Supplementary Information

Entrapment of Prostate Cancer Circulating Tumor Cells with a Sequential Size-Based Microfluidic Chip

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1. Fabrication

The CTC-HTECH was designed with seven channels connected by six rows of constriction channels. On different rows of constriction channels, we added one or two larger trapping chambers in the constriction channel to create more obstacles for cancer cell entrapment. The microfluidic channels were fabricated by polydimethylsiloxane (PDMS) soft-lithography, followed by PDMS-glass bonding after plasma treatment. Forty microchannels with constriction channels (width: 8μm; height: 8μm; length: 100 μm/each) and trapping chambers (width: 30 μm; height: 30 μm; length: 40 μm/each) were connected in parallel in each row.

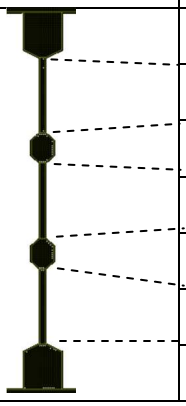
The molds for microfluidic channels were fabricated on a silicon wafer with two layers of SU-8 (SU-8 3005 and SU-8 3025, MicroChem, Newton, MA) photolithography on a clean and dehydrated silicon prime wafer. The first layer SU-8 3005 was spin coated at 2000 rpm with an acceleration of 400 r/s for 30 s. This SU-8 layer was ~8 μm in thickness. Then, the wafer was soft baked at 65°C for 10 min and followed by 95°C for 35 min. After the wafer with uncured SU-8 was cooled down to room temperature, the wafer was moved to a mask aligner (Karl Suss MA-6, SUSS MicroTech, Inc., Corona, CA) and covered with a negative photo mask with patterned cavity and channel structures. The exposure time was set to 40.7 s at 8.6 mW/cm^2, which provided a total dose of 350 mJ/cm^2 of i-line (365 nm) UV on the wafer. After the first UV exposure, the wafer was transferred to a hot plate for post exposure bake at 65°C for 10 min and followed by 95°C for 30 min. After the wafer cooled down to room temperature after the post bake, the wafer was immersed in SU-8 developer (MicroChem, Newton, MA) for 5 min to remove the uncured SU-8. The wafer with the first layer SU-8 pattern with the constriction channel structures was cleaned by isopropanol and DI water, then dried with a nitrogen gun.

Tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (TFOCS, Fisher Scientific) was coated on the surface of the molds for the easy release of PDMS. 0.3 mL of TFOCS was dropped on the surface of a petri-dish, with the mold placed next to the droplets. Then, the petri-dish was moved into a vacuum chamber for 30 min. The TFOCS fully evaporated and formed a Teflon-like surface on the SU-8 mold. After the mold was prepared, standard PDMS replica molding was conducted to fabricate microchannel. PDMS pre-polymer (SYLGARD® 184 silicone elastomer, Dow Corning, Midland, MI) and curing agent (SYLGARD® 184 silicone elastomer curing agent, Dow Corning, Midland, MI) mixture with a weight ratio of 10:1 was poured on the silicon with the SU-8 mold. The mixture was then placed in a vacuum container for 30 min to remove all the air bubbles. The degassed PDMS mixture was poured onto the mold and placed in a 65°C oven for 24 hours for the solidification of PDMS. The PDMS channels were then bonded to a glass slide after air plasma treatment using plasma cleaner.

2. Pressure and Flow in CTC-HTECH

The detailed dimension of the microchannel array can be obtained from the schematic design.

Table S-1. Dimensions of one microchannel.

	Width (W, μm)	Height (H, μm)	Length (L, μm)
	50	30	60
	8	8	100
	30	30	40
	8	8	100
	30	30	40
	8	8	100
	50	30	60

The pressure drop across the microfluidic channels are:

$$\Delta p = \frac{a\mu QL}{WH^3} \tag{1}$$

where

$$a = 12\left[1 - \frac{192H}{\pi^5 W}\tanh\left(\frac{\pi W}{2H}\right)\right]^{-1} \tag{2}$$

Accordingly, the fluid resistance is

$$R_h \approx \frac{12\mu L}{wh^3(1 - 0.63h/w)}, \qquad h < w \tag{3}$$

The fluid resistance across one microchannel with 3 constriction channels and 2 trapping chambers is 3.6×10^5Pa·s/(μL). With 40 parallel microchannels, the fluid resistance is $R_h/40$. So, assuming all channels are open and no large cell is trapped, the total fluid resistance across one row will be around 9×10^3Pa·s/(μL). During our experiments, we keep a constant pressure of 500 mbar across the CTC-HTECH. This varies the flow rate from around 330 μL/min for a single row configuration to 55 μL/min. In this calculation, we assume that pressure drop in large interconnecting channels are negligible and that all the parallel channels in each row experience the same pressure difference. To prove this point, a COMSOL simulation was performed. The results shown in Figure S1 confirm the validity of our assumption. Regardless of the number of rows used, the major drop in the pressure is seen in the small channels possessing the cell trapping chambers. The large interconnecting channels act as wires in electrical circuits forcing all the nodes having the same potential (pressure in this case) while allowing each parallel resistor (channel) in the row to experience a different current (flow rate) depending how resistive that path is.

