trap the magnetic particles in the continuous microfluidics channel flow, the magnetic force must be greater in comparison to the drag force experience by the magnetic microbeads $\left(\vec{F}_m > \vec{F}_d\right)$. In this work the microchannel is defined with one inlet and outlet. A chamber of circular-shaped of 0.75 mm is designed as a main domain for trapping magnetic microbeads flowing in the continuous microchannel flow. In order to determine the velocity experience by the magnetic microbeads in the continuous microfluidics flow, a finite element analysis (FEA) is conducted. The velocity obtained from the simulation is then used to calculate the drag force experience by the magnetic microbeads. The model geometry used in this FEA is as Figure 1.

The physics used to solve this problem is single phase laminar fluid flow which governed by the Navier-Stokes equation. The fluid used is water at 25 degree Celsius with density, ρ of 997.13 kg/m^3 and dynamic viscosity, μ of 8.91 x 10^{-3} Pa.s. A three dimensional (3D) analysis of *x*-, *y*- and *z*-component of geometry and physics are involved. The stationary study is selected for this analysis. The fluid flow is considered Newtonian, incompressible and steady in the analysis. The flowing flow in the microchannel is drives by the pressure drop, *P* prescribed between the inlet and the outlet. No-slip wall conditions are applied to other boundaries. Theoretically, the maximum velocity at the center of the microchannel is calculated by manipulating the relationship between the volumetric flow rate with pressure drop of Poiseuille flow in a rectangular channel of height less than its width $(h < w)$ [12]. The maximum velocity in a rectangular channel is expressed as Eq. (3).

$$u_{max} = \frac{3Q}{2A(1-0.63\alpha)} \quad (3)$$

From Eq. (3), Q is defined as volumetric flow rate, A is the cross-sectional area and α is the microchannel aspect ratio (*height*/*width*). Validation of the numerical results was done by comparing the theoretical and the simulation maximum velocity in the microchannel. A 5.3 percent different between the theoretical and simulation results for the maximum velocity value was obtained. Therefore the FEA 3D is considered satisfactory in modeling the microchannel flow.

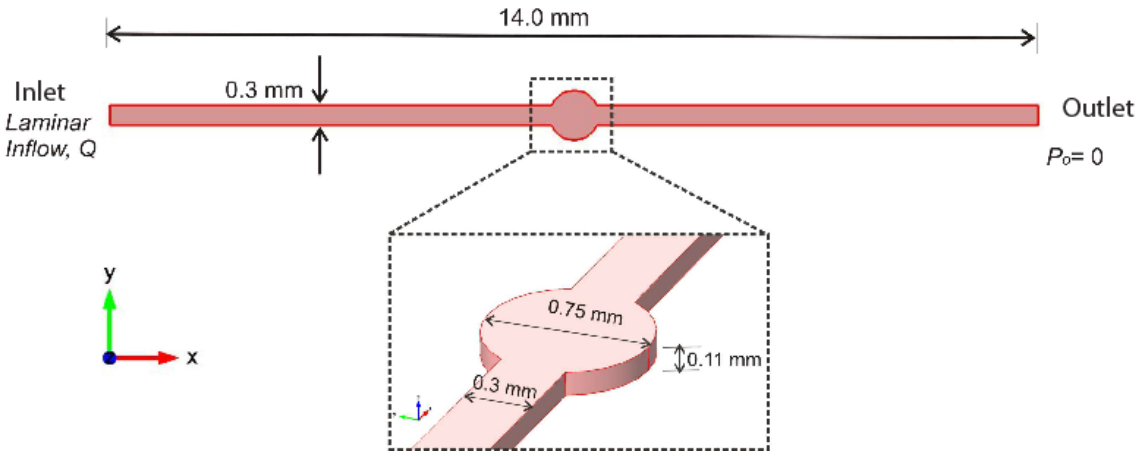

Fig. 1. FEA simulation model of the microchannel with trapping chamber of 0.75 mm

