ISOLATION OF CIRCULATING TUMOR CELLS FROM WHOLE BLOOD USING IMMUNOMAGNETIC NANOBEADS AND LATERAL MAGNETOPHORESIS

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ABSTRACT

This paper presents a circulating tumor cell (CTC) microseparator for isolation of CTCs from human whole blood using immunomagnetic nanobeads and lateral magnetophoresis. Experimental results showed that the CTC microseparator isolates approximately 90% of spiked CTCs in human peripheral blood at a flow rate of up to 5 ml/h and purifies to approximately 97%. By the CTC microseparator, CTCs from peripheral blood of patients with breast and lung cancers were isolated and the results were compared with those of healthy donors. Using a fluorescence-based viability assay, the viability of CTCs isolated from peripheral blood of patients with cancer was observed. In addition, the effectiveness of the CTC microseparator for subsequent genetic assay was confirmed by RT-PCR amplification of cancer-specific genes using CTCs isolated from patients with cancer.

KEYWORDS

Circulating tumor cells, Cell separation, Immunomagnetic nanobeads, Lateral magnetophoresis,

INTRODUCTION

To increase the throughput and prevent a contamination caused by red blood cells (RBC), our previous work [1] presented experimental results of CTC isolation using blood with RBC lysis. However, RBC lysis procedure inevitably involves centrifugation and washing steps, which are the main causes for loss or destruction of a significant proportion of CTCs [2], thereby decreasing the separation recovery and purity. In addition, because membrane specific makers could be damaged by the RBC lysis procedure, immunoaffinity of CTCs may be weakened, thereby reducing the separation performance. To avoid the problem, in this research we isolated CTCs from whole blood, which is treated only by cocktailing anti-epithelial cell adhesive molecule (EpCAM) antibodies and immunomagnetic nanobeads without RBC lysis procedure. The CTC isolation was achieved by the lateral magnetophoresis [1] generated by a microscaled ferromagnetic wire array inlaid on the bottom substrate of microchannel (Figure 1) to maximize magnetic force acting on target cells.

RESULTS AND DISCUSSION

Experimental results showed that the CTC microseparator isolates approximately 90% of CTCs spiked into blood at a flow rate of up to 5 ml/h (Figure 2(A)). The recovery rate is also measured for various the number of CTCs spiked into healthy blood (Figure 2(B)). The result showed that the recovery rate was consistent as 90% for various spiked the number of CTC. By fluorescence observation of CTCs (green color) and normal nucleated blood cells (blue) (Figure 3(A)), the purity of isolated CTCs was measured as approximately 97% for various sample flow rate of 2 to 5 ml/h (Figure 3(B)). To verify the advantage of the CTC microseparator for subsequent genetic assays, RT-PCR amplification using isolated CTCs were performed and compared with results using blood sample spiked with CTCs (Figure 3(C)).

