

Supplementary information

1. Tumor cells

T-cell acute lymphoblastic leukemia cells (CCRF-CEM cells), and human pancreatic cells (PAN-1 and MIAPaCa-2) were purchased from American Type Culture Collection (ATCC). All cells used were cultured in an incubator at 37 °C with 5% CO₂. CCRF-CEM cells were grown in RPMI 1640 medium (ATCC) supplemented with 100 units mL⁻¹ penicillin-streptomycin (Cellgro, Manassas, VA, USA) and 10% fetal bovine serum (FBS; heat-inactivated; Gibco). PAN-1 cells and MIAPaCa-2 cells were cultured in DMEM medium (ATCC) with the same supplement.

A solution of 1 % (w/v) bovine serum albumin (BSA) (Fisher) and 0.05% (w/v) Tween-20 (Fisher) in PBS was used for re-suspending cells for cell capture.

2. Surface modification of microchannels using fibrin

Fibrin gel was formed by fibrinogen (Sigma) and thrombin (Sigma) through a three-step reaction. First, with thrombin, fibrinopeptides were released from fibrinogen to generate fibrin monomer. Second, intermediate polymers were formed by noncovalent interactions of fibrin monomers. Third, intermediate polymers aggregated into fibrin polymer.¹ Substrate-immobilization was established through thrombin-fibrinogen reaction. The reaction was optimized by comparing the following two protocols:

- a) After device been washed, thrombin and fibrinogen were introduced into the device together and incubated.
- b) Thrombin was first introduced onto the channel surfaces by physical adsorption, followed by incubating with fibrinogen. We found that the substrate was immobilized with fibrin polymers.

To compare these two protocols, a sample of 10⁵/mL CCRF-CEM cells in PBS buffer was introduced into the device treated with one of the two methods. With protocol (a), only a few CCRF-CEM cells were captured (<10%). The possible reason is as follows. When thrombin was first mixed with fibrinogen, enzymatic reactions immediately took place and a thick layer of fibrin could form away from the channel surfaces, which was then washed away. In contrast, with protocol (b), thrombin was first absorbed onto the glass substrate. As a result, the fibrin was generated on the surface, and cannot be easily washed away. We used the protocol (b) for the rest of experiments.

The concentrations of fibrinogen and thrombin were also optimized to get high capture efficiency. A fibrinogen solution was prepared fresh in 0.1 M citric acid-sodium citrate buffer (CA) buffer (pH 6.6) containing 25 mM CaCl₂. A

thrombin solution was prepared fresh in deionized water. We found that if the concentration of thrombin was more than 20 U/mL, a thick layer of fibrin was generated and it clogged the microchannels.

To generate fibrin inside microchannels, the device was washed by ethanol (300 μ L, 2 μ L/s), and then by 0.1 M CA buffer (300 μ L, 2 μ L/s). Then, the device was incubated with thrombin (2.5 U/mL) for 30 min. After washing with 0.1 M CA buffer, fibrinogen (200 μ g/mL) was introduced into the device, followed by incubation for 45 min. Finally, PBS buffer (2 μ L/s) was introduced into the device to stop the reaction. After being washed by PBS, the device should be ready for cell capture experiments.

Coomassie Blue R-250 (Fisher) stain solution was prepared according to manufactory's menu. It was then introduced into the devices, and incubated overnight. A device using only fibrinogen or thrombin was tested as the negative control. The devices were washed with PBS buffer before images were taken. Images of the devices were shown in Figure S1.

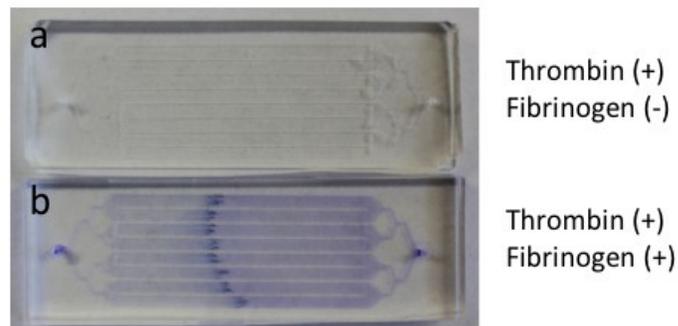


Figure S1. Images of the devices with incubation of (a) thrombin, without fibrinogen, (b) thrombin and fibrinogen.

The results in Figure S1 proved the generation of a layer of fibrin gel. A thrombin solution itself in (a) was essentially washed away by a buffer, thus there was no blue staining when Coomassie Blue was introduced afterwards. In contrast, fibrin gel was formed after introducing thrombin and fibrinogen solutions into the channels. The fibrin gel could not be washed away fully, leaving them to be stained in (b) when Coomassie Blue was introduced afterwards. The dark blue trace in the middle is likely related to the loose fibrins accumulated during the washing process.

3. Tumor cell capture in blood samples

To evaluate the cell capture efficiency of fibrin-immobilized microchannels for tumor cells in blood samples, CCRF-CEM cells were pre-stained with Dil (Invitrogen, Carlsbad, CA, USA) in red color, and then spiked in whole healthy human blood (Novi, MI, USA) that contains the anticoagulant ethylenediaminetetraacetic acid (EDTA). After cell capture and device washing, DAPI (4,6-diamidino-2-phenylindole, Invitrogen) was introduced into the device to label the nonspecifically captured white blood cells.

At the end of the experiment, the microchannel was washed with PBS at flow rate of 1.2 $\mu\text{l/s}$, followed by acquiring fluorescent images for the determination of the number of cells captured. For cell capture experiments performed in PBS buffer, the same condition was used.

Note that the target cells and the WBC on the device were washed away if the washing flow rate is too high ($>3.5 \mu\text{l/s}$).

4. Surface modification using both aptamer and fibrin

First, the device was washed as above. After incubating with thrombin, the device was washed with 0.1 M CA buffer, and then incubated with a solution of avidin (1 mg/mL) and fibrinogen (200 $\mu\text{g/mL}$) in 0.1 M CA buffer for 30 min. Then, biotin-sgc8 (20 μM) aptamer was introduced in PBS buffer, allowing the reaction for 30 min. PBS buffer was used for washing after each of these steps. Cells of $10^5/\text{mL}$ in PBS buffer were then pumped into the device. For comparison, sgc8 aptamers were immobilized on device surfaces using an established method of biotin-avidin chemistry.²

The sequence of biotinylated sgc8 aptamer is 5'-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GAT TTT TTT TTT- 3'-biotin.

References:

- (1) Scheraga, H. A. *Biophys Chem* **2004**, *112*, 117.
- (2) Sheng, W. A.; Chen, T.; Katnath, R.; Xiong, X. L.; Tan, W. H.; Fan, Z. H. *Anal Chem* **2012**, *84*, 4199.