2.2 LoC Magnetic Separator

In this work, the fabricated high magnetic gradient LoC magnetic separator has been tested for its trapping efficiency. This simple and easy fabricated LoC magnetic separator comprising of spiral-shaped magnet wire coil, V-shaped nickel ferrite ($Ni_{80}Fe_{20}$) magnetic core and a microfluidics channel [10]. The V-shaped magnetic core is fabricated by KOH anisotropic wet etching of bulk micromachining and $Ni_{80}Fe_{20}$ electroplating processes. The magnetic core is able to guide and concentrate the magnetic beads at the microchannel trapping chamber due to its high magnetic field and gradient generated. The detail fabrication processes of this high gradient LoC magnetic separator are as in [10]. Microfluidics channel has been successfully fabricated using replica molding technique using polydimethylsiloxane (PDMS) polymer materials. A trapping chamber at the microchannel center is designed to minimize the fluid velocity and thus lowering down the hydrodynamics drag forces on the magnetic microbeads. The PDMS microchannel fabrication is as described in [12].

2.3 Magnetic Microbeads Trapping Efficiency

The performance of this high magnetic gradient LoC separator was examined in its ability to trap and separate a 4.5 μm magnetic microbeads. In this work, a smooth surface polystyrene magnetic microbeads (Spherotech, USA) are used [13]. To prove the magnetic microbeads trapping efficiency, the number of the beads are counted first before the sample entering the microchannel fluidic inlet and secondly the counting was done on the recovered or the not trapped magnetic microbeads at the fluidic outlet. The magnetic microbeads was counted by a hemocytometer. Counting microbeads using hemocytometer is considered inexpensive and easy method with the aid of optical microscope. Furthermore, a good quality data can be obtained with the analyst level of expertise [14]. The coefficient of variance, CV in counting magnetic microbeads using hemocytometer is between 2.19 - 9.30 % in comparison to 0.09 - 7.08 % using highly expensive automated counter of TC10^{TM} Bio-Rad Laboratories, USA [16]. The hemocytometer used in this calculation is the Neubauer improved type. The trapping rate or its efficiency calculation using Eq. (4) is taken from Celeromics Technical Note [17].

$$Trapping\ Efficiency = \frac{Total\ number\ of\ trapped\ microbeads}{Total\ number\ of\ injected\ microbeads}\ x\ 100\ \% \quad (4)$$

where Total number of trapped microbeads = Total number of injected microbeads - Total number of microbeads counted at the outlet.

Prior to the trapping experiment, 2.0 μL magnetic microbeads suspension is diluted 2500 times by mixing with 5.0 mL deionized (DI) water. A 10 μL volume of the beads and DI mixture is pipetted and injected into the hemocytometer counting chamber. In this study, the center grid system (counting chamber number 3) of the Neubauer improved hemocytometer comprises of 25 square grid is used for the magnetic microbeads counting. Taking into account the volume of the counting chamber and the dilution of the magnetic microbeads suspension, the concentration of the magnetic microbeads per volume in mL is given as in Eq. (5) [19]

$$Concentration = \frac{Total\ microbeads\ counted}{25 \times Dilution} \times 250000 \quad (5)$$

In this study, a dilution of 1 to 2500 or 0.0004 of the magnetic microbeads is done. The step by step of the magnetic microbeads counting are done by the procedure given by [18]. For ease of the magnetic microbeads counting, the online hemocytometer is used [20]. The magnetic microbeads counting results are shown in Table 1.

Table 1
Counting of the 4.5 μm magnetic microbeads using hemocytometer

	2.5 μm (beads/mL)	4.5 μm (beads/mL)
Minimum	5.47719×10^9	2.07583×10^9
Maximum	6.7527×10^9	2.57603×10^9
Average	6.03992×10^9	2.3822×10^9
Median	5.96489×10^9	2.43848×10^9

