Supporting Information

**Uelx Europaeus Agglutinin-I-Based Magnetic Isolation for Efficient and Specific Capture of SW480 Circulating Colorectal Tumor Cells**

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1. Additional Experimental Section

1.1 Chemicals and Materials.

The carboxyl-terminated-Fe$_3$O$_4$ magnetic nanospheres (CSMN Beads-1000, termed as MBs) were purchased from Shanghai So-Fe Biomedicine Co., Ltd. (Shanghai, China). Fluorescein ullex europaeus agglutinin I (termed as FL-UEA-I) and uelx europaeus agglutinin I (termed as UEA-I) were purchased from Vector Laboratory Ltd. (Burlingame, CA, USA). Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (termed as EDC) and N-hydroxysulfosuccinimide sodium salt (termed as sulfo-NHS) were purchased from Aladdin Industrial Co., Ltd. (Shanghai, China). Triton X-100, Hoechst 33342, Rhodamine B, Calcein AM and propidium iodide (PI) were purchased from Sigma-Aldrich (USA). Alexa Fluor 568-labeled anti-CK19 monoclonal antibody (ab203445) and Alexa Fluor 488-labeled anti-CD45 monoclonal antibody (ab197730) were purchased from Abcam Company (Shanghai, China). The culture medium McCoy’s 5A and Leibovitz’s L-15 were obtained from Jiangsu KeyGEN BioTECH Co., Ltd. (Jiangsu, China). Bovine serum albumin (termed as BSA), 4-morpholineethanesulfonic acid hydrate (termed as MES), N-(2-hydroxyethyl) piperazine-29-(2-ethane-sulfonic acid) (termed as HEPES), 4% polyformaldehyde, Tween-20, fetal bovine serum (FBS) and trypsin were purchased from Beijing Dingguo Biotechnology Ltd. (Beijing, China). Human colon cell lines (SW480, HCT116) were obtained from Shanghai Cell Bank of Chinese Academy of Sciences (Shanghai, China), and the normal colon cell line (NCM460) was obtained from Shanghai Honsun Biological Technology Co., Ltd. (Shanghai, China). The fresh
whole blood samples were obtained from The First Hospital of Jilin University (Institutional Review Board approval number 19K060-001) with the anticoagulant sodium heparin and patients’ informed consents were obtained. Other reagents of analytical grade were purchased from Beijing Chemical Reagents Company (Beijing, China). Milli-Q water with a resistivity of 18.2 MΩ cm was used all through the experiments.

1.2 Characterization

Scanning electron microscope (SEM) micrographs were performed on field emitted SEM (XL 30 ESEM FEG, FEI Co., USA). Fluorescence spectra were obtained from a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., France). The fluorescence images of cells were performed with a Nikon Ti-S fluorescent microscope (Nikon, Tokyo, Japan). The confocal fluorescence images of the cells were performed with a confocal microscope (C2, Nikon, Tokyo, Japan). The fluorescence images of the MBs and MB-UEA-I were observed by Davinch Invivo HR (Davinch K, Korea).

1.3 Preparation of MB-UEA-I.

0.5 mg MBs were washed with 1 mL MES (0.1 mol L⁻¹, pH 6.0, 3 times), then redispersed in 1 mL MES including EDC (50 mmol L⁻¹) and sulfo-NHS (50 mmol L⁻¹) solution to activate the carboxyl group with gentle shaking at room temperature (RT) for 1 h. The activated MBs were separated using a magnet. After washed with 1 mL HEPES (0.01 mol L⁻¹, pH 7.2, 3 times), the activated MBs were incubated with 25 µg UEA-I in 1 mL HEPES under sustained oscillating at RT for 3 h. For removal of
excess UEA-I, the UEA-I modified MBs (MB-UEA-I) were separated by using a magnet, washed with 1 mL HEPES (3 times), redispersed in 1 mL HEPES, and stored at 4 °C. MB-UEA-I-FL were prepared as previously described except use of Fluorescein modified UEA-I (FL-UEA-I).

