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Flowing-Fluid Mediated Detachment Method for Releasing Micro-Particles from Micro-Wells

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Abstract

This paper presents a computational simulation to design a microfluidic device that combines two main functions together—immobilizing and releasing for a single cell study. The idea is that the releasing device will be fabricated as the main channel located over the wells and the second channels (buffer channels) located at the bottom of the wells. During immobilization, the fluid in the main channel will flow over the wells, while the fluid from buffer channels will slowly be injected to keep the cells floating. In the releasing process, the fluid flow in the main channel will be slowed down. Meanwhile, the stronger fluid flow is released from the buffer channels to push the cells out of the wells. A computational software, in which cells were modeled as solid particles, was employed to help examine flow behavior. From the study, the appropriate flow rate—generating force equivalent to the particle's weight of various sizes of the particle— in buffer channel would be found in order to immobilize the cells and then release the cells out of the wells. We wholeheartedly believe that if this technique is achieved, it could be used to clearly analyze cancer diseases that result from the abnormality and the change of cell biology in both animals and humans.

Keywords: Microfluidics, Single cell, Cancer, Immobilization, Cell releasing

1. Introduction

The analysis of biological cancer cells is gathered by collecting samples of tissue cells. The tissue samples have several cells. Based on the behavior of the cellular heterogeneity, there are various possible outcomes. Therefore, a single cell study is necessary to clearly analyze cancer cell behavior, and could provide a precise result from a study. Microfluidic system is recently developed to address this problem. This technology allows us to analyze a single cell more clearly compared to the conventional methods. Microfluidic technology has been used for decades among various methods in the field for a single cell analysis. In addition, this technique appears to be more popular in other fields as well besides in biological study.

Over the past years, the group of Microfluidics at Chulalongkorn University has developed several techniques of both sorting and trapping devices. The team using the sorting technique is responsible for separating particles by using the spiral microchannel method [1]. From the study, particles could clearly be seen separating from the inlets to the outlets in the microchannels. In the trapping process, it was focused on trapping particles in wells. The wells were designed to be a triangular shape because this shape was previously reported to be able to appropriately create a votex that helps the particles go down into a well [2]. The result of our study was impressive as 62.5% of the total particles were trapped in the wells.

However, the releasing technique has not been addressed and established yet. This technique focused on immobilizing and releasing particles out of wells.

According to the group of Yamaguchi, the experiment focused on a single cell manipulation and injection by fabricating two main parallel channels which were connected by a drain channel in the middle of device. These parallel channels allowed a cell suspension and flow to inject the cell separately [3]. Similarly, the group of Chanasakulniyom presented cell proliferation and migration by designing and fabricating perpendicular channels. The device had two main channels as well. However, these two channels were perpendicular in horizontal axis. The second channel and the well were designed in one piece without having a drain channel linking the first and second channel [4].

Both designs greatly inspired our idea in this releasing project. We decided to use the concepts of injection and the design of Chanasakulniyom et al. combined together. This technique will become our next challenge and a new discovery. Dealing with the challenge, a risk of cell viability and its property are taken into our considerations. Consequently, flowing fluid mediated detachment was chosen as the releasing method because there is no external forces which affect the cell biological and physical properties. In addition, our device will be able to immobilize the cell by keeping the cell floating in the middle of the well in order to avoid a wall effect that might change cell's biological and physical properties. Moreover, this microfluidic technique is inexpensive due to simplicity of the design and no need of external equipments.

In order to reduce a risk of failure in experiment and fabrication, computational software is needed to simulate the behavior of flow in the main and buffer channels. A computational software (COMSOL Multiphysics 5.0), in which cells were modeled as solid particles, was employed in order to help examine flow behavior—streamlines, pressure, velocity distributions and total force. They were found under various flow rates. The aim of this study is to find the appropriate flow rate in the buffer channels—generating force as equal to the particle's weight—as well as to examine the devices' feasibility for immobilizing and releasing.

