Tumor cell capture patterns around aptamer-immobilized microposts in microfluidic devices

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Circulating tumor cells (CTCs) have shown potential for cancer diagnosis and prognosis. Affinity-based CTC isolation methods have been proved to be efficient for CTC detection in clinical blood samples. One of the popular choices for affinity-based CTC isolation is to immobilize capture agents onto an array of microposts in microchannels, providing high CTC capture efficiency due to enhanced interactions between tumor cells and capture agents on the microposts. However, how the cells interact with microposts under different flow conditions and what kind of capture pattern results from the interactions have not been fully investigated; a full understanding of these interactions will help to design devices and choose experimental conditions for higher CTC capture efficiency. We report our study on their interaction and cell distribution patterns around microposts under different flow conditions. Human acute lymphoblastic leukemia cells (CCRF-CEM) were used as target cancer cells in this study, while the Sgc8 aptamer that has specific binding with CCRF-CEM cells was employed as a capture agent. We investigated the effects of flow rates and micropost shapes on the cell capture efficiency and capture patterns on microposts. While a higher flow rate decreased cell capture efficiency, we found that the capture pattern around microposts also changed, with much more cells captured in the front half of a micropost than at the back half. We also found the ratio of cells captured on microposts to the cells captured by both microposts and channel walls increased as a function of the flow rate. We compared circular microposts with an elliptical shape and found that the geometry affected the capture distribution around microposts. In addition, we have developed a theoretical model to simulate the interactions between tumor cells and micropost surfaces, and the simulation results are in agreement with our experimental observation. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.5000707>]

I. INTRODUCTION

Circulating tumor cells (CTCs) are cancer cells that are shed from a primary tumor and enter the blood stream of patients, and they have great potential for cancer diagnosis and prognosis.^{1,2} However, the number of CTCs in patient's peripheral blood is extremely low, normally a few CTCs out of a billion of healthy blood cells.³ Therefore, the detection and characterization of CTCs have been a challenge.

Microfluidics is one of the techniques that offer advantages for CTC enrichment and isolation, including higher detection sensitivity and lower cost.⁴ Through these years, various

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microfluidic devices have been developed for CTC isolation. They can be divided into two major categories: physical-property-based and affinity-based CTC isolation. Physical-property-based isolation makes use of the CTC physical properties, such as cell sizes, elasticity, and electro-polarity.^{5–18} Although these methods have shown merits for CTC isolation, they must address a well-known fact that CTCs are very heterogeneous including their physical properties.^{19–21} For instance, the size of CTCs (typically 10–20 μm) could overlap with the size of some leukocytes (typically 6–20 μm).²² On the other hand, affinity-based methods rely on the specific interactions between biomarkers on CTCs and capture agents (e.g., antibody). An epithelial cell adhesion molecule (EpCAM) is one of the most common biomarkers used for CTC isolation. The first report of using microfluidics for isolating CTCs was based on EpCAM-coated microposts,²³ and many other EpCAM-based microfluidic devices have been employed for CTC isolation later on.^{24–32}

While antibodies are widely used for CTC isolation, aptamers have also been explored as alternatives to capture tumor cells.^{33–40} Aptamers are single-stranded oligonucleotides that can specifically bind to target cells by folding into unique secondary or tertiary structures. Our previous work demonstrated that a microfluidic device with aptamer-coated microposts showed a tumor cell isolation efficiency of 95% with a cell purity of 81%.³⁶ To improve the CTC isolation performance of a microfluidic device, the structure of the device is very important. Micropost-based devices have been investigated by several research groups and proved to be highly efficient with various designs and optimization. Toner and his colleagues optimized the hydrodynamic efficiency of their device through changing the distance between microposts.²³ Dickson *et al.* randomized the alignment of different arrays of microposts to improve the probability of CTC capture.⁴¹ Kirby's research group altered the shift distance between different columns of microposts to increase encounter frequency between CTCs and microposts.⁴² Nagrath and others designed microposts with varying shear rates to achieve high throughput and capture efficiency.²⁷ Liu *et al.* used microposts with a triangle shape as structures for cell separation.⁴³ Guttman and his colleagues fabricated tilted posts to enhance cell capture probability.⁴⁴ Howard *et al.* created rotated concave-shaped microposts for rare cell capture.⁴⁵ Toner and others employed nanoporous arrays to mimic microposts to increase cell interception probability.⁴⁶ Buřík *et al.* compared microposts with different sizes to look for optimal micropost dimensions.⁴⁷ When optimizing the designs of these devices, researchers have focused on certain parameters, including the flow rate, shear stress, micropost radius, and distance between microposts, and tried to find the empirical correlation between these parameters and the CTC capture efficiency. The actual interaction between CTCs and microposts and how the interaction changes under these parameters are often not fully studied even though they directly affect the device performance.

In this work, we report our studies on the interaction between cancer cells and aptamer-immobilized microposts in a microfluidic device. We evaluate the distribution pattern of tumor cells captured around microposts and study the effects of the flow rate on the distribution pattern. The cell capture mechanism, which is closely related to the cell capture efficiency of the microfluidic device, is discussed. In addition, a theoretical model is used to simulate the interaction between aptamers and the receptors on tumor cells. Modeling sheds light on the aptamer-protein conjugation, tumor cell motion, and cell capture process. The insight obtained could help to design future microfluidic devices for CTC isolation. Note that the methodology and knowledge about the interaction between tumor cells and capture agents in a flow environment are not limited to micropost-based devices; they can be extended for studying the interactions between cells and other micro-features in a microfluidic device.

