EFFICIENT ISOLATION OF TUMOR CELLS IN WHOLE BLOOD USING APTAMERS IMMOBILIZED IN A DEVICE

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ABSTRACT

This paper describes the incorporation of DNA aptamers with a microfluidic device for the isolation of cancer cells from whole blood. Aptamers with specific binding to leukemia or colorectal cells were used as an alternative to antibodies that are often used for circulating tumor cell (CTC) sorting. The microfluidic device contained unique geometry and design of micropillars, and showed better performance than those in the literature. We applied the platform to whole blood analysis, and demonstrated the detection of 10 cancer cells in 1 mL of blood. The device was capable of processing 1 mL of blood within 28 minutes.

KEYWORDS

circulating tumor cell (CTC), aptamer, whole blood, enrichment, cancer.

INTRODUCTION

Cancer is one of the leading causes of death and it accounts for millions of deaths every year worldwide. To treat it successfully, early diagnosis of cancers is the key. Circulating tumor cells (CTC) in the peripheral blood are promising biomarkers for early-stage cancer detection. However, CTC are extremely rare in the bloodstream (about 10 CTC in 1 mL of blood), making their detection and characterization technically challenging. To address this, efforts have been made by several research groups, including ours.[1-4]

The unique contribution of this work includes three folds. The first distinction is the incorporation of aptamers, replacing antibodies that were used in most efforts. DNA aptamers were generated by cell-SELEX (Systematic Evolution of Ligands by EXponential enrichment) and they showed selective and strong binding to target tumor cells.[5] The second contribution is the fabrication of unique device geometry to achieve high capture efficiency in <30 min. to process 1 mL of sample. The third one is the capability of the device for isolation of 10 colorectal tumor cells from 1 mL of non-processed whole blood.

EXPERIMENTAL

Device Fabrication. The device was designed to be in the size of a microscope slide as shown in **Figure 1**. The device consists of >59,000 micropillars, which enhanced the probability of the interactions between aptamers and target cancer cells. The geometric design of the micropillar array was inspired by the deterministic-lateral-displacement-based particle separation,[6] in which the flow streamlines are distorted to enhance cell-micropillar interactions. Glass devices were fabricated according to the procedures reported previously.[7] The device was functionalized with aptamers through two-step surface modification as shown in **Figure 2a**: physical adsorption of avidin onto the glass surface and immobilization of biotinylated aptamers via biotin-avidin interaction. Target cancer cells were captured due to the specific binding between cell surface receptors and aptamers.

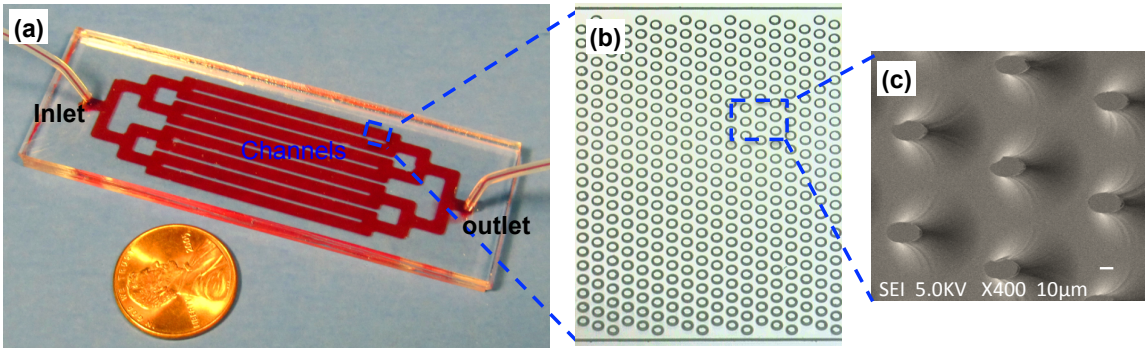

Figure 1. (a) *Picture of a CTC sorting device consisting of 1 inlet, 1 outlet, and 8 parallel channels connected through bifurcation. The size of the device is 1" × 3". **(b)** Exploded view of a portion of one channel containing a large array of micropillars. **(c)** Scanning electron microscope (SEM) image of elliptical micropillars in the glass substrate. The curvature sides of micropillars are due to isotropic etching of the glass. The micropillars are in elliptical shape, with a dimension of 30 µm (major axis) × 15 µm (minor axis) × 32 µm (height). The distance between adjacent pillars is 80 µm and each column of the micropillars is shifted down 26.7 µm to have total shift of 80 µm after every 3 columns.*

