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An ensemble of aptamers and antibodies for multivalent capture of cancer cells†

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Abstract

We developed an optimized ensemble of aptamers and antibodies that functions as a multivalent adhesive domain for the capture and isolation of cancer cells. When incorporated into a microfluidic device, the ensemble showed not only high capture efficiency, but also superior capture selectivity at a high shear stress (or high flow rate).

The enrichment and capture of cancer cells and particulates including bacteria and viruses from peripheral blood are of great importance for clinical disease diagnosis, therapy monitoring, biological studies, and drug development.^{1–3} Circulating tumor cells (CTCs) are cancer cells circulating in the peripheral blood and having the potential to spread iatrogenically into blood vessels, which is an early step in the cascade of events leading to metastasis.⁴ CTCs captured from a blood sample potentially provide an accessible source for detection, characterization, and monitoring of cancers.⁵ However, isolation of these cells is a significant technological challenge due to their rarity and low recovering rate following traditional batch purification techniques.^{2,6–10} Recent microfluidics-based CTC devices using monovalent capture agents, including antibodies^{11–13} and nucleic acid aptamers^{14,15} offer a promising platform for isolating cancer cells from complex cellular fluids with high efficiency,^{16,17} sensitivity^{18,19} and throughput.^{12,20,21} Furthermore, multivalent binding has played an important role and has increased the binding avidities by one to nine orders of magnitude.²²

In this work, we report the incorporation of multivalent binding surfaces into microfluidic devices. We hypothesized that aptamers and antibodies, due to their differences in sizes, would enable effective capture by binding to cell surface markers in a cooperative manner, leading to a higher cell capture efficiency than with antibody or aptamers alone. As shown in Fig. 1, aptamers possess a substantially smaller size (2–3 nm in diameter, 8–15 kDa

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molecular weight) than antibodies (12–15 nm in diameter, 150 kDa molecular weight), thereby enabling multivalent binding. Due to the morphology of the cell and its surface structure with nano-scale microvilli and filopodia,²³ the aptamer–antibody ensemble can increase the accessibility of receptors on cell surfaces and the frequency of interactions between the receptors and capture agents, permitting cell capture (even under high flow rates). The advantages of increased binding avidity through the multivalent effect can generate enhanced local topographic interactions between the microchannel surface and nano-scale cellular surface component,^{23,24} significantly improving the isolation/detection of rare cells, such as CTCs. Thus, we immobilized microfluidic channels with an ensemble of aptamers and antibodies to create multivalent binding surfaces (Fig. 1).

To confirm the multivalent binding, we measured the cell capturing behaviours of the aptamer–antibody ensemble in a microfluidic device that contains micro-pillar arrays (ESI†). We isolated human leukaemia cells (CCRF-CEM) using an ensemble of sgc8 aptamers and anti-PTK7 (protein tyrosine kinase-7) antibodies. Both aptamers and antibodies exhibited strong binding to PTK7 that is over-expressed in many human cancer cells (including CCRF-CEM cells).²⁵ We immobilized both antibodies and aptamers onto microfluidic channels to form a multivalent affinity surface. The affinity surface inside the device was prepared using an established method of avidin and biotin reactions.¹⁵ We strategically mixed biotinylated anti-PTK7 with biotinylated sgc8 aptamers in a certain ratio and introduced them to an avidin-immobilized surface. The specific binding of the aptamers and target CCRF-CEM cells was verified using flow cytometry (ESI†). As shown in Fig. 2a and b, the ensemble of antibodies and aptamers enhanced the capture efficiency of CEM cells compared with antibodies alone. The cell capture efficiency was calculated by dividing the number of the target cells captured by the number of target cells introduced into the device.

One important feature of the antibody–aptamer ensemble is its versatility. Specifically, the density of the capture reagents on a surface can be easily tuned by varying the ratio of antibody-to-aptamer. Microfluidic devices containing eight parallel channels with a micropillar array inside were used for proof-of-concept studies. The ratio of the antibody to the aptamer was studied first by comparing the capture efficiency of different ratios of anti-PTK7 and sgc8 aptamer immobilized onto the device. Mixtures containing different ratios were prepared (ESI†), and each mixture was introduced into the device and immobilized on the channel surfaces before cell capture experiments. The capture efficiency of each mixture at different flow rates ranging from $1.0 \mu\text{L s}^{-1}$ to $3.0 \mu\text{L s}^{-1}$ (corresponding shear stress from 0.32 dyn cm^{-2} to 0.96 dyn cm^{-2} on the wall) was compared (Fig. 2c). Under the flow rates studied, the ratio of 1 : 300 containing $5.0 \text{ mg } \mu\text{L}^{-1}$ antibodies and 10 mM aptamers showed the highest capture efficiency among all conditions. We hypothesize that a number of aptamers are needed to accommodate each antibody molecule due to their difference in size, and their collaborative interactions with receptors on cell surfaces resulted in higher capture efficiency. Under such a ratio, the aptamers and antibodies immobilized on the surface likely worked in the most efficient, cooperative way that produced the highest capture efficiency. We used this ratio for the following cell capture studies.