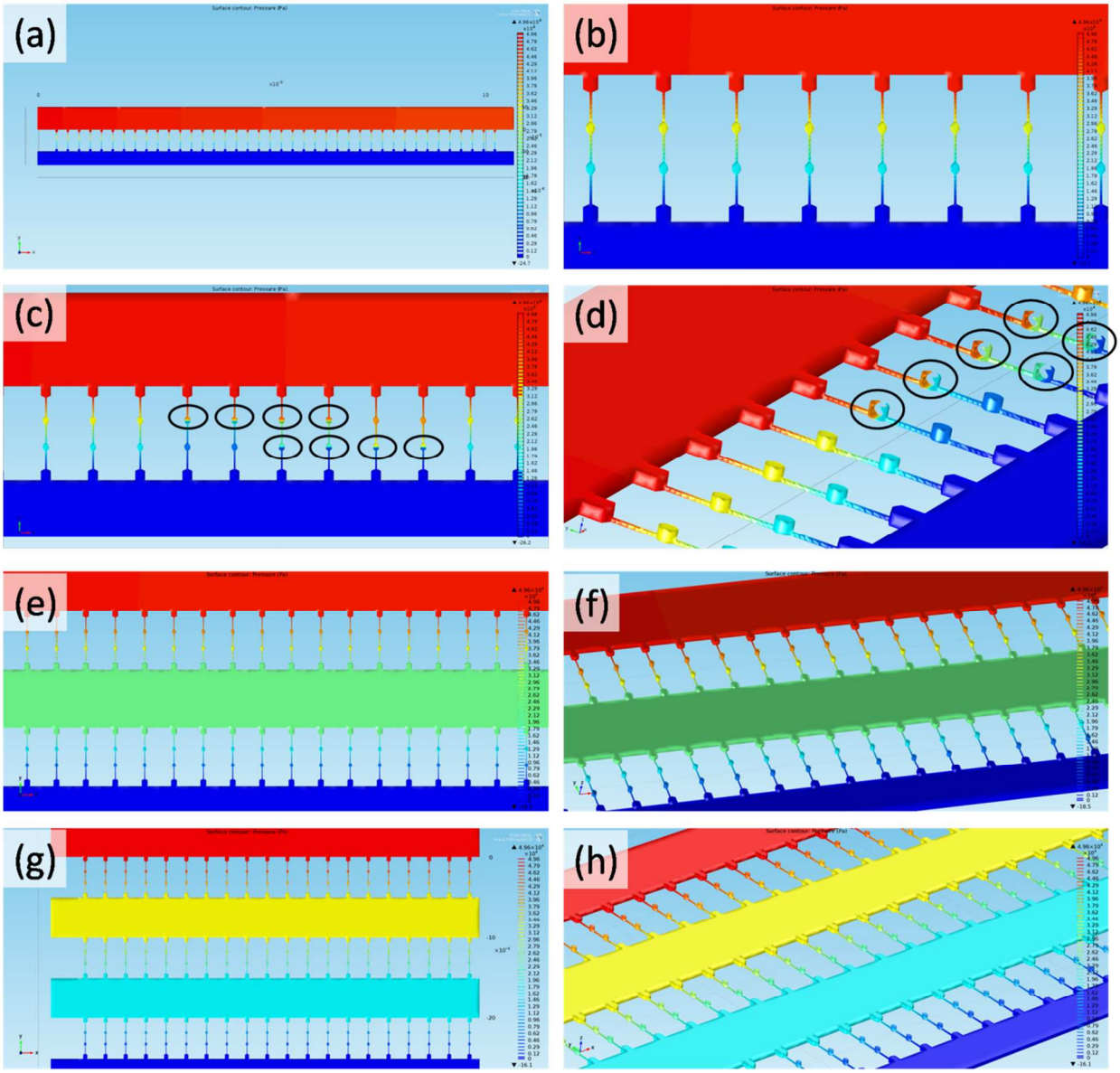


Figure S1. COMSOL simulation of pressure distribution along the CTC-HTECH chip: (a) the pressure (Pa) distribution at row ①; (b) enlarged pressure distribution near microchannels;(c) enlarged pressure distribution at row ① with cells trapped in trapping chambers (highlighted in black circles); (d) enlarged pressure distribution near the microchannel with and without cells trapped in trapping chambers (channels with cells are highlighted in black circles); (e) pressure distribution on two rows of microchannels; (f) enlarged pressure distribution on two rows of microchannels; (g) pressure distribution on three rows of microchannels; (f) enlarged pressure distribution on three rows of microchannels.

