Supporting information

Adhesion analysis of single circulating tumor cell on base layer of endothelial cells using open microfluidics

*Sifeng Mao,† Qiang Zhang,† Haifang Li, Wanling Zhang, Qiushi Huang, Mashooq Khan, and Jin-Ming Lin**

Department of Chemistry, Beijing Key Laboratory of Microanalytical Methods and Instrumentation, MOE Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Tsinghua University, Beijing 100084, China.

† These authors contributed equally to this work.

**Corresponding author. Email: jmlin@mail.tsinghua.edu.cn*

Content

Materials and Methods

Reagents

0.25% Trypsin (Corning, USA), Caco-2 cells (National Infrastructure of Cell Line Resource, China), U-87 MG cells (National Infrastructure of Cell Line Resource, China), HepG2 cells (National Infrastructure of Cell Line Resource, China), HUVEC cells (National Infrastructure of Cell Line Resource, China), HBMEC cells (National Infrastructure of Cell Line Resource, China), Minimum Essential Medium (Corning, USA), Penicillin Streptomycin Solution (Corning, USA), 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate (Dil, Sigma Aldrich, USA), Phosphate Buffer Saline (Corning, USA), PDMS prepolymer and curing agent (Sylgard 184, Dow corning, USA).

Microfluidic device fabrication and operation

The geometry of the microfluidic device was designed by Adobe Illustrator software (Adobe Systems Incorporated, USA), and then the pattern was printed to generate a photo mask. The PDMS chip was manufactured by soft lithography: SU-8 2050 negative photoresist (Microchem, USA) was spread on a clean silica wafer (Tianjin Silica, China) using a spin coater, and then they were soft baked on a hotplate. After cooling down to room temperature, the wafer was covered with the photo mask, exposed to UV light, and then developed with developing solution (Microchem, USA). The obtained wafer was hard baked on a hotplate to stabilize the geometry of photoresist. The mixture of PDMS prepolymer and curing agent (Sylgard 184, Dow corning, USA) with a weight ratio of 10:1 was poured on the wafer mold. After baked at 80 ℃ for 2h, the PDMS can be peeled off from the wafer mold and punched at proper positions by a needle. The obtained PDMS replica and another PDMS chip with no pattern were cleaned and modified by oxygen plasma (PDC-32G, Harrick Plasma, Ithaca, NY) treatment, and then they were adhered to each other to generate the final microfluidic device (LSCE).

Numeric simulation

Comsol Multiphysics 5.3a (Comsol, USA) was utilized to carry out 3-D simulations of

the fluid behaviors, and the parameters are almost the same as real experiment. Two rectangular apertures (cross section: 50 μm × 80 μm) were separated with a center distance of 150 μm. The gap between the surface of substrate and the tip of both apertures was set at 50 μm. The liquid was regard as water with a density of 999.7 kg/m^3 and a viscosity of 0.001 Pa·s. Physical fields of Laminar Flow and Transport of Diluted Species were coupled to simulate the hydrodynamic properties and concentration distribution. The flow rate of both apertures were set according to experiments and other entrances and exits of the model were set as pressure-driven flow.

Cell culture and adherence of CTCs on ECs

U-87 MG cells were cultured in minimal essential medium (MEM, Corning, USA) with Earle's Salts and L-glutamine supplemented with 10% fetal bovine serum (FBS, Corning, USA), nonessential amino acids, 100 units/mL penicillin, and 100 units/mL streptomycin. Caco-2 cells, HUVEC cells and HepG2 cells were cultured in Roswell Park Memorial Institute 1640 (RIPI 1640) with 10% fetal bovine serum (FBS, Corning, USA), nonessential amino acids, 100 units/mL penicillin, and 100 units/mL streptomycin. As for the sample preparation of cell-cell experiment, the upper cells were stained by 10 μM DIL (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate) in PBS (Phosphate Buffer Saline) solution for 30 min in incubator and the HUVEC cells serving as base were cultured in petri dish until they form a layer. Then the stained upper cells suspension was added to base cell layer, following by incubation for 3 h. Finally, the surrounding solution was replaced by fresh cell culture medium for use in experiments.

