REUSABLE MICROFLUIDIC CHIP FOR CELL CAPTURE AND RELEASE USING SURFACE-IMMOBILIZED APTAMERS

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ABSTRACT

We present a microfluidic platform for capture and subsequent trypsin-induced release and recovery of CCRF-CEM cells using DNA aptamer molecules immobilized on a glass substrate. Cells are specifically captured by the surface-immobilized aptamer molecules. Upon purification of the captured cells, they are released by introducing trypsin, which disrupts the cell-aptamer interaction, from the surface and collected for further detection and analysis. We have also demonstrated that the aptamer-modified surface is reusable and capable of cell enrichment. Our approach can be potentially used in rarefied cell screening and capture applications, such as cancer diagnostics.

Keyword: Aptamer, Cells Capture, Enrichment, Cancer Diagnostics

INTRODUCTION

Microfluidics has been actively used to capture, purify, enrich and isolate rare cells for cancer diagnostics [1]. They have commonly relied on affinity microfluidic surfaces comprised of receptors, such as antibodies [1], lectins [2], and aptamers [3, 4] for cell capture. Although effective, many existing approaches lack the ability to release captured cells and hence, regenerate the device for further reuse. A few examples that do however employ air bubbles [4] or trypsin [5], but fail to demonstrate device regeneration. To address this limitation, we present a reusable microfluidic chip for the capture, release and recovery of cells using affinity surfaces. The specific cell capture is achieved by using highly selective aptamers, which bind to target cells at room temperature. Additionally, our preliminary observations suggest that consecutive sample introduction enables the enrichment of captured cells, thus increasing their density on the aptamer-modified surface. Subsequently, we introduce trypsin to reversibly disrupt the aptamer-cell interaction, and thus perform cell release and recovery. Additional cell solutions are then introduced to the microchamber to demonstrate reusability of the aptamer-modified surface. To establish inter-device consistency, cell capture is performed across four similar devices. As a proof-of-concept, we utilize this method to capture and release CCRF-CEM cells, which are T-lymphoblasts present in the peripheral blood of individuals with acute lymphoblastic leukemia.

DESIGN AND SURFACE MODIFICATION

The chip consists of a glass substrate supporting a PDMS microfluidic chamber, which encloses a patterned DNA-aptamer surface (of sg1c8c: 5'-NH2-TTT TTT TTT TAT CTA ACT GCT GCG CCG CCG GGA AAA TAC TGT ACG GTT AGA-3') specific to CCRF-CEM cells. The microfluidic aptamer layer was generated as follows [6]. Glass slides were cleaned in Nano-Strip overnight. Afterwards, they were silanized by immersion in a solution of 1% 3-Aminopropyl(diethoxy)methylsilane (3-APS) and anhydrous ethanol (1 h), twice washed in ethanol, and baked for 1 h at 120°C. The slides were then placed in a solution of 0.5% p-Phenylenediisothiocyanate (PDC, Fig. 1A) dissolved in a solution consisting of 10% v/v of pyridine and 90% v/v of anhydrous N,N-dimethylformamide for 4 h to form a PDC-grafted surface (Fig 1B). After which, an amino-modified sg1c8c aptamer solution (20 μM) was locally introduced to the slides and incubated overnight (25 °C). Subsequently, slides were kept at 37 °C in a humidity chamber containing 300 mM K2HPO4 (pH 9.0) for 2 h (Fig. 1C). Any unreacted PDC groups were inactivated with 1M ethanolamine-HCl (pH 8.5) (Fig. 1D). Following a wash with 4 M Urea, 15 mM EDTA and deionized water, a microfabricated PDMS chamber was aligned and attached to the glass slide (Fig. 2). Nonspecific adsorption was prevented by blocking with BSA.