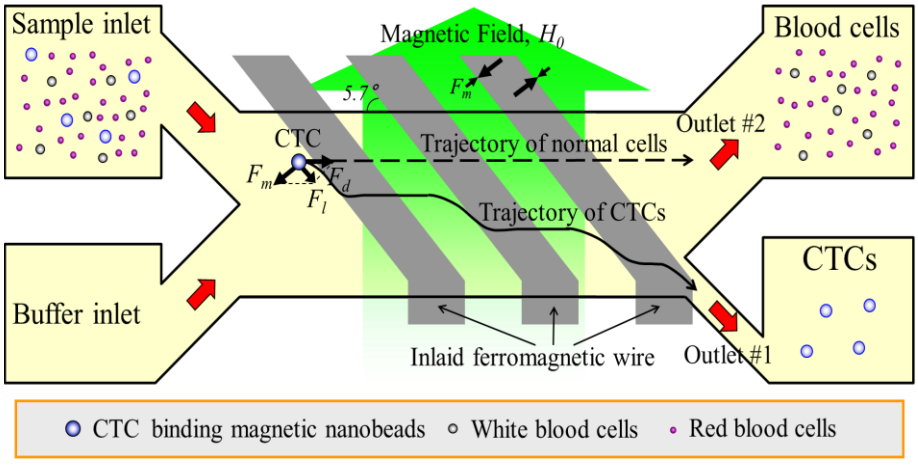

Figure 1. Perspective view of the CTC microseparator, including the inlaid ferromagnetic wire array placed at an angle of θ (5.7°) to the direction of fluid flow under an applied external magnetic field H_0. The magnetic force acting on CTCs is generated by a high magnetic field gradient induced near around the ferromagnetic wire array with the external magnetic field.

Meanwhile, CTCs were isolated by the CTC microseparator from 200 μl of peripheral blood of patients with breast (n=3) and lung (n=3) cancers (Figure 4(A)) and identified through an immunofluorescence image analysis (Figure 4(B)). To measure viability of the isolated CTCs, they were observed by a fluorescence-based viability assay (Figure 4(C)). The viability assessment showed that 100 % of the isolated CTCs from peripheral blood of patients with cancer is alive, which is a significant evidence that the majority of CTCs lives in circulating system. Finally, detection of a lung cancer-specific gene (TTF-1) using isolated CTCs (Figure 4(D)) confirmed that the present CTC microseparator is effective for CTC-based molecular assays.

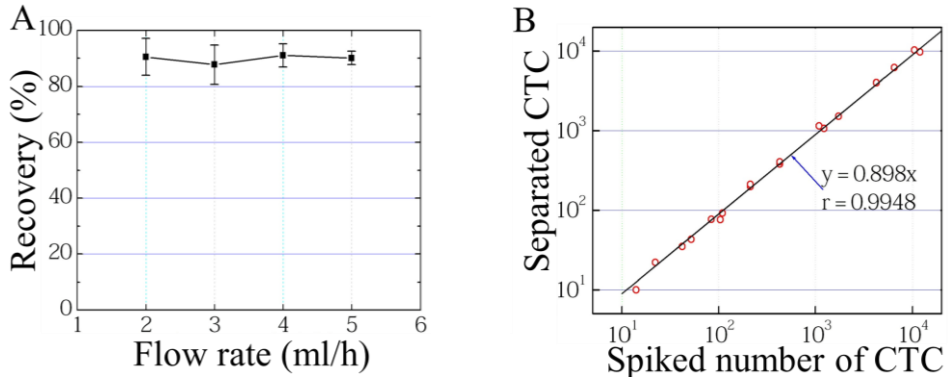

Figure 2. (A) Recovery rate of the CTC microseparator for various sample flow rates. Breast cancer cell lines (SKBR-3) are separated by the CTC microseparator at each sample and buffer flow rates of 2 to 5 ml/h with an external magnetic field of 0.2 T. The error bars represent one standard deviation calculated from three data sets. (B) Regression analysis of separation recovery rate for varying SKBR-3 cell concentration between 10 and 10^4 cells at sample and buffer flow rate of 4 ml/h.

