# ISOLATION OF CIRCULATING TUMOR CELLS WITH HIGH RECOVERY AND PURITY BY CELL SIZE AMPLIFICATION AND A MIRO SLIT FILTER HAVING EXTREMELY HIGH ASPECT RATIO

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## ABSTRACT

We have developed a novel circulating tumor cell (CTC) isolation platform for high recovery and purity. It is composed of two processes: 1) selective size amplification of cancer cells using microbeads conjugated with anti-EpCAM to discriminate the size between WBCs and CTCs more clearly, 2) filtration of CTCs using a micro slit filter chip. We designed a micro slit filter having very long single rectangle shape with dimension of 27900 μm × 8 μm (aspect ratio = 3488) to prevent clogging. Clogging causes unwanted aggregation of cells, sticking of WBCs and decrease of purity. An automatic fluid control system was also implemented to conduct the whole fluidic procedures including cell separation and chemical treatments for cell analysis with high reproducibility. We could separate the size amplified CTCs with 91 % recovery and 52 % purity from 5ml of normal whole blood spiked with 100 MCF-7 cells at the flow rate of 100 μl/min.

## KEYWORDS

Circulating tumor cell, size amplification, slit filter, high aspect ratio.

## INTRODUCTION

Circulating tumor cells (CTCs) have been paid attention in cancer diagnostics because of their valuable clinical implications in metastatic cancers. However, CTCs are so rare in whole blood (1~ 10 CTCs/ml) that it has been challenged to isolate CTCs with high recovery and purity. Purity is getting more significant as needs for molecular analysis of CTCs are emerging these days[1]. Various separation methods using different properties of CTCs from other blood cells have been introduced and implemented to microfluidic platforms[2]. Size and antibody specific affinity have been the mostly used separation properties. Although each method has its own advantages, they still don't satisfy both high recovery and high purity at the same time because the characteristic difference between CTCs and normal blood cells is not clear enough for complete separation. The immunomagnetic separation method using anti-epithelial cell adhesion molecule (anti-EpCAM), such as CellSearch system, yields poor recoveries (~70 %) due to cell loss during washing steps. On the other hand, the size-based filtration yields low purity (~0.1 %) because of the overlapping sizes of CTCs and white blood cells (WBCs). In this paper, we developed a novel separation method complementing these weak points. As shown in Fig. 1, CTCs are amplified by microbeads bound with proteins which bind to CTCs specifically. We used the anti-EpCAM as a binding protein. The amplified CTCs make the size difference between CTCs and WBCs more clearly. A micro-slit filter having extremely high aspect ratio rectangular shape was developed to capture the amplified CTCs more safely without clogging.