2. Design and Plan

The idea was that the releasing device will be fabricated as shown in Fig. 1a. The trapping principle is simple as shown in Figs. 1b-c as the cells fall down in the wells due to a recirculation flow. During immobilization (Fig. 2d), the fluid in the main channel flows over the wells, while the fluid from buffer channels will be slowly accelerated until an appropriate flow rate is reached in order to keep the cells floating. In the releasing process (Fig. 2e), the fluid flow in the main channel will be slowed down. Meanwhile, the stronger fluid flow is released from the buffer channels in order to push the cells out of the wells.

According to the previous data of trapping device, the appropriate flow rate was fixed at 0.1 mL/hr (velocity approximately equal to 540 µm/s) which this flow rate will be employed in the main channel in this study. The next step was to employ a solid particle with different sizes in the middle of the well. In this case, the flow rates in the buffer channels were varied into several cases. After that, total forces were evaluated over a surface of the solid particle at different flow rates in the buffer channels. If these total forces were generated equivalent to the polystyrene beads' weight—10, 15 and 20 µm, the beads would be floating in the middle of the wells. For the releasing, the flow rate in the buffer channel will be increased beyond the flow rate during the immobilization in order to release the particle out of the well.

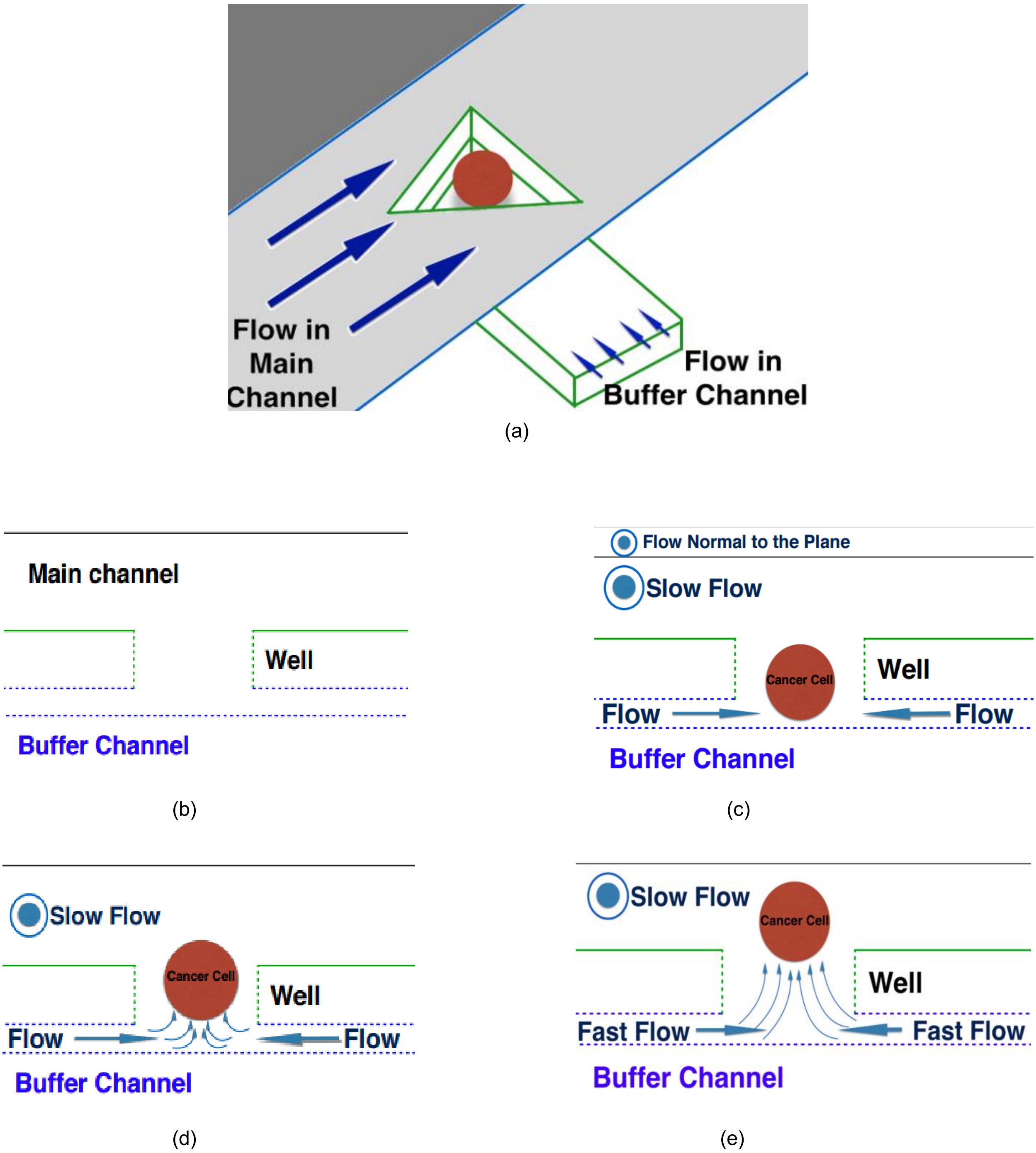


Fig.1 Working principle of immobilizing and releasing device; (a) schematic picture of the device and flow direction of both main and buffer channels (b) vacancy well, (c) trapping, (d) immobilizing, (e) releasing.

3. Simulation

3.1. Boundary Conditions and Materials

In computational simulation, 3-D simulation and single-phase flow were selected. The medium was assumed to be water as it has been studied in the past with density of 1000 kg/m^3 and viscosity of 0.001 Pa.s and the flow was considered as laminar flow. Furthermore, no slip boundary condition for the walls and the stead-state Navier-Stokes equation for incompressible fluids which is defined as

$$\rho(u\cdot\nabla)u=\nabla\cdot[p+\mu(\nabla u+(\nabla u)^T]+F=0 \quad ....(1)$$