Adhesion analysis of single-circulating tumor cells on endothelial cells

LSCE was immobilized at a XYZ scaffold (Sigma KOKI Co., Ltd.), and the cell sample was placed on the XY stage of microscopy (Leica DMI 4000 B, Wetzlar, Germany). LSCE was moved into the field of view of microscopy, above the culture dish. Then the target cell was moved into the target area, and the height of LSCE was adjusted to shorten the distance of LSCE and the target cell to about 50μm. The pump for aspiration and injection were started successively, and the data recording was begun at the same

time. After the extraction of the target cell, LSCE was elevated at first, then another target cell was moved into the target area, finally LSCE was lowered to set the gap as 50 μm and the pumps and data recording were initiated.

Supplementary Figures

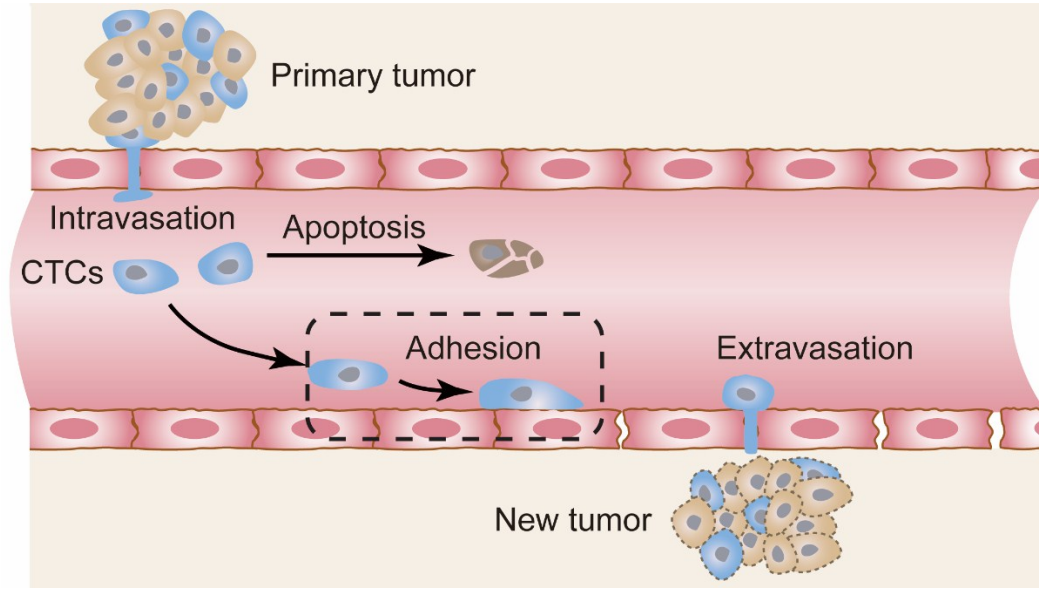

Figure S1. CTCs adherence to the vascular internal wall in tumor metastasis.

