PATTERNED NANOMAGNETS ON-CHIP FOR SCREENING CIRCULATING TUMOR CELLS IN BLOOD

Yu-yen Huang[1], Peng Chen[1], Kazunori Hoshino[1], Chun-hsien Wu[1], Nancy Lane[2], Michael Huebschman[2], Jonathan Uhr[2], Konstantin Sokolov[1], Eugene Frenkel[2], Xiaojing Zhang[1]

[1]The University of Texas at Austin, USA, [2]The University of Texas Southwestern Medical Center, USA

ABSTRACT

Circulating tumor cells (CTCs) are considered potential indicators for prognosis and clinical management. However, detection of CTCs is very challenging due to its rarity (the ratio per CTC to leukocyte is $1:10^7$-10^9). We have previously developed a microchip-based immunomagnetic screening system for the isolation of CTCs from whole blood samples. The system has been successfully applied in cancer patient sample screening. Here, we report arrays of directly patterned metallic nanomagnets on a channel substrate that locally enhance magnetic field and further improve system's capture capability. The proposed screening system can be a powerful platform for the cancer management.

KEYWORDS

Circulating tumor cell, microchip, immunomagnetic, nanomagnets

INTRODUCTION

Detection of circulating tumor cells (CTCs) has been considered an important indicator in assessing the response to cancer treatments and thus provides possible treatment strategies [1]. Due to the rarity of CTCs (the ratio per CTC to leukocyte is $1:10^7$-10^9), it is challenging to separate CTCs from patient whole blood sample [2].

With the advent of microfabrication technology, several microchip-based tools have been proposed for the capturing of rare CTCs. We have developed a microchip-based immunomagnetic screening system for the separation of CTCs [3]. The developed system has been successfully applied in the screening of cancer patient samples [4].

Figure 1 illustrates the CTC screening system. A polydimethysiloxane (PDMS)-based microchannel is fixed on a glass coverslip. As blood flows through the microchannel, magnetic nanoparticle-labeled CTCs are attracted to the microchannel substrate by external magnetic force during the screening process. Nanoparticle-labeled cancer cells and free nanoparticles flowing in the microchannel tend to be captured on a channel substrate around boundaries of the adjacent permanent magnets. In the previous design, permanent magnets only provided a macroscopic magnetic field distribution to attract target nanoparticle-labeled cancer cells towards high field gradient areas between neighboring magnets [3]. This can be further optimized through fine tuning the magnetic field at local level.

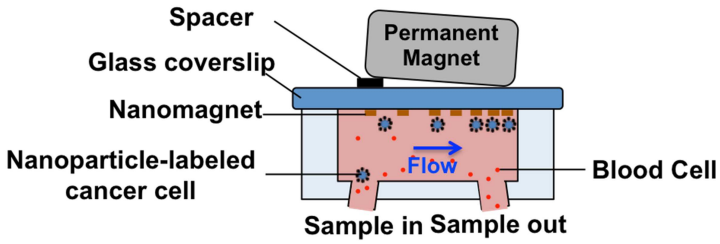

Figure 1. Schematic of the CTC screening microchip cross-section.

Aggregation of nanoparticles can interfere with the identification process or even damage the captured CTCs. Target cells rolling on the weaker magnetic field areas can also escape from the microchannel without being captured by the stronger magnetic field. Integration of microchip devices with magnetic nanostructures has been proposed for separating red blood cells (RBCs) and white blood cells (WBCs) from whole blood samples [5]. In this paper, we integrate the developed microchip-based immunomagnetic screening system with metallic nanomagnets to uniformly distribute target CTCs, free nanoparticles, RBCs, and WBCs on the glass substrate. Therefore, the capture capability of the screening system can be further improved.

THEORY

Patterned nanomagnets on channel substrates can provide locally enhanced magnetic field in the microchannel. Ferromagnetic material (nickel) is deposited on the channel substrate to introduce localized magnetic forces. Figure 2(a) shows the schematic of localized magnetic forces induced by the permanent magnets, which are alternately arranged, and nanomagnets. Figure 2(b) shows that high magnetic field gradients occur at the edges of the nanomagnet. The magnetic flux density passing through the nanomagnet in the x-direction is greatest when the nanomagnet width (w) is larger than the height (h) [5].

