# Supporting Information for

# Efficient detection and single cell extraction of circulating tumor cells in peripheral blood

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## 1. Materials

The following materials were used in the present study: 3-mercaptopropyl trimethoxysilane (95%, MTPMS); Anhydrous ethanol; N-y-maleimidobutyryloxy succinimide ester (4-Maleimidobutyric acid N-hydrosuccinimide, GMBS); Streptavidin (SA); Biotinylated anti-human EpCAM antibody (Anti-EpCAM); Fetal bovine serum (FBS); Fluorescein diacetate (FDA); propidium iodide (PI); Dulbecco's Modified Eagle's Medium (DMEM); PE-labeled anti-cytokeratin (CK); FITC-labeled anti-human CD45; 0.25% Trypsin-EDTA; Tween-20; Dimethyl sulfoxide (DMSO); Anhydrous ethanol; 1X PBS (Hyclone, 0.0067 M $PO_4^{3+}$); 4, 6-diamidino-2-phenylindole dihydrochloride (DAPI); Polyvinyl pyrrolidone (PVP); Zinc acetate; Sodium alginate; Sodium iodide; Glycol; Sodium chloride; Calcium chloride; Lithium niobate; SU-8 2100; S1813; Gold; Chromium; and Deionized water;

## 2. Experimental Section

### *2.1 Surface acoustic wave device introduction*

The acoustic surface wave was usually produced by applying the appropriate electric field to the piezoelectric substrate, in turn, the piezoelectric material produced the mechanical stress of propagation. A typical acoustic surface wave device contained at least one set of interdigital transducer (IDT). The structure of IDT determined the bandwidth and directionality of the resulting surface acoustic wave. By changing the number, interval, and aperture (overlapping length) of the interdigital transducer, we can modify the characteristics of the resulting surface acoustic wave.

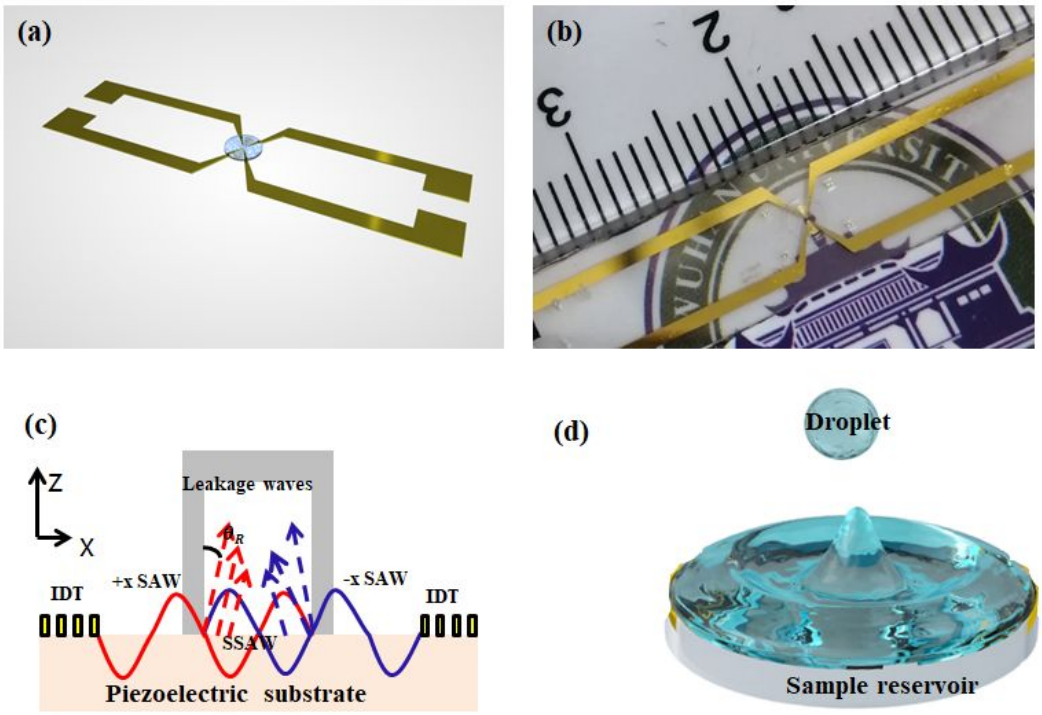


Figure S1 (a) A schematic of surface acoustic waves. (b)A photograph of the device which consist of FIDTs and a circular sample reservoir. (c) The working mechanism of the focused interdigital transducer to generate droplet. (d) A schematic of nozzleless droplet generation with focused acoustic beams.