II. EXPERIMENTAL METHODS

A. Reagents and materials

Sylgard 184 reagents, ethanol, Dulbecco's phosphate buffered saline (DPBS) with 0.9 mM calcium chloride and 0.49 mM magnesium chloride, DPBS without calcium chloride and magnesium chloride, DPBS containing 1% bovine serum albumin (BSA), and Tween 20 were purchased from Thermo Fisher Scientific (Hampton, NH). Aptamer sgc8 was synthesized in house, and its

sequence is 5'-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GAT TTT TTTTTT-3'-biotin.³⁶

The CCRF-CEM cell line (human acute lymphoblastic leukemia cells) was purchased from American Type Culture Collection (ATCC, Manassas, VA). The cells were cultured in RPMI medium 1640 (ATCC) supplemented with 10% fetal bovine serum (FBS; GIBCO of Thermo Fisher) and 100 units/ml penicillin-streptomycin (Cellgro, Manassas, VA) using an incubation condition of 37 °C with 5% CO₂.

B. Device fabrication

As shown in Fig. 1(a), our device consists of four pairs of microchannels connected to one inlet and one outlet.³⁶ Each microchannel is 2.1 mm wide, 60 μ m deep, and 45 mm long, and it contains an array of microposts. The microposts have the same height as the depth of microchannels. Two kinds of microposts, in a circular or elliptical shape, were studied, as shown in Fig. 1(b). A circular micropost has a diameter of 90 μ m, while an elliptical micropost has a

FIG. 1. (a) Picture of a micropost-based microfluidic device consisting of 1 inlet, 8 microchannels, and 1 outlet. [(b), left] Layout of circular microposts in the microchannel. The distance between the centers of two microposts in each column is 150 μ m; there is a 50- μ m shift in the position of microposts from one column to the next; thus, three columns are considered as a period. [(b), right] An array of elliptical microposts in a microchannel; they are arranged in a similar way as in circular microposts. (c) Surface functionalization of microposts and microchannels by physical adsorption of avidin, followed by conjugation with biotinylated sgc8 aptamers through biotin-avidin chemistry. When a cancer cell flows through the microchannel, it can be captured through interactions between the surface receptors on the cancer cell and the aptamer. (d) Image of microposts with a set of tiles marked. Each tile corresponds to one micropost inside. The exploded view of one micropost is shown on the right, indicating the distance between the center of a capture cell and the center of the micropost. When the distance is larger than the sum of the cell radius and the micropost radius, the cell is not captured by the micropost.

major axis of 120 μm and a minor axis of 90 μm . The distance between the centers of two microposts, either vertically or horizontally, is 150 μm , leaving a 60- μm gap between the edges of two microposts. There is a 50- μm shift for microposts between two columns; this shift design is inspired by the concept of deterministic lateral displacement (DLD) when cells flow through microposts.^{48,49} There are a total of 32 274 microposts in the device.

The devices were fabricated using soft lithography.⁵⁰ In brief, the layout of the device was designed using AutoCAD, and then a dark-field transparency film was printed by a vendor (CAD/Arts Services, Inc., OR). The transparency film was taped onto a 4 in. $\times$ 4 in. glass plate, which was used as a photomask. To create a silicon master, a silicon wafer was first coated with SU8 negative photoresist (MicroChem, MA). The features in the photomask were then transferred to the SU8 layer by photolithography. Before use, the silicon master was treated with trichloro(1H,1H,2H,2H-perfluorooctyl)silane to help the device release later on. The polydimethylsiloxane (PDMS) pre-polymer was prepared by mixing the base and the curing agent at a ratio of 10:1, and the resultant mixture is poured onto the silicon master after bubble removal. After curing at 55 $^{\circ}\text{C}$ for 4 hours, the PDMS substrate was peeled off the silicon master and trimmed into the size of a microscope slide. The PDMS substrate was then bonded directly with a glass microscope slide.

Before use, the device was functionalized with aptamers using two steps as shown in Fig. 1(c). First, avidin was immobilized onto both microposts and microchannels through physical adsorption. The second step was to flow a biotinylated aptamer solution into the device so that the surfaces were immobilized with aptamers through biotin-avidin conjugation chemistry.

C. Experimental procedure

A syringe pump (KD scientific) with a 1-ml syringe was connected to the inlet of the device via polymer tubing. The outlet was connected to a waste reservoir. Pure ethanol solution of 300 μl (about 3 times of the inner volume of the device) was first pumped into the device to wet all channels, followed by washing with 300 μl of DPBS. The device was then functionalized with the sgc8 aptamer as discussed above. Before introducing tumor cells, the microchannels of the device were passivated with DPBS containing 1% BSA and 0.05% Tween 20 to reduce non-specific binding. CCRF-CEM cells were suspended in DPBS at a concentration of 5.0×10^5 cells/ml, and 300 μl of this cell sample was pumped through the device. A tiny magnetic bar was placed inside the syringe, being stirred to prevent cell settling during pumping. The microfluidic device was placed on the stage of an Olympus IX71 microscope and monitored using a scientific-grade CCD camera (Hamamatsu C4742-80-12AG).

