MICROFLUIDIC DETECTION OF CIRCULATING TUMOR CELLS (CTC) USING SIDE FILTRATION-BASED CAPTURE

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ABSTRACT

Highly sensitive detection of CTCs is difficult because they are extremely rare (i.e. 1~5 CTC / 10⁹) in blood. Recently various devices were developed implementing a method of either affinity-based or size-based separation. There are some drawbacks of each method. Antibody-based capturing has low sensitivity if CTCs express less target surface antigen and the size of CTCs overlaps with that of normal blood cells. Hence we utilized a filter on the side of the channel for introducing a deformability in separation principle. The capturing result of this method demonstrated a higher capture rate (95%) and purity (99%) than conventional size-based filtration because easily-deformed CTCs stuck to the holes more tightly than normal blood cells.

KEYWORDS: Circulating Tumor cells (CTC), Side filtration, Micro-fluidic, Size capture

INTRODUCTION

Detection of CTCs is a promising method for both diagnosis and clinical management of cancer patients through monitoring treatment efficacy before metastasis occur.[7] However, CTCs are extraordinarily rare (estimated at one CTC per billion normal blood cells) which make it difficult to develop a suitable assay.[8] CTC detection techniques can be classified as affinity-based techniques (i.e., antibody-based enrichment) and non-affinity based techniques (i.e., physical separation).[10] But, due to less expression of an antigen (EpCAM) on some tumor cells, affinity-based method may not detect some tumor cells and size-based filtration methods have low purity or loss of tumor cells.

Hence, side filter detection method was development to improve the low purity of size-based filtration. The filters of device are installed on the side of main channel to wash out the captured cells that were not deformed into the trap and also to avoid direct strong flow. This approach could significantly enhance the detection of CTCs using filtration for cancer diagnosis and prognosis.

THEORY and EXPERIMENTAL

This chip comprised 20 co-centric square channels connected to central inlet and an outer outlet in the chip (Fig. 1a). Blood sample injected into the inlet flows through the main channel. At side channel the cells smaller that the holes in the filter just exit to the next main channel, but the cells bigger than the filter trapped in holes. The large tumor cells were trapped by the side filter and small blood cells escaped through filters. The microfluidic chip was fabricated by soft lithography process (Fig. 1c). The thickness of each filter is 25~100 μm and distance between each filter is 100~300 μm. Width of main channel is 100~300 μm. Various gaps of filter hole (8~16 μm) were used to be optimized to the various sizes of tumor and blood cells.

Figure 1 Design and principle of micro side filtration chip. (a) Diagram is schematic drawing of the micro chip with trajectory of whole blood sample. (b) Red box is about stream line of flow of cells (tumor cells mixed blood cells) in micro side filter. (c) This image is a PDMS micro chip. It is optical micrograph of the filter array fabricated via soft lithography at side of main channel. Height of micro filtration is 40 μm.

Figure 2. Image of captured tumor and blood cells in micro filtration. Width and gap of filtration are 50 μm, 12μm, respectively. (a) This photo shows the tumor cells caught in the filter. Large tumor cells clogged filters by deformation of cell membranes. At this point, almost flow is in the main channel. (b) The small blood cells just exited filters. And then, the side flow continues flow in filter. The scale bar represents 20 μm.
Figure 3. Capture efficiency of cells on micro chip with various geometric dimensions: The tumor and blood cell numbers were $10^6$, $10^4$ respectively. (a) It is a graph of captured cells for gap of filtration. The size of the filter was from $8 \mu m$ to $16 \mu m$, using flow rate was $0.5 \sim 1.5$ ml/hr. Used cells are the blood cells (Jurkat) and tumor cells (MCF-7). In this experiment, the gap of the filter optimized as 12 $\mu m$ (red box). (b) Comparison of capture efficiency of tumor cells for change of width of filters: The size of filtration was 12 $\mu m$. In this result, the width of the filter optimized as 50 $\mu m$ (red box). (c) Graph shows that capture efficiency of cells for change of width of a main channel. Here, width and gap of filters fixed as 50 $\mu m$ and 12 $\mu m$ respectively. In this graph, the width of the channel was optimized as 300 $\mu m$ (red box).

Figure 2 shows the cells captured in each filters. Figure 2a shows the tumor cells (MCF-7) captured in a micro filter, and Figure 2b are blood cells (Jurkat) captures in a micro-filter. The filter on the side of the direction of the flow enabled the cells avoid large shear flow. Normal blood cells are smaller than the tumor cells.

RESULTS AND DISCUSSION

At first the size of filter and the channel structure were optimized (Fig 3). Figure 3a shows the capture efficiency of side-filter. Large tumor cells (MCF-7) were captured more frequently than blood cells (Jurkat). Little number of Jurkat cells were captured in the gap. Flow rate did not have a significant impact on the cell trapping. But, if flow rate is less than 0.5 ml/hr, most of the cells adhered to the channel floor or wall. On the contrary, when the flow rate was more than 1.5 ml/hr, the cells were damaged. Capture yields were analyzed when the width of filters and width of channel was changed (Fig. 3b, c). When the width of the filter and channel was increased, more tumor cells were captured. The optimized filter gap and width were 12 $\mu m$ and 50 $\mu m$, respectively. In addition, the channel width was 300 $\mu m$.

Figure 4 shows the capturing effect of the number of spiked cancer cells. Figure 4a shows the number of captured cells, when the number of tumor cells ranged from 3 to $10^3$ spiked in white blood cells ($10^5$ Jurkat cells). It is show that the number of spiked cells corresponded well to that of captured cells. The number of tumor cells increased with the spiked cells, but the captured number of Jurkat cell was almost constant. Figure 4b shows the capture efficiency and purity of rare tumor cells spiked in Jurkat cell suspension with a concentration of $10^6$/ml as well as in whole blood. We also obtained high purity of tumor cells with whole blood sample, even though the capture efficiency (70%) was lower than that of test with cell lines. We guess the decrease in purity was caused by the effect of white and red blood cells in whole blood.

Figure 4. Capturing of mixing MCF-7 cells in blood cell line and whole blood. (a) The number of spiked cells corresponded well to that of captured cells. It is the experimental results from 1050 micro side-filters. Injected tumor cells are $10^3 \sim 10^5$, and Blood cells are one million. (b) Graph shows capture efficiency and purity of tumor cells in Jurkat cell line ($10^6$) and whole blood (1ml). The tumor cell numbers $10^7$. 
Finally, we carried out separation of CTCs from the whole blood of patients. The blood samples were extracted from metastatic cancer patients. The amount of blood in each experiment was 1 ml. The captured cells were immune-stained to identify the kind of captured cells. Figure 5 shows a fluorescence image of tumor cell and blood cells. Figure 6 shows the number of detected CTCs from each nine cancer patients.

CONCLUSION
This study demonstrated that side filtration, size capture, and micro flow-based detection was efficient in isolating tumor cells. In comparison with the existing microfluidic devices for CTC separation, the side filtration chip has many advantages such as a simple, high efficiency and high purity. It was demonstrated capture of circulating tumor cells in the whole blood of metastatic cancer patients.

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