where u is the fluid velocity, p is the fluid pressure, ρ is the fluid density, and μ is the fluid dynamic viscosity, ∇ is the del operator, F is the external forces applied to the fluid [5].

3.2. Defining Geometry

In order to avoid computational time consuming, a computational domain was modeled with a length of 1,200 µm, a width of 80 µm and a height of 160 µm. The well size was modeled as a triangle shape with each side of 40 µm and a depth of 15 µm as the main channel where was located over the wells. As for the buffer channel, it was modeled with a length of 40 µm and a height of 5 µm which was located at the bottom of the well. After finishing modeling geometry, meshing was the next step of this process.

3.3. Meshing

The program provides nine built-in size parameter sets [6]. Distribution of meshing was selected and separated into three domains—the solid particle, the well and the edges of the well. The number of meshes in these three domains was increased until the results were stable. A validation and grid independence tests were done.

3.4 Simulating results

The results were focused on total forces over the surface. The total hydrodynamic forces on the surface of the sphere were able to be evaluated by the sum of integral of pressure distribution and viscous stress is defined as

$$F_{hydro}=F_{pressure}+F_{viscous} \quad ....(2)$$

4. Computational Results

4.1 Immobilizing Process

To improve the results of simulation, the technique of the grid independence was implemented by using smaller size of meshes for calculation. After the results from the grid independence were stable, the next step was to find the appropriate flow rate and total force in vertical direction. In this case, polystyrene beads were used as a replica model of a cancer cell in different diameters—10, 15 and 20 µm. The net force from a gravitation effect of the different size of polystyrene beads were calculated which was written as

$$F_{net}=mg-\rho V_{disp}g \quad ....(3)$$

where F_{net} is a net force (N) due to gravitational effect, m is a mass of the object (kg), ρ is a density of the fluid (kg/m^3), V_{disp} is the volume of the displaced body of liquid (m^3) and g is the acceleration due to gravity 9.807 (m/s^2).