3. Control group

Control group experiment using mouse whole blood without LNCaP-C4-2 spiked.

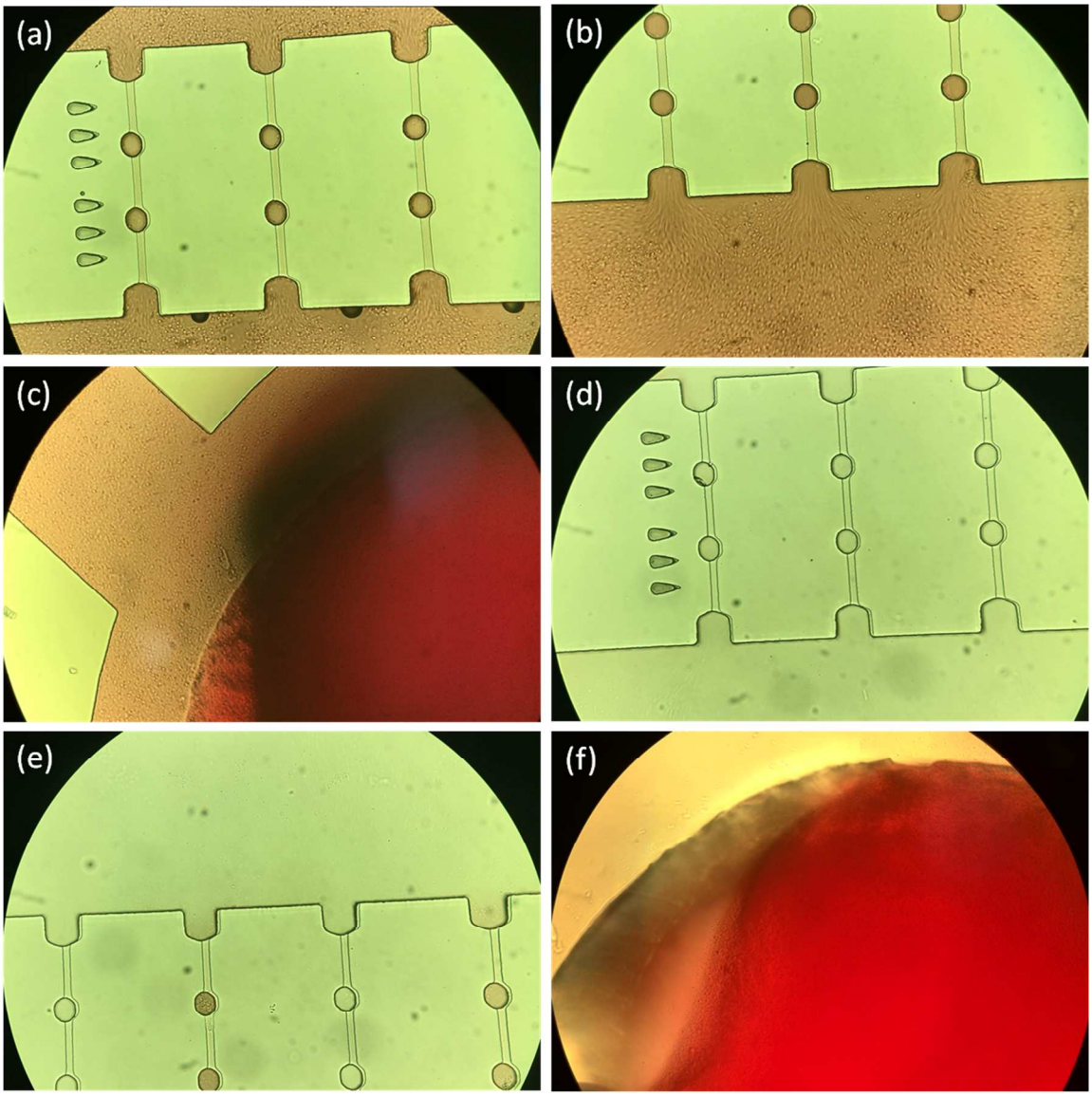


Figure S2. Image of the control group of mouse whole blood going through the CTC-HTECH: (a) mouse whole blood enters the inlet; (b) mouse whole blood pass through microchannels; (c) mouse whole blood at the reservoir of outlet 6; (d) the inlet switched to LNCaP-C4-2 culture medium to remove the blood cells; (e) image of microchannels after 10 min rinsing; (f) the image of outlet 6 reservoir after 10 min rinsing.