AN INTEGRATED MICROFLUIDIC PLATFORM FOR NEGATIVE SELECTION AND ENRICHMENT OF CIRCULATING TUMOR CELLS

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ABSTRACT

Circulating tumor cells (CTCs) are indicators for cancer diagnosis and prognosis. In this work, a new integrated microfluidic system capable of un-biased and sensitive "negative" enrichment of CTCs was developed. By using anti-human CD45 coated magnetic beads, leukocytes were removed by external magnetic force, leaving behind an enriched target cell population. The results demonstrated that the developed chip was capable of performing CTC negative enrichment with comparable performance as the conventional benchtop method while offering significant advantages in rapid incubation and potential for automation, suggesting the developed chip is promising for standardized CTC isolation platform.

KEYWORDS: Circulating tumor cells, Negative enrichment, Microfluidics

INTRODUCTION

CTCs have emerged as a promising indicator for cancer diagnosis and prognosis because they are essentially a "liquid biopsy" of tumor that can be directly acquired from the bloodstream [1]. Common methods for isolating CTCs, which rely on tumor surface marker-specific antibodies such as EpCAM, are limited to the detection of epithelial cell-type cancers and can suffer from antibody binding to non-target cells. Thus, a "negative selection" strategy, wherein the non-target cells are depleted, presents an alternative way for un-biased and sensitive CTCs isolation. Furthermore, un-labeled CTCs are more amenable for downstream molecular and cellular analyses [2]. Equally importantly, leveraging the automation and precision of microfluidics can further improve the negative selection method. Motivated by these reasons, a new integrated microfluidic system capable of automatic and rapid enrichment of CTCs was developed in this study.

EXPERIMENTAL

The basic concept of CTC negative enrichment is illustrated in Figure 1. To diminish the non-target cells, red blood cells in blood sample was first removed by hemolysis. After washing out the debris, anti-human CD45 coated magnetic beads, which recognizes leukocytes, were added and incubated with blood samples. Magnetically-captured leukocytes were then removed by external magnetic force, leaving behind an enriched target cell population. Additional rounds of leukocyte depletion could be performed to improve the purity of target cell population.

The entire protocol was performed in a new microfluidic chip (Figure 2). It contained a polydimethlysiloxane (PDMS) air control layer, a PDMS liquid transport layer and a glass substrate, and harbored several transportation units (Figure 2). Two units were especially critical for on-chip operation: the micromixer, which was responsible for the mixing, incubation, and washing, and the micropump, which served to transport magnetic beads to the cell suspension. Leukocytes/CTCs suspension, anti-CD45 coated beads and wash buffer were pre-loaded to the indicated reservoirs and serial depletion of leukocyte was processed following the schematic illustration in Figure 3. $CD45^+$ cell depletion efficiency was calculated. CTC cell recovery rate from the microfluidic chip was further calculated by counting the recovered cell numbers.

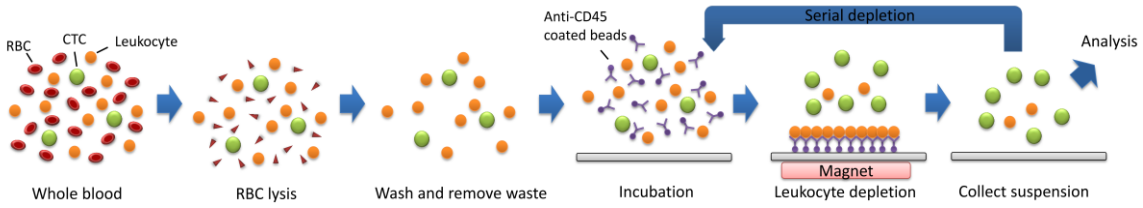

Figure 1: Schematic diagram of the CTC negative enrichment process.

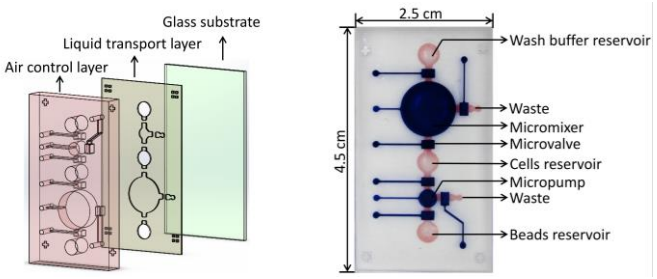

Figure 2: Microfluidic CTC enrichment device. An exploded view (Left) and a photograph (Right). Red color and blue color indicated the liquid transport channel and the air control channel, respectively.

