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Spatially selective release of aptamer-captured cells by temperature mediation

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Abstract

Isolation of cells from heterogeneous biological samples is critical in both basic biological research and clinical diagnostics. Affinity-based methods, such as those that recognise cells by binding antibodies to cell membrane biomarkers, can be used to achieve specific cell isolation. Microfluidic techniques have been employed to achieve more efficient and effective cell isolation. By employing aptamers as surface-immobilised ligands, cells can be easily released and collected after specific capture. However, these methods still have limitations in cell release efficiency and spatial selectivity. This study presents an aptamer-based microfluidic device that not only achieves specific affinity cell capture, but also enables spatially selective temperature-mediated release and retrieval of cells without detectable damage. The specific cell capture is realised by using surface-patterned aptamers in a microchamber on a temperature-control chip. Spatially selective cell release is achieved by utilising a group of microheater and temperature sensor that restricts temperature changes, and therefore the disruption of cell–aptamer interactions, to a design-specified region. Experimental results with CCRF-CEM cells and sgc8c aptamers have demonstrated the specific cell capture and temperature-mediated release of selected groups of cells with negligible disruption to their viability.

1 Introduction

Specific cell isolation is important in basic biological research and clinical diagnostics. Antibodies that are specific to cell membrane proteins are most often employed to achieve this goal. For example, magnetic-activated cell sorting (MACS) and fluorescence-activated cell sorting (FACS) are highly attractive because of their high specificity to target cells [1, 2]. The MACS method relies on the presence or absence of magnetic forces to recognise different cell types. Although it is amenable to high-throughput operations, there is generally no difference between the magnetic forces generated by microbeads with different surface-modified antibodies specific to different target cells [3]. Hence, MACS is a single-parameter cell isolation method, and lacks the capability to distinguish and sort multiple types of cells. On the other hand, FACS uses different species of antibodies with different fluorescent

labels to recognise target cells. Multiple characteristics of cells can be monitored, and thus different cell types can be separated and collected simultaneously [2]. However, the application of FACS is restricted by its relatively low yield and complex and expensive experimental instrumentation.

Microfluidic technologies have been developing to enable more efficient and effective cell isolation with improved sensitivity and resolution, minimised sample and reagent consumption, lower cost and the capability of automation and point-of-care [4]. To achieve specific cell isolation, antibodies are always employed [5, 6]. For example, the isolation of rare circulating tumor cells from whole blood samples has been achieved in a microfluidic device with micropillars that are functionalised with anti-epithelial cell adhesion molecule antibodies [6]. Unfortunately, antibodies are not always stable, and are expensive and time-consuming to develop [7]. In addition, in order to achieve molecular and functional analysis [8] or cell based therapeutics [9], cells must be released with minimal contamination and negligible disruption to their viability. However, the interaction between antibodies and antigens are not reversible under normal physiological conditions [10, 11]. Cells are hence typically released from antibody-functionalised surfaces using trypsin to digest antibody-specific cell membrane proteins [12], or varying the substrate hydrophobicity to detach hydrophobically anchored antibodies [13]. Tryptic digestion is not efficient, only applicable to a small portion of biomarkers involved in affinity cell capture [14], and may influence cell viability and phenotypic properties [15, 16]. Meanwhile, temperature dependent substrate property alteration cannot cause the dissociation of antibodies from the antigens, leaving the antibodies attached to the cell membranes [13]. Therefore there is a strong need for methods that allow rapid and non-destructive release of cells from affinity surfaces.