Figure S1(a) and Figure S1(b) shown the schematic and photograph of the device. The preparation of acoustic surface wave devices with eight pairs of electrode structures and sample reservoir through a two-step lithography process. We chose lithium niobate as the piezoelectric substrate, and gold was chose as electrode. The device has good light transmission which is beneficial for the observation of biological samples. Figure S1(c) and Figure S1(d) shown that resulting FSAW propagates on the substrate, enters the liquid in the sample reservoir in the form of a leaking surface sound wave (LSAW). Then applied a pulsed RF signal, the dynamic acoustic pressure profile will suspend the liquid-air interface as a liquid mound and overcome the surface tension of the sample fluid, dripping liquid droplets into the air.

### *2.2 Device fixation*

Before the experiment start, rotated the amplifier button slowly to the end, observed the power meter display without a large fluctuation, adjusted and stabilized the placement of the joint, and set all the parameters. Fixed the focused interdigital transducer device on the microscope (IX71,Olympus), aspirate 2.5ul 2% OSA to a circular reservoir. Then adjusted the voltage to 100mvpp, rotated amplifier to the maximum position, holding a clean glass slide in the appropriate position above the device, pressed the start key to start ejecting droplets. This process can be used to determine whether the device is stable, meanwhile, the size and morphology of hydrogel sphere were preliminarily determined.

### *2.3 Alginate preparation*

0.2g sodium alginate was dissolved in 20mL deionized water, and then the sodium iodide (0.0161g) was dissolved in 0.302mL deionized water to obtain 0.25mol/L sodium iodide aqueous solution, subsequently mixing two types of solutions. Added 0.2g of ethylene glycol and stirred for 10 minutes. Then added 0.5g of sodium chloride and stirred for 5 minutes. We used 40mL anhydrous ethanol to precipitate products. After two times of precipitation, the product is then dissolved and finally dried in refrigeration machine for 24 hours.

### *2.4 Electrospinning method and device introduction*

Electrospinning is a new, simple and inexpensive technology that is used to fabricate micro-nanometer fiber materials. The prepared nanofiber has highly specific surface area, long diameter, three-dimensional porous structure, high porosity, and high uniformity. The substrate can be well applied to capture and release of circulating tumor cells.

The electrostatic spinning device consists of three parts as following: high voltage DC power, injection wire spraying device, and receiving device. Under the action of a high voltage electric field, the precursor solution formed a conical surface. The solution was jetted out from the device surface by increasing the voltage. Solvent evaporation allowed a nanofiber with a disordered random arrangement to be obtained on the receiving device. Nanofibers are affected by the following factors: precursor solution concentration, viscosity and surface tension; working environment, temperature, humidity, and gas flow rate; working electrostatic spinning parameters; operating voltage; distance between the needle and receiving plate; spray needle pipe diameter; and precursor fluid injection speed. Optimization of each parameter will generate the target nanofiber.

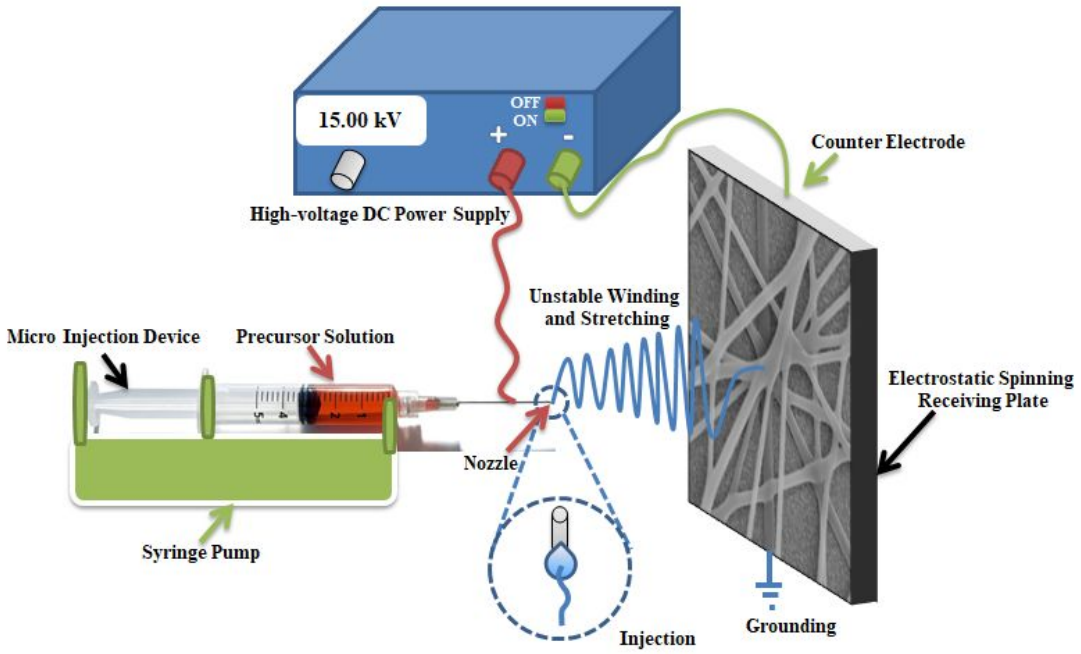


Figure S2 Schematic of the electrospinning device.