We conducted cell capture assays under controlled dynamic flow conditions in the device to determine the minimum time required to achieve cell capture. We examined the cell capture performances for 10^3 mL^{-1} target CEM cells that were mixed with 10^6 mL^{-1} human lymphoma Ramos cells (as a negative control) in PBS buffer at different flow rates. The capture efficiency of CEM cells decreased as the flow rate increased (Fig. 2d). The purity of captured cells had no statistically significant difference between adjacent flow rates ($\text{ESI}^\dagger$). The cell purity was calculated by dividing the number of the target cells captured by the number of total cells captured (i.e., both target and control cells). Flow rates under $2.0 \mu\text{L s}^{-1}$ enabled both high capture efficiency and cell purity using the antibody–aptamer ensemble. Therefore, the optimal flow rate of $2.0 \mu\text{L s}^{-1}$ for the antibody–aptamer ensemble was obtained.

Control experiments using identical devices and conditions with antibodies or aptamers alone were also conducted ($\text{ESI}^\dagger$). As we theorized, higher capture efficiencies and purities were obtained using the antibody–aptamer ensemble than using antibodies or aptamers alone, especially at a high flow rate. As shown in Fig. 3, the capture efficiency of CEM cells using anti-PTK7 alone is comparable with that using the sgc8 aptamer alone, but both are significantly less than the antibody–aptamer ensemble, especially at a higher flow rate. This suggests that the device with the ensemble is able to capture cells at a much higher shear stress than a device immobilized with monovalent agents. This finding is significant because high capture efficiency is essential for isolating rare cells in peripheral blood. Moreover, devices reported to date using monovalent antibodies or aptamers showed relatively low capture efficiency at higher shear stress.^{9,11,26} Also note that the antibody–aptamer ensemble resulted in significantly higher cell purity than the antibody alone or the aptamer alone, at all flow rates, because more target cells are captured by the ensemble. Therefore, this antibody–aptamer ensemble offers an approach with enhanced capture efficiency and cell purity.

We also demonstrated the use of the ensemble for the capture of human leukemia cells in blood. To test the cell capture performance of the ensemble at low concentrations, we prepared a cell suspension ranging from 10 to 10^3 cells per mL of CEM cells in both PBS buffer and human whole blood. The results showed that the ensemble has a capture efficiency of 490% for all cases at the flow rate of $2.0 \mu\text{L s}^{-1}$ (Fig. 4). The antibody–aptamer ensemble generated a multivalent binding effect on the surface of microchannels, significantly enhancing the interactions between cell surfaces and capturing molecules on the device and leading to higher capture efficiency. In addition, with a flow rate of $2.0 \mu\text{L s}^{-1}$ (7.2 mL h^{-1}), 1 mL of blood sample can be processed within 15 min, which offers sufficient throughput for clinical applications. The system offers more benefit at higher flow rates and maintains a target cell capture efficiency of 460% for all flow rates up to $3.0 \mu\text{L s}^{-1}$.

Although we used pre-stained cancer cells for the demonstration of this antibody–aptamer ensemble, it is possible to adapt it to a real world sample. Without pre-staining, tumor cells are still captured on the device. Immunocytochemistry is then employed to identify and validate tumor cells. For example, fluorescently labelled anti-cytokeratin, anti-CD45, and

nucleus-staining dye DAPI are used to validate CTCs in our previous work²⁸ and FDA-approved methods.^{6,8}

We have demonstrated the enhanced capture efficiency of cancer cells in unprocessed peripheral blood using an ensemble of aptamers and antibodies in a microfluidic device. The unique topology of the multivalent affinity agents on the channel surface resulted in high-performance cell isolation at a high flow rate. This antibody–aptamer ensemble enabled 490% capture efficiency at a flow rate of $2.0 \mu\text{L s}^{-1}$, which is much higher than those of the reported studies to date.^{11,27} The results show that the ensemble of aptamers and antibodies has a potential to isolate and enumerate CTCs for clinical applications such as cancer diagnosis, prognosis, and monitoring the progress of therapeutic treatment, as well as for subsequent molecular analysis, which can facilitate the development of effective chemotherapeutic drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Schematic showing an ensemble of antibodies and aptamers: multiple receptors on the cell membrane can bind strongly via cooperative, multivalent interactions to the channel surfaces immobilized with aptamers and antibodies. The drawing is not to scale.

Fig. 2.

(a, b) Representative images of the target CEM cells (green) and control Ramos cells (red) captured using (a) an antibody–aptamer ensemble or (b) anti-PTK7 alone. The effects of antibody-to-aptamer ratio (c) and the flow rate (d) on the cell capture efficiency.

Fig. 3.

Comparison of the antibody–aptamer ensemble with antibody or aptamer alone for cell capture: (a) capture efficiency of 10^3 mL^{-1} CEM cells (target) and 10^6 mL^{-1} Ramos cells (negative control) in PBS buffer; (b) capture purity under the same conditions. Error bars represent one standard deviation ($n = 3$).

Fig. 4.

Calibration plot of cancer cell capture from whole blood and a buffer solution containing different cell concentrations (flow rate at $2.0 \mu\text{L s}^{-1}$); solid lines represent linear fitting. Error bars represent one standard deviation ($n = 3$). The inset is a representative image of captured CEM cells (DiI+, DAPI+) from whole blood; the DAPI+ cells (blue only) are non-specifically captured white blood cells.