Aptamers, which are oligonucleotides that bind specifically to target molecules, have the potential to resolve these problems. Aptamers can be selected from a randomised oligonucleotide library using a synthetic process [17]. Compared with antibodies, aptamers are stable, designable and amenable to chemical modifications [18]. Meanwhile, the binding between aptamers and target molecules is reversible because of conformational changes caused by temperature variations [19, 20]. In addition, recent advances in synthetic aptamer development have resulted in aptamers for multiple cellular targets, such as acute lymphoblastic leukaemia (ALL) precursor T cells [21], liver cancer cells [22] and stem cells [23]. These aptamers bind to cell membrane proteins by hydrogen bonds, hydrophobic interactions, van der Waals interactions, aromatic stacking or their combinations. Such affinity binding allows the aptamers to capture target cells specifically [24, 25]. Therefore aptamers are attractive alternatives to antibodies as affinity ligands for cell isolation. For example, aptamers targeting prostate-specific membrane antigen have been used in a microfluidic system to separate LNCaP cells from a heterogeneous cell mixture [26]. Release of aptamer-captured cells has been accomplished by methods such as exonuclease degradation of aptamers [27], air bubble dislodging [28] and temperature stimulation [19]. Unfortunately, the use of exonuclease is inefficient because of the slow diffusive transport of enzymes and the low enzymatic reaction rate, whereas the use of air bubbles may damage cells and generate dead volumes leading to low cell release efficiency. We recently demonstrated that these issues could be addressed by temperature-mediated cell release [19],

although the release was not spatially selective in that all captured cells were released from the entire microfluidic surfaces.

This paper presents a microfluidic device with a surface selectively functionalised with cell-specific aptamers and integrated microheaters with temperature sensors to achieve specific cell capture and temperature-mediated release of selected groups of cells. Aptamers are patterned on design-specified regions of the chip surface, and the heat generated by the microheaters is restricted to each aptamer-functionalised chip area. Target cells can be captured by the surface-patterned aptamers with high specificity. A moderate temperature change is then produced using one group of microheater and temperature sensor to reversibly break cell-aptamer binding in the selected chip area, allowing the release and retrieval of viable target cells from this region for downstream applications. After the temperature change is reversed, the aptamer-functionalised surface recovers its binding affinity to target cells. The device has been applied to CCRF-CEM cells, a human ALL T cell line and sgc8c, an aptamer specific to these cells [29], to demonstrate its capability for specific capture and non-destructive, spatially selective temperature-mediated release of target cells.

2 Experimental

2.1 Principle

The principle of aptamer-based specific cell capture and spatially selective temperature-mediated cell release is as follows. Cell specific aptamers are first patterned on design-specified regions of the surface of a temperature-control chip. A cell suspension containing target cells is introduced into the device. Target cells located on the aptamer modified regions are captured specifically by the patterned aptamers (Fig. 1a), whereas those situated outside the aptamer-functionalised surface are not captured and removed by a Dulbecco's phosphate-buffered saline (D-PBS) wash (Fig. 1b). Next, the temperature of a specific region is increased to change the conformational structure of aptamers, by activating the microheater. Thus, the binding strength between target cells and aptamers is decreased. Cells within this region can then be easily washed away and collected, whereas cells in other regions are not affected (Fig. 1c). After the temperature is reversed, aptamers recover their ability to capture cells. In addition, this moderate temperature change does not affect cell viability [19]. For a demonstration, the microfluidic device is functionalised with the aptamer sgc8c for specific capture and temperature-mediated release of CCRF-CEM cells, a human acute lymphocytic leukaemia cell line.

2.2 Design and fabrication

The microfluidic device used for specific cell capture and spatially selective temperature-mediated cell release consisted of a tapered microchamber (2.7 mm in length, 2.2 mm in width and 20 μm in height) situated on a microchip with four groups of serpentine-shaped heaters (linewidth: 50 μm) and serpentine-shaped temperature sensors (linewidth: 20 μm) (Fig. 2).

The microchip was fabricated using standard microfabrication techniques. Briefly, a chrome (~10 nm)/gold (~200 nm)/chrome (~10 nm) thin film was first deposited and patterned to form microresistive heaters, which were then passivated by approximately 1 µm silicon dioxide using plasma-enhanced chemical vapour deposition (Fig. 3a). The microheaters generated joule heat when subjected to a DC voltage. Next, an additional chrome (~10 nm)/gold (~200 nm)/chrome (~10 nm) thin film was deposited and patterned to form the temperature sensors, which were also passivated by approximately 1 µm silicon dioxide (Fig. 3b). Subsequently, the microchip was incubated with 4% (v/v) 3-mercaptopropyl trimethoxysilane (3-MPTS) in ethanol for 30 min at room temperature, followed by an ethanol wash. The microchip was then treated with 2 mM 4-maleimidobutyric acid N-hydroxysuccinimide ester (GMBS) in ethanol for 20 min at room temperature, followed by another ethanol wash and drying by nitrogen. Afterwards, the microchip was incubated with 100 µg/ml streptavidin in D-PBS at 4°C overnight, and a polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning Inc. Midland, MI) membrane with through openings (diameter: 400 µm) was manually attached onto the microchip surface, to which the biotinylated sgc8c aptamers were immobilised through biotin–streptavidin interaction. After peeling off the PDMS membrane, only aptamers immobilised on the microchip remained, and those modified on the membrane were removed (Fig. 3d). Finally, the microchamber was fabricated from PDMS using standard soft lithography methods (Figs. 4a and b), and then attached onto the microchip (Fig. 4c). A fabricated and packaged microfluidic device is shown in Fig. 4d.