2.4 Experimental Set-up

Trapping efficiency of 2.5 μm and 4.5 μm diameter magnetic microbeads was conducted using the fabricated microchannel and LoC magnet system. The testing set-up comprises of a microsyringe pump to supply the magnetic microbeads liquid into the microchannel, an optical microscope (Olympus, Germany) with image analysis software (Analysis) to observe the magnetic microbeads flowing and trapping, a programmable power supply to supply electric current to the electromagnet system and a stop watch to measure the time taken for collecting the sample. The experimental set-up used in this study is as shown in Figure 2. An improvement was done for the fluidics connection whereby substitution of the PTFE barbed sleeve used as the fluidic inlet and outlet with a micropipette tip of 0.1-10 μL volume (Diamond®, Gilson, USA). This improvement was made for the ease of injecting and collecting the magnetic microbeads samples. In the trapping experiment, samples of 10 μL is needed for the hemocytometer counting. The duration needed for collecting the sample is based on the volumetric flow rate use in the experiment. Therefore, for 1.0 μL/min volumetric flow rate experiment, a duration of 10 minute is required for collecting the 10 μL sample.

Prior to the trapping efficiency experiment, purging or degassing of the microfluidics channel was done. The purging procedure was conducted to free the channel from air bubbles for a steady microfluidics flow. Agitation of the magnetic microbeads in the syringe was also conducted before each of the testing repetition. The agitation is to prevent the magnetic microbeads agglomeration and sedimentation. A volumetric flow rate of 1 to 60 μL/min were used in the magnetic microbeads

trapping efficiency experiment with four numbers of repetition. Direct current of I_{DC} = 1.0 A was used for the electromagnetic system during the experiment.

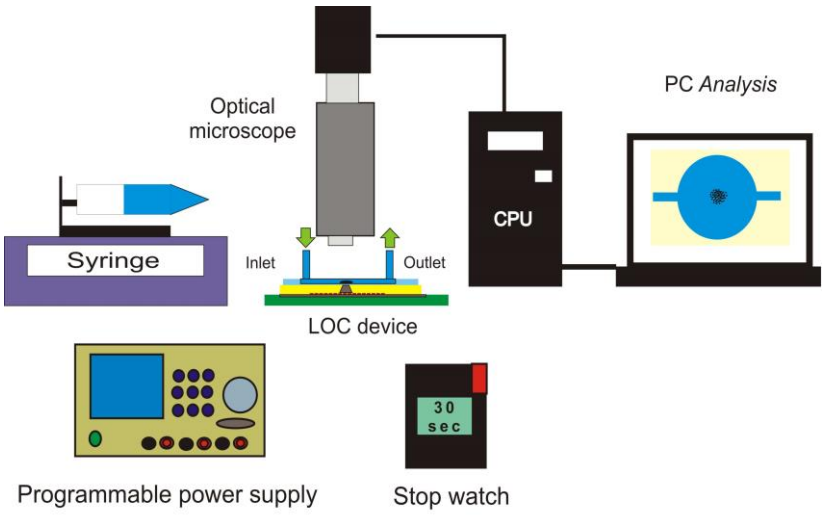

Fig. 2. Magnetic microbeads trapping efficiency experimental set-up

3. Results

Magnetic microbeads trapping was successfully demonstrated with fluid flow rate in the range of 1 to 10 µL/min and 1 to 60 µL/min for 2.5 µm and 4.5 µm microbeads respectively. A direct current of I_{DC} = 1.0 A was supplied to the electromagnetic system. Figure 3 shows the magnetic microbeads trapping efficiency with the sample volumetric flow rate supplied into the microchannel. Almost 100 % trapping efficiency for 4.5 µm magnetic microbeads was obtained with a flow rate of 1.0 µL/min. Furthermore, 94.9 % trapping efficiency was recorded for 2.5 µm magnetic microbeads. This is expected as the hydrodynamics drag forces on the magnetic microbeads are considered minimum in comparison to the magnetic forces on the beads. In addition, the trapping efficiency is consider efficient with approximate thickness of 140 µm PDMS layer between the electromagnetic source and the microchannel flow. The trapping efficiency decreases with the increase of the volumetric flow rate. The same trend was observed in the previous studies by Ramadan *et al.,* and Fulcrand *et al.,* [21-23].

