Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Protein Corona-coated Immunomagnetic Nanoparticles with Enhanced Circulating Tumor Cells Isolation

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Experimental Section

Materials and Reagents

Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium (RPMI 1640), 0.25% trypsin-EDTA, fetal bovine serum (FBS), fluorescein isothiocyanate-conjugated anti-human CD45 (FITC-anti-CD45), eFluor 570-conjugated anti-human Cytokeratin 18 (eFluor 570-anti-CK18) and commercial streptavidin-conjugated Dynabeads were obtained from Thermo-Fisher (USA). Biotinylated anti-human EpCAM antibody and Alexa Fluor® 488 Anti-EpCAM antibody were purchased from Abcam (USA). FITC-conjugated Affinipure Goat Anti-Mouse IgG(H+L) was purchased from proteintech (China). Iron chloride hexahydrate, ethylene glycol, sodium acetate trihydrate, glutaraldehyde (GA) and ethanolamine were obtained from Aladdin-Reagent (China). Bicinchoninic acid (BCA) assay kit, calcein acetoxymethyl ester/propidium iodide (AM/PI) cell viability assay kit, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine,4-chlorobenzenesulfonate Salt (DiD), 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO), 4’,6-diamidino-2-phenylindole (DAPI) and RIPA lysis buffer and red blood cell (RBC) lysis buffer were purchased from Beyotime (China). Cell Counting Kit-8 (CCK-8) was obtained from Dojindo (Japan). The other reagents used were obtained from Solarbio (China). All of the aqueous solutions in this work were prepared using deionized water purified with a purification system (Millipore, USA).

Cells Culture and Clinical Blood Samples

Human cervical cancer cell line (Hela), human breast cancer cell line (MCF-7), human colon cancer cell line (HCT116), human T lymphocyte cell line (Jurkat) and the mouse monocyte macrophage cell line (RAW264.7) were purchased from ATCC. Jurkat cells and other cells were cultured in RPMI 1640 or DEME medium, respectively, supplemented with 10% FBS and 1% penicillin/streptomycin at 37 ℃ in 5% CO_2 incubators. Whole human serum (HS) was obtained from Second Affiliated Hospital of Tianjin university of TCM and used within a month. Clinical blood samples from health donors and breast cancer patients were obtained from Tianjin Medical University Cancer Institute and Hospital.

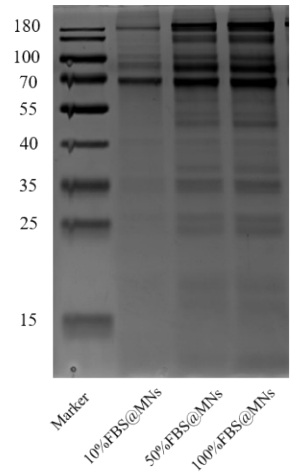

Fig S1. SDS-PAGE protein analysis of 10%FBS@MNs, 50%FBS@MNs, 100%FBS@MNs.

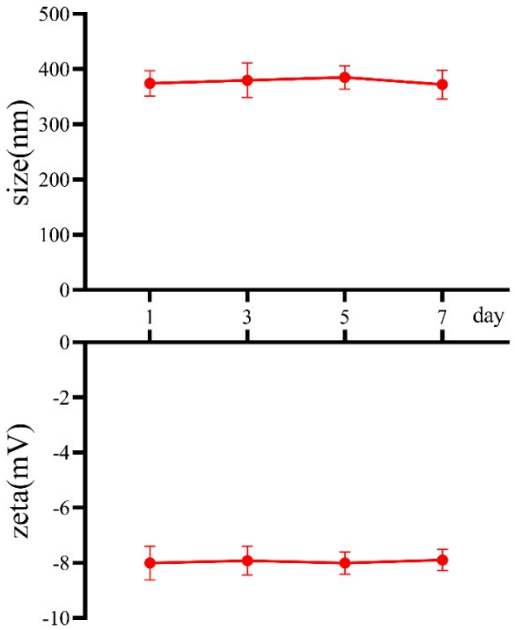

Fig S2. Mean diameter and zeta potential of C-MNs in PBS over 7 days.

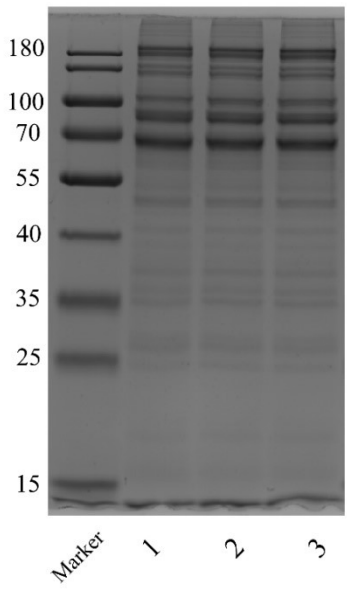

Fig S3. The repeatability of protein corona formation.