2.3 Materials

3-MPTS, GMBS, streptavidin and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (St. Louis, MO). 5,5', 6,6'-Tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1), RPMI-1640 media, fetal bovine serum (FBS), penicillin–streptomycin (P/S, penicillin 10 000 units/ml, streptomycin 10 000 mg/ml) and D-PBS were purchased from Invitrogen (Carlsbad, CA). CCRF-CEM was obtained from the American Type Culture Collection (ATCC, Manassas, VA). The biotinylated sgc8c aptamer (biotin-5'-(triethylene glycol)₉-AT CTA ACT GCT GCG CCG CCG GGA AAA TAC TGT ACG GTT AGA-3', $K_d = 0.78$ nM) was synthesised and purified with high-performance liquid chromatography by Integrated DNA Technologies (Coralville, IA).

2.4 Experimental setup

Closed-loop temperature control of each aptamer-modified region was achieved by using the corresponding integrated temperature sensor and heater with a proportionalintegral-derivative algorithm implemented in a LabVIEW (National Instruments Corp., TX) program on a personal computer. The sensor resistances were measured by a digital multimeter (34 420 A, Agilent Technologies Inc., CA) through a 4-way mechanical switch. The microheaters were connected to a DC power supply (E3631, Agilent Technologies Inc., CA) through another 4-way mechanical switch. The microfluidic device's inlet was connected to a syringe driven by a syringe pump (KD210P, KD Scientific Inc., MA). The outlet was connected to a microcentrifuge tube in order to collect released cells. Unless indicated otherwise, all phase contrast images of cells captured on the microchip surface were taken

using an inverted epifluorescence microscope (Diaphot 300, Nikon Instruments Inc., NY) with a CCD camera (Model 190CU, Micrometrics, NH) (Fig. 5).

2.5 Experimental procedure

CCRF-CEM cells were incubated with complete culture media that consisted of RPMI-1640 media supplemented with 10% FBS and 1% P/S, and were kept at 37°C in a humidified incubator containing 5% CO₂. The cells were collected through centrifugation, resuspended at 1×10^8 cells/ml in complete culture media with 1 mg/ml BSA and kept on ice.

During the cell capture experiments, the microfluidic device was first treated with 1 mg/ml BSA in D-PBS for at least half an hour. Then, a suspension of CCRF-CEM cells was introduced into the microchamber at 1 µl/min for 2 min, followed by a D-PBS wash at 5 µl/min. An image of the microchip surface was then taken and used to show the specific capture of cells onto the aptamer-modified surface.

In the spatially selective temperature-mediated cell release experiments, the microchamber was rinsed with complete culture media with 10 µg/ml JC-1 at 5 µl/min, and a selected region on the microchip was heated using the integrated heater via closed-loop temperature control for 20 s. An image was then taken and used to show the selective release.

To test cell viability, the retrieved cells in complete culture media with 10 µg/ml JC-1 were kept at 37°C in an incubator with 5% CO₂ for 1 h, and a fluorescent image was taken with an inverted microscope (IX81, Olympus Corp., PA) equipped with a digital camera (C8484, Hamamatsu Corp., NJ).