D. Image processing

Since we need to enumerate numerous cells captured around tens of thousands of microposts, we developed an image processing program using Gaussian blur and Hough transform as detailed in the [supplementary material](#) and Fig. S1.⁵¹ The program could identify the position of each micropost as well as cancer cells around it. A score-based evaluation was used to ensure that the circle Hough transform correctly detects cells. The evaluation score is based on absolute brightness of the center of a cell, relative brightness of the center to other portions, variation in radial brightness from the center outward, brightness of the cell edges, and the brightness symmetry. Each score criterion was assigned with a weight factor that was fine-tuned by manually examining a sample of the processed images and verifying its correct identification of true cells, as well as false positives and false negatives. The following formula is used for tuning of weight factors:

$$\text{Accuracy (\%)} = \frac{\text{Number of cells detected} - \text{false positives}}{\text{Number of true cells}} * 100\%. \quad (1)$$

In addition, an internal accuracy optimization was employed for each image to improve accuracy further. The optimization used an internal evaluation based on the knowledge that

cells could not enter a micropost, and thus, any cells detected within a micropost were false positives. As a part of the automated processing, the analysis was re-iterated internally, with proportional adjustments to the parameters until a threshold number of false positives were detected. After optimization, the program could achieve a cell detection accuracy as high as 92.1%. The false positive rate reduced to 5%.

To initiate the imaging process, a set of tiles was created with each tile corresponding to the area surrounding the micropost as shown in Fig. 1(d). The positions of cells in each tile were recorded with respect to the position of the micropost. Whether a cancer cell was captured by the micropost was determined by the distance between the cell's center and the micropost's center. The distance should be not more than the sum of the micropost radius, R_0 , and the cell radius, R , as shown in the exploded view of a micropost in Fig. 2. Only when the cell center was located within the designated zone, the cancer cell was considered as captured by the micropost. The cells detected with a distance larger than $R_0 + R$ were considered as captured by the channel walls.

III. THEORETICAL MODEL

Since we did not observe significant deformation of cancer cells in this study (because of the low Reynolds number in microchannels that will be discussed later), we modeled a cell as a rigid sphere. Consider a cancer cell flowing over a flat surface; its movement is likely affected by several factors: the free flow velocity, the hydrodynamic force from the ambient fluid, the exerted force from other sources, and the thermal fluctuation. As a result, the motion of the cell is described by the Langevin equation as previously discussed⁵²

$$\mathbf{U} = \mathbf{U}_\infty + \mathbf{M}(\mathbf{F}^S + \mathbf{F}^B) + k_B T \nabla \mathbf{M} + \mathbf{g}_t, \quad (2)$$

where $\mathbf{U}$ is the six-dimensional velocity vector at the cell center, including three translational components and three rotational components, $\mathbf{U}_\infty$ is the velocity vector of the free flow at the cell center, $\mathbf{M}$ is the mobility matrix, $\mathbf{F}^S$ is the shear force vector including three force

FIG. 2. Theoretical model of the interactions between a cancer cell and aptamers immobilized on a micropost. The cell is modeled as a rigid sphere, while the receptors (PTK7) are modeled as the strings distributed on the cell surface with a length r_0 . Each PTK7 receptor forms a half sphere on the cell surface, which is called a capture zone. When an aptamer appears within the capture zone, an aptamer-PTK7 bond forms with certain probability as discussed in the text.

components and three components of torque, $\mathbf{F}^B$ is the bond force acting on the cell, k_B is the Boltzmann constant, T is the ambient temperature, and g_t is the Gaussian white noise at time t .

In a non-dimensional form, the radius of the cell, R , is used as a length scale. The inverse of the shear rate, $\frac{1}{\dot{\gamma}}$, is the timescale; $6\pi\mu R^2\dot{\gamma}$ is the force scale, where μ is the dynamic viscosity of the buffer solution. Thus, the non-dimensional Langevin equation is depicted as

$$\Delta\mathbf{X} = Pe[\mathbf{U}_\infty + \mathbf{M}(\mathbf{F}^S + \mathbf{F}^B)]\Delta t + \nabla\mathbf{M}\Delta t + \mathbf{g}(\Delta t) + O(\Delta t^2), \quad (3)$$

where $\Delta\mathbf{X}$ is the displacement increment of the cell at a time step Δt and $O(\Delta t^2)$ is the high order residual term that is negligible. Note that the dimensionless Péclet Number, $Pe = 6\pi\mu R^3\dot{\gamma}/k_B T$, which shows the importance of the convective transport rate over the diffusive transport rate, ranges from 1.6×10^3 to 1.2×10^6 in this work.

