Effect of Blood Cell Sedimentation on Immunomagnetic Isolation of Circulating Tumor Cells in Microfluidic Channels

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ABSTRACT

Isolation and detection of circulating tumor cells (CTCs) from blood can reveal critical information for cancer diagnosis and prognosis [1]. Immunomagnetic assay, together with microfluidic technology, provides a highly efficient CTC screening method. In this paper, we investigate the impact of the red blood cells (RBCs) sedimentation during the process of isolating CTCs in the immunomagneic microchip system that we previously developed [2]. Both computational model and experimental studies are carried out to characterize RBCs sedimentation and its effect on CTC capturing in two different microchip orientations, upright and inverted. Colo205 cells (human colon adenocarcinoma cell) are spiked into healthy blood samples for screening experiment. The experimental results confirm the theoretical models and simulations, both indicating that inverted operation is optimal with higher capture rate of 75%.

KEYWORDS

Blood cell sedimentation, Circulating tumor cells, Immunomagnetic assay, Microfluidic

INTRODUCTION

The clinical and biological significance of circulating tumor cells (CTCs) in metastatic cancer have been widely investigated. Studies of CTCs require effective separation of target cells, which is especially challenging because of their extreme rareness in blood (1 ~10^7 -10^9 blood cells) [3].

Various methods have been proposed to separate CTCs from blood samples [4], among which the immunomagnetic assay stands out due to its high sensitivity, specificity and simplicity [2]. It utilizes functionalized magnetic nanoparticles, conjugated with antibodies that can specifically label the cancer cells in blood. While the blood sample flows through the Polydimethylsiloxane (PDMS) microchannel, the labeled cancer cells will be separated by the magnetic force, which is generated by the permanent magnets placed outside the channel (shown in Figure 1). After capture, we use immunofluorescence technique to identify the captured cells based on the cell morphology and fluorescence intensity information.

Theoretical study of the blood cell isolation has been a topic of interests in applied physics [5, 6]. In this paper, we take the non-Newtonian biological fluid nature of blood into account, and build a partial viscos model to describe the impact on the CTC isolation from the blood environment. In addition, we developed a simplified while effective model to process large amount of cells with high accuracy without consuming significant computational power.

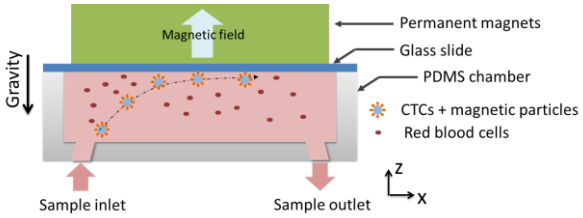

Figure 1. Microchip based immunomagnetic CTC isolation system. With the influence from the hydrodynamic force and gravitational force, magnetic force dominates the motion of CTCs and attracts them to the substrate of the microchannel for isolation.

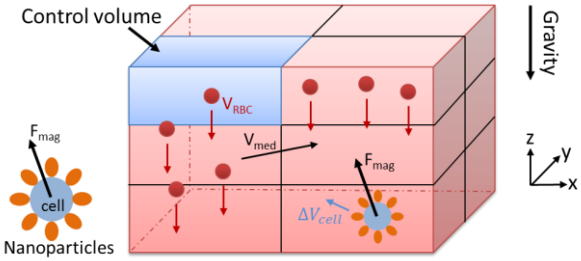

Figure 2. Schematic of RBC sedimentation and partial viscosity model of CTC isolation. RBC sedimentation in each control volume is in the vertical direction, whose magnitude depends on the local RBC volumetric concentration $\boldsymbol{\rho_{RBC}}$.

THEORY

Before we study the relation between red blood cells (RBCs) sedimentation and the CTCs capture process, we first build a model to describe the motion of RBCs [7]. The flow field is segmented into multiple cubic control volumes. For each time step, local volumetric RBCs concentration is dynamically calculated using the RBCs flux from neighboring control volumes, using Equation (1).

$$\Delta\rho_{RBC}(x,y,z) = (\Phi_x^+ + \Phi_x^- + \Phi_y^+ + \Phi_y^- + \Phi_z^+ + \Phi_z^-)\Delta t \qquad (1)$$

To investigate the effect of RBCs sedimentation on CTC separation, we build a partial viscous model, attributing the drag force on CTCs to both the medium and RBCs, as illustrated in Figure 2. Therefore, the total drag force experienced by the cell is

$$F_{drag} = 6\pi\Delta V_{cell}\eta_{med} + 6\pi R_{cell}(\Delta V_{cell} - \Delta V_{RBC})\Delta\eta_{RBC}(\rho_{RBC}) \qquad (2)$$