1.4 Optimization of MB-UEA-I concentration and incubation time

The MB-UEA-I concentration and incubation time were optimized to achieve the best performance for capturing tumor cells. First, 100 SW480 cells were treated with different concentrations of MB-UEA-I (0.0125, 0.025 and 0.05 mg mL\(^{-1}\)) in 1 mL complete culture medium (L-15 + 10 v/v% FBS) with continuous shaking at 37 °C for 60 min. And 100 SW480 cells were also treated with MB-UEA-I (0.05 mg mL\(^{-1}\)) in 1 mL complete culture medium with continuous shaking at 37 °C for different incubation time (15, 30, 45 and 60 min). The same volume of cell suspension was distributed among three wells in a 48-well plate to calculate the mean of cells spiked into the complete medium. Then the captured cells were separated by a magnetic scaffold, and the uncaptured cells scattered in the solution were placed in 48-well plate and counted using the inverted microscope to calculate the capture efficiency.

1.5 SEM observation.

Samples of MBs, MB-UEA-I and the cells captured by MB-UEA-I or uncaptured cells scattered in the solution were prepared for SEM imaging with a standard procedure. The SEM samples of the cells were prepared according to the previous report.[S1] Simply, the cells were fixed with 2.5% glutaraldehyde in PBS at room temperature for 1 h and at 4 °C for 12 h, respectively. Then the samples were
dehydration by the gradient ethanol (30%, 50%, 70%, 90%, 95% and 100%) stepwise for 10 min of every step at room temperature. The cells were dropped onto the clean silicon wafer for freezing in the refrigerator, followed by dried with a vacuum freeze-drier. The dried samples were gold-coated by a sputter and scanned under SEM.

1.6 Cell viability and proliferation of captured cancer cells.

The cell viability was studied by Calcein AM and PI co-staining. Specifically, the MB-UEA-I captured SW480 cells were incubated with Calcein AM (2 μmol L\(^{-1}\)) and PI (4.5 μmol L\(^{-1}\)) at 37 °C for 30 min, washed with 1 mL PBS (3 times), and then transferred to image with fluorescent microscope. 200 cells were counted to calculate the cell viability. For proliferation study, the 3 \(\times\) 10\(^4\) MB-UEA-I captured SW480 cells in 1 mL L-15 were seeded in a 48-well plate and incubated for various time (12 h, 24 h, 72 h, reached confluence, reached confluence after one passage and reached confluence after five passages), respectively. Then, the cells were incubated with Hoechst 33342 (4 μg mL\(^{-1}\)) and Rhodamine B (10 μg mL\(^{-1}\)) for 30 min, washed with 1 mL PBS (3 times), then imaged by fluorescent microscope. Besides, 3 \(\times\) 10\(^4\) MB-UEA-I captured SW480 cells in 1 mL L-15 were seeded into the 48-well plate and cultured for different time (1, 2, 3 and 4 days). At each time point, the cell numbers were counted and normalized with seeded cell number, respectively. The viability and proliferation of normal cultured SW480 cells were also examined as control.

1.7 Three-color immunocytochemistry (ICC) identification.

The three-color ICC was performed according to the previous report.\(^{[S2]}\) Briefly,
the captured cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% Triton X-100 (10 min) and blocked with 1% BSA in 0.05% PBST (1 h) at room temperature. Then the cells were then stained with 10 µg mL\(^{-1}\) AF568-CK19 and AF488-CD45 at 4 ºC overnight. Finally, the stained cells were nuclei-stained by Hoechst 33342 (4 µg mL\(^{-1}\)) at 4 ºC for 10 min. Further identification and enumeration of CTCs were performed using the confocal fluorescence microscope. The Hoechst 33342\(^+\)/CK19\(^+\)/CD45\(^-\) cells were enumerated as CTCs, and the Hoechst 33342\(^+\)/CK19\(^-\)/CD45\(^+\) cells were enumerated as white blood cells (WBCs).
2. Additional Figures S1-S7

