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Electronic Supplementary Information

Integration of aligned polymer nanofibers within a microfluidic chip for efficient capture and rapid release of circulating tumor cells

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Part of experimental details:

Materials. Branched polyethylenimine (PEI, M_W = 25 kDa), poly (vinyl alcohol) (PVA, M_W = 85-124 kDa), 2-methacryloyloxyethyl phosphorylcholine (MPC), L-cysteine (Cys), and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (St. Louis, MO). Glutaraldehyde (GA) with a concentration of 25% in aqueous solution, triethylamine (Et_3N), hydrogen peroxide (H_2O_2, 30 wt%), dichloromethane, and anhydrous methanol were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Copper(I) bromide (CuBr), 2,2′-bipyridine (BPY), 2-bromoisobutyryl bromide (BIBB) and potassium carbonate (K_2CO_3) were supplied by J&K Chemical Co., Ltd. (Beijing, China). PEGylated folic acid (PEG represents polyethylene glycol) with one end of sulfhydryl group (SH-PEG-FA, M_W = 2000) was provided by Shanghai Pengsheng Biotechnology Co., Ltd. (Shanghai, China). Regenerated cellulose dialysis membranes with a molecular weight cut-off (MWCO) of 1000 were received from Yanyi Biotechnology Corporation (Shanghai, China). Calcein-AM and PI were obtained from KeyGen Biotech. Co., Ltd. (Nanjing, China). 4’,6-Diamidino-2-phenylindole (DAPI) was from Bestbio. Co., Ltd. (Shanghai, China). Anti-cytokeratin 7 antibody-cytoskeleton marker (Alexa Fluor® 568) and fluorescein isothiocyanate (FITC)-labeled anti-CD45 were from Abcam. Biotech. Co., Ltd. (Shanghai, China). Polydimethylsiloxane (PDMS) base and curing agent were from Dow Corning (Midland, MI). Negative photoresist SU-8 was obtained from MicroChem (Newton, MA). Red blood cell (RBC) lysis buffer was obtained from Biosharp Biotech. Co., Ltd. (Shanghai, China). HeLa cells (a human cervical carcinoma cell line), KB cells (a human oral squamous epithelium tumor cell line), SKOV-3 cells (a human ovarian carcinoma cell line), as well as a folate receptor negative cell line of A549 cells (a non-small cell lung carcinoma cell line) were provided by Institute of Biochemistry and Cell Biology (the Chinese Academy of Sciences, Shanghai, China). Dulbecco’s Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were obtained from Gibco (Carlsbad, CA). Water used in this study was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with a resistivity higher than 18 MΩ cm. All chemicals were used as received.

Characterization. The morphology of the nanofibrous mats was observed using scanning electron microscopy (SEM, JEOL JSM-5600LV, Tokyo, Japan) with an accelerating voltage of 5 kV. The samples were sputter coated with a gold film with a thickness of 10 nm before observation. The nanofiber diameter distribution was analyzed using ImageJ software (https://imagej.nih.gov/ij/download.html). At least 200 nanofibers from different SEM images were measured for each sample. Fourier transform infrared (FTIR) spectra were collected on a Nicolet Nexus 670 FTIR spectrophotometer (Thermo Nicolet Corporation, Madison, WI) in the wavenumber range of 400-4000 cm^{-1}. Thermal gravimetric analysis (TGA) was carried out using a TG209 F1 (NETZSCH Instruments Co., Ltd., Selb/Bavaria, Germany) under nitrogen atmosphere with a temperature range of 30-700 ^{o}C. ^{1}H NMR measurements were performed on an Avance 400 NMR spectrometer (Bruker Corporation, Switzerland). Samples were dissolved in D_2O or DMSO-d6 before analysis.

Hemocompatibility Tests. The hemocompatibility of nanofibers were evaluated *via* hemolysis and dynamic clotting assays according to protocols described in our previous work.[1] Human whole blood stabilized with heparin from healthy adult volunteers was kindly provided by Shanghai General Hospital (Shanghai, China) after approval by the ethical committee of Shanghai General Hospital. For hemolysis assay, the fresh whole blood was centrifuged and washed with PBS to completely remove serum and obtain RBCs. Then, the obtained RBCs were diluted 150 times with PBS. Nanofibers with a mass concentration of 4 mg/mL were tested. For dynamic clotting assay, the whole blood was used and the concentration of the nanofibrous mat was also set at 4 mg/mL for different nanofibers.