The key parameter in Eq. (3) that affects the cell movement is the bond force $\mathbf{F}^B$. The bond force is caused by the conjugation between the sgc8 aptamers immobilized on the micropost surface and the PTK7 receptors on the CCRF-CEM cell. PTK7 is a protein known to interact with the sgc8 aptamer to form a specific bond.⁵³ We employed bond dynamics to simulate the interaction between CCRF-CEM cells and sgc8 aptamers. As shown in Fig. 2, each PTK7 receptor is modeled as a string on the surface of the cancer cell, and N_r represents the density of receptors on the cell surface. For each individual PTK7 receptor, there is a capture zone with a radius r_0 . Aptamers are modeled as an array of points, which are evenly distributed on the micropost surface, with aptamer-aptamer distance l . When the PTK7-aptamer distance is smaller than r_0 , a bond is likely formed. The bond is modeled as a spring, with a spring constant, K , producing an elastic bond force. The probability for the formation of a bond, P_{on} , is expressed as⁵⁴

$$P_{on} = 1 - e^{-k_a\Delta t}, \quad (4)$$

where k_a is the association rate of the PTK7-aptamer bond and Δt is a unit time step. During the interaction between a cancer cell and a micropost surface, certain probability exists that the bond breaks due to external forces acting on them. The bond-breaking probability for an existing bond, P_{off} , is given by⁵⁴

$$P_{off} = 1 - e^{-k_d\Delta t}, \quad (5)$$

where k_d is the dissociation rate of the binding sites, and it is sensitive to the external force acting on the bond. The relationship between k_d and the external force is given as⁵⁴

$$k_d = k_0 e^{\frac{F_e}{F_t}}, \quad (6)$$

where k_0 is the dissociation rate without an external force, F_e is the external force acting on the bond, and F_t is the thermal fluctuation force that can be expressed as⁵⁴

$$F_t = \frac{k_B T}{x_c}, \quad (7)$$

where x_c is the reactive compliance.

To employ these equations to model the interactions between cells and aptamers on a micropost, we used several parameters from either our own measurement or the literature. The average radius of CCRF-CEM cells was measured from cells' optical images using ImageJ (available from <http://www.rsweb.nih.gov/ij/>), giving their radius $R = 7.0 \pm 1.5 \mu\text{m}$. The diameter of the sgc8 aptamer is about 3 nm (Ref. 55), the length of PTK7 is about 13 nm,⁵⁶ and the capture zone radius, r_0 , was thus estimated to be 16 nm, the sum of PTK7 and the aptamer length. The PTK7 density on the cell surface is given as $N_r = 1300/\mu\text{m}^2$.⁵⁷ The aptamer-aptamer distance, l , was calculated to be 28.2 nm as explained in the supporting material. An

unstressed dissociation rate constant for aptamer-PTK7 binding is $k_o = 0.04$ Hz.⁵⁸ The association rate was estimated to be 0.5 Hz (see supporting material). The compliance distance is $x_c = 2 \times 10^{-11}$ m.⁵⁹ The spring constant of the aptamer-PTK7 was estimated to be 0.075 N/m.⁶⁰ In addition, all experiments were conducted at room temperature, $T = 297$ K. The flow rate was from 0.2 μ l/s to 1.5 μ l/s, corresponding to the flow velocity in the range of 0.45 mm/s to 3.38 mm/s. The buffer (DPBS) viscosity is estimated as the water viscosity which is 10^{-3} Pa $\cdot$ s. The respective buffer density is estimated to be 1 g/ml. These parameters, their values, and sources are compiled in Table I.

IV. RESULTS AND DISCUSSION

A. Effects of the flow rate

To establish a baseline, we studied the effects of the flow rate on the cell capture efficiency of the device. Homogeneous samples containing CCRF-CEM cells at 5.0×10^5 cells/ml were infused into the device using a syringe pump. The device was immobilized with sgc8 aptamers as discussed in Sec. II. Using the dimensions of the device and fluid properties, the Reynolds number in the microchannel was calculated to be from 0.048 to 0.360 for the flow rate from 0.2 μ l/s to 1.5 μ l/s. The Reynolds Number is defined as $Re = \frac{\rho u (2R_0)}{\mu}$, with u being the average flow velocity in the microchannel. At these low Reynolds numbers, fluid flow should be laminar and no flow separation is expected to occur at the rear edge of a micropost.⁶¹ This laminar flow contour around the micropost should provide efficient interactions between cells and aptamers on the microposts in the device. Nevertheless, with the increasing flow rate, the total number of cells captured on both microposts and channel surfaces decreases as shown in Fig. 3(a). Note that only cells in one region (containing 504 microposts) of the device were counted, and the same region was used in this set of analysis. This region is in the middle between the inlet and the outlet, and it was chosen because (1) it contains sufficient microposts for statistical analysis and (2) the total number of cells captured in the region is possible for manual enumeration, which is necessary for checking the accuracy of the automatic image processing method discussed above. It should also be pointed out that cell capture efficiency is not evenly distributed along a microchannel, generally highest at the inlet and lowest at the outlet as reported previously.⁶²

Representative images of a few posts with cells captured at different flow rates are shown in Fig. 3(b). There are two reasons behind the trend observed: (1) as the flow rate increases, the interaction time between cells and aptamer-coated surfaces is reduced, leading to lower bonding probability between them; (2) the shear force in the flow increases with the increasing flow rate, leading to higher bond-breaking probability between the cells and the aptamers.