Trapping efficiency of 98 % and 89.4 % was observed at volumetric flow rate of 5 µL/min and 10 µL/min respectively for 4.5 µm magnetic microbeads. Doubling the flow rate to 20 µL/min resulting a dropped of trapping efficiency to 81.3 %. The lowest achieved trapping efficiency of 63.6 % was obtained at the flow rate of 60 µL/min. For 2.5 µm magnetic microbeads, a significant trapping efficiency drop from 72 % to 40 % was observed at 5 µL/min and 10 µL/min respectively. No further trapping experiment was continued at flow rate lower than 10 µL/min. Depending on the type of biological cells to be separated, a different trapping efficiency range is required. This will determine a successful analysis of the biological cells in the subsequent procedure. The decreasing trend of the trapping efficiency with the higher volumetric flow rate is expected as the greater hydrodynamics drag forces experienced by the magnetic microbeads and the low field value of the magnetic forces supplied from the electromagnet system. In addition, a good bonding between the magnetic microbeads and the biological cells, the type and size of the magnetic beads use will also contributed to biological cells trapping and separation efficiency.

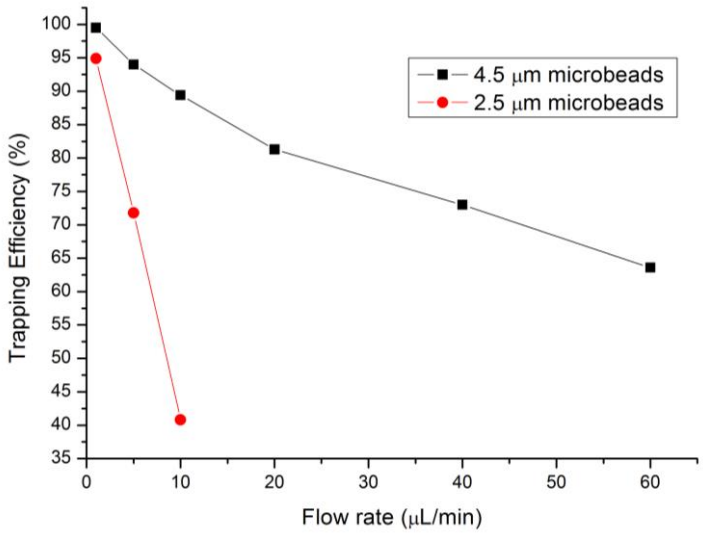

Fig. 3. Trapping efficiency of the 2.5 μm and 4.5 μm magnetic microbead with different flow rate supply into the microchannel

In this study, a magnetic and hydrodynamics forces comparison has been conducted using the 2.5 μm and 4.5 μm magnetic microbeads using the data obtained from the FEA simulation. The comparison is conducted to determine the magnetic microbeads trapping possibilities in four different depth of the microchannel which are at the x-, y- and z-coordinate of (0, 0, -5), (0, 0, -55), (0, 0, -105) and (0, 0, -110). The velocity surface plot of the four locations in the microchannel at volumetric flow rate of 1 μL/min are shown in Figure 4(a), (b), (c) and (d). A significant drop of the fluid velocity can be clearly seen from the figure due to the trapping chamber design configuration. The maximum velocity magnitude in the middle of the microchannel chamber are 7.5 x 10^{-5} m/s, 40 x 10^{-5} m/s, 7.5 x 10^{-5} m/s and 0 m/s corresponding to the four different depth. All the velocities at the microchannel chamber corresponding to Reynolds number less than 1 where flow is completely laminar. Table 2 shows the magnetic and hydrodynamics drags forces at the four different depth of the microchannel where the z = -110 μm is the bottommost microchannel wall. The magnetic forces from the electromagnetic system on the 2.5 μm and 4.5 μm magnetic microbeads at different location in the microchannel were determined from the magnetic FEA study done [10].