Protein Adsorption Assay. The fibrinogen (Fg) and bovine serum albumin (BSA) were adopted as model proteins to characterize the protein resistance property of the nanofibrous mats. Firstly, the concentration-absorbance (at 280 nm) calibration curve of Fg (or BSA) solution was obtained using UV-vis spectrophotometer. Round nanofibrous mats with a diameter of 14 mm were placed into 24-well plates and 500 μL of phosphate buffered saline (PBS, pH = 7.4) was added into each well and equilibrated for 24 h at room temperature. Then, the PBS solution was sucked out and 1 mL of Fg (or BSA) in PBS solution with a concentration of 1 mg/mL was added to each well. After incubation in a

constant-temperature shaking bed at 37 °C for 1 h, the nanofibrous mats were withdrawn and the absorbance of Fg (BSA) solution was measured by UV-vis spectrophotometer. And the protein absorption rate was calculated by equation (S1):

$$Absorption\ rate\ (\%) = \frac{C_I - C_F}{C_I} \times 100\% \quad \text{(S1)}$$

where C_I is the initial concentration of Fg (or BSA) solution, and C_F the final concentration of Fg (or BSA) solution after incubation for 1 h. The protein concentrations were calculated according to the concentration-absorbance standard curve of Fg (or BSA) solution. For each fibrous mat, 5 parallel specimens were tested.

Blood Cell Resistance Assay. Fresh human blood was lysed to remove RBCs using the RBC lysis buffer according to the manufacturer's instruction. After centrifugation, the precipitated white blood cells (WBCs) were stained with Calcein-AM to have a concentration of 10^6/mL. A circular nanofibrous mat (diameter = 14 mm) was placed in each well of a 24-well plate and the mats were equilibrated at room temperature for 24 h in 500 μL of PBS solution. After that, 500 μL of WBC suspension with a concentration of 10^6/mL was added into each well of the 24-well plate and incubated at 37 °C for 1 h. The WBC suspension was aspirated and the nanofibrous mats were washed three times with PBS (500 μL for each time). The WBCs adhered to the nanofibrous mats were then observed and counted under fluorescence microscope. For each sample, the number of adhered WBCs was counted from five different fields at a 200× magnification. The process was repeated using three independent samples.

Statistical Analysis. One-way ANOVA statistical method was adopted to analyze the experimental results. A value of 0.05 was selected as the significance level, and the data were marked with (*) for $p < 0.05$, (**) for $p < 0.01$, and (***) for $p < 0.001$, respectively.