Cell Enrichment. CCRF-CEM cells (CCL-119, T cell line, human acute lymphoblastic leukemia), Ramos cells (CRL-1596, B-cell, human Burkitt's lymphoma), and HCT 116 cells (colorectal carcinoma) were purchased from

American Type Culture Collection (ATCC). DNA aptamers were synthesized in house. Aptamer sequences were as follows, sgc8, 5'-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GAT TTT TTT TTT-3'-biotin; TD05, 5'- AAC ACC GTG GAG GAT AGT TCG GTG GCT GTT CAG GGT CTC CTC CCG GTG TTT TTT TTT T- 3'-biotin; KCHA10, 5'-ATC CAG AGT GAC GCA GCA GGG GAG GCG AGA GCG CAC AAT AAC GAT GGT TGG GAC CCA ACT GTT TGG ACA CGG TGG CTT AGT TTT TTT TTT T-3'-biotin. The specific binding between aptamers and target cells is illustrated in **Figure 2b**. A washing buffer was prepared by adding 4.5 g/L glucose and 5 mM $MgCl_2$ in DPBS and it was for rinsing cells. A binding buffer was prepared by supplementing the washing buffer with yeast tRNA (0.1 mg/mL), bovine serum albumin (BSA) (1 mg/mL), and it served as the medium for cell-aptamer interactions.

Before experiments, cells were rinsed with the washing buffer and resuspended at 10^6 cells/mL The cells were stained with Vybrant DiI or Vybrant DiD by following the manufacturer's instructions. To initiate cell capture experiments, 1 mL of a mixture of CEM cells (target) and Ramos cells (control) in the binding buffer was pumped into the channel at a flow rate of 600 nL/s. At the end of the experiment, the microchannel was washed three times, followed by taking fluorescent images for the determination of the cell concentrations.

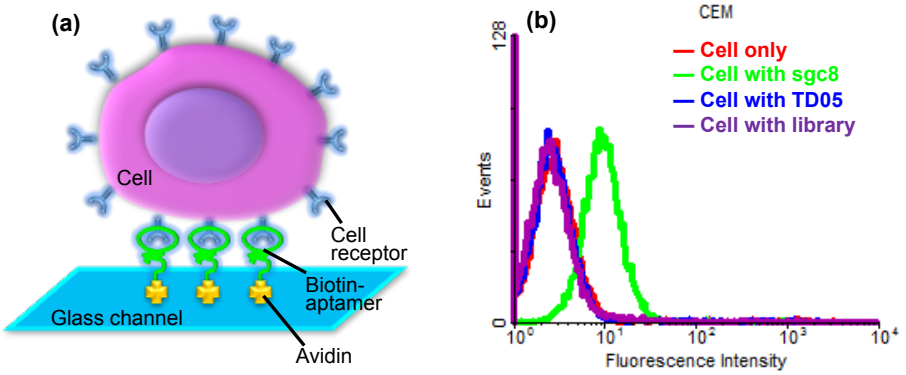

Figure 2. ***(a)*** *Scheme of capturing cancer cells in the device. Avidin is immobilized on the surface of the microchannels/micropillars via physical adsorption, followed by immobilization of biotinylated aptamers through biotin-avidin chemistry. Target cancer cells are then captured via the interaction between the aptamers and the receptors on cell surface.* ***(b)*** *Flow cytometry analysis of CEM cells conjugated with fluorescently labeled sgc8 aptamers. Compared to cells only, a large shift in the fluorescence signal was observed for those cells conjugated with sgc8 aptamers, indicating specific binding between the target CEM cells and sgc8 aptamers. A random single strand DNA library or TD05 aptamer had non-specific binding with CEM cells, thus showing a tiny shift in the fluorescence signal compared to cells only.*

RESULTS AND DISCUSSION

Isolation of lymphocytes. The performance of the microfluidic device was demonstrated first by sorting leukemia cells: CCRF-CEM cells that function as target cancer cells and Ramos cells that function as control cells. Biotinylated sgc8 aptamers have specific binding with CCRF-CEM cells, and they were immobilized onto the surfaces of the micropillars/microchannels. A cell mixture containing 10^6 CEM cells and 10^6 Ramos cells in 1 mL of buffer was used as a sample. To differentiate these two types of cells during imaging, CEM and Ramos cells were pre-stained with Vybrant DiI (red) and DiD (blue), respectively.