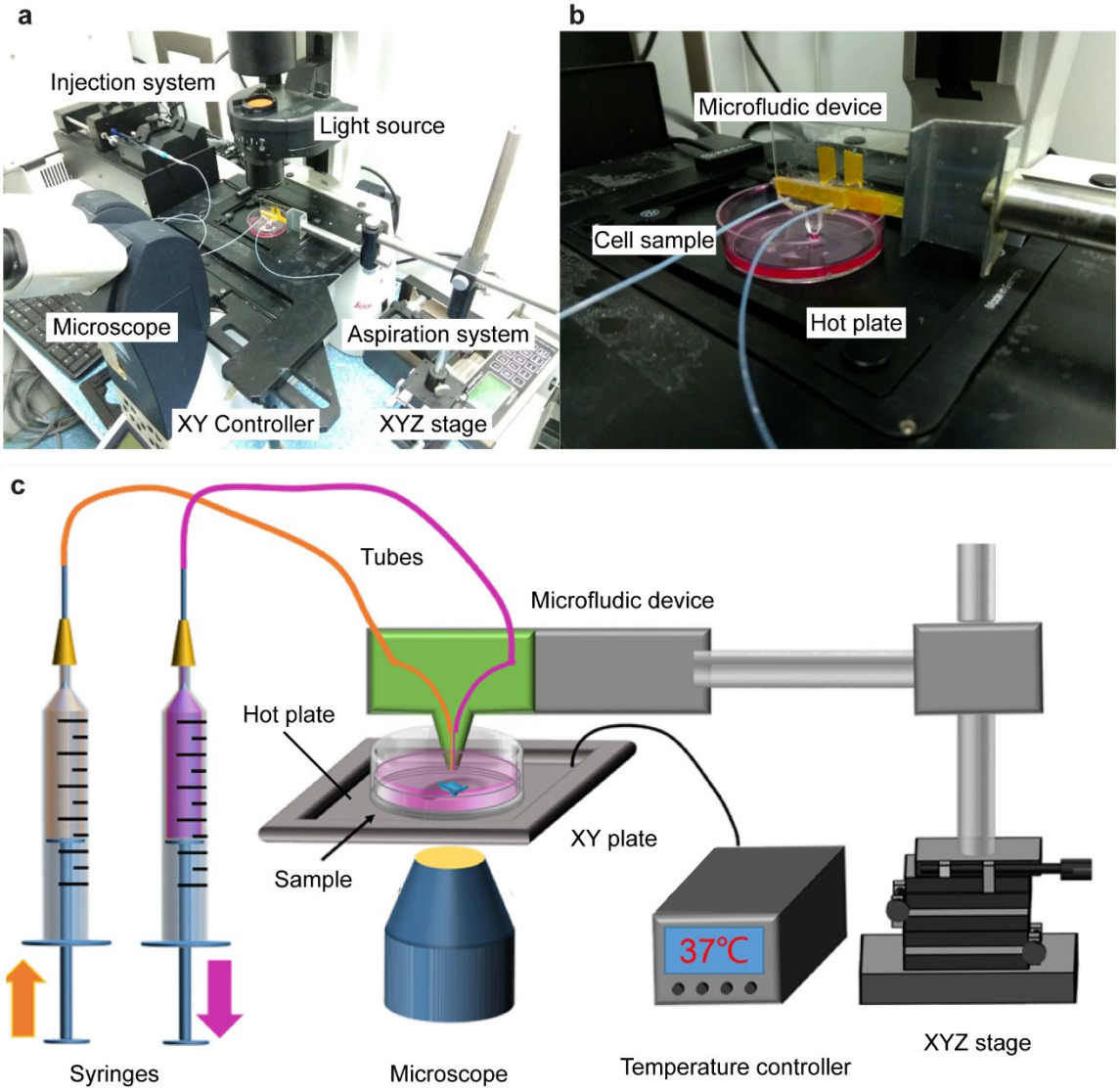

Figure S2. **Device and setup of system.** (a) Operation system for adhesion strength measurement of single cell. (b) Interface between device and cell sample. (c) Scheme of the operation system.

