A CTC-MICROSEPARATOR FOR ISOLATION OF CIRCULATING TUMOR CELLS USING LATERAL MAGNETOPHORESIS AND MAGNETIC NANOBEBDS

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ABSTRACT
This paper introduces a high-speed high-performance CTC-microseparator for isolation of circulating tumor cells (CTCs) from human peripheral blood. The isolation is achieved through lateral magnetophoresis [1], generated by a ferromagnetic wire array inlaid on a glass substrate, and magnetic nanobeads, specifically binding to epithelial-derived CTCs. From healthy peripheral blood spiked with CTCs, the CTC-microseparator isolated 78.7% of the spiked CTCs and its purity was >95%. The overall isolation procedure was completed within 10 min per 1 ml blood. In addition, RT-PCR amplifications using the isolated CTCs were successfully performed. From peripheral blood of patients in breast and lung cancers, CTCs were isolated and identified through an immunofluorescent image analysis. From the experiment, we observed that all the cells isolated by the CTC-microseparator are CTCs.

KEYWORDS: Circulating tumor cells (CTCs), Cell separation, Lateral magnetophoresis, Magnetic nanobeads

INTRODUCTION
Due to the extremely low concentration of CTCs in blood, such as one or two CTCs per 10 billion blood cells, previous separation methods isolate CTCs with low recovery rate (~20-60%) [2] and very low purity (0.01-0.1%) [3], which is prohibitive to their capacity to monitor response to treatment in a dynamic fashion and for early cancer detection. A further problem arises that the low purity enormously increases processing time to identify CTCs from the enriched cells. Therefore, in this paper, we introduce a CTC-microseparator, which can isolate CTCs in a high-speed (<10 min per ml), high-recovery rate (~78.7%), and high-purity (>95%) including immunomagnetic beads coated with anti-EpCAM antibodies and lateral magnetophoresis. Most of all, CTCs isolated by the CTC-microseparator can be used directly for CTC-based molecular analysis.

THEORY
CTCs with bound magnetic nanobeads behave as paramagnetic particles. Consider a high-gradient magnetic field generated near an inlaid ferromagnetic wires (Figure 1(a)). As the CTCs with bound magnetic nanobeads are passing over the ferromagnetic wire array placed at an angle (θ) to the direction of flow (Figure 1(b)), they experience magnetic force $F_m$ with hydrodynamic drag force $F_d$. The lateral magnetic force $F_l$ on CTCs is a vector sum of the magnetic force and the drag force. Lateral displacement is created by the lateral magnetic force and thus can be controlled by the angle. Consequently, with an external magnetic field, the CTCs bound the magnetic nanobeads are forced laterally and flow into outlet #1 and meanwhile, normal blood cells in the peripheral blood waste out into outlet #2 (Figure 1(b)).

Figure 1: Schematic views of (a) the ferromagnetic wire array inlaid on a glass substrate with a high-gradient magnetic field and (b) the CTC-microseparator, including the ferromagnetic wire array placed at an angle of θ to the direction of flow.
EXPERIMENTAL

The CTC-microseparator was fabricated by using 0.7-mm-thick borofloat™ glass slides and SU8-to-glass adhesive bonding technique (Figure 2). Instrumental setup included stacked two Nd-Fe-B permanent magnets, generating an external magnetic field of 0.14 T, and two syringe pumps, providing controlled laminar flow in the microchannel. The two syringe pumps were used to drive the RBC lysate blood sample and PBS buffer with the same flow rates (from 2 to 5 ml/h).

Healthy human peripheral blood samples were drawn from healthy laboratory personnel. Cancer blood samples were drawn from patients in lung and breast cancer. All specimens were collected in Vacutainer tubes containing the anticoagulant EDTA, and processed within 24 h. To determine the recovery rate and purity of the CTC-microseparator, breast cancer cell line (SKBR-3) in a range of 10^4-10^6 cells were fluorescently dyed and spiked into 1 ml healthy human peripheral blood. RBCs in peripheral blood were lysed using RBC lysis buffer and washed twice with PBS containing 2% (v/v) FBS to remove dead cells and debris. Then, nucleated cells were resuspended in 1 ml PBS. According to instruction of manufacture, the blood sample was mixed with anti-EpCAM antibodies and magnetic nanobeads in sequential, and incubated on ice for 20 min and 15 min, respectively.

![Figure 2: A photograph of the fabricated CTC-microseparator. The inset shows that CTCs, spiked into peripheral blood, are flowing into the outlet #1 with an external magnetic field, while normal blood cells are flowing into the outlet #2](image)

RESULTS AND DISCUSSION

Recovery rate of the CTC-microseparator was measured as 78.7% for various sample flow rate from 2 to 5 ml/h (Figure 3(a)) and number of CTCs spiked into 1 ml blood (Figure 3(b)). To obtain the enrichment rate, we used a flow cytometry. Initial percentage of CTCs spiked into healthy peripheral blood was measured as 0.16% (Figure 3(c)) and after enrichment, percentage of CTCs reached to 83% (Figure 3(d)), which indicates that the enrichment rate of the CTC-microseparator is 520-fold. In addition, RT-PCR amplifications using the isolated CTCs were performed and compared with RT-PCR results using the crude blood sample (Figure 4). From the RT-PCR result, we can conclude that the present CTC-microseparator is a powerful device for CTC-based molecular assay. From 2 ml peripheral blood of patients in breast (n=3) and lung (n=1) cancers, CTCs were isolated (Figure 5(a)) and identified (Figure 5(b)) through an immunofluorescent image analysis. The experimental results explained that specificity of the CTC-microseparator is 100%, defined by the ratio of cytokeratin+ (CTCs) to CD45+ cells (hematologic cells).

![Figure 3: (a) Recovery rate from outlet #1 as a function of flow rate. The error bar represents one standard deviation calculated from three data sets. (b) Regression analysis of separation efficiency for varying CTC concentration. Flow cytometry profiles of (c) crude blood sample spiked with CTCs (normal blood cells : CTCs ≈ 10000 : 16, i.e., 0.16%), and (d) cells enriched by the CTC-microseparator (17 : 83, i.e., 83%). The induced cells were labeled with the monoclonal antibody 5E11-FITC](image)
CONCLUSION

In this study, we presented the CTC-microseparator for highly efficient isolation of CTCs from human peripheral blood based on magnetic nanobeads coated with anti-EpCAM antibodies and the lateral magnetophoresis. In experiment, 78.7% of the spiked CTCs were collected from the outlet #1 and its purity was >95%. In addition, through a subsequent cDNA synthesis and PCR procedure with isolated CTCs, we further verified that the CTC-microseparator was effective for subsequent molecular analysis. Consequently, the experimental results explains that the CTC-microseparator is an practical device for fast and high-recovery and enrichment for CTCs from peripheral blood.

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REFERENCES


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