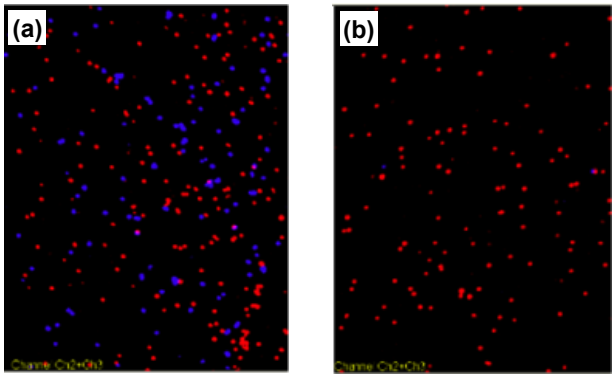

Figure 3. ***(a)*** *Image of a cell mixture consisting of target CEM cells (stained with a red fluorescent dye) and Ramos cells (blue) before sorting.* ***(b)*** *Confocal image of cells captured in the device after processing 1 mL of the cell mixture through the device. Since all cells are essentially the target CEM cells in red, efficient isolation of tumor cells was obtained.*

Figure 3a shows an image of the cancer cell mixture prior to sorting in the device. The mixture of CEM and Ramos cells were introduced into the device immobilized with sgc-8 aptamers. After washing, target CEM cells were captured while Ramos cells were washed away. **Figure 3b** shows a confocal image of cells captured after sorting. These images show qualitatively that significant enrichment of the cancer cells was obtained through the microfluidic device.

We studied the effects of different device geometries and flow rates on the isolation of target leukemia cells from a binary mixture. The channel depths ranged from 24 μm to 40 μm and the flow rate changed from 300 to 2100

nL/s. The capture efficiency of >95% was obtained at the optimum device geometry and flow rate.

Tumor Cell Isolation from Whole Blood. To illustrate the potential of the device for clinical applications, we spiked colorectal carcinoma cells, HCT 116 cells, into whole blood that was used as received. We evaluated the isolation of HCT 116 cells from whole blood at concentrations of 10,000, 1,000, 100 and 10 cells/mL. One mL of non-processed whole blood was introduced into the microfluidic device at a flow rate of 600 nL/s. Capture efficiencies of >95% were achieved in all cases, and a calibration curve between the number of the cells spiked and the number of the cells captured is shown in **Figure 4**. The results show that the device has a potential to detect CTC in clinical samples since the number of CTC in 1 mL of peripheral blood of cancer patients is often in the range of 1-100.

The post-isolation analysis included cell viability study, which showed that (94 ± 2)% of cells were viable after being processed through the device using the optimal flow rate and channel depth. As a result, these cells can go through cell proliferation and other cellular studies such as apoptosis.

In addition, we addressed the question of low throughput of a typical microfluidic device by connecting 8 microchannels through bifurcation (Figure 1). The width of each channel is 2 mm. With the optimal flow rate of 600 nL/s, the time required to process 1 mL of whole blood in the device is 28 minutes, which is favorable compared with hours of operation required in the benchmark instruments.

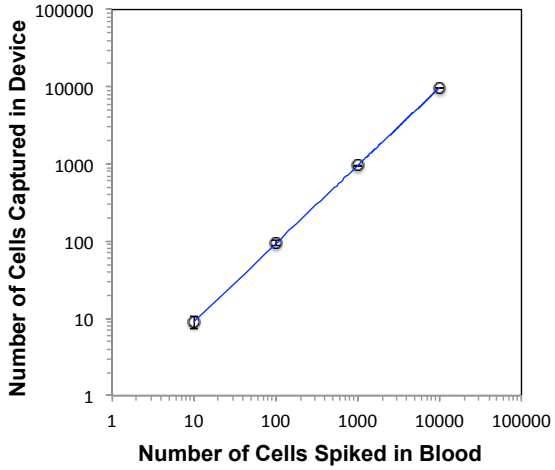

Figure 4. *Calibration curve for the isolation of colon tumor cells (HCT-116) from whole blood at concentrations of 10,000, 1,000, 100 and 10 cells/mL. Both axes are in the logarithmic scale. The error bars represent one standard deviation of 6 repeats for 10-cell samples and 3 repeats for other cell numbers. Capture efficiencies of >95% were achieved in all cases.*

CONCLUSION

We demonstrated using a DNA aptamer-enabled, micropillar-based microfluidic device to isolate cancer cells in non-processed peripheral blood. The unique geometry of the micropillar array in the device resulted in the high-performance cell isolation. This microfluidic device enabled the isolation of as few as 10 tumor cells from 1 mL of non-processed whole blood with >95% capture efficiency within 28 minutes. The advantages of such a device over the other methods include rapid analysis, no pre-treatment of blood samples, and low detection limit. As a result, the device has a potential to be used for clinical applications such as cancer diagnosis, prognosis, and monitoring the progress of therapeutic treatment.

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