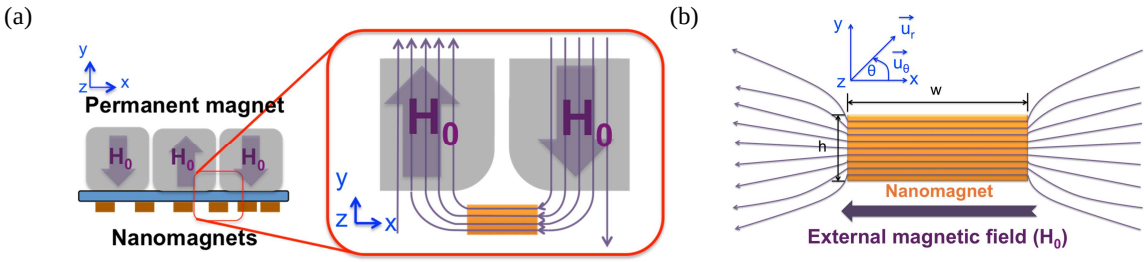

Figure 2. Schematic of nanomagnets patterned on the microchannel substrate. (a) Locally enhanced magnetic field generated by the nanomagnet, which is magnetized by permanent magnets placed on top of the capture substrate (b) Magnetic flux density travels through a nanomagnet located between two permanent magnets in the x-direction. A uniform external magnetic flux density (H_0) deforms around the nanomagnet and generates a high magnetic field gradient.

The magnetic force generated by a nanomagnet acting on a nanoparticle can be calculated as [5]

$$\vec{F}_{NP} = -\frac{2k\Delta\chi V_{NP}a^2}{\mu_0 r^3}\left[k\left(\frac{w}{h}\right)\frac{a^2}{r^2}+\cos 2\theta\right]B_i H_0 \vec{u}_r - \frac{2k\Delta\chi V_{NP}a^2}{\mu_0 r^3}(\sin 2\theta)B_i H_0 \vec{u}_\varphi, r > a \quad (1)$$

Where

$$B_i \approx 2\left(\frac{w}{h}\right)H_0, \quad k = \frac{\mu_m - \mu_B}{\mu_m + \mu_B}, \quad \Delta\chi = \chi_{NP} - \chi_B$$

B_i is the inner magnetic flux density of the nanomagnet

χ_{NP} and χ_B are the susceptibilities of the nanoparticle and the buffer solution

μ_{NP} and μ_B are the permeabilities of the nanoparticle and the buffer solution

V_{NP} is the volume of the nanoparticle

a is the lateral dimension of the nanomagnet

r and θ are the cylindrical coordinates of the distance and angle

H_0 is the external magnetic field

$\vec{u}_r$ and $\vec{u}_\theta$ are unit vectors for the distance and angle in the cylindrical coordinate.

MATERIALS AND METHODS

The nanomagnets are designed as rectangular prisms with thickness (h): 200 nm, width (w): 8 μm, and length (a): 24 μm and cubes with thickness (h): 200 nm, width (w): 20 μm, and length (a): 20 μm. Figure 3(a) shows the dimensions of a microchannel. Figure 3(b) shows the design of nanomagnet arrays.

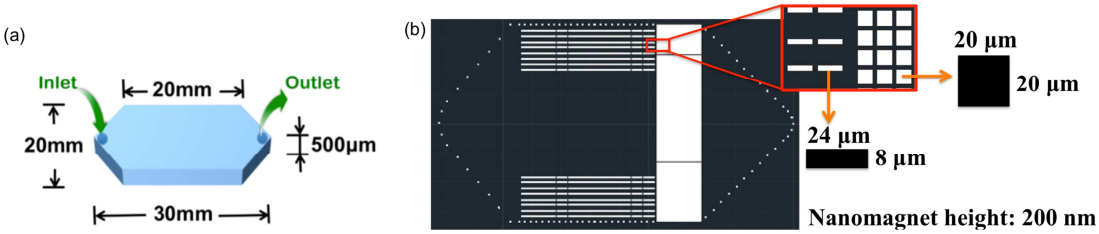

Figure 3. Nanomagnets patterned directly on the channel surface. (a) Dimensions of the microchannel. (b) Design of nanomagnet arrays.

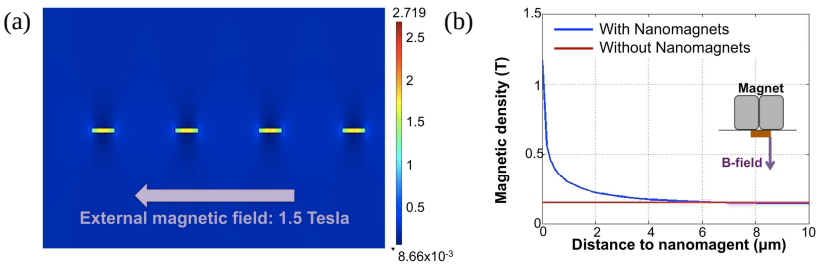

Figure 4. Simulations of the field enhancements from the nanomagnets. (a) Localized magnetic field induced by an array of nanomagnets placed in the uniform magnetic field generated by the permanent magnets. (b) Simulation of magnetic flux density with and without a nanomagnet.

Figure 4(a) shows the distribution of the total magnetic field generated by an array of nanomagnets placed in a uniform external magnetic field (0.15 Tesla). The nanomagnets induce a localized magnetic field. Simulation results show that magnetic field intensity increased 8 times locally near a single nanomagnet, as shown in Figure 4(b).