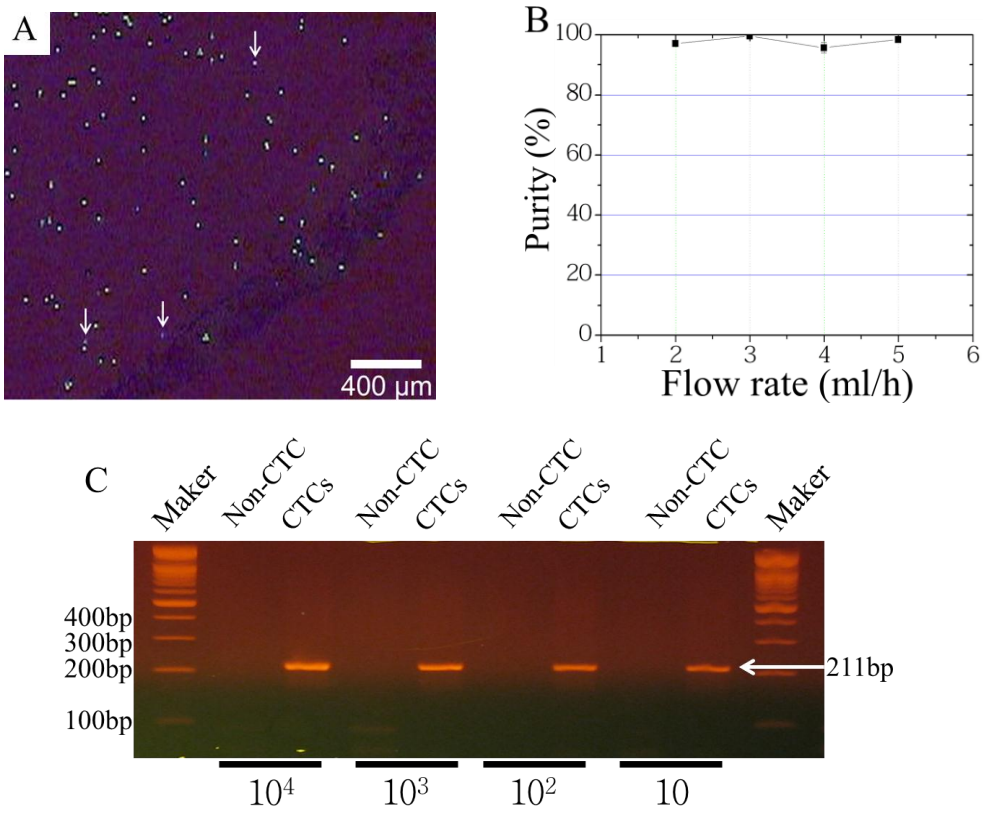

Figure 3. (A) A photomicrograph of CTC separated into outlet #1. They are stained by fluorescent dyes of green color for CTCs and blue color for normal hematologic cells. The arrows (↓) identify normal blood cells. (B) Separation purity of the CTC microseparator at various sample flow rate. 10^3 of SKBR-3 cells are spiked into the blood sample and isolated through the CTC microseparator for varying flow rates of 2 to 5 ml/h. The error bars represent one standard deviation calculated from three data sets. (C) RT-PCR amplification of KRT19 transcript using blood samples spiked of 10^4 to 10 of CTCs (written "Non-CTC") and cells isolated from the each samples (written "CTC").