Theoretically, trapping of the magnetic microbeads are possible when the magnetic force is greater than the hydrodynamics drag force, $F_m > F_d$. In the proposed microchannel with trapping chamber design, magnetic microbeads trapping is possible at the depth of z = - 105 μm and at the bottommost of the channel depth of z = -110 μm where the hydrodynamics forces are minimum and zero. The design of V-shaped magnetic core enable a generation of non-homogeneous and concentrated magnetic field from the electromagnet system. This localized effect in the continuous microchannel flow, contributed to the high trapping efficiency of the magnetic microbeads [24]. In addition, the efficient trapping at the very low flow rate is contributed by the effect of the trapping chamber design and long entrance length of 7 mm to the trapping chamber as shown in Figure 5.

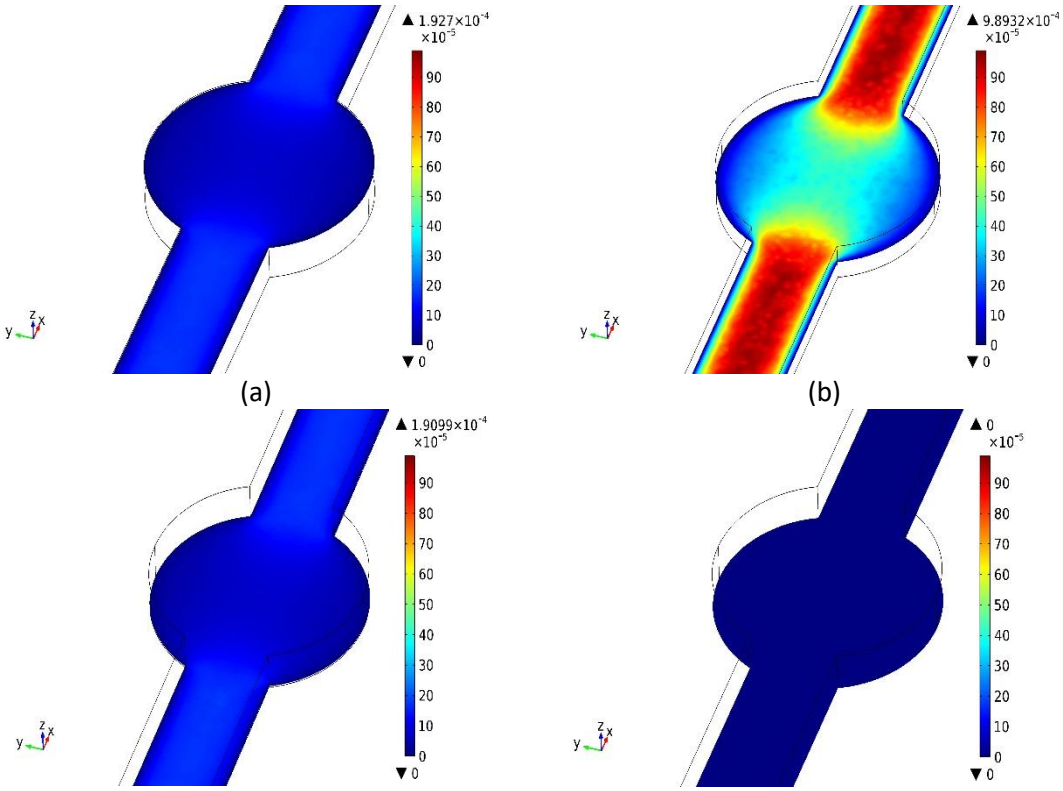

Fig. 4. Velocity in m/s surface plot at the microchannel trapping chamber at the depth of (a) 5 μm (b) 55 μm and (c) 105 μm (d) 110 μm

Table 2
Comparison of the magnetic and hydrodynamics drags forces at four different depth in the microchannel