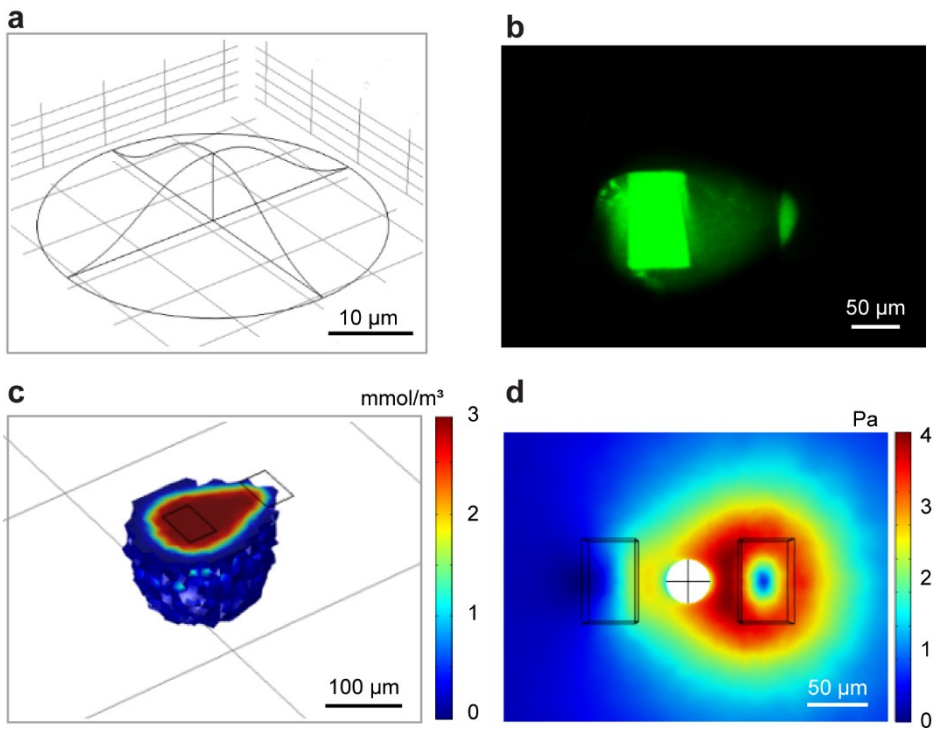

Figure S3. Numeric simulation. (a) Cell model in the simulation. (b) Experimental fluorescence image observed from the bottom of culture dish. (c) 3D simulation of diffusion region of trypsin without the obstruction of substrate. d) Distribution of shear stress on the surface of substrate.

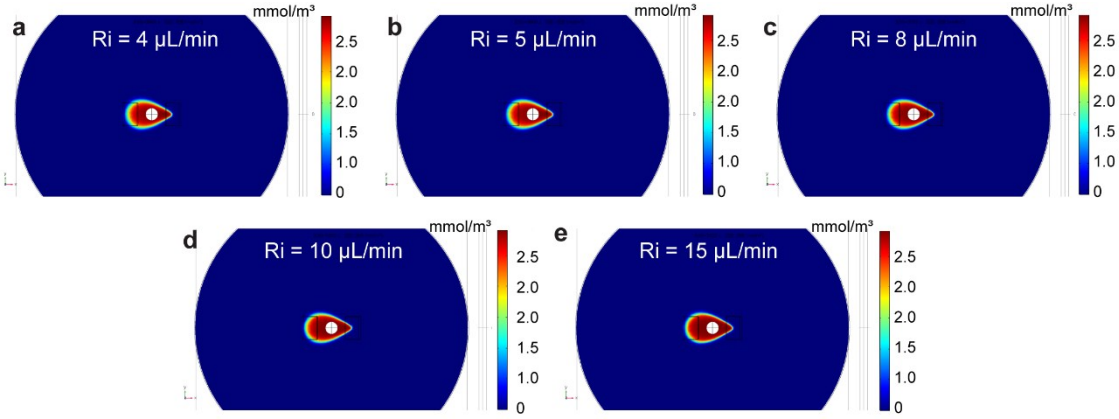

Figure S4. Concentration distribution of trypsin at the substrate under different flow rates (a) 4 µL/min, (b) 5 µL/min, (c) 8 µL/min, (d) 10 µL/min, (e) 15 µL/min.

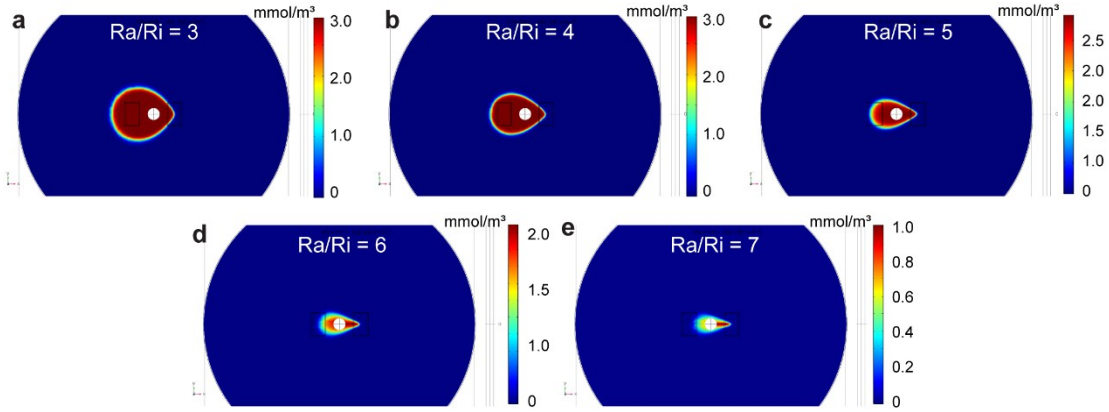

Figure S5. Concentration distribution of trypsin at the substrate under different flow ratios (a) Ra/Ri = 3, (b) Ra/Ri = 4, (c) Ra/Ri = 5, (d) Ra/Ri = 6, (e) Ra/Ri = 7.

Supplementary movie S1 Adhesion strength analysis of single-Circulating Tumor Cells on Endothelial Cells