APTAMER-FACILITATED HIGH-EFFICIENCY CANCER CELL SORTING IN A MICROPPOST-BASED MICROFLUIDIC DEVICE

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ABSTRACT
We have developed a micropost-based PDMS (polydimethylsiloxane) microfluidic device for high-efficiency cancer cell sorting. The device consists of eight parallel channels with >59,000 microposts. The device is first modified with aptamers, followed by pumping a mixture of two different cancer cells into the microchannel. Target cancer cells are captured with above 96% efficiency while control cancer cells are washed away. The micropost structure facilitates the interaction between the cancer cells and the aptamer-functionalized substrate, thus the cancer cell capture efficiency increases significantly compared with our previous flat channel device.

KEYWORDS: Cell sorting, Aptamer, Micropost, Circulating tumor cells, Polydimethylsiloxane

INTRODUCTION
Cancer is a leading cause of death worldwide and accounts for millions of deaths every year. In order to have a promising treatment, early diagnosis of cancer by detecting circulating tumor cells (CTCs) in patient blood is of great significance. CTCs are cells detached from a primary tumor and circulate in the bloodstream that can serve as important cancer biomarkers. However, they are rare, comprising only 1 out of 108 hematologic cells in 1 mL blood sample. Methods that can sensitively and accurately detect CTCs are, therefore, in great demand. Among all the tools developed till now, microfluidic chips with high-affinity ligands have provided an easy and inexpensive way to capture and detect CTCs.

Recently, Nagrath et al. have developed a silicon microchip for capturing CTCs with 65% efficiency using antibodies as capturing ligands.1 Other groups have employed microfluidic device with different structures to increase the capture efficiency of CTCs.2 3 4 Our groups have developed a prototype double-sided tape glass device and a single flat channel PDMS device and successfully utilized them for capturing target cancer cells in a mixture of two different cancer cells.5 The excellent selectivity of these two devices came from the selective binding to target cells of DNA-aptamers generated by cell-SELEX6 (Systematic Evolution of Ligands by EXponential enrichment). The single flat channel device was proved to capture target cells with >80% efficiency at a flow rate of less than 200 nL/s with 30 s incubation for each 3 μL cell pulse. Then we extended our design to an S-shaped flat channel PDMS device which achieved multiplexed detection of three different cancer cell lines simultaneously.7 Compared to silicon device, the PDMS devices are easy to fabricate, with a decreased channel height for better cell capture. In addition, high affinity and selectivity aptamers were utilized instead of antibodies. Herein, we have designed and fabricated an aptamer-functionalized micropost-based PDMS device. More than 96% capture efficiency of target cancer cells was obtained at a flow rate of 300 nL/s without incubation due to enhanced aptamer-cell interaction provided by the microposts.

EXPERIMENTAL

Device design. As shown in Figure 1, the microscopic slide-sized PDMS microfluidic chip consists of >59,000 elliptical microposts and eight parallel channels with effective bifurcation. The device enables equal distribution of flow and avoids physical trapping of cells. The PDMS microchannel is reversibly bonded with a 1”×3” microscopic glass slide. The cell capture starts from physical adsorption of avidin onto glass surface, followed by immobilization of biotinylated aptamer through biotin-avidin chemistry, and ends with target cells captured via aptamer-receptor interaction.

Cell capture assay. One channel volume of avidin at 1 mg/mL in phosphate buffered saline (PBS) was first introduced into the device followed by incubation for 1 min and then rinsed three times with binding buffer (4.5 mg/mL glucose, 5 mM MgCl2, 0.1 mg/mL yeast tRNA and 1 mg/mL bovine serum albumin (BSA) in 1× Dulbecco’s PBS). After that, biotinylated sgc8 aptamer with a polyT10 linker (biotin-T10-sgc8: 5′-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GAT TTT TTT TTT-3′-Biotin) was introduced into the channel and incubated for 1 min, and then rinsed with the binding buffer. Finally, a mixture of labeled CEM (CCRF-CEM cells, CCL-119, T cell line, human acute lymphoblastic leukemia, ATCC) and Ramos cells (CRL-1596, B-cell, human Burkitt’s lymphoma, ATCC) in the binding buffer with 50% Histopaque-1119 (Sigma Aldrich) were pumped into the channel. Histopaque-1119 was used to create similar viscosity of fluids as whole blood as well as to provide the buffer same density as the cells to prevent cells from settling while continuously pumping. Confocal microscope (Olympus FV500-IX81 confocal microscope) images were taken to determine the initial concentration of both target CEM cells and control Ramos cells. Then the microchannel was rinsed with the binding
buffer, and confocal microscope images were taken again to determine the cell concentration after washing. Images were imported into ImageJ (NIH) to count the cells. Capture efficiency was determined by dividing the target cells captured by total target cells introduced into the channel.

![Diagram](image)

Figure 1: (A) The design of the microscopic slide-sized micropost device with eight parallel channels; (B) The 10× microscope image of the microposts array; (C) The scheme of aptamer immobilization and cell capture, biotinylated aptamer was immobilized through biotin-avidin chemistry.

RESULTS AND DISCUSSION

Cell capture. Figure 2 shows confocal microscope images of target cells captured in the micropost chip before and after washing. A mixture of CEM and Ramos cells were introduced into the sgc8 aptamer modified channel. After washing, target CEM cells were captured with > 96% efficiency, while control Ramos cells were washed away.

![Images](image)

Figure 2: (A) Representative image of mixed CEM and Ramos cells spiked in binding buffer introduced into the sgc8 aptamer functionalized micropost channel under the confocal microscope; target CEM cells were labeled with red dye (Vybrant DiI cell-labeling solution, Invitrogen), control Ramos cells were labeled with blue dye (Vybrant DiD cell-labeling solution, Invitrogen); (B) Representative confocal image of target CEM cells captured with >96% capture efficiency, control Ramos cells were washed away.

High capture efficiency. Compared with previous prototype device and flat channel device, the micropost device increased capture efficiency of cancer cells significantly. Figure 3 compares the micropost device with previous prototype device and the flat channel device. It proves that the microposts structure in the channel significantly increases the capture efficiency of cancer cells. This is due to that the microposts 1) increase the inner surface area to volume ratio and 2) increase the probability of encounter between the cells and aptamer coated surface.
Figure 3: Capture efficiency of micropost device compared with previous prototype device and single flat channel device for CEM cell capturing. The micropost device improved the capture efficiency significantly. Error bars show standard deviations (n=3). The flow rates here were all 200 nL/s.

Flow rate effect. To investigate the flow rate effect on the cell capture efficiency, flow rates from 200 nL/s to 1200 nL/s were tested. As illustrated in Figure 4, the capture efficiency decreases gradually as flow rate increases, because the increased flow rate reduces the duration of cell-micropost interaction and increases flow induced shear forces. The capture efficiency maintains over 80% at flow rate of 600 nL/s while the previous flat channel device requires flow rate less than 200 nL/s with 30 s incubation every 3 μL cell pulse to maintain the same capture efficiency. Compared with previous devices, the micropost device sorts cancer cells at a higher speed and achieves higher throughput.

CONCLUSION
A micropost-based PDMS microfluidic device was fabricated and applied for cancer cell sorting with high efficiency and high throughput. Aptamers with high affinity were functionalized onto the device to selectively capture the target cancer cells. Since the method is invasive and requires no pretreatment of cancer cells, it could serve as a useful tool for cancer diagnostics.

REFERENCES

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