

# A NOVEL FULLY AUTOMATED CENTRIFUGAL MICROFLUIDIC PLATFORM WITH MASSIVE VOLUME CAPABILITY TO ISOLATE CIRCULATING TUMOR CELLS

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## ABSTRACT

Since circulating tumor cell(CTC) studies have shown many clinical implication, technologies enabling detection and analysis of the CTC have been briskly demonstrated. Here, we present a novel fully automated centrifugal microfluidic platform to isolate CTCs, capable of high efficient density based separation and dealing with large volume. Over 90% of cancer cells were recovered while less than 60 WBCs were remain after ~80-minute process starting with whole blood insertion into the device.

**KEYWORDS:** Centrifugal microfluidics, Cell separation, Circulating tumor cell

## INTRODUCTION

Circulating tumor cells(CTCs) have many clinical implication and the detection of CTC is of great importance for cancer prognosis and personalized therapy[1]. Although various methods have been introduced for the CTC isolation[2], low purity of the CTC hamper the downstream molecular analysis. In addition, most of them include manual steps that impede the sample consistency and user convenience. Selective capture and sedimentation of CTC from blood cells using EpCAM coated bead and density gradient medium(DGM) have been demonstrated with high recovery rate and purity[3]. This method basically requires fluids transfer, removal, mixing and centrifugation so that the centrifugal microfluidics is eligible to fulfill automation.

## THEORY

Most applications of centrifugal platforms have focused on immunoassays and clinical chemistry using blood plasma and dealt with only small blood volumes[4]. On the contrary, two big challenges faced with CTC application, handling large volume and blood cell manipulation, have never been studied in disc platforms. In particular, plasma separation is difficult because the direction of the blood separation is perpendicular to the gravitational direction, thereby instant remixing between separated blood cells and plasma occurs. In our observation, separated blood cells spread from the bottom thus, we made a triangular obstacle structure not only to move targets to the blood cell zone but also retard the