Electrospinning technology was used to obtain ZnO nanofiber substrate. The electrostatic spinning devices (Figure S2) included three parts: high voltage DC power, injection wire spraying device, and receiving device. Under high pressure, the precursor

solution was deposited on the receiving substrate in disorderly random arrangement to form a nanofiber network membrane.

### *2.5 Preparation of precursor liquid*

Zinc acetate was used as the zinc source material, and polyvinyl pyrrolidone (PVP) was used as the polymer skeleton material. Anhydrous ethanol was the solvent and volatile auxiliary agent. The preparation process was as following: 1) 1.0 g of Polyvinyl pyrrolidone (PVP) was dissolved in 6 g of ethanol and 3 g of deionized water by stirring at room temperature to form a uniform and transparent viscous liquid; 2) 2.8 g of zinc acetate was added to the above solution at 60 ℃ with stirring for 30 min; 3) the distance between the nozzle and receiving device was adjusted to 13 cm; 4) the voltage was set at 15 kV; and 5) the flow rate was set at 0.3 μl/min. The prepared nanofiber substrate was heated at 70 ℃ for 12 h and then annealed at 500℃ for 120 min to remove organic matter to obtain ZnO nanofibers.

### *2.6 Circulating tumor cell preparation*

The MCF-7 cell line was obtained from the Hubei Key Laboratory of Tumor Biological Behaviors (Hubei, People's Republic of China). Dulbecco's Modified Eagle's Medium (DMEM) as purchased from BD Biosciences (San Jose, CA, USA) and supplemented with 5% v/v fetal bovine serum, 100 units/mL penicillin, 0.01 mg/mL insulin, 100 μg/mL streptomycin, and 2 mM L-glutamine. The cell line was cultured at 37°C under a humidified 5% $CO_2$ atmosphere.

### *2.7 Surface modification*

ZnNFs substrate was washed with anhydrous ethanol and dried in an oven at 30 ℃. Anhydrous ethanol was used to prepare 4% MPTMS solution, which was added to the substrate and incubated for 1 hour (h) at room temperature. The substrate was then washed three times with anhydrous ethanol and DMSO respectively. The GMBS (1

mg/mL in DMSO) coupling agent was then added and incubated for 45 min at room temperature. The platform was washed three times with DMSO and PBS respectively. Streptavidin (50 μg/mL) was then added, and the platform was placed in refrigerator at 4 ℃ overnight. Finally, 0.1 mg/mL epithelial cell adhesion factor antibody solution was added and incubated for 2 h at room temperature. The capture experiment was then subsequently performed. The modification process of the ZnO nanofiber substrate to capture and release CTCs was displayed in Figure S3.