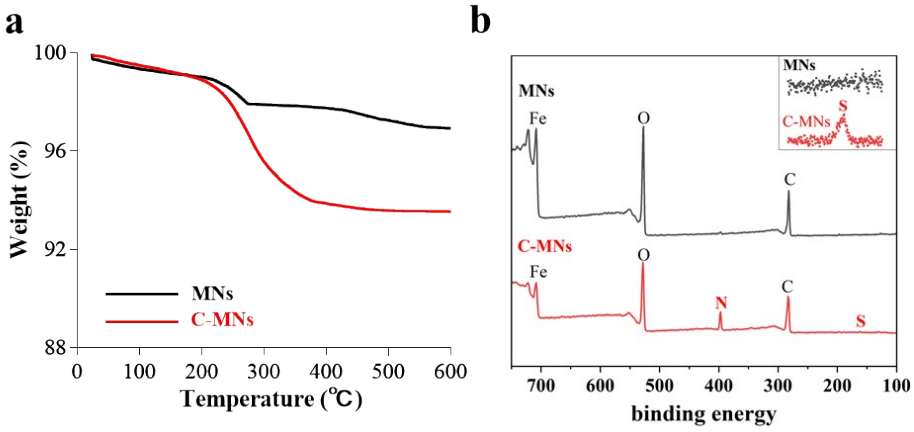

Fig S4. (a) Weight loss thermograms of MNs and C-MNs. (b) XPS spectrum of MNs and C-MNs. The insert is S element of MNs and C-MNs.

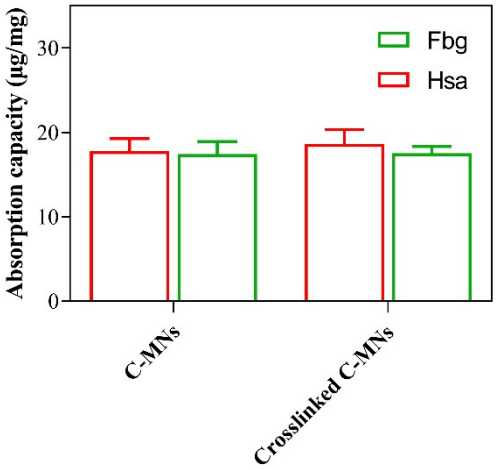

Fig S5. The protein adsorption of C-MNs with or without crosslinking.

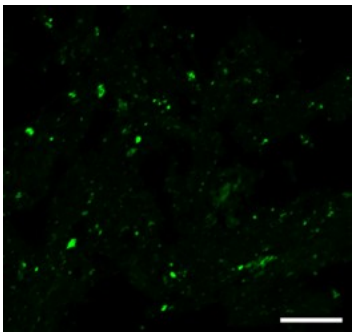

Fig S6. CLSM image of FITC-IgG labeled IC-MNs. Scale bar, 50 μm.

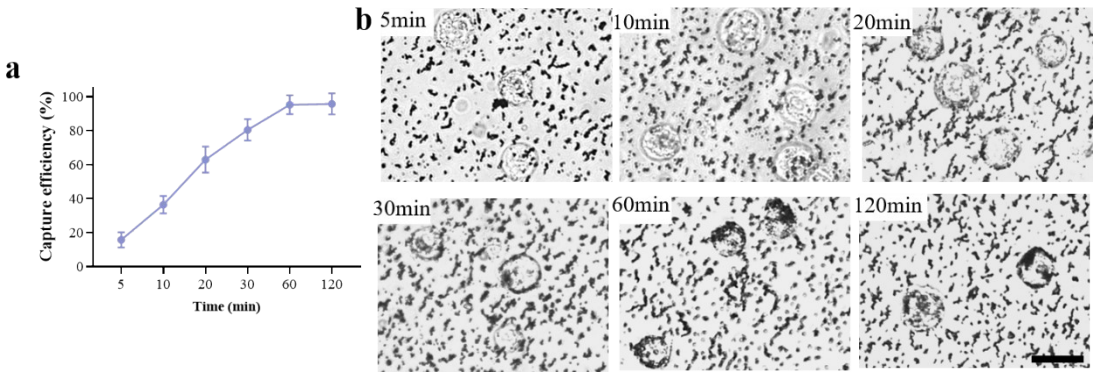

Fig S7. (a) Cell capture efficiency and (b) microscopic bright field images of MCF-7 cells bound by the IC-MNs with different incubation times. Scale bar, 50 μm.

