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Optimization of Microfluidic Immunomagnetic Chip for Circulating Tumor Cell Capture

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The enumeration of circulating tumor cells (CTCs) has proved valuable for early detection and prognosis in cancer treatment. In this work, an optimized design of a microfluid-based immunomagnetic chip is proposed to separate CTCs from whole blood. Unlike most reported immunomagnetic-based methods in which the magnetic field is applied perpendicular to the flow direction, here, the magnetic field is applied parallel to the flow direction, which makes the hydrodynamic force not the dominant force to hamper cell capture. Experimental validation showed that this novel chip can be operated at a flow rate of up to 6 ml/h with 90% CTC capture efficiency.

1. Introduction

Circulating tumor cells (CTCs) are widely considered to be an important factor in metastasis and have been found in patients having many different types of cancer, such as breast, lung, pancreatic, prostate, liver, and colon cancer. The isolation of CTCs has attracted a great deal of attention because the number of CTCs isolated can be useful for cancer diagnosis and prognosis. However, the isolation of intact CTCs is still challenging owing to their extremely low concentrations in blood, as few as one cell per 10^9 hematologic cells, which hampers any medical and molecular analysis.

Immunomagnetic-based assays, in which specific cells are labeled with antibody-functionalized magnetic carriers and separated by a magnetic field, have been widely utilized in the isolation of CTCs from blood. Microchip-based immunomagnetic separation has also been applied to CTC detection. However, in most of these chips,[(1)] the motion of an immunomagnetic labeled cell is highly dependent on the competition between the magnetic force and the drag force. To achieve high capture efficiency, the separation must be carried out at a low flow rate to reduce the drag force. In this case, the separation time is increased, which leads to a loss of cell viability.

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Here, we demonstrate a microchip-based immunomagnetic separation of tumor cells with advantages such as rapid separation and high performance. An array of magnetic microposts is fabricated inside the microchip to generate a strong magnetic force when magnetized by external permanent magnets. Unlike most reported immunomagnetic-based methods in which the magnetic field is applied perpendicular to the flow direction, here, the magnetic field is applied parallel to the flow direction. Therefore, the hydrodynamic force is no longer the dominant force to hamper cell capture. A theoretical framework and numerical model are developed for the demonstration and predication of immunomagnetic separation. An experimental validation is also performed to demonstrate that this novel microchip could be used to isolate CTCs at a high flow rate with a high capture efficiency.

2. Materials and Methods

2.1 *Theory and design*

Because magnetic nanobeads coated with anti-EpCAM antibodies bind to epithelium-derived CTCs, the CTCs can be treated as paramagnetic particles. Therefore, the magnetic force that acts on a CTC is expressed as[2]

$$F_{\mathrm{mag}} = N \cdot \frac{4\pi R_{\mathrm{p}}^3}{3} \cdot \frac{\Delta x_{\mathrm{p}}}{2\mu_0} \nabla B^2, \tag{1}$$

where $\mu_0 = 4\pi\times10^{-7} T{\cdot}m{\cdot}A^{-1}$ is the magnetic permeability of a vacuum, B is the magnetic field intensity, N is the number of magnetic nanoparticles attached to the cell, R_{p} is the radius of the magnetic nanoparticles, and $\Delta\chi_{\mathrm{p}}$ is the volumetric susceptibility of the magnetic nanoparticles. We adopt the conditions of $R_{\mathrm{p}} = 50$ nm and $\Delta\chi_{\mathrm{p}} = 5$.[2]

At low Reynolds numbers, the inertial effects can be neglected, and a fully developed laminar flow is assumed. Therefore, Stokes' law is appropriate for determining the drag force on a sphere, as given by

$$F_{\mathrm{drag}} = 6\pi\eta R_{\mathrm{c}}(\mu_{\mathrm{c}} - \mu_{\mathrm{f}}). \tag{2}$$

Here, R_{c} is the effective cell radius, η is the fluid viscosity, and μ_{f} is the fluid velocity. The immunomagnetic system is defined by a balance of the magnetic and drag forces, resulting in the following dimensionless parameter,

$$\Pi_1 = \frac{F_{\mathrm{mag}}}{F_{\mathrm{drag}}}. \tag{3}$$

As described in the literature,[3] the capture efficiency depends on the ratio of these two forces. In the case of $\Pi_1 > 1$, the relative magnetic force is stronger than the drag force, which increases the probability of CTC capture. In contrast, the magnetic force has a minimal effect on the CTCs and cells prefer to flow out of the microchannel. Figure 1 shows a schematic illustration of our microchip-based immunomagnetic isolation system. An array of soft magnetic microposts is fabricated inside the microchannel. Compared with the standard magnetic microfluidic chip, which typically

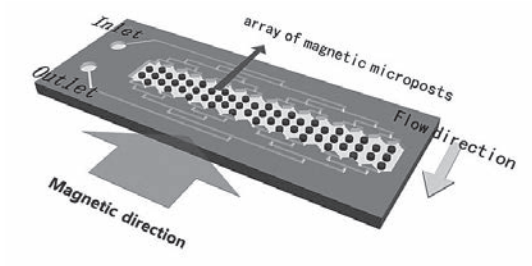


Fig. 1. Schematic of the microchip-based immunomagnetic isolation system.

has a narrow and long channel, our chip is designed in such a way that the cross-sectional area and, consequently, the width of the channel are increased, while the length is decreased with the magnetic field applied parallel to the flow direction.