**Figure S1.** (a) Calibration curve of FL-UEA-I. (b) The fluorescence spectra of 25 µg FL-UEA-I before (i) and after (ii) reaction with 0.5 mg MBs. The solution volume is 0.5 mL.
Figure S2. Capture efficiencies of spiked SW480 cells in complete culture medium as a function of various concentrations of MB-UEA-I (a) and incubation time (b), respectively. Error bars mean standard deviations (n = 3).
Figure S3. Fluorescence images of Hoechst 33342 pre-stained SW480 cells, Rhodamine B pre-stained HCT116 cells and Rhodamine B pre-stained NCM460 cells incubated with MB-UEA-I, and Hoechst 33342 pre-stained SW480 cells incubated with MBs. The scale bars are 50 µm.
Figure S4. SEM micrographs of MB-UEA-I after interaction with (a) SW480 cells, (b) HCT116 cells and (c) NCM460 cells, (d) MB after interactions with SW480 cells. The scale bars are 2 μm.
Figure S5. Fluorescence images of the 4 captured cells (a-d) from 6 Hoechst 33342 pre-stained SW480 cells spiking in 5 mL healthy whole blood. The scale bars are 10 μm.
Figure S6. Viability analysis of captured cells. Fluorescence images of the control cells (a) and the captured cells (b) stained with Calcein AM and PI. (c) Quantitative viability analysis of control cells and captured cells. The normal cultured cells were employed as control samples. The scale bars are 50 µm. Error bars mean standard deviations (n = 3).
Figure S7. The folds of increased cell numbers as a function of incubation days. The time point before proliferated in the plate was deemed as 0 day. The results are shown as fold changes relative to the numbers of those at 0 day. The normal cultured cells were employed as control sample. Error bars mean standard deviations (n = 3).
3. Additional Table S1

Table S1. Quantitative detection of CTCs in clinical blood samples from colorectal cancer patients and healthy donors.

| Sample No. | Cancer Type | Gender | Age | Metastasis | Volume processed/mL | CTCs |
|------------|-------------|--------|-----|------------|---------------------|------|
| 1          | Colorectal  | M      | 85  | No         | 1                   | 12   |
| 2          | Colorectal  | M      | 72  | No         | 1                   | 20   |
| 3          | Colorectal  | F      | 69  | No         | 1                   | 12   |
| 4          | Colorectal  | M      | 85  | No         | 1                   | 11   |
| 5          | Colorectal  | F      | 68  | No         | 1                   | 16   |
| 6          | Colorectal  | M      | 73  | No         | 1                   | 10   |
| 7          | Colorectal  | M      | 57  | No         | 1                   | 8    |
| 8          | Colorectal  | M      | 63  | Yes        | 1                   | 5    |
| 9          | Colorectal  | M      | 60  | Yes        | 1                   | 3    |
| 10         | Colorectal  | F      | 78  | Yes        | 1                   | 5    |
| 11         | Colorectal  | M      | 58  | Yes        | 1                   | 7    |
| 12         | Colorectal  | M      | 40  | Yes        | 1                   | 6    |
| 13         | Colorectal  | M      | 52  | Yes        | 1                   | 3    |
| 14         | Colorectal  | M      | 49  | Yes        | 1                   | 5    |
| 15         | Healthy     | M      | 69  | No         | 1                   | 0    |
| 16         | Healthy     | M      | 51  | No         | 1                   | 0    |
| 17         | Healthy     | F      | 49  | No         | 1                   | 0    |
| 18         | Healthy     | F      | 59  | No         | 1                   | 0    |
| 19         | Healthy     | M      | 63  | No         | 1                   | 0    |
4. Additional References

[S1] Wu, L. L.; Tang, M.; Zhang, Z. L.; Qi, C. B.; Hu, J.; Ma, X. Y.; Pang, D. W. Chip-Assisted Single-Cell Biomarker Profiling of Heterogeneous Circulating Tumor Cells Using Multifunctional Nanospheres. *Anal. Chem.* **2018**, *90*, 10518-10526.

[S2] Chen, L.; Peng, M.; Li, N.; Song, Q.; Yao, Y.; Xu, B.; Liu, H.; Ruan, P. Combined use of EpCAM and FRα enables the high-efficiency capture of circulating tumor cells in non-small cell lung cancer. *Scientific reports* **2018**, *8*, 1188.