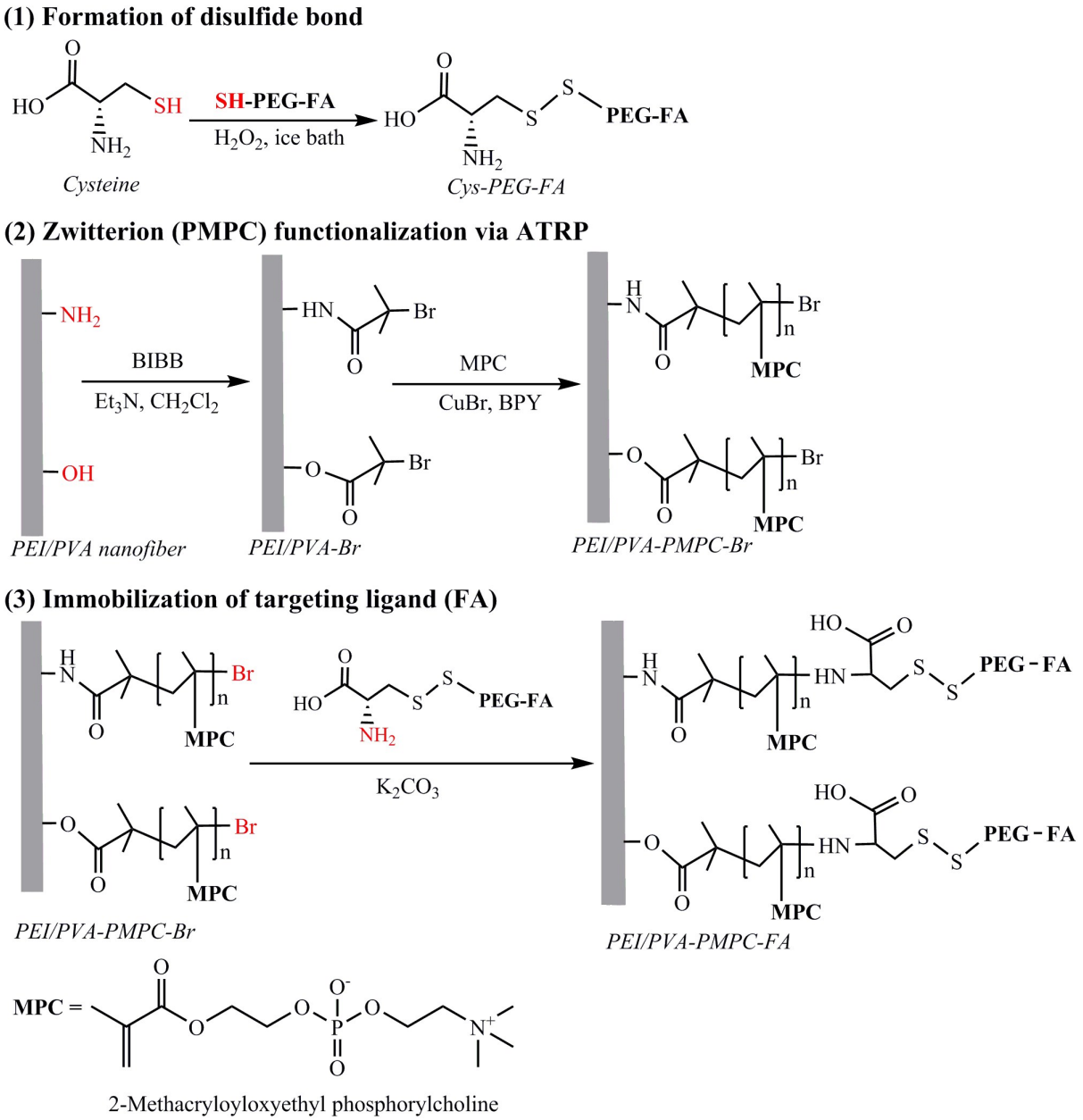

Figure S1. Schematic illustration of the formation of PEI/PVA-PMPC-FA nanofibers.

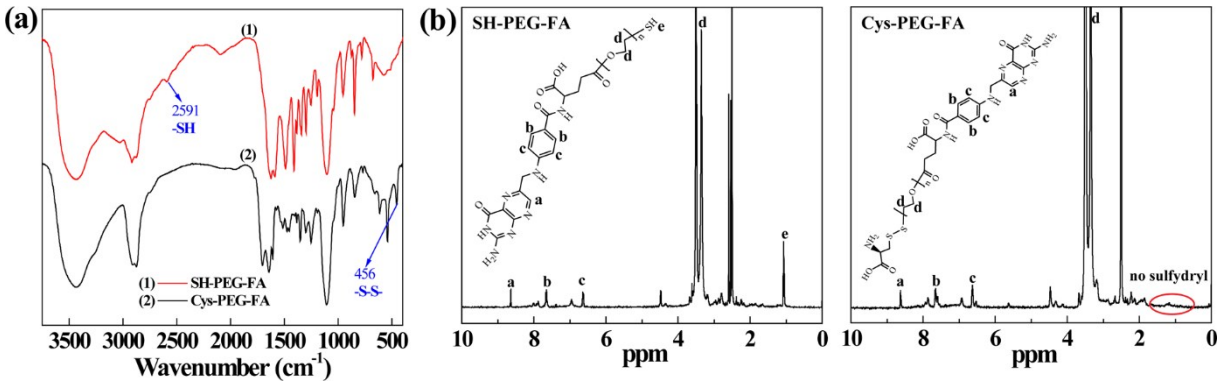

Figure S2. FTIR (a) and ^{1}H NMR (b) spectra of SH-PEG-FA and Cys-PEG-FA.