TABLE I. Parameters used in the theoretical modeling.

Parameter	Meaning	Value
R	Cell radius	7 ± 1.5 μ m
$\dot{\gamma}$	Shear rate	8 – 150 Hz
T	Temperature	297 K
μ	Dynamic viscosity	10^{-3} Pa $\cdot$ s
K	Elastic constant	0.075 N/m
k_a	Association rate	0.5 Hz
k_o	Unstressed dissociation rate	0.04 Hz
r_0	Capture radius	16 nm
l	Aptamer-Aptamer distance	28.2 nm
x_c	Reactive compliance	0.02 nm
N_r	PTK7 surface density	1300/ μ m ²
R_0	Micropost diameter	45 μ m
ρ	Buffer density	1 g/ml

FIG. 3. (a) The total number of cells captured in one region of the device at different flow rates. (b) Images showing cell capture patterns around microposts at different flow rates. (c) The ratio of cells captured by microposts to the total number of cells captured by both microposts and microchannel surfaces as a function of the flow rate. (d) Schematic illustrating the momentum interception effect of the micropost.

B. Cell distribution between microposts and channel walls

We compared the distribution of cells captured by microposts and by channel walls and studied the effect of the flow rate on the distribution. Figure 3(c) shows the ratio of the number of cells captured around the microposts to the total number of cells captured by the device (i.e., by both microposts and channel walls) as a function of the flow rate. The increase in the ratio with the increasing flow rate can be explained by two phenomena: momentum interception and gravity effect. The momentum interception effect is significant for this design since microposts function as obstacles in the flow. Fluid dynamic analysis has shown that a higher Reynolds number (due to a higher flow rate) increases the cell-micropost encounter efficiency.⁶³ The encounter efficiency, η is defined as $\eta = \frac{b}{2R_0}$, where R_0 is the radius of the micropost and b is the upstream span of the cells that encounter the micropost, as shown in Fig. 3(d). The encounter efficiency represents the fraction of intercepted flow in a bulk flow. Considering the situation where cells are evenly distributed in the bulk flow, η thus reflects the encounter probability of a cell to a micropost. Spielman showed in 1977 that⁶³

$$\eta = \frac{R_0^2}{2 - \ln(Re)} \quad \text{for } Re < 1. \quad (8)$$

Equation (8) clearly indicates that an increasing flow rate, thus an increasing Reynolds number, results in a higher cell-micropost encounter efficiency. In other words, the momentum interception effect is more significant at a higher flow rate, producing more cell capture on microposts.

The second factor for explaining the increase in the ratio with an increasing flow rate is the gravity effect. Due to the density difference between CCRF-CEM cancer cells and the infused medium, the suspended cells slowly settle down as they flow through the microchannel. The faster the cells move, the less time for them to descend while having more opportunities to interact with microposts before reaching the device bottom. As a result, cells are less likely to be captured by channel walls.

C. Cell distribution pattern around microposts

It is reasonable to assume that more cells are captured on the front half of a micropost than on its back half because cells collide directly with the front half of the micropost. Our recorded videos suggest that the cells captured on the back half are mostly from the front half, i.e., unstopped cells rotating around the micropost from the initial contact at the front half before being captured on the back half. We also found that the cell distribution pattern around microposts varies with different flow rates; as flow velocity increases, the ratio of the number of cells captured on the front half of the micropost to the number of cells captured on the back half (shortened as “front-to-back”) also increases.

Figure 4(a) shows the ratio of front-to-back as a function of the flow rate for devices containing circular microposts. The ratio increases slowly and then speeds up with an increasing flow rate. The ratio is as low as 4.85 when the flow rate is $0.2 \mu\text{L/s}$ and is as high as 13.01 when the flow rate reaches $1.5 \mu\text{L/s}$. As shown in Fig. 3(a), the overall cell capture efficiency decreases with the increasing flow rate. Therefore, the result in Fig. 4(a), as well as the images in Fig. 3(b), shows that the cell capture capability of the front-half of microposts decreases much slower than that of the back-half of microposts as the flow rate increases. This phenomenon can be explained by two possible reasons. One is the momentum interception as discussed above. As suggested by Eq. (8), the cell-post encounter efficiency increases as the Reynolds number increases.

FIG. 4. (a) The ratio of the number of cells captured on the front half of the micropost to the number of cells captured on the back half as a function of the flow rate for a device containing circular microposts. (b) The cell capture pattern around circular microposts at a flow rate of $0.5 \mu\text{L/s}$ (blue line) and at a flow rate of $1.2 \mu\text{L/s}$ (red line). The concentric circles represent the scale of the capture ratio (see the text for the definition), from 0 to 0.04. The angles around the micropost are marked on the outside of the largest circle, with the flow stagnation point at 0° . (c) The front-to-back ratio as a function of the flow rate for a device containing elliptical microposts. (d) The dislodge force distribution around a circular micropost at flow rates of $0.5 \mu\text{L/s}$ (blue line) and $1.2 \mu\text{L/s}$ (red line). The horizontal dashed line corresponds to the twice aptamer-PTK7 bond strength.