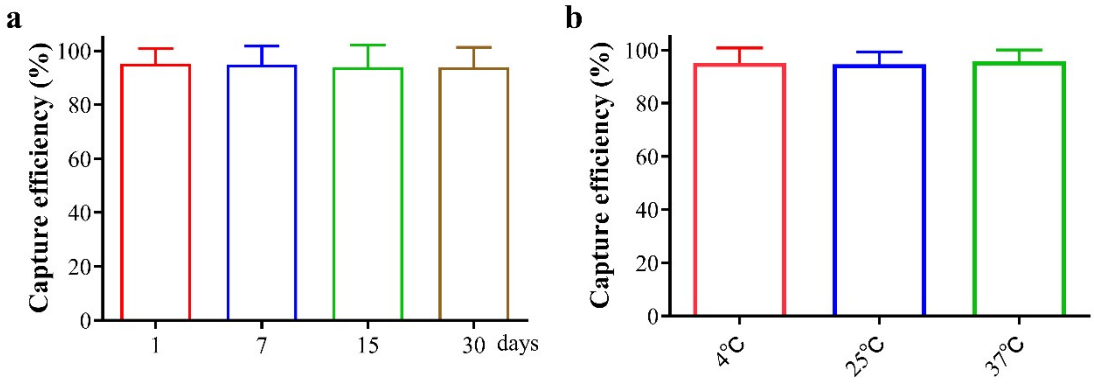

Fig S8. (a) The capture stability of IC-MNs stored in PBS solution. (b) The CTCs capture efficiency of IC-MNs at different temperatures.

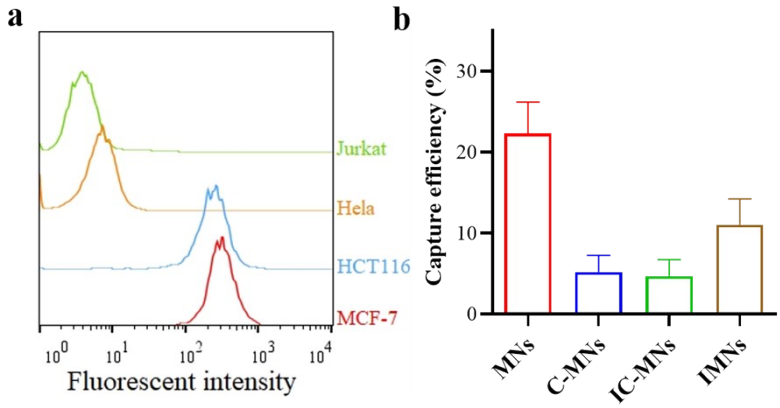

Fig S9. (a) Different expression of EpCAM levels of four cell lines were tested by flow cytometry. (b) The capture efficiency of Jurkat cells using different MNs.

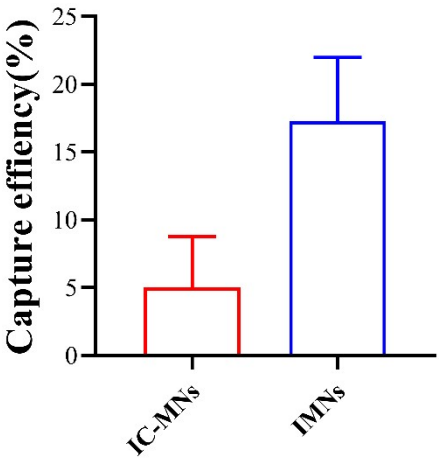

Fig S10. The capture efficiency of WBCs by IC-MNs or IMNs.

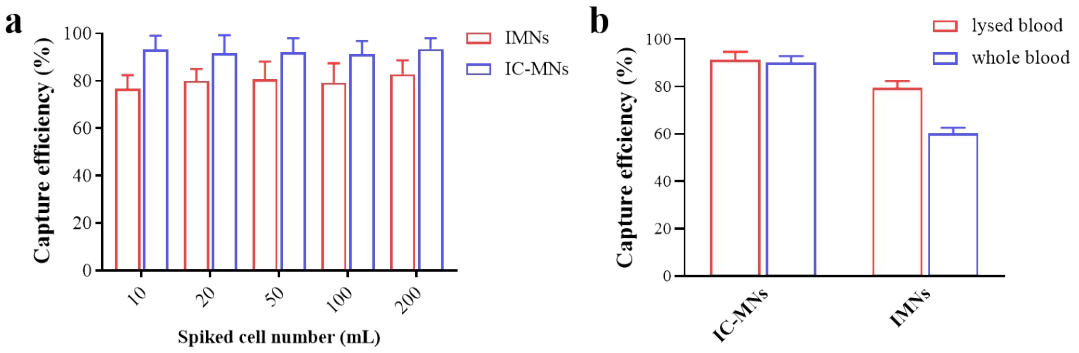

Fig S11. (a) Capture efficiency of IMNs or IC-MNs based on different quantity of MCF-7 cells spiked in PBS. (b) The capture efficiency of MCF-7 cells spiked in whole blood or lysed blood obtained from healthy donors using IMNs or IC-MNs.