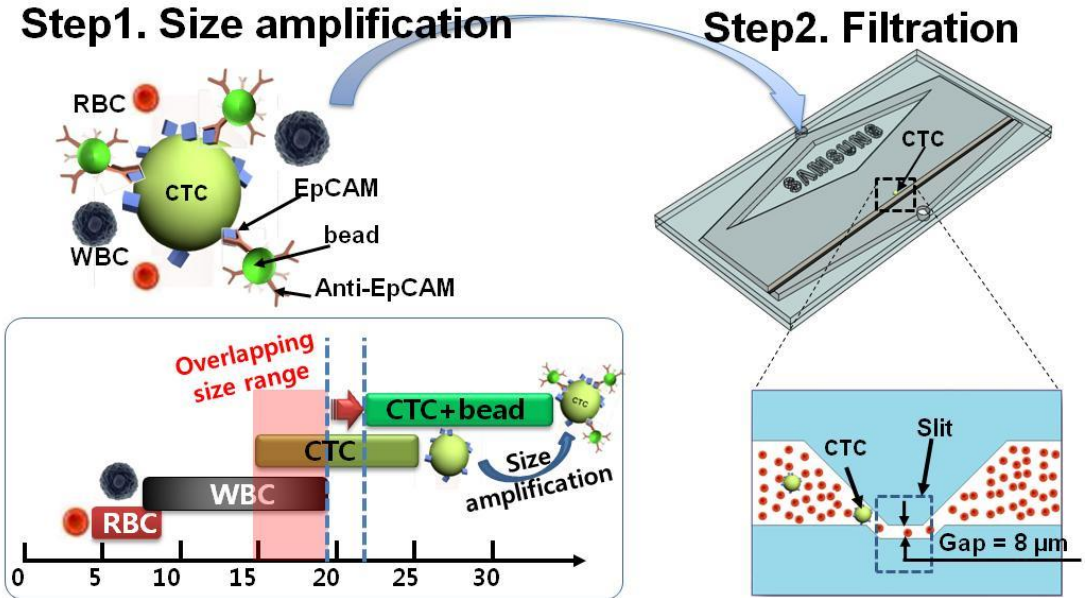


Fig. 1. *Schematic diagram of CTC separation. The 3 μm microbeads conjugated with anti-EpCAM are mixed in whole blood to increase size of CTCs. Size-amplified CTCs are selectively filtered by micro slit filter having extremely high aspect ratio rectangular slit shape. Size amplification of CTCs provides not only easy filtration with high purity but also stable capture of CTCs owing to rigid microbeads.*

## DESIGN & FABRICATION

We designed 8 um gap micro slit structure having very long single rectangle shape with dimension of 27900 µm×8 µm (AR=3488) to prevent clogging and fabricated an automatic fluid control system to reduce manual error and enhance the reproducibility. Fig. 2 shows details of the micro slit filter chip such as dimension, fabrication method and fabrication results. The extremely high aspect ratio rectangular shape slit was simply formed by bonding of two quartz wafers having simple wet etched structures. Considering the amplified size of CTCs bound with beads as 26 µm and the number of CTCs as 100 cells, the variation of slit width due to the captured CTCs only changes less than 9.3 %. There are still plenty of other spaces for fluid flow so that cells can bypass the previously captured CTCs, which results in prevention of clogging. This self bypassing behavior was observed using the buffer spiked with 100 MCF-7 cells (Fig. 3). Furthermore, since the fluidic resistance is inversely proportional to the channel area, the variations of fluidic resistance due to the captured CTCs are also negligible.