![Fig. 1: Aptamer immobilization. (A) Structural formula of PDC. (B) PDC-functionialized substrate surface. (C) Aptamer-functionalized substrate surface. (D) Blocking of remaining PDC group with ethanolamine-HCl.](image-url)
EXPERIMENTAL METHODS AND RESULTS

CCRF-CEM cells were obtained from American Type Culture Collection and then cultured in RPMI medium 1640 supplemented with 10% Fetal Bovine Serum and 100 IU/mL penicillin-streptomycin. This culture media was subsequently used in all experimental washing steps. Immediately before experiments, cells were aliquoted in varying concentrations. Trypsin solution was prepared with 2.5 g/L of trypsin and 0.38 g/L of Na4EDTA in Hanks’ Balanced Salt Solution.

Selective microfluidic capture of CCRF-CEM cells was achieved by introducing a dilute cell solution (4×10⁶ cells/mL) into the chamber (Fig. 3A); washing removed nonspecifically bound cells, leaving a distinctive circular pattern of cells. The flow rates used for sample introduction and washing were respectively 1 and 5 µL/min in all experiments. Since cells were only present on the aptamer-functionalized surface (Fig. 3B), we concluded the capture to be specific.

To test the effect of cell concentration on capture density, a concentrated cell solution (10×10⁶ cells/mL) was then introduced to the microchamber (Fig. 3C). Note that the density of captured cells was nearly 7,000 cells/mm², which was significantly higher than the dilute solution case (around 420 cells/mm²). This is significantly more effective cell capture than a previously reported publication [3], which demonstrated only 300 cells/mm². Furthermore, this result suggested that cell enrichment by the aptamer surface was possible. Hence, we tested this hypothesis by continuously introducing a dilute cell solution for 300 s. As more sample passed through the microchamber, captured cells increased (Fig. 4A-D). Such an enrichment scheme would lead to denser cell clusters, thereby allowing more sensitive detection of rare cancer cells in cancer diagnostics applications.

To observe trypsin-induced release of captured cells, 200 µL of trypsin solution at 37 °C was injected into the cell-laden chamber (Fig. 3B) at 10 µL/min. A following observation of the chamber revealed a substantial removal of CCRF-CEM cells, indicating successful release (Fig. 3D). We conjecture that the cell release is due to the hydrolyzation of CCRF-CEM cell membrane proteins at the binding site, which deteriorates specific interaction with the sg8c aptamer. To verify the reusability of the aptamer-surface, three subsequent experimental cycles were performed in the device consisting of first introducing a dilute cell solution to the aptamer surface, and then washing with warm trypsin (Fig. 5A). Note that following the first cycle, a similar captured cell density was observed for all succeeding cycles (~400 cells/mm²). Moreover, the maximum difference in captured cell density was c.a. 100 cells/mm² (between first and second cycles), a difference of roughly 25%. Hence, given these results, regeneration of cell capture function in the microfluidic device can be both effective and consistent.
We finally tested the capture efficiency using multiple devices with aptamer-modified surfaces. Four separate cell solutions, derived from a single culture and of equal concentration, were introduced to the aptamer-modified microfluidic chambers of four chips in parallel. After washing, each device was observed for cell attachment, and cell capture density was then measured (Fig. 5B). The densities of captured cells on different chips were similar (around 1300 cells/mm$^2$), with a maximum deviation of 20%. Thus, the immobilization strategy and aptamer spot generation scheme were reproducible. This is important for practical applications in cancer cell detection and diagnostics where robust and reproducible assays ensure accurate diagnoses.

**CONCLUSION**

We have performed specific cell capture, efficient trypsin-induced release and regeneration on aptamer-functionalized microfluidic surfaces. Experiments demonstrated highly effective capture of CCRF-CEM cells (up to 7000 cells/mm$^2$), while efficient release of captured cells was possible by introducing a warm trypsin solution. We also verified that the aptamer-surface was reusable and capable of cell enrichment. These results confirm the effectiveness of this method to be potentially used as a practical platform for clinical applications such as cancer diagnostics.

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