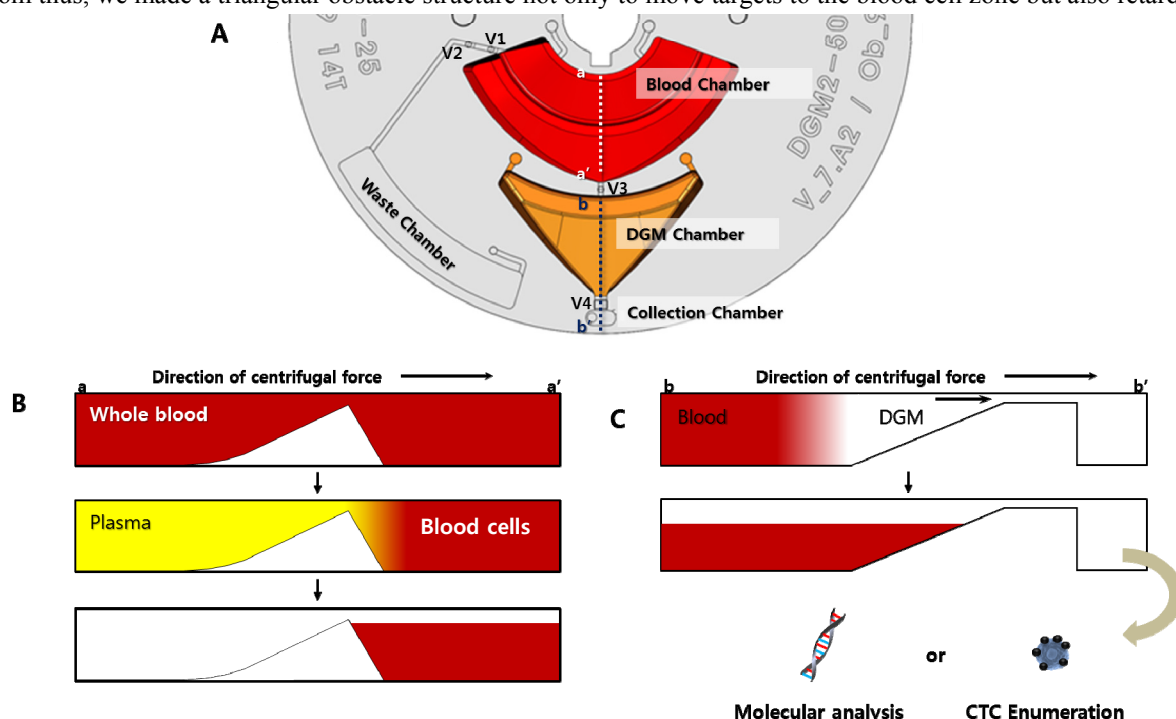


Figure 1. (A) Schematic diagram of the centrifugal microfluidic platform for CTC isolation. (B) Triangular obstacle structure for centrifugal separation of large volume sample. (C) Density gradient medium(DGM) chamber for density filtration.

diffusion of the blood cells(Fig. 1B).

Figure 1A shows schematics of our device for CTC isolation. Blood chamber separate and remove plasma from whole blood to waste chamber through centrifugation. Mixing process for CTC-microbead binding is also performed in blood chamber by shaking. After mixing process, sample is transferred to DGM chamber and only CTC-microbeads and remnant microbead penetrate the DGM layer(Fig. 1C).

## EXPERIMENTAL

To maximize recovery rate, we investigated design of the DGM chamber with computational fluid dynamics as function of chamber angle (Fig. 2). As the angle is smaller, particles are difficult to move to the collection chamber because their major trajectories were along with the boundary walls as well as secondary flows were severely formed, deciding the angle with 50°.

Five mL of blood with pre-stained(CellTracker® Green) cancer cell (MCF-7, HCC827) spiked in were used to demonstrate our system. Next to adding EpCAM coated microbead(4.5um Dynamicrobeads® Epithelial Enrich), the sample was introduced to the disc platform and processed in the rotor system(Fig. 3A). For the first step, plasma was removed via centrifugation (Fig. 3B,C). Triangular obstacle structure in the middle of blood chamber prevented blood cells to escape blood cell zone by diffusion after centrifugal depletion. This structure make the disc platform affordable for large sample volume required for CTC detection while other centrifugal microfluidic platforms are hardly deal with. After sufficient mixing for EpCAM bead binding to cancer cell, the sample was transferred to the DGM chamber. Bead bound cancer cell selectively penetrate the DGM(86% Percoll,  $d=1.118\text{g/cm}^3$ ) and gathered to the collection chamber while other blood cells remain above DGM layer(Fig. 2D). As the most critical process for recovery rate, we investigated optimal condition of the DGM chamber in terms of G-force and time. Once the target cells collected, enumeration was performed using 3D-flow filter chip[3].

## RESULTS AND DISCUSSION

G-force and time were positively affected on recovery rate as they increased. Over 90% of recovery rate was achieved when centrifugal condition of the DGM chamber was exceed 300g for 150 sec. Based on the result, 450g for 300sec was applied to give sufficient force and time for sedimentation to CTCs bounded with only a few microbeads. As shown in figure 4, more than 90% of spiked MCF-7 and HCC827 was recovered from 5mL blood and less than 60 WBCs were remained in isolated sample.

Detection of genetic mutation in CTCs via minimally invasive liquid biopsies allows monitoring of changes in genetic abnormalities, which could be useful in designing individualized treatment for patients. However, to analyze genome or transcriptome of isolated CTC, severe WBC contamination(i.e., low purity) has to be overcome. Our system showed minute residual WBCs which is enough to perform molecular analysis toward personalized cancer treatment.