3 Results and discussion

3.1 Temperature control

To evaluate spatially selective heating through on-chip temperature control, we present results from a numerical simulation using a three-dimensional steady-state heat transfer model in COMSOL Multiphysics® (COMSOL, Inc., Los Angeles, CA) [19]. The model considers heat conduction in the solid materials as well as forced convection due to the buffer flow (flow rate: 5 µl/min), while neglecting natural convection because of relatively small temperature changes and the small length scales of the chamber. The temperature distribution on the lower surface of the microchamber is shown in Fig. 6a. It can be seen that the temperature increase generated by each microheater is restricted to the surrounding area as desired, indicating the feasibility of temperature-mediated release of a selected group of cells.

Prior to performing the experiments, we first characterised the temperature sensors using an environmental test chamber (Model 9023, Delta Design Inc., CA) maintained at a series of temperatures which were measured with a high accuracy temperature reference probe (Model 5628, Fluke Calibration, UT) [19]. The resistance (R) of the temperature sensor varied linearly with temperature (T). The dependence was well represented by the relationship $R = R_0[1 + \alpha(T - T_0)]$, where R_0 is the sensor resistance at a reference temperature T_0 , and α is the temperature coefficient of resistance (TCR) of the sensor.

Fitting this relationship to the measurement data allowed the determination of TCR, which was used to determine the temperature in the region directly above the sensor from the measured resistance during experiments. A typical microtemperature sensor in this design had a measured resistance of 22.66 Ω at a reference temperature of 25.3°C with a TCR of $2.16 \times 10^{-3} \text{ } 1/^{\circ}\text{C}$ (Fig. 6b).

Further verification of selective heating in the aptamer-patterned regions is provided by on-chip temperature control using the integrated thin-film heaters and temperature sensors. We experimentally monitored the temperature of all four heating regions with continuous D-PBS infusion (flow rate: 5 $\mu\text{l}/\text{min}$) to simulate the cell release process, when the temperature of heating region 1 was controlled in a closed loop by the integrated heater via feedback from the temperature sensor. The temperature of region 1 increased from room temperature to 48°C rapidly ($\sim 10 \text{ s}$) and remained within approximately 0.2°C of the desired temperature setpoint (48°C) for the entire heating duration, whereas those of regions 2–4 all remained below 28°C during the whole process (Fig. 6c). This implies that the temperature change in one region does not significantly affect the temperatures in other regions, and thus enables spatially selective temperature-mediated cell release.

3.2 Specific cell capture and temperature-mediated selective cell release

We first tested the patterning of aptamers onto the surface of a microchip. Fluorescently labelled biotinylated ssDNA was used to functionalise the microchip, which was then observed under a fluorescent microscope. As shown in Fig. 7, only the area exposed to reagents, which was in the through opening region, showed bright green fluorescence, indicating the feasibility of immobilising aptamers onto design-specified regions of a microchip.

To demonstrate spatially selective cell capture, a CCRF-CEM cell suspension of 5×10^6 cell/ml with 1 mg/ml BSA was introduced into the devices with immobilised aptamers at 1 $\mu\text{l}/\text{min}$ for 2 min, followed by a D-PBS wash at 5 $\mu\text{l}/\text{min}$ for 1 min. CCRF-CEM cells only became attached to the aptamer functionalised surfaces (Fig. 8a), and not to the bare surface, confirming spatially selective cell capture. Owing to the manually performed surface modification process, aptamers were not precisely immobilised onto the surface above the microheaters. Therefore the shape of aptamer-captured CCRF-CEM cell patterns did not strictly follow the envelope of the microheaters.

To test temperature-mediated release of a selected group of cells, the cell laden chamber was then rinsed with complete culture media with 10 $\mu\text{g}/\text{ml}$ JC-1 at 5 $\mu\text{l}/\text{min}$, while the temperature in regions 2 and 3 increased to 48°C in series, by using the integrated heaters. It can be seen that only the cells within regions 2 and 3 became detached from the aptamer-surface, which may have been caused by conformational changes of the aptamer structure [19], whereas negligible cell release was observed in other regions (Fig. 8b). Next, we further increased the temperature in region 4, and observed noticeable cell release in this region, and cells in region 1 were not affected (Fig. 8c). These results indicate the success of temperature-mediated release of selected groups of cells.