The second reason for the increased ratio of front-to-back is the presence of the “stagnation region.” There is a stagnation point at the center of the front half of each micropost, where the flow velocity is zero.⁶⁴ Around the stagnation point, there is a low speed region (“stagnation region,” Fig. S2) where the velocity magnitude is close to zero ($<10^{-6}$ m/s). The capture probability in this stagnation region is similar for different flow rates. However, the capture probability in other regions of the micropost is significantly reduced with the increasing flow rate. This significant increase in the ratio of front-to-back in the stagnation region from a low flow rate (0.5 μ l/s) to a high flow rate (1.2 μ l/s) is clearly demonstrated in Fig. 4(b), which will be explained in detail later on. The bulge in the cell distribution diagram between 30° and 330° at 1.2 μ l/s is much bigger than that at 0.5 μ l/s.

We compared circular microposts with elliptical microposts because of their difference in the overall shape and possible effects on the front-to-back ratio. Figure 4(c) shows the ratio of front-to-back as a function of the flow rate for a device containing elliptical microposts. Again, the front-to-back ratio increases with an increasing flow rate; this trend is similar to that in a device containing circular microposts. However, for every flow rate, the front-to-back ratio of elliptical microposts (i.e., the values in the y-axis) is smaller than that of circular microposts; the ratio already reaches a plateau at 1.2 μ l/s. A possible explanation is that a long interaction distance between a cell and the surface over the top and bottom of an elliptical micropost [Fig. 1(b)] results in more capture probability on the back half of an elliptical micropost than that of a circular micropost. It will be more understandable after the cell capture mechanism is discussed in Sec. IV D.

The effects of the flow rate on the front-to-back ratio are further illustrated in the cell distribution pattern around a micropost, as shown in Fig. 4(b). This pattern was created by using our image processing program as described in Sec. II. This pattern was obtained by averaging about 96 822 microposts in three devices. During the process, we considered every 5 degrees as a unit zone (equivalent to 4.54 μ m around a micropost), and the circumference of a micropost is divided into 72 zones. The degree of the unit zone was chosen after studying 1°, 3°, and 5° and then verifying the counting accuracy of the program by comparing it with manual counting. For easy visualization, we used concentric circles in Fig. 4(b) to represent the capture ratio, which is defined as a ratio of the cells captured in each unit zone over the total number of cells captured by all zones in the devices. It is understandable that the cell distributions on the top half and on the bottom half are essentially symmetric. As expected, more cell capture took place at the stagnation point (0°), indicated by a peak at both flow rates. More cell capture took place in the stagnation region, indicated by the bulge in the front as explained above. For a flow rate of 0.5 μ l/s, the capture ratio is essentially the same (about 0.02) from 5° to 75° and then starts to decrease slowly. For a flow rate of 1.2 μ l/s, the capture ratio is high from 10° to 45° (about 0.03) and then starts to decrease rapidly. If we compare these two flow rates, the capture ratio in the front region (45° to 315°) increases with the increasing flow rate, while the capture ratio in the other region decreases with the increasing flow rate. For the same flow rate, we compared the cell capture pattern in different channel regions. As shown in Fig. S3, the cell capture pattern around the micropost in the first half of the device (near the infusion inlet) and that in the second half (near the outlet) were very similar. This result was expected because the flow velocity pattern was uniform along the microchannel.

We conducted hydrodynamic analysis around the micropost for better understanding the cell capture pattern we obtained. Considering a stationary cell attached to a micropost, the dislodge force the cell experiences is mainly from the ambient flow. The dislodge force can be expressed as⁶⁵

$$F_d = \frac{32}{3} \pi \mu R U, \quad (9)$$

where U is the velocity of ambient flow. Note that F_d is different from the shear force F^S given in Eq. (2). F^S is the shear force the cell experiences when it is still moving around the micropost, whereas F_d is for a stationary cell attached to the micropost. Using Eq. (9), we obtained

the distribution of dislodge force in different regions of the micropost as shown in Fig. 4(d). The horizontal line represents twice the value reported for the PTK7-sgc8 bond strength (92 pN).⁶⁰ For the flow rate of 0.5 $\mu\text{l/s}$, the hydrodynamic force is smaller than 92 pN until the angle reaches 78°. For the flow rate of 1.2 $\mu\text{l/s}$, the hydrodynamic force is smaller than 92 pN until the angle reaches 43°. These angles roughly agree with 75° and 45° for two flow rates when the capture ratio starts to decrease as discussed above. The rapid decrease in the capture ratio after 45° for the flow rate of 1.2 $\mu\text{l/s}$ in Fig. 4(b) can be explained by the rapid increase in the dislodge force for the same flow rate in Fig. 4(d).

We also observed a peak in the cell capture ratio at 90° (and 270°). Although the fact that the peak for the flow at 1.2 $\mu\text{l/s}$ is smaller than the peak for the flow at 0.5 $\mu\text{l/s}$ makes sense, the presence of peaks seems to disagree with the fluid mechanics theory, which says that the velocity and shear force at 90 degrees are the highest. To understand the observation, we must consider the interaction between a cancer cell and the aptamer-functionalized micropost as shown in the video. When a cell touches the micropost, it will move and rotate around the micropost. Sgc8-PTK7 bonds may form or break during this process, and the movement of the cell is altered. Since the formation or breaking of sgc8-PTK7 bonds can be random as discussed in Sec. IV D, a cell stops at any unit zone behind its initial position (including the initial position). The position at 90° is the last location where both collision and collection (stopped after collision in the initial location) can take place. Any unit zone after 90° will not have collision. In addition, any cell in the streamlines $\sim 7 \mu\text{m}$ (the radius of the tumor cells used) above the micropost could collide with the position at 90°.