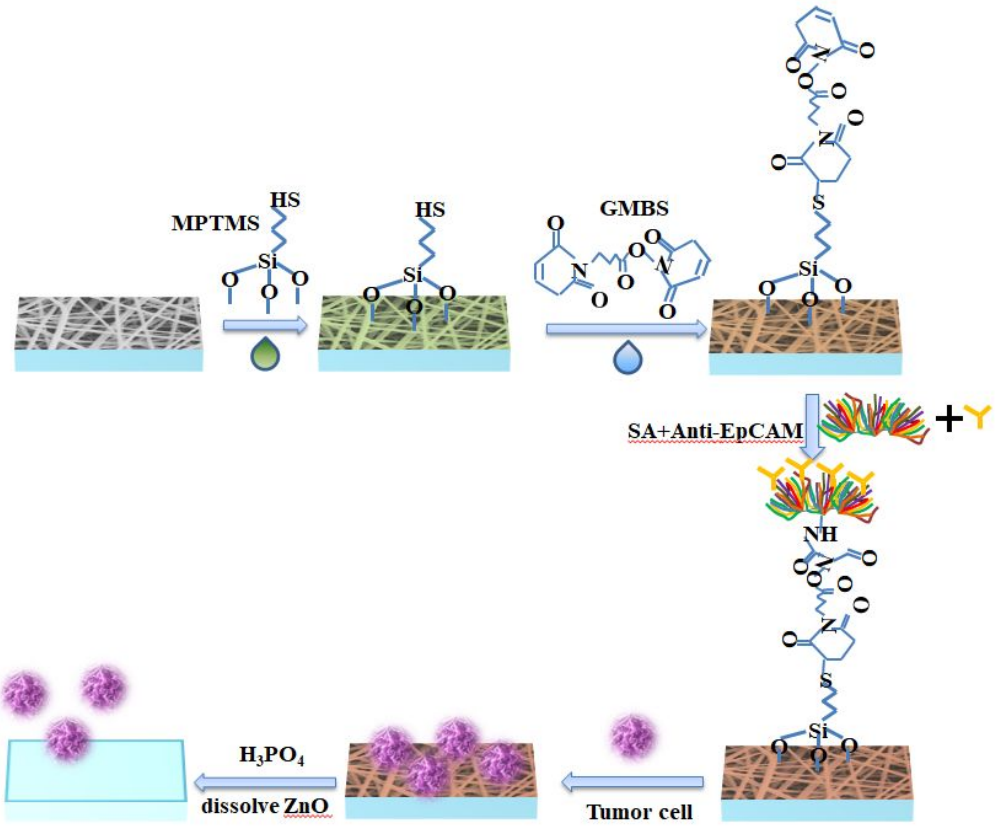


Figure S3 Modification process of the ZnO nanofiber substrate to capture and release circulating tumor cells.

***2.8 Cell capture and release***

MCF-7 cells were selected as the target cells to perform the capture and release experiments. The substrate was divided into chips (area of 1x1 $cm^2$). After the silanization reaction, protein-crosslinking reaction, addition of modified biotinylated

SA, and addition of anti-EpCAM, a 100 μl cell suspension was added to the substrate. After incubation in a 37 °C and 5% $CO_2$ incubator, the captured cells were counted using a fluorescence microscope (IX71, Olympus, Japan). After obtaining high capture efficiency, different concentrations of phosphoric acid (0, 15, 25, 75, 125, and 175 mM) were used to dissolve the ZnO nanofiber substrate to release the captured cells. The release effect was observed, and the release efficiency was calculated.

***2.9 Cell propagation and culture***

The X71 microscope was used to observe the growth status of cells. UV light was used for 30 min to sterilize the biological operator station. The culture liquid was removed from the culture bottle, and PBS was used to rinse the bottle and remove dead cells. To digest the adherent cells, 1 ml of pancreatic enzymes was added, and the enzymes were removed by centrifugation. Cells were resuspended with DMEM.

Half of the cells were removed, and the remaining cells were placed back into the culture bottle with 5 ml of DMEM, which was then placed in an incubator (37℃ and 5% $CO_2$).

***2.10 Blood sample treatment***

The breast cancer patient blood samples were obtained from the Department of Hematology and the Department of Oncology (Zhongnan Hospital of Wuhan University). All samples were stored in 2 ml anticoagulant tubes (EDTA-K 2, violet cap) and treated within 6 h. All the patients which involved in the experiment have been informed of the purpose of the sample and received informed signature.

The specific processing steps were as follows:

1)Balanced salt solution (1:1) was used to dilute blood samples.

2)Diluted blood samples were added dropwise into 2 mL of lymphocyte separation fluid to maintain clear page boundaries.

3)Samples were then centrifuged at 1800 g for 30 min, which divided the blood samples into a serum layer, mononuclear cell layer, lymphocyte separation liquid layer and erythrocyte layer.

4) Mononuclear cell layers were collected and washed with PBS to obtain monocytes.

***2.11 Three color fluorescence method***

To exclude interference from other cells, three color fluorescence staining was used verify that the captured cells were CTCs. The following stating process was used:

1)Using PBS to wash the ZnNFs substrate for three times, 4% PFA was used to fix the cells for 10 min.

2)The platform was cleaned with PBS, and 0.3% Triton X-100 was used to permeabilize cells for 10 min.

3)The substrate was washed with PBS, and 1 mL of blocking solution was used to block the cells.

4)After removal of the blocking solution, the immunofluorescence protein mixture (10 μL PE-CK+ 10μLFITC-CD45+ 80 mL blocking solution) was added to the cells, which were then incubated at 4 ℃ for at least 6 h.

5)To stain the nuclei, 100 μl of 0.1μg/mL DAPI was added to the cells. The substrate was washed with PBS and then visualized using the X81 microscope to identify and count the target cells.