Under a Quasi-Static motion assumption ($F_{mag} = F_{drag}$), the instant velocity of the CTCs is completely dependent on the local magnetic field ($F_{mag} = \frac{V\Delta\chi_{cell}}{2\mu_0}\nabla B^2$), flow field and local viscosity, and can be described as

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$$V_{cell} = \frac{F_{mag} + 6\pi R_{cell} \Delta V_{RBC} \Delta \eta_{RBC}}{6\pi R_{cell}(\eta_{med} + \Delta \eta_{RBC})} + V_{med} \qquad (3)$$

For each step time $t \to t + \Delta t$, the position $(D_{x,y,z})$ of the cell can be updated according to

$$D_{x,y,z}(t + \Delta t) = D_{x,y,z}(t) + V_{cell}\Delta t \qquad (4)$$

here $\Delta\rho_{RBC}$ is the RBC volumetric concentration (ρ_{RBC}) change in time Δt; $\Phi_{x,y,z}^{+/-}$ is the RBC flux in one neighboring side of the control volumes in six directions; ΔV_{cell} is the velocity difference between the cell and the medium; η_{med} is the viscosity coefficient of the buffer solution; R_{cell} is the radius of the cells; ΔV_{RBC} is the RBC sedimentation velocity, which only works in the direction perpendicular to the substrate; $\Delta\eta_{RBC}(\rho_{RBC})$ is the partial viscosity coefficient due to the RBC volumetric concentration, which is obtained through experimental measurement; $\Delta\chi_{cell}$ is the magnetic susceptibility of the cells; B is the local magnetic flux density.

EXPERIMENT: EFFECT OF MEDIUM VISCOSITY ON CELL MOTION

RBCs sedimentation, as indicated in the model, will eventually result in different viscosities. In order to address its effect on CTC capture, we record and study the cell motion in the near-surface region in different viscous mediums using the system shown in Figure 3(a). PBS buffer solution and 5% Polyvinyl alcohol solution (PVA), which has a higher viscosity, are chosen for comparison. Colo205 cells are spiked into PBS/PVA solutions for observation experiment. Cell motion videos are divided into frames and stacking on each other to form the trajectory as shown in Figure 3(b), from which cell instant velocities and planar positions can be directly extracted. From the moment the cells becoming visible to their firm stop, the time (35s) and distance (980μm) needed in PVA are twice as those in PBS solution (20s, 400 μm) as shown in Figure 3(c).

According to our model, higher medium viscosity generates higher drag forces. The cells are in similar magnetic environment (we focus on the same spots on the glass slide), this essentially changes the acceleration of the cells in both vertical and horizontal directions. Therefore, the cells approach the substrate slower, giving them more time to move forward, hence increasing the opportunities of the cells slipping away from the channel without being captured.

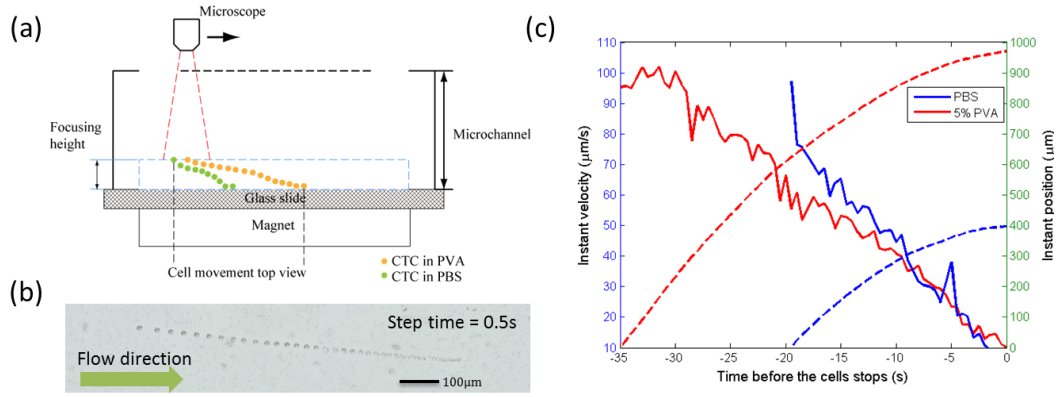

Figure 3. System set-up and cell trajectory results. (a) Schematic of the measurement system. The microscope focus on the substrate of the channel at the same planar position, therefore the cells can be considered to be in similar magnetic environment, with same initial height. (b) The Colo205 cell trajectory calculated by stacking different frames pictures on top of each. (c) Experimental results of the instant velocities and positions of the cells moving in PBS and PVA separately.