Coordinate of the trapping chamber (x, y, z)	Forces	Magnetic microbeads diameter		Comparison	Trapping Possibility
		4.5 μm	2.5 μm		
(0,0,-5)	F_m	7.0 x 10^{-14}	1.90 x 10^{-14}	$F_m < F_d$	No
	F_d	2.66 x 10^{-12}	1.48 x 10^{-12}	$F_m < F_d$	No
(0,0,-55)	F_m	1.8 x 10^{-13}	3.00 x 10^{-14}	$F_m < F_d$	No
	F_d	1.52 x 10^{-11}	8.44 x 10^{-12}	$F_m < F_d$	No
(0,0,-105)	F_m	3.1 x 10^{-13}	5.1 x 10^{-14}	$F_m < F_d$	No
	F_d	2.66 x 10^{-12}	1.48 x 10^{-12}	$F_m < F_d$	No
(0,0,-110)	F_m	5.0 x 10^{-13}	8.0 x 10^{-14}	$F_m > F_d$	Yes
	F_d	0	0	$F_m > F_d$	Yes

The trapping of the magnetic microbeads is possible due to the longer residence time of the magnetic microbeads at very low flow rate. Moreover, the greater area circular trapping chamber design has the effect in lowering microbeads velocity. Therefore, greater trapping efficiency from the LoC magnetic separator is achieved in the trapping chamber. The effect of the microchannel design with trapping efficiency of magnetic microbeads has also been observed by the work of Wu *et al.,* [25].

The major drawback for an active technique LoC separator is low throughput. A low volumetric flow rate is needed as the magnetic force is only in the order of 250 pN to nN [8]. In addition, there will be a decreased in magnetic field and its gradient with distance between the magnetic field source

and the microfluidics flow. The range of the microfluidic flow of microliter per minute used in this study is comparable with the works of Smistrup *et al*., Lund-Olesen *et al.,* and Fulcrand *et al.,* [26-28]. Currently, a direct comparison with this works is not possible to be done as there are some differences in LoC magnetic separator system been used. The distinction includes the microchannel design and dimension, size of the magnetic microbeads used and the distance between the magnetic system and the microfluidics flow. In this study, efficient magnetic microbeads trapping has been quantitatively determined using a high gradient magnetic field system and microfluidics circular-shaped trapping chamber as LoC magnetic separator. This novel design system is expected to be used for future biological cells separation application.

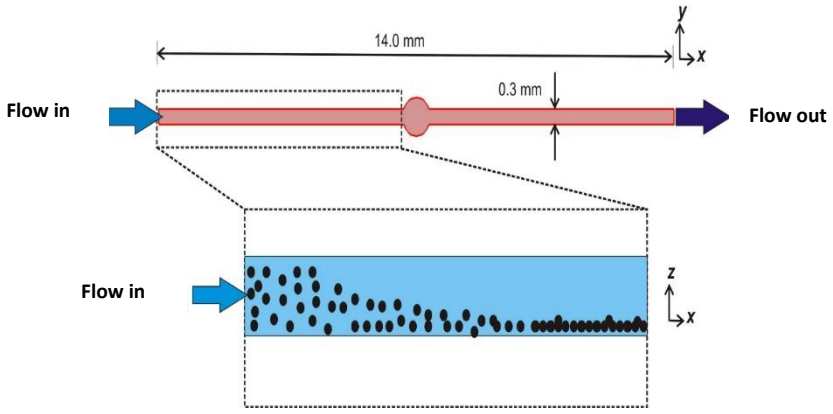

Fig. 5. Illustration of the efficient magnetic microbeads trapping at very low flow rate due to the effect of the trapping chamber design and long entrance length of 7 mm to the trapping chamber.

4. Conclusions

In conclusion, a highly efficient magnetic microbeads trapping was proven using this novel design of the LoC magnetic separator. The trapping efficiency of 99.5 % to 94.5 % using micron-sized magnetic beads of nominal diameter of 2.5 μm and 4.5 μm was demonstrated in continuous microchannel flow of 1.0 μL/min. The bottommost location of the trapping chamber with lowest drag force values makes the magnetic microbeads trapping possible in the hydrodynamics flow. Therefore, the microfluidics channel with chamber design had facilitated low hydrodynamics drag force on the magnetic beads and resulted high efficiency trapping. With the great performance of the LoC magnetic separator, the functional biological cells labelled with magnetic microbeads is predicted to be trapped and separated for biological studies and next generation POCT clinical diagnostics device application.

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