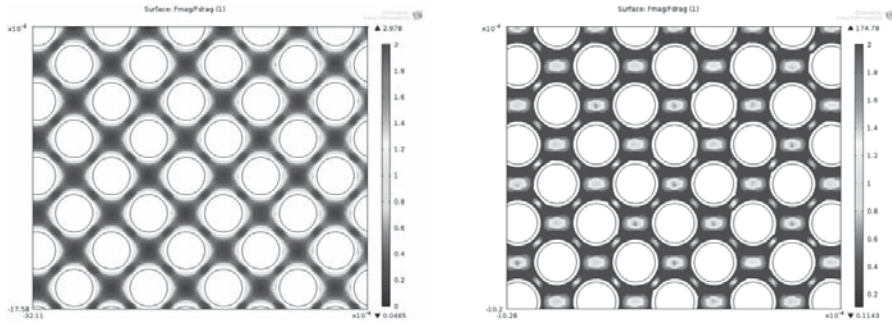
2.2 *Fabrication of microchip-based immunomagnetic system*

The microchip was fabricated using electroplating and rapid prototyping as reported elsewhere.[4] Briefly, an array of nickel microposts is fabricated on a glass slide by photolithography and electroplating. A polydimethylsiloxane (PDMS) microchannel bonded to it is used for blood sample flows. Target cancer cells are labeled with commercial magnetic nanoparticles (Veridex Ferrofluid™, LLC) that are functionalized with anti-epithelial cell adhesion molecules (anti-EpCAMs). As the blood sample flows through the microchannel with an external magnetic field applied parallel to the flow direction, nanoparticle-labeled cancer cells are separated from blood flow, captured on the microposts, and successively stained with DAPI, anti-cytokeratin, and anti-CD45. Images were recorded under an inverted microscope (IX 71, Olympus) equipped with a cooled CCD (DP70, Olympus).

3. Results and Discussion

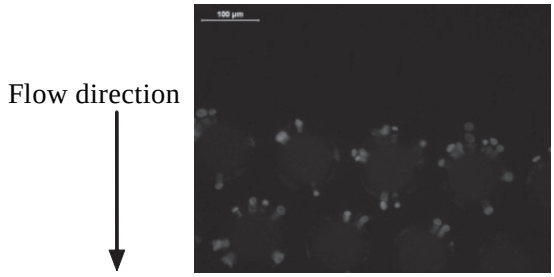
To capture CTCs in a microchip, the magnetic force has to be sufficiently high so as to hold the cell against the drag force. Therefore, a dimensionless parameter Π_1 is introduced to delineate the probability of CTC capture. To simulate our experiments, we use the COMSOL Multiphysics Software. Capture models in which the magnetic field is perpendicular and parallel to the flow direction are investigated. All the models are set at the same flow rate of 2 ml/h with the other simulation conditions set as reference. The main results are presented in Fig. 2.

As shown in Fig. 2, our immunomagnetic chip has a larger area of $\Pi_1 > 1$ than the standard immunomagnetic chip. Therefore, our immunomagnetic chip has a higher probability of capturing CTCs inside the microchannel. Moreover, in our design, the flow velocity is in the same direction as that of the magnetic force, which gives the cell an initial velocity when it moves to the microposts driven by magnetic force. Thus our immunomagnetic separation chip is capable of isolating CTCs with high capture efficiency. To verify the simulation results, cancer cell lines (SW620) in the range of 100 –1000 cells were spiked into 1 ml of human peripheral blood and were flowed into our



(a) (b)

Fig. 2. (a) Numerical simulation of Π_1 in a standard immunomagnetic chip. (b) Numerical simulation of Π_1 in our design.



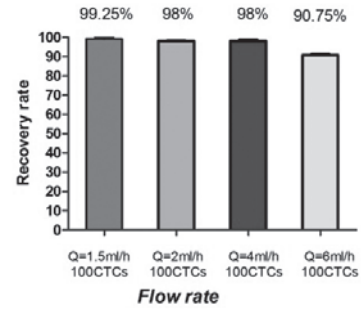


Fig. 3 (left). Fluorescence image of captured CTC cells on microposts.
Fig. 4 (right). Recovery rates of CTC microseparator at various sample flow rates.

new immunomagnetic chip at a flow rate of 6 ml/h. Figure 3 is a fluorescence image that shows that cancer cells stained with DAPI and CK were successfully captured by our immunomagnetic chip.

To investigate the purity of the CTC microseparator under different flow rates, the CTCs are separated by the CTC microseparator at a flow rate of 1.5−6 ml/h. Figure 4 shows the recovery rate of the CTC microseparator determined by the observation of CTCs inside the channel at various sample flow rates. The recovery rate is defined as the ratio of the cells collected inside the microfluidic channel to the cells spiked in the blood. It can be seen that our CTC microseparator isolated approximately 90% of CTCs spiked into blood samples with a flow rate of up to 6 ml/h. This flow rate is faster than those reported previously with a microchannel-based assay.[5]

4. Conclusions

The reported immunomagnetic microchip provides a practical method by which to isolate CTCs from peripheral blood quickly and with high recovery and purity. The ease of use and versatility of the CTC microseparator allow for its extensive applicability in the separation of various rare cells. This novel immunomagnetic chip could be further advanced as a microscaled automated platform for CTC-based cellular and molecular assays.

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