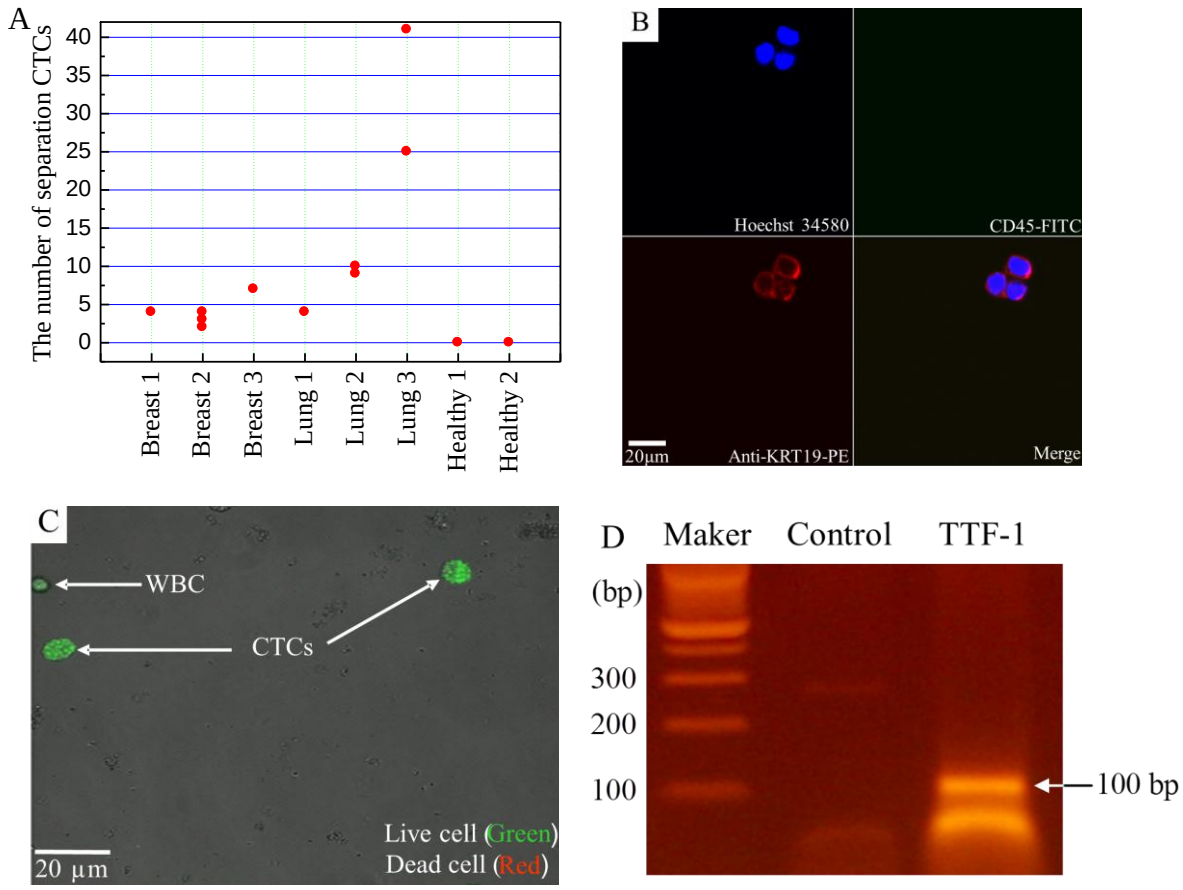

Figure 4. (A) Frequency of CTCs per 200 µl of peripheral blood of patients with breast (n=3) and lung (n=3) cancers. (B) Photomicrographs of CTCs stained by immunofluorescence dyes of Hoechst 34580 for DNA content, anti-KRT19-PE for CTCs, and anti-CD45-FITC for hematologic cells. (C) Viability CTCs, measured by a fluorescence-based viability assay. Live cells are stained by Calcein-AM (green color) and dead cells by EthD1 (red). (D) RT-PCR amplification of thyroid transcription factor-1 (TTF-1) transcript using CTCs isolated from a patient with lung cancer.

CONCLUSION

In this study, we presented the CTC microseparator, which enables to isolate CTCs from peripheral blood of patients with breast and lung cancers based on the lateral magnetophoresis and immunomagnetic nanobeads coated with anti-EpCAM antibodies. In experiment, the CTC microseparator continuously separates approximately 90% of CTCs from human blood at a sample flow rate of up to 5 ml/h. Separated purity for CTCs spiked into 200 µl of peripheral blood was about 97%. CTCs from the isolated cells were identified by an immunofluorescence image analysis, which is used as cytokeratin 19-positive cells were scored as CTCs, whereas CD45-positive cells were counted as contaminating normal hematologic cells. It is most notable that the CTC microseparator takes less than 15 min to complete the total CTC separation from 200 µl of peripheral blood. In addition, viability of CTCs measured with a fluorescence-based viability assay is an evidence to show that the CTCs are alive in the circulating system. In other words, the CTC microseparator can isolate viable CTCs. RT-PCR assay using isolated CTCs confirmed that the CTC microseparator is effective for CTC-based molecular level assay. Consequently, the experimental result explains that the CTC microseparator is a practical device with which to isolate CTCs from peripheral blood quickly and with high recovery and purity.

REFERENCES

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