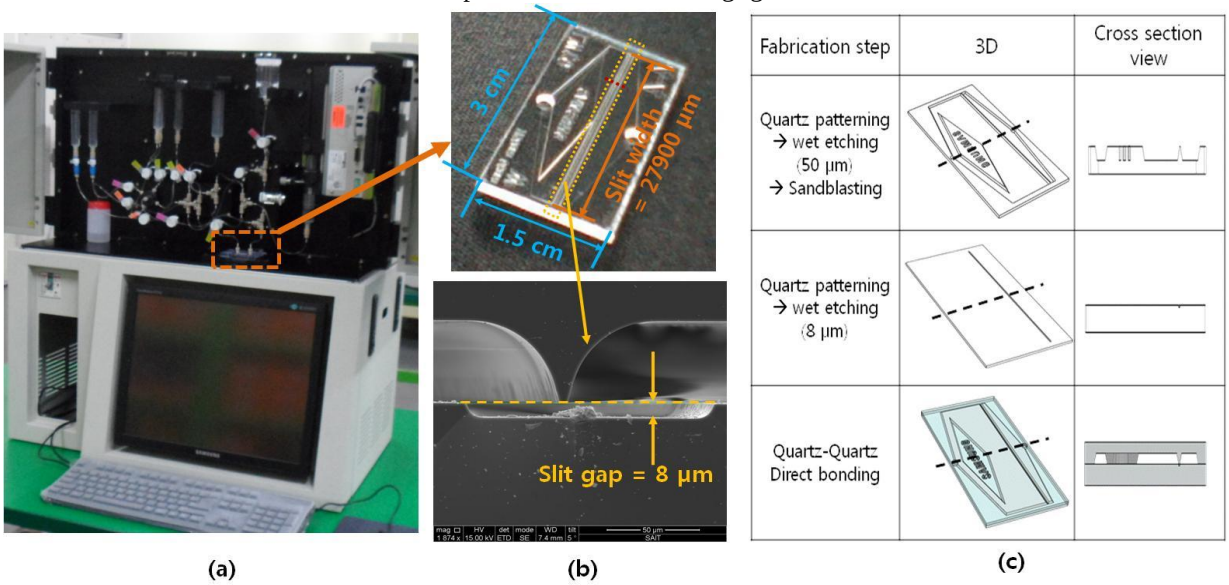


Fig. 2. *Details of the CTC separation platform. (a) Full automatic fluidic control system, (b) Photograph of the fabricated micro slit filter and SEM image of the cross section view of slit, (c) Fabrication procedures.*

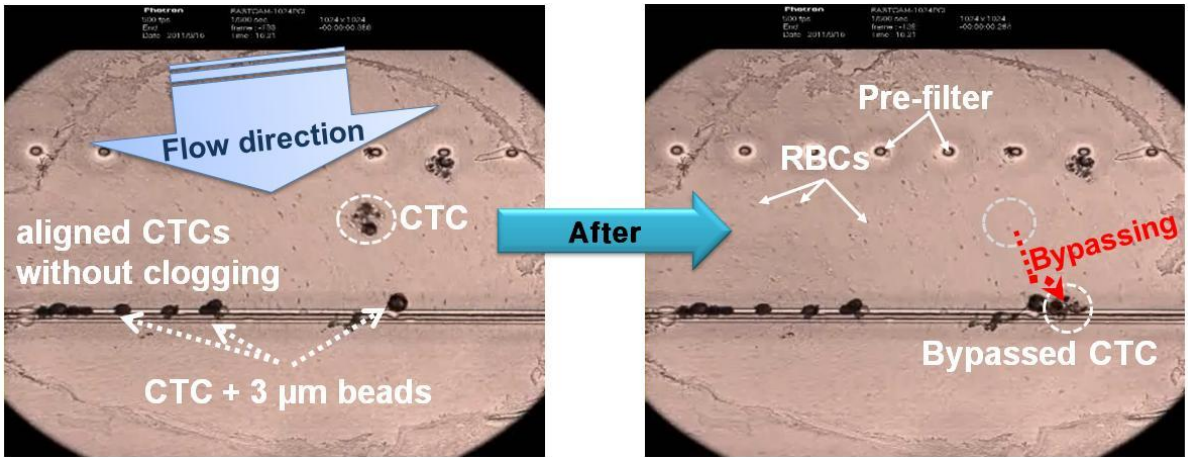


Fig. 3. *Photographs of self bypassing*

## EXPERIMENT AND RESULTS

As a circulating tumor cell model, one hundred MCF-7 breast cancer cells were spiked into 5ml of normal whole blood. The spiked whole blood was centrifuged and the plasma layer was removed. Buffer was added and mixed well to dilute the condensed blood cells. After that, 3 µm carboxylate modified melamine microbeads ($10^9$) conjugated with anti-EpCAM was added to increase the size of MCF-7 breast cancer cells. The pretreated blood sample was loaded to the automatic fluid control system and the whole procedures for CTC separation such as priming, filtration, washing, fixation, permeabilization, staining were conducted automatically. 4 % paraformaldehyde and 0.01% Triton X-100 reagent were used for fixation and permeailization, respectively. And, mixture of 4',6-diamidino-2-phenylindole (DAPI), anti-cytokeratin PE (CK; CAM 5.2, BD Biosciences, CA) and CD45 FITC (BD Biosciences, CA) was used for the staining agent. The stained cells were observed with Olympus IX81-ZDC inverted microscope. CK positive and CD45 negative cells were counted as CTC. Since cells are not rigid, the recovery rate varies with respect to the flow rate during filtration. As shown in Fig. 4, most bare MCF-7 cancer cells are passed through the 8 µm filter by cell deformation at the flow rate ranged from 50 µl/min to 1000 µl/min, but the size amplified MCF-7 cancer cells were stably captured with over 92% recovery rate until 100 µl/min.