EXPERIMENT: EFFECT OF BLOOD SEDIMENTATION ON CTCS CAPTURE

Using our model, we are able to calculate the RBC sedimentation pattern in the microfluidic channel under two different channel orientations, regarding different directional relation between the magnetic force and gravitational force. Figure 4 shows the development of the RBC sedimentation in both upright and inverted channels. The glass slide substrate, where the cells are expected to be captured, gradually gets covered in the upright channel, while it stays uncovered in the inverted case. This thick layer of RBCs creates a high viscosity zone, which makes it more difficult for the CTCs to get close and be captured by the channel. In addition, we are also able to theoretically track the trajectories of cells in the microchannels with two orientations, as illustrated in Figure 5.

To verify the theoretical models, we carry out blood screening experiments with the same settings in the simulation by placing the microchannel upright and inverted. Again Colo205 cells were spiked into healthy blood samples for screening experiment. Figure 6(a) shows the design of the microfluidic channel, and blood sample filling the channel. The immunofluorescent images of the captured cancer cells are presented in Figure 6(b). Figure 6(c) is the picture of the automated screening system that can change the channel orientations under the control of a LabView program. Comparisons between the counts and coordinates of the captured cells obtained through the simulation and experiments yields high consistency, as shown in Figure 7. In upright channel, the capture rate

(= $\frac{\text{Number of captured cells}}{\text{Total number of spiked cells}}$) is 40%, while it goes up to 75% in the inverted channel.

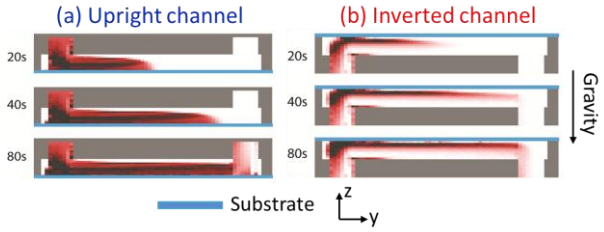

Figure 4. Simulation results of the RBC sedimentation in the (a) upright and (b) inverted channels at the center plane for time points of 20s, 40s and 80s. The upright channel in (a) show thicker sedimentation layer on the substrate surface.

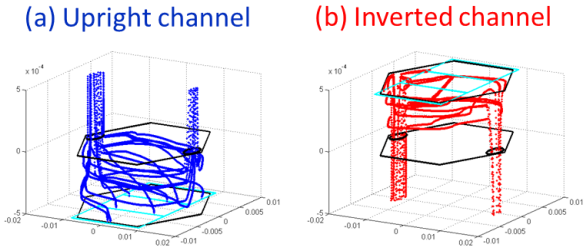

Figure 5. Theoretical calculation of cell trajectories within the microchannel in both (a) upright (b) inverted channels. The positions of the inlet/outlet holes, and the magnets are shown.

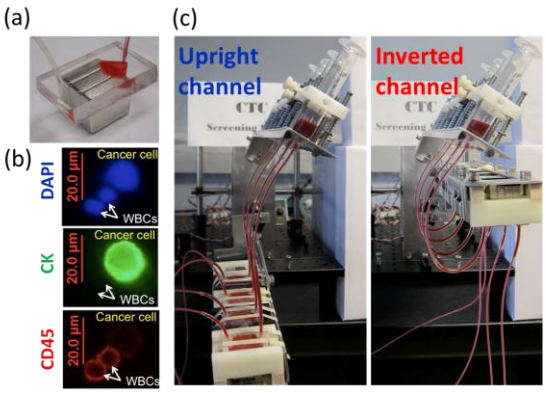

Figure 6. Microchip based CTC screening system. (a) The microfluidic chip, where the blood sample is flowing through the microchannel. (b) Immunofluorescet images of the captured cells, which are stained with CK, CD45 and DAPI for identification. (c) Automated screening system with motion control. The system is controlled by a Lab-view program to switch the orientation of the microchannels.

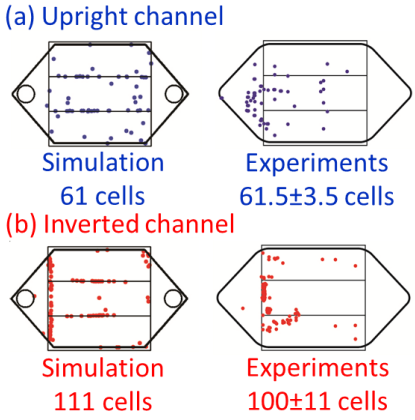

Figure 7. Captured cell counting and mapping on the glass slide form both simulation and experiments. (a) Upright channel, (b) Inverted channel. Total number of cell released was 150 (Colo205 cells spiked into healthy blood samples).

In conclusion, we investigate the effect of blood cell sedimentation on immunomagnetic isolation of CTCs in microfluidic chips. We develop a theoretical model to describe the sedimentation motion of RBCs and characterize the impact of RBC sedimentation on CTC isolation process using immunomagnetic microchip system. The experimental results verified the theoretical design calculation, which indicated the preferable design of inverted microchannel orientation to achieve higher capture rate.

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