D. Simulation of cell-micropost interactions

We first simulated the flow field without considering the presence of tumor cells by setting up a creeping flow model in COMSOL. The geometry of the microposts in the microchannel was created; while no slip condition was applied at the wall and micropost surface, a parabolic velocity profile was set at the inlet and zero pressure boundary was at the outlet. Figure 5(a) shows a closed-up view of the model, in which h is the gap between two microposts and U_0 is the bulk flow velocity. The resulting velocity field at the flow rate of 1.2 $\mu\text{l/s}$ is shown in Fig. 5(b). Based on the simulated flow field, the velocity distribution around the circular post was obtained as shown in Fig. 5(c). Due to low flow velocity in the microchannel, no flow separation was observed. Shear rate distribution along the cell center path line indicated by the red circle in Fig. 5(a) was obtained as shown in Fig. 5(d). The upper and lower points (90° and 270°) of the micropost experience the highest shear rate. It mainly results from the high flow velocity near these two points. At the front and the back of the micropost, the shear rate is low because the flow velocity at these two points is low.

We then simulated the interaction between cancer cells and aptamer-functionalized microposts using the theoretical model discussed in Sec. III. Since the cell capture pattern on the top half and the bottom half of the micropost is approximately symmetric, we simulated only the top half for simplicity and then compared the simulation results with our experimental observation.

Considering a cancer cell that follows a streamline in the microchannel, its movement will be altered when the cell encounters a micropost. As soon as the distance between the cancer cell center and the micropost surface is smaller than $R + r_0$ (Fig. 2), the interaction between the cell and the micropost starts. We used the non-dimensionalized Langevin equation [Eq. (3)] to simulate the motion of the cancer cell during the interaction. Because the Pe number is large in this study, the thermal fluctuation terms in Eq. (3) can be ignored and the non-dimensionalized Langevin equation can be simplified as

$$\Delta X = Pe[U_\infty + M(F^S + F^B)]\Delta t. \quad (10)$$

In Eq. (10), the calculation of the mobility matrix, M , and the shear force vector, F^S , has been reported in the literature.^{66,67} The free flow velocity, U_∞ , is simulated using COMSOL as

FIG. 5. (a). The free flow field simulation setup in COMSOL Multiphysics. A parabolic velocity profile is set as the inlet boundary condition, with the non-slip condition at the surfaces of microposts and zero pressure condition at the outlet. (b). The velocity field at a flow rate of $1.2 \mu\text{L/s}$. (c). Free flow velocity magnitude at the cell center line (the red line circle in A, in the absence of cells) around a micropost at various flow rates from $0.2 \mu\text{L/s}$ to $1.5 \mu\text{L/s}$. (d). Shear rate distribution at the cell center line around a micropost at various flow rates from $0.2 \mu\text{L/s}$ to $1.5 \mu\text{L/s}$.

discussed above, to represent the flow velocity at the cell center path in the absence of a cell. The bond force vector, $\mathbf{F}^B$, is modeled as an elastic force and torque (see Fig. S4 and the related text). The time step, Δt , is set as small as 0.1 ms , and thus, the longest distance a cell could move within the time frame is $0.34 \mu\text{m}$, corresponding to a rounding angle of 0.43° . Such a short surface can be considered as an infinitesimal flat surface for the Langevin equation.

We used the standard Monte Carlo technique to determine the formation and breaking of a bond between PTK7 molecules on a cancer cell and aptamers on a micropost when the distance between the cancer center and the post surface is smaller than $R + r_0$. Briefly, a random number in the interval $[0,1]$ is generated by a pseudorandom number generator, denoted as P_{rand} . At each time step Δt , ligands (aptamer) in the contact area are examined if they are located in the capture zone of a receptor (PTK7). If the ligand-receptor distance $l_0 < r_0$ is satisfied, the bond formation probability P_{on} is calculated using Eq. (4). When $P_{\text{on}} > P_{\text{rand}}$, a ligand-receptor bond is formed and the bond force, $\mathbf{F}^B$, is calculated. Using Eqs. 5 and 6, P_{off} can be calculated with $\mathbf{F}^B$ plugged in. If $P_{\text{off}} \geq P_{\text{rand}}$, $\mathbf{F}^B$ is clear to zero and no bond force is added to the Langevin equation. If $P_{\text{off}} < P_{\text{rand}}$, $\mathbf{F}^B$ will be added to the Langevin equation [Eq. (10)] and the motion of the cancer cell will be changed.