Although the slit gap is much smaller than the size amplified CTCs, CTCs began to evade due to the deformation at the flow rates over 100 µl/min. We chose the 100 µl/min flow rate as an optimal filtration condition because the purity is proportional to the flow rate. The higher flow rate is, the better purity becomes.

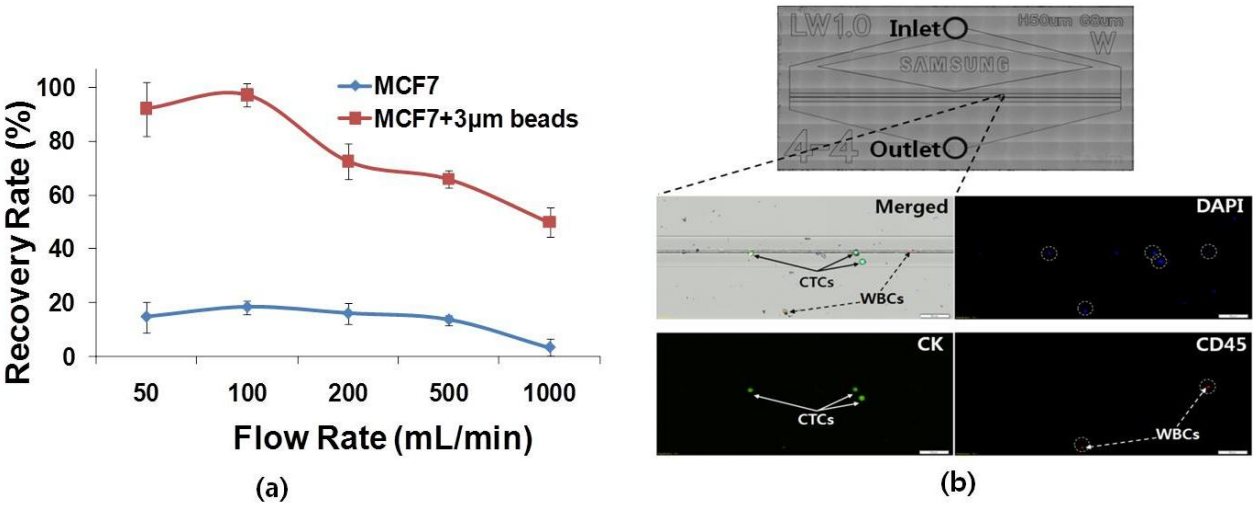


Fig. 4. *Recovery results. (a) Recovery rate along with flow rate, (b) Microscopic images after filtration and staining process of CTC*

We confirmed recovery rate and purity at the optimal flow rate using buffer and whole blood. The highest recovery rate was 95.6 % at 100 µl/min with buffer solution (100 MCF-7 cells in 5 ml PBS). When using whole blood spiked with MCF-7 cancer cells, it is notable that the recovery rate was also maintained over 91% with 52% purity without any clogging (Fig. 4 and Table 1).

Table 1. *Recovery and purity rates of spiked MCF-7 cancer cells at buffer and whole blood conditions.*

| sample | Input CTC Conc. (cells/ml) | Captured CTC Conc. (Cells/ml) | Standard deviation | CV(%) | Recovery rate (%) | Purity rate (%) |
|---|---|---|---|---|---|---|
| Buffer | 103.5 | 99 .0 | 13.0 | 13.1 | 95.6 | - |
| Whole Blood | 110.2 | 100.4 | 5.7 | 5.8 | 91.1 | 52.0 % |

## CONCLUSION

We have demonstrated a novel separation method showing high recovery rate and purity at the same time through the combination of cell size amplification and filtration method. As we expected, the specific cell size amplification method makes the size difference between cancer cells and WBCs so clearly that we could separate cancers cells with high recovery rate (91.1 %) similar to other membrane filter separation method and much better purity (52.0 %) than any other separation method. Owing to the micro slit structure of extremely high aspect ratio rectangular shape, clogging problem was successfully prevented. Furthermore, the unique very long slit structure makes the trapped cancer cells align along with the slit, which makes the scan task easier and faster.

## REFERENCES

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