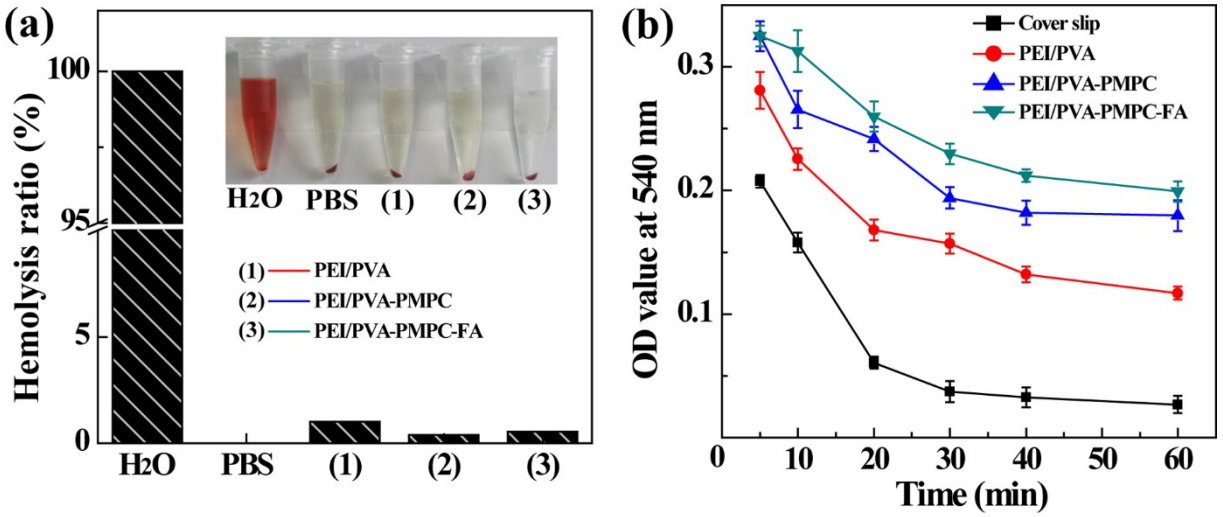

Figure S3. (a) Hemolysis percentage of HRBCs exposed to H_2O, PBS, and PBS containing nanofibrous mats (4 mg/mL) for 2 h. Inset shows a digital photograph of HRBCs after centrifugation. (b) OD values at 540 nm for anticoagulant assay of coverslips, PEI/PVA, PEI/PVA-PMPC and PEI/PVA-PMPC-FA nanofibrous mats at different time intervals.

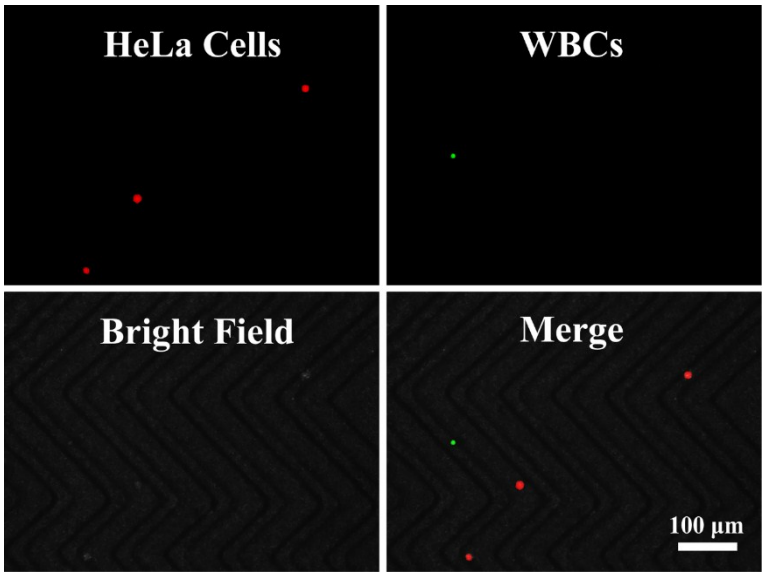

Figure S4. Fluorescence microscopic images of HeLa cells (red) and WBCs (green) captured using the microfluidic chip at a flow rate of 2 mL/h.