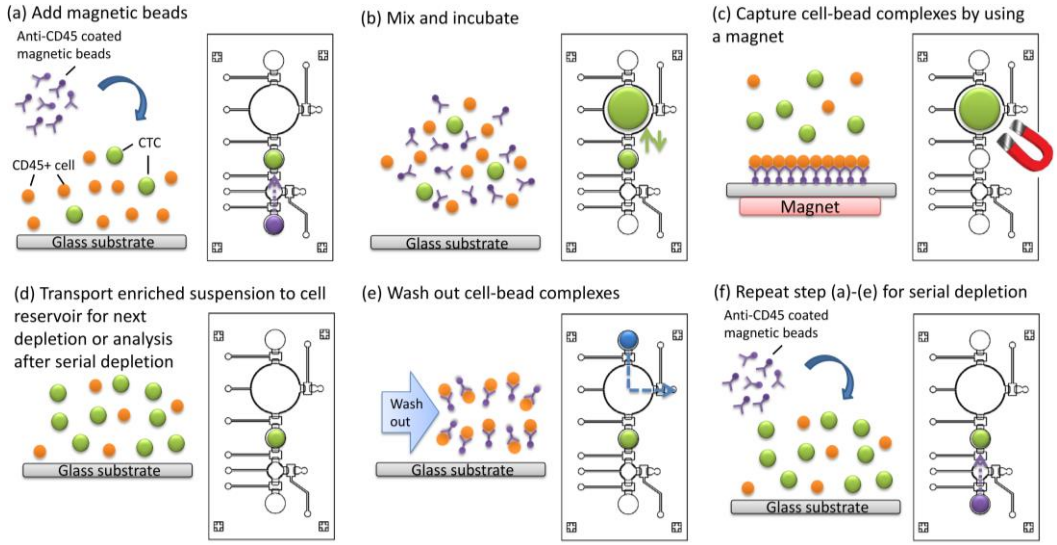

Figure 3: Overview of the experimental protocol on the integrated CTC enrichment chip. (a) Addition of anti-CD45 magnetic beads; (b) Incubation; (c) Collection of cell-bead complexes; (d) Transportation of enriched suspension for next depletion or analysis after serial detection; (e) Removal of cell-bead complexes; (f) Repeat step (a)-(e) for serial depletion.

RESULTS AND DISCUSSION

Effective negative enrichment for CTCs in the microfluidic device was successfully achieved by maximizing the recovery and viability of target cells, as well as the depletion of non-target cells. The dead volume in the microfluidic chip was found to be negligible, which is important for maximizing the recovery of target cells. This is verified as the volume of reagents that were drawn into the micromixer and pumped back into the outlet chamber was comparable to the volume of reagents that simply remained in the outlet chamber (Figure 4a). Cells remained viable during the mixing and incubation step under an actuation frequency of 0.05 Hz. However, further increasing the pumping frequency to 0.5 Hz damaged cell viability (Figure 4b). $CD45^+$ depletion efficiency reached ~79.7% when bead/cell ratio was 7 (Figure 5a). The on-chip $CD45^+$ cell depletion efficiency was 78.9% under 0.05 Hz mixing for 10 min at room tem-

perature (Figure 5b). As a comparison, conventional benchtop method required 30 min to deplete a comparable 80.9% of $CD45^+$ cells. Notably, the microfluidic incubation time was shortened by 66.7% and the depletion could be processed automatically. The average recovery rate of CTCs from the chip from various initial input populations of CTCs was ~80.0% (Figure 5c). These results clearly demonstrate this microfluidic chip's capacity for achieving effective CTC negative enrichment.

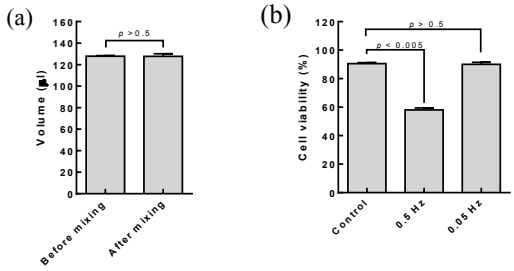

Figure 4: Characterization of CTC negative-enrichment chip. (a) Pumping rate of the transportation unit. (b) Volume recovered from the chip after mixing/pumping was similar to that without mixing/pumping. (c) Cells remained viable under a pumping frequency of 0.05 Hz.

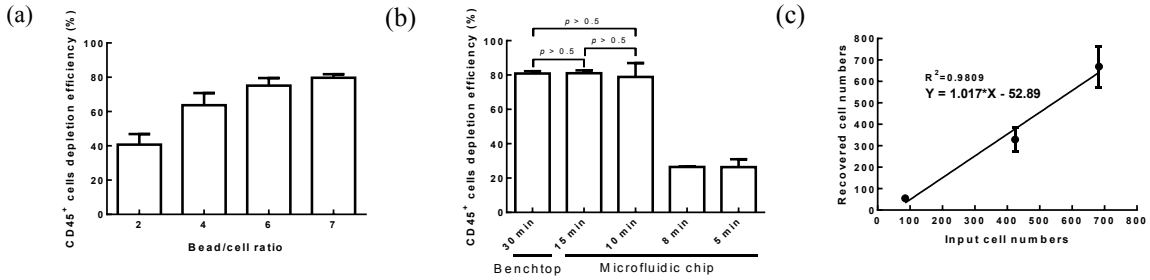

Figure 5: Parameter optimization of $CD45^+$ cells depletion and evaluation of CTC recovery rate. (a) The efficiency of $CD45^+$ cell depletion reached to ~79.7% when bead/cell ratio was 7. (b) On-chip $CD45^+$ cell depletion efficiency for 10 min was comparable to that using conventional benchtop method (c) The average cell recovery rate across various initial input cell population was ~80%.

CONCLUSION

In summary, we demonstrated an integrated microfluidic chip capable of performing CTC negative enrichment with comparable performance as the conventional benchtop method while offering significant advantages in rapid incubation and potential for automation. These promising results, coupled with the potential for performing multiple rounds of depletion to increase the purity of CTC recovery, suggest that the developed chip could become an effective tool for standardized CTC isolation.

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