To verify the reusability of the aptameric surface, another CCRF-CEM cell suspension with the same concentration was introduced into the same device at 1 $\mu\text{l}/\text{min}$ for 2 min. Following a D-PBS wash at 5 $\mu\text{l}/\text{min}$ for 1 min, similar densities of captured cells were observed in all the regions (Figs. 8a and d), implying that the microfluidic device with aptamers is reusable.

3.3 Cell viability assay

To enable downstream (e.g. tissue engineering and cell-based therapeutic) applications [9, 30], the released and retrieved cells must be viable. To evaluate cell viability, released cells in complete culture media with 10 $\mu\text{g}/\text{ml}$ JC-1 from multiple devices were collected and incubated at 37°C with 5% CO_2 for 1 h, centrifuged and resuspended in 10 μl of complete culture media. JC-1 exists as a monomer in cytoplasm exhibiting green fluorescence and it accumulates in underpolarised healthy mitochondria showing red fluorescence, whose intensity decreases along with mitochondrial depolarisation during apoptosis or death of cells [31, 32]. The released cells showed bright red fluorescence (Fig. 9), indicating that they were still viable and the temperature-mediated cell release process did not affect cell viability. In addition, to further decrease the potential cell damage, releasing cells at lower temperature is possible using appropriately selected aptamers [33].

4 Conclusion

Spatially selective capture, release and retrieval of cells on affinity surfaces are important in basic biological research and clinic diagnostics. We have developed a microfluidic device for specific cell capture and selective temperature-mediated cell release using cell-specific aptamers and integrated microheaters and temperature sensors. The device consists of a microchamber situated on a microchip with four groups of microheaters and temperature sensors. Aptamers are patterned on design-specified regions of the chip surface, and the heat generated by the microheaters is restricted to each aptamer-functionalised chip area. A moderate temperature change using one group of microheater and temperature sensor breaks the cell-aptamer binding in the selected chip area, allowing the release and retrieval of target cells from this region without detectable cell damage. The microchip is first characterised experimentally and numerically to ensure spatially selective temperature control. Further experiments show specific capture of CCRF-CEM cells using the aptamer sgc8c, followed by efficient and spatially selective temperature-mediated release. In addition, the aptamer functionalised surface is shown to be reusable and the retrieved cells are viable. The results demonstrate that our approach can potentially be used for cell purification from biological samples and selective cell retrieval for downstream analysis.

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Fig. 1.
Principle of specific cell capture and spatially selective temperature-mediated cell release
a Cell capture at room temperature
b D-PBS wash to remove non-target cells
c Temperature-mediated release of a selected group of cells

Fig. 2. Schematic of the microfluidic device for specific cell capture and spatially selective temperature-mediated selective cell release

Fig. 3.

Microchip fabrication and aptamer immobilization

a Deposition, patterning and passivation of gold heaters*b* Deposition, patterning and passivation of gold temperature sensors*c* Attachment of a PDMS membrane with through holes onto the microchip, and functionalisation of biotinylated aptamers*d* Removal of the PDMS membrane

Fig. 4.
 Microchamber fabrication and bonding
a Fabrication of SU-8 mould
b Casting of PDMS microchamber
c Bonding of the PDMS microchamber onto the microchip
d Photograph of a fabricated microfluidic device, and micrograph of the microheaters and sensors

Fig. 5.
Experimental setup for specific cell capture and spatially selective temperature-mediated cell release

Fig. 6.

On-chip temperature control

a Simulated temperature distribution on the lower surface of the microchamber, showing spatially selective heating

b Linear temperature dependence of the resistance of the temperature sensor

c Time-resolved tracking of the temperature of each heating region inside the buffer filled chamber.

Fig. 7.
Immobilisation of aptamers in design-specified regions of the chip surface

Fig. 8.

Specific cell capture and spatially selective temperature-mediated cell release

a CCRF-CEM cells were captured by the aptamer functionalised surface

b Temperature-mediated cell release in regions 2 and 3

c Temperature-mediated cell release in region 4

d Specific cell recapture on the same aptamer functionalised surfaces

Fig. 9.
Micrograph of JC-1 stained cells following cell capture and temperature-mediated cell release performed on a microfluidic device