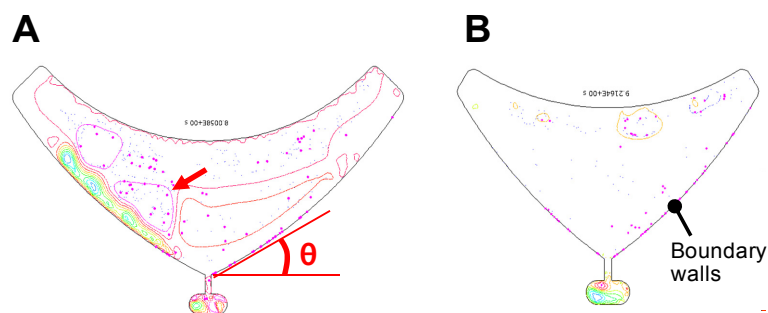


Figure 2. CFD simulation to investigate fluid behavior as angle of the DGM chamber. (A)The chamber angle, 33°. (arrow: secondary flow) (B) 50°

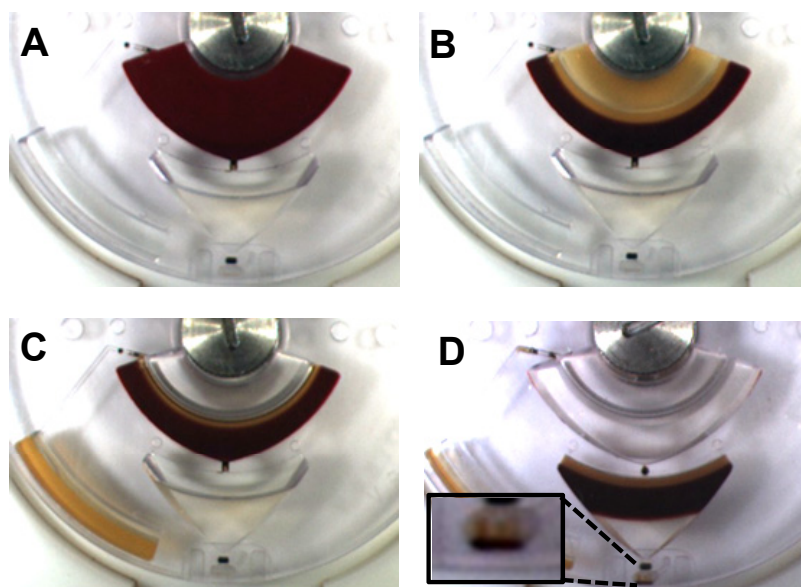


Figure 3. Automated CTC isolation using the TOS disc system. (A) 5 mL of blood plus microbeads and DGM were injected. (B) After 10 min, the plasma layer was formed over the triangular obstacle. (C) The plasma was completely removed by opening the V1 valve. (D) After shaking the disc for bead- binding process (1 hr), the CTCs and microbeads were accumulated in the collection chamber (inset).

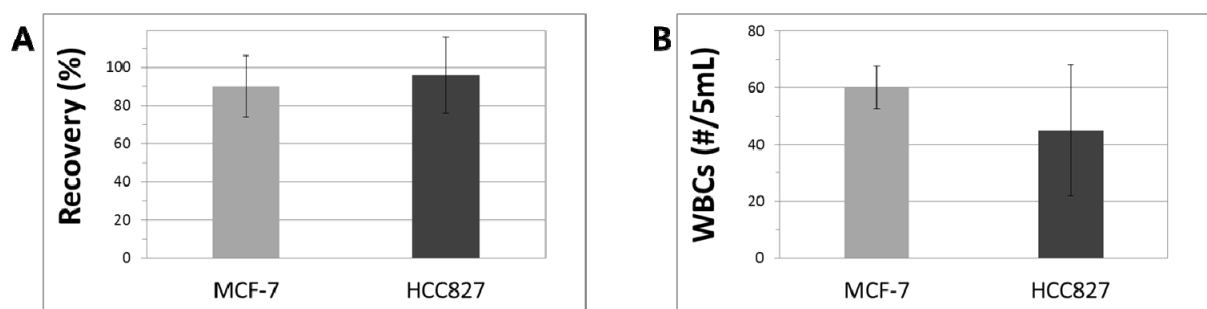


Figure 4. Recovery rates and purities of the MCF-7 and HCC827 cancer cells. 100 cells were spiked in 5mL whole blood respectively.

## CONCLUSION

We developed a novel centrifugal microfluidics based CTC isolation platform that possesses large-volume capability and fully automated system with high recovery rate. Our platform also showed high purity indicating that it is capable of downstream molecular analysis for personalized cancer treatment.

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