According to the data in Table 1, the results showed that increasing flow rates in the range of 0-1.87 nL/hr resulted in an increasing of the total hydrodynamic force. The magnitude of the hydrodynamic force also depended on a particle size. For instance, at the flow rate of 1.87 nL/hr, the hydrodynamic force exerting on a particle with the size of 10, 15 and 20 µm was equal to 0.45, 1.39 and 4.26 pN, respectively.

Table 2 represents the flow rate in buffer channels where the net force due to gravitational effect equal to hydrodynamic force of each size of solid particles. It was found that the flow rate in buffer channels should be equal to 1.07 nL/hr, 1.18 nL/hr and 0.92 nL/hr in order to immobilize the solid particle of 10, 15 and 20 µm, respectively. The results suggested that the flow rate in buffer channels should be precisely controlled in order to achieve the floating of cells.

Table 1 Comparison of total hydrodynamic forces in a vertical direction with different diameters of a solid at various flow rates in buffer channels

Flow rate in buffer channels (nL/hr)	Total hydrodynamic force (pN)		
	10 µm	15 µm	20 µm
0	0	0	0
0.62	0.15	0.46	1.42
1.25	0.30	0.91	2.85
1.87	0.45	1.39	4.26

Table 2 Flow rates in buffer channels when the floating of cells occurs.

Diameters (µm)	Flow rate (nL/hr)	$F_{net} = F_{hydro}$ (pN)
10	1.07	0.26
15	1.18	0.87
20	0.92	2.05

The conclusion was made that the flow rate which could keep the particles floating in the well for 10, 15 and 20 µm should be in an order of magnitude of 1 nL/hr. Figure 2 shows the linear increment of hydrodynamic force due to the change of flow rate in buffer channels as well as the appropriate flow rates that should be for floating the cells in micro-wells when the flow rate in the main channel is fixed at 0.1 mL/hr.

4.2 Releasing Process

In the releasing process, the fluid flow in the main channel was slowed down. Meanwhile, the stronger fluid flow is released from the buffer channels in order to push the cells out of the wells. This purpose of this process was to find the appropriate flow rate in the buffer channel to release particles out of the wells. Due to the data from the immobilizing process, the results showed that the particle of 10, 15 and 20 µm would be able to be released out of micro-wells with the flow rates in buffer channels higher than 1.07, 1.18 and 0.92 nL/hr, respectively.

5. Discussion

According to the data for total force and various flow rates, there are possibilities for immobilizing and releasing particles. These possibilities are based on both flow rates in main and buffer channels.

As shown in Figs. 3 a-c, the streamlines passed through the solid particle of 15 and 20 µm smoothly. However, recirculation behind the particle occurred for the solid particle of 10 µm. For this reason, the larger size of particles relatively to the size of micro-well could reduce recirculation and may be able to stabilize the floating of particle in the well.

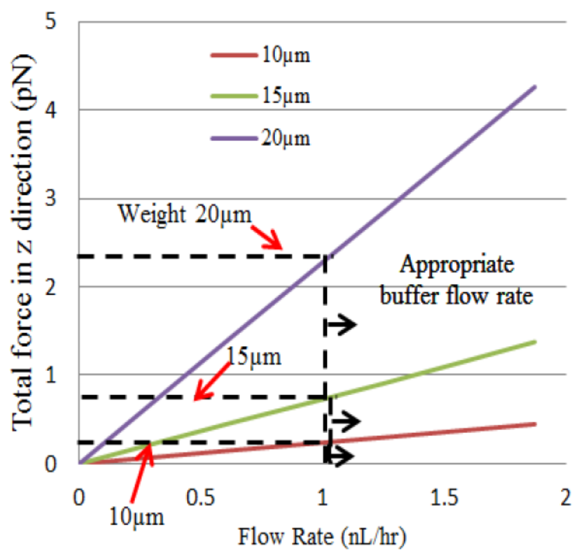
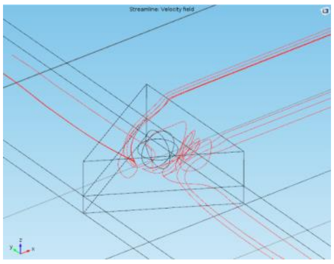
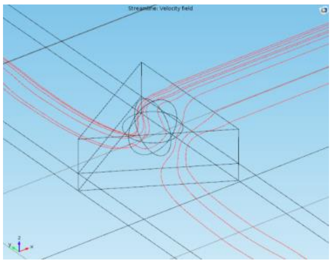