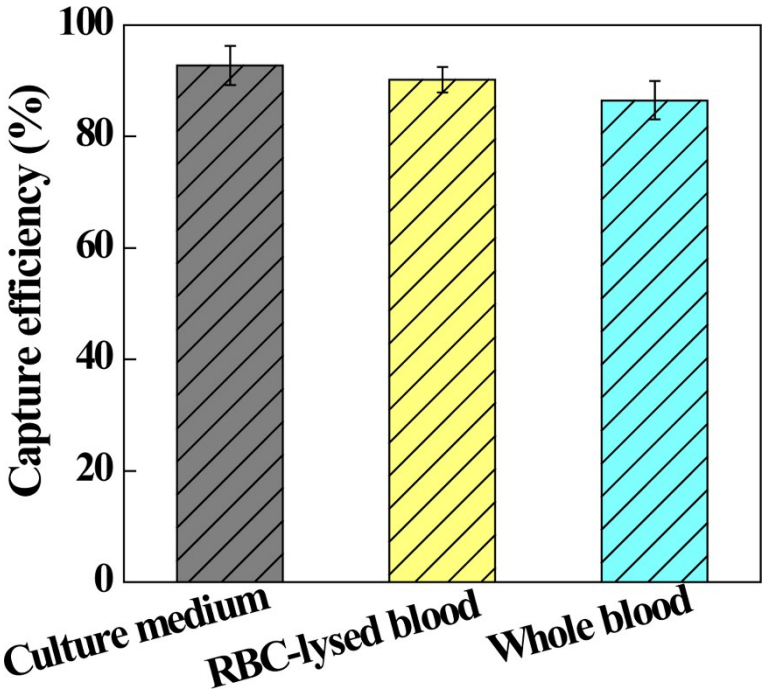

Figure S5. Capture efficiency of HeLa cells captured from culture medium, RBC-lysed blood, healthy human whole blood, respectively. The HeLa cells with a density of 200/mL were spiked into three medium, and then the HeLa cells were captured using our microfluidic chip at a flow rate of 2 mL/h.

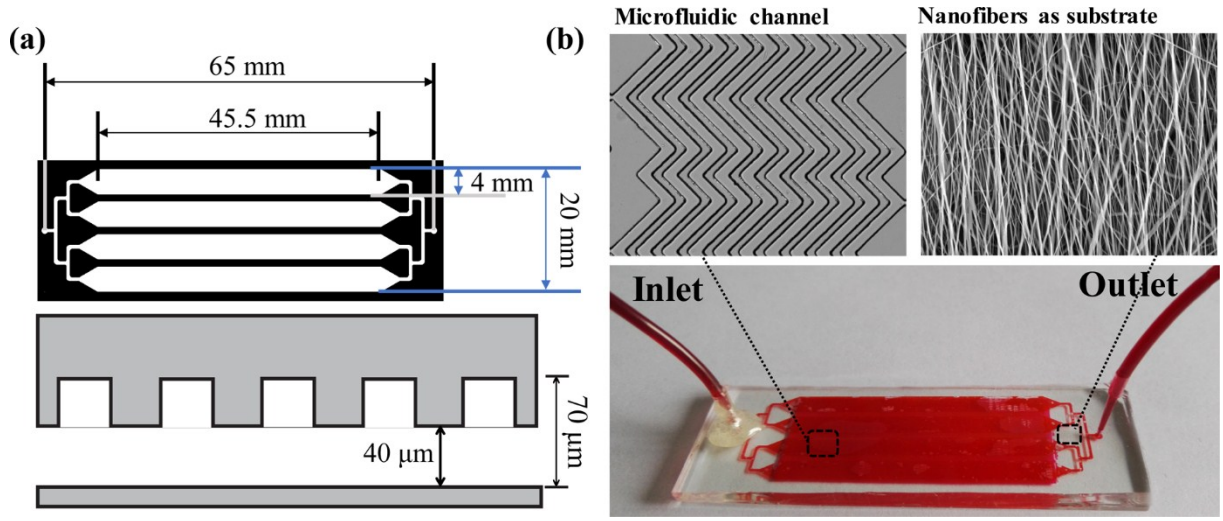

Figure S6. (a) The structure and dimension of the designed microfluidic channel. (b) Photograph of the nanofiber-integrated microfluidic chip. Upright and upleft panels show the magnified micrograph of the channel and the SEM image of nanofibers.

References

1. Y. L. Zhao, S. G. Wang, Q. S. Guo, M. W. Shen and X. Y. Shi, *J. Appl. Polym. Sci.*, 2013, **127**, 4825-4832.