The fabrication process of nanomagnets is shown in Figure 5(a). Patterns of nanomagnets are defined by the spin-coated photoresist. Next, Cr/Ni metal is thermally deposited on a photoresist-patterned glass substrate. Lift-off technique is then used to fabricate arrays of patterned nanomagnets.

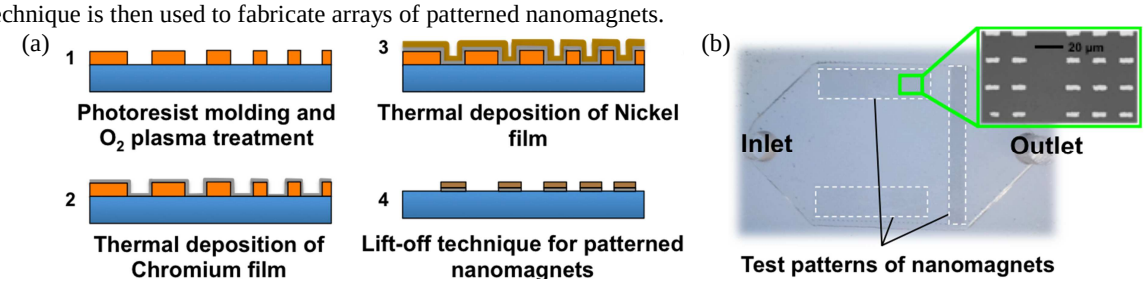

Figure 5. Microchip fabrication. (a) Patterning process of nanomagnets on a glass substrate. (b) Microchannel integrated with test patterns of thin-film nanomagnets.

An array of nanomagnets is patterned on the top-side, bottom-side, and the end of channel substrate to locally enhance the magnetic field for the capturing of target cells. Figure 5(b) shows the top view of the PDMS microchip bonded to a glass subtrate patterned with nanomagnets. The inset in Figure 5(b) shows an array of nanomagnets patterned on three sections in a microchannel.

For system operation, nanoparticle-labeled CTCs are introduced into a microchannel by a syringe pump. CTCs are then attracted to the microchannel substrate by external magnetic force during the screening process. Captured CTCs are permanently fixed on the channel substrate followed by an immunofluorecent staining and identification process. Specially, the microchip integrated with nanomagnet-patterned channel substrate, as shown in Figure 1, has been tested for spiked experiments. BT20 cells (human breast cancer cell line) and Colo205 cells (human colon cancer cell line) were spiked in blood sample for screening experiments. Figure 6 shows an example of captured cancer cells on nanomagnets. In spiked experiments, metallic nanomagnets achieved high capture rates of 85% and 81% for BT20 cells and Colo205 cells, respectively.

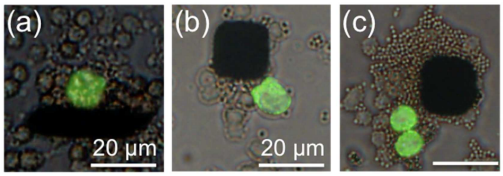

Figure 6. Cell captures experiments. (a) A BT20 cancer cell is directly captured on one of patterned nanomagnets. (b) A Colo205 cancer cell is captured on a nanomagnet. (c) A cluster of two Colo205 cells is attracted to a nanomagnet.

In conclusion, we developed the design methodology in combination with nanofabrication techniques to pattern the microchannel directly with metallic nanomagnets. After being magnetized, nanomagnets can enhance the local magnetic field, providing a near-field capture force for CTCs. The consistency of simulation with experimental results shows the great promise of this compact microchip for high throughput screening systems towards cancer prognosis and personalized therapy.

REFERENCES

[1] J.W. Uhr and K. Pantel, *Controversies in clinical cancer dormancy*, PNAS 2011, 108, p. 12396, (2011).

[2] A.L. Allan and M. Keeney, *Circulating tumor cell analysis: technical and statistical considerations for application to the clinic*, Journal of Oncology 2010, (2010).

[3] K. Hoshino, Y-Y Huang, N. Lane, M. Huebschman, J.W. Uhr, E.P. Frenkel and X.J. Zhang, *Microchip-based Immunomagnetic detection of circulating tumor cells*, Lab on a Chip 11, p. 3449, (2011).

[4] Y-Y Huang, K. Hoshino, N. Lane, M. Huebschman, K. Sokolov, J.W. Uhr, E.P. Frenkel and X.J. Zhang, *Micro-chip based Immunomagnetic assay for circulating tumor cells*, Cancer Prevention & Research Institute of Texas, USA, Austin, TX, (2011).

[5] K-H Han and A. Bruno Frazier, *Continuous magnetophoretic separation of blood cells in microdevice format*, J. Appl. Phys. 96, p. 5797, (2004).

CONTACT

Dr. John X.J. Zhang Telephone: 512-475-6872 or Email: john.zhang@engr.utexas.edu