Supporting Information

A Size Amplified Immune Magnetic Microbeads Strategy in Circulating Tumor Cells Rapid Detection

Hongyan Zhang,[1,a,b] Yanhong Wang,[1,b] Qingling Li,[a] Fumiao Zhang,[b] and Bo Tang*[a]

a. College of Chemistry, Chemical Engineering and Materials Science, Collaborative Innovation Center of Functionalized Probes for Chemical Imaging, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Shandong Normal University, Jinan 250014, P. R. China

b. College of Life Science, Shandong Normal University, Jinan 250014, P. R. China

Corresponding Author: Bo Tang

*Fax: (86)531 86180017. Email: tangb@sdnu.edu.cn.

Ethics Statement. Human blood samples were obtained from participants in Shandong Normal University (Shandong, China). In all cases, informed written consent was obtained from all participants. We are especially grateful to all study participants and hope that their contributions will help advance cancer diagnosis, via improving CTC capture techniques. All experiments were performed in compliance with the relevant laws and institutional guidelines, and the academic committee of Shandong Normal University has approved the experiments.

Culture and Preparation of MCF-7 Cells. The commercially available breast carcinoma cell line, MCF-7 (KeyGEN Biotech, Nanjing, China) was maintained in Dulbecco's modification of Eagle's medium Dulbecco (DMEM) supplemented with 10% fetal bovine serum, cultivated at 37℃ and incubated in 5% CO_2 conditions. Adherent cells were harvested by trypsinization.

Preparation of the Immune-magnetic Microbeads. The carbodiimide (EDC)/N-hydroxysuccinimide (NHS) coupling strategy was used to covalently link the anti-EpCAM antibodies (Beijing Boosen Biological Technology Co., Ltd., Beijing, China) to the magnetic microbeads. 200 μL COOH-magnetic microbeads (3 μm, Tianjin Beisile Chromatography Technology Development Center, Tianjin, China) were activated by incubating with 250 μL solution of EDC/NHS for 30 min.

Following 5 washes with phosphate buffer saline (PBS), 150 μL anti-EpCAM antibodies (10 $\mu g \cdot mL^{-1}$) were added into the activated magnetic microbeads. After 6 hours incubation at room temperature, magnetically separated microbeads were washed 5 times to remove un-bound antibodies. 0.025% sodium azide was added to the immune-magnetic microbeads, and stored in PBS at 4℃ until use.

Capture Device and Procedure. Polydimethylsiloxane (PDMS) sheets were synthesized using two components of silicone elastomer 184 (50 g: 5 g) (Base and curing agent sylgard ® 184,

Dow Corning, Midland, American). A hole (diameter = 3.5 mm) was punched in the center of each PDMS sheet. A Nuclepore Track-Etch membrane (8 μm of pore diameter, GE Healthcare Life Sciences, Fairfield, USA) was clamped by two PDMS sheets, and the two holes in the PDMS sheets were kept overlaid. A syringe imbibed with MCF-7 cells or human blood sample was placed on the hole of the upper PDMS sheet, and the hole of the underlying PDMS sheet was connected to a TS-2A/L0107-2A Syringe Pump (LongerPump®, Baoding, China). The MCF-7 cells or human blood samples were filtered through the membrane inhaled by the Syringe Pump (200 μL/min), and then the membrane was put on the magnetic separation rack to perform the magnetic separation. Figure S1 showed the devices of membrane filtration and magnetic separation.

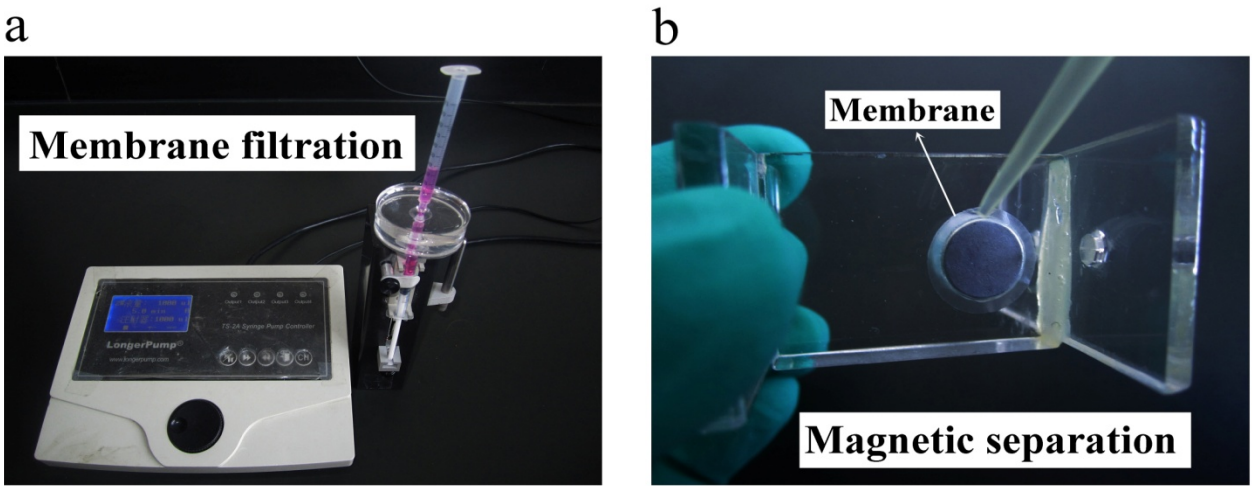

Fig. S1 Devices of the CTCs capture and purification

Capture and Detection of MCF-7 Cells. 50 μL immune-magnetic microbeads were added to 100 μL culture media spiked with MCF-7 cells, incubated at room temperature, magnetically separated, washed 5 times with PBS, 150 μL PBS was added, and then the cells were observed by microscope. The numbers of magnetic microbeads surrounding each of the MCF-7 cells were used to measure the efficiency of cell incubation.

50 μL immune-magnetic microbeads were added to 100 μL culture media or 1 mL human blood spiked with MCF-7 cells, incubated at room temperature, filtered through membrane, and leukocytes retained on the membrane were further magnetically separated. The membrane was then observed under the inverted fluorescence microscope (Leica Microsystems Ltd., Wetzlar, Germany). The capture efficiency and capture purity of MCF-7 cells spiked in human blood were used to evaluate the effect of the overall method.