The motion of the cell is expressed by the Langevin equation with the accumulation of time steps. After the calculation of time steps, we obtain the total displacement of the cell along the micropost surface as well as the velocity of the cell. When the velocity of a cell reduces to zero, the cell is considered to be captured on the micropost surface. The capture position of the cell is determined by the initial position and the displacement of the cell. The initial position of the cell can be any position on the front half of the micropost. To statistically simulate this mechanism, we released the same number of cells at every 0.36° from 0° to 90° .

We observed that some cells are not captured by a micropost as in the video. Instead of stopping on the post surface, these cells detach from the micropost after a period. The detachment of cells from the micropost is complicated and hardly predicted. To simplify the detachment process, we propose two detachment mechanisms. Mechanism A is shown in Fig. 6(a), in which a cell comes into contact with the micropost at the position of the θ angle, then separates from the original streamline due to its interaction with the micropost, and continues to move around the back half of the micropost. This cell is considered to detach from the micropost when it reaches 180° even if it is still not stopped. This detachment mechanism tends to take place at a slow flow rate.

Detachment mechanism B is illustrated in Fig. 6(b), in which a cell has a tendency of following the same streamline in a fast flow. Again, a cell comes into contact with the micropost at the position of the θ angle, moves around the micropost, and detaches from the micropost at the symmetric position (angle of $180^\circ - \theta$) by following the initial streamline.

The simulation results we obtained are shown in Fig. 6, in which the experimental results are also shown for comparison. Figure 6(c) shows the simulation results using detachment

FIG. 6. (a) Detachment mechanism A of cells from the micropost. A cell comes into contact with the micropost at the θ angle position, separates from the initial streamline while interacting with the micropost, and goes around the back half of the micropost towards 180° . (b) Detachment mechanism B of cells from the micropost, in which a cell comes into contact with the micropost at the θ angle position, runs around the micropost, and detaches from the micropost (at the $180^\circ - \theta$ position) by following the initial streamline. (c) Comparison of experimental results with simulation using detachment mechanism A for a flow rate of $0.5 \mu\text{L/s}$. (d) Comparison of experimental results with simulation using detachment mechanism B for a flow rate of $1.2 \mu\text{L/s}$. (e) The same experimental results of (c) with simulation using detachment mechanism B. (f) The same experimental results of (d) with simulation using detachment mechanism A.

mechanism A for a flow rate of $0.5 \mu\text{l/s}$, while Fig. 6(d) shows the simulation results based on detachment mechanism B for a flow rate of $1.2 \mu\text{l/s}$. For comparison, we also performed simulations using detachment mechanism B for a flow rate of $0.5 \mu\text{l/s}$ [Fig. 6(e)] and performed simulations using detachment mechanism A for a flow rate of $1.2 \mu\text{l/s}$ [Fig. 6(f)]. It is evident that a better agreement between the simulation and the experimental results is observed in Figs. 6(c) and 6(d) than in Figs. 6(e) and 6(f). They can be explained by the following reason. At a low flow rate, a cell interacts with the micropost for a longer time. Thus, it is more likely for the cell to deviate from the initial streamline. In contrast, at a high flow rate, the interaction time is much shorter, and thus, the cell is more likely to follow the initial streamline.

Using these simulation and detachment mechanisms, we predicted the cell capture pattern around elliptical microposts. Figure S5 shows the simulation of the cell capture pattern and experimental verification using elliptical micropost devices. The simulation was found to be in general consistence with the experimental results. Therefore, we can draw two important conclusions from these results in both Figs. 6 and S5. The first conclusion is that the flow rate affects the detachment mechanism of the cells from the microposts. The second conclusion is that it is valid to use the Langevin equation by assuming a smooth surface in an infinitesimal region of a micropost. Our simulation has been validated experimentally with the variation in velocity, shear rate, and vorticity in different regions of the micropost. Since the assumption of the infinitesimal smooth surface can be theoretically applicable to any shape, we can infer that our theoretical model can be extended to microposts with different shapes such as squares, diamond, concave, and so on. With flow parameters in the microchannel from software such as COMSOL, cell capture distribution in such kinds of microchannels can be obtained using our theoretical model.

V. CONCLUSION

We have explored the interaction between cancer cells and microposts in microfluidic devices and studied the cell capture behavior at different flow rates. We have found that a higher flow rate resulted in a higher ratio of cells captured around microposts in relation to the total cells captured. To our knowledge, this is the first report to study and compare cells captured on microposts with cells captured by channel walls. In addition, we found that the cell distribution pattern on the micropost varied with the flow rate, having a higher front-to-back ratio with the increasing flow rate.

We have also developed a theoretical model to study the effects of micropost shapes on the cell distribution pattern. The model was able to simulate the interactions between protein receptors on cancer cells and aptamers immobilized on microposts. Our theoretical model gives an insightful explanation about how a cancer cell collides with a micropost, moves and rotates around the micropost surface, and forms bonds with capture agents (aptamers) on the surface of the micropost. Although micropost-based devices were used in this report, our theoretical model is expected to be applicable to other microfeatures in microfluidic devices.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for additional parameters used for the theoretical modeling and the programming information for image processing. The stagnation zone was illustrated using the COMSOL simulation. The method for calculating the sgc8-PTK7 bond force was discussed. In addition, we presented the comparison between the experimental results of the cell capture pattern around an elliptical micropost and the simulations at the corresponding conditions.

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