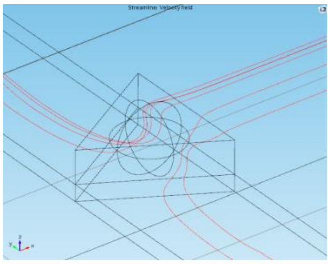
Fig. 2 Vertical hydrodynamic forces versus flow rates for 10, 15 and 20 µm particles.



(a)



(b)



(c)

Fig. 3 Streamlines from computational results at the flow rate of 1 nL/hr in buffer channels for different sizes of particle; (a) 10 µm, (b) 15 µm, (c) 20 µm.

Another interesting issue is the appropriate flow rate in buffer channels that could float a particle. A simulation for particles with a diameter between 5 to 20 µm was performed further in order to investigate an effect of the particle size on this flow rate where the net force due to gravitational effect equal to hydrodynamic force. The results are shown in Fig. 4 comparing between two cases, i.e. with and without micro-well. With the well, when the size was increased, the required flow rate was increased as well until the particle size of around 14 µm. At this size, the required flow rate was around 1.2 nL/hr. Beyond this particle size, the required flow rate reduced.

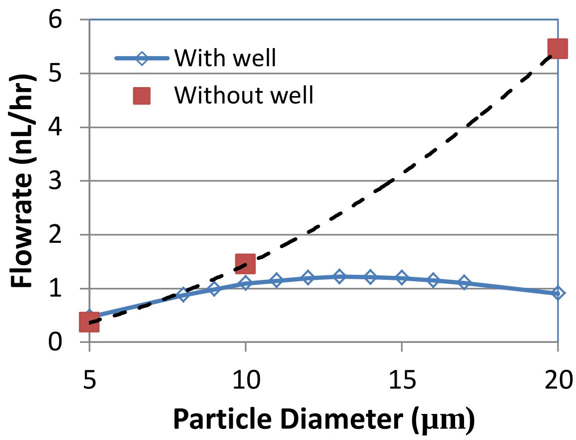


Fig. 4 Flow rate in buffer channels when hydrodynamic force equal to the net force due to a gravitational effect for different particle sizes ranging from 5-20 µm.

According to the case when the particle floating in a non-confined space, the flow rate required for the floating increased with the increasing of particle size. This suggested the strong effect of the ratio of particle and the micro-well dimensions. When the particle size is still small comparing to the micro-well, two cases showed a similar trend. However, when the particle size became larger, e.g. 10 µm, the difference of the required flow rate between two cases was observed.

This might be an effect of flow phenomena that was different between the two cases, and the reason that caused the reducing of required flow rate for large particle must be investigated further.

6. Conclusion

The goal of this project is to fabricate the releasing device which consists of two functions—immobilizing and releasing process. In order to do so, the design of releasing device—main channel, triangle wells and buffer channels were modeled based on the data from previous experiments on a trapping device. Furthermore, the appropriate flow rates in the buffer channels were found in order to immobilize (generating force equivalent to the particle's weight) various sizes of the particle and release (increasing force over the particle's weight). From simulation results, it suggested that for different sizes of particles on 10, 15 and 20 µm, the critical flow rates in buffer channels for the fixed flow rate in the main channel of 0.1 mL/hr should be 1.07, 1.18 and 0.92 nL/hr, respectively.

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