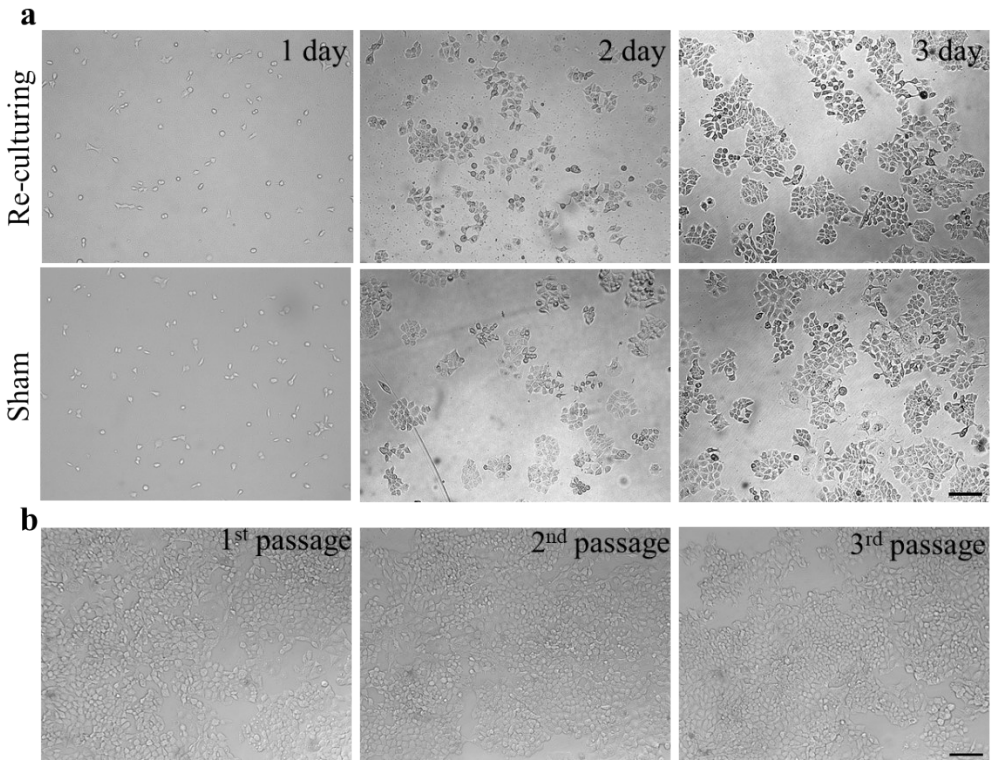

Fig S12. (a) Microscopic images of released or untreated MCF-7 cells were cultured after different days. (b) Microscopic images of detached MCF-7 cells were cultured after different passages.

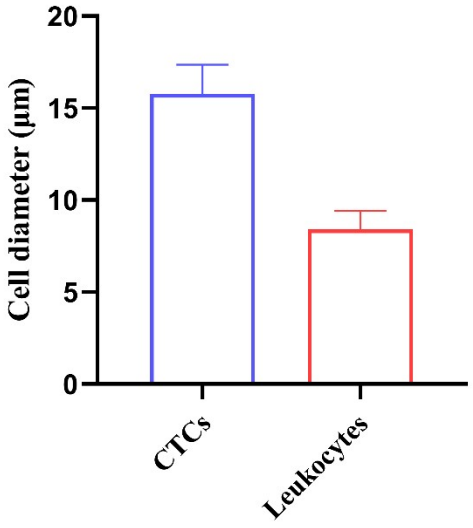

Fig S13. The mean size of the captured CTCs and leukocytes from the breast cancer patients by using IC-MNs or IMNs.

Table S1. Complete blood panel analysis of the blood samples from the human treated with PBS or PBS containing IC-MNs.

Group	Unit	Control	IC-MNs
WBC	10^9/L	5.77±1.71	5.80±1.67
RBC	10^{12}/L	4.78±0.06	4.83±0.08
PLT	10^9/L	204.67±37.06	195.00±28.89
HGB	g/L	140.00±8.49	141.33±8.73
HCT	%	39.57±2.10	39.87±2.32
MCV	fL	82.67±3.58	82.43±3.52
MCH	pg	29.27±1.48	29.23±1.31
MCHC	g/L	353.67±3.30	354.33±0.94
RDW	%	12.07±0.17	11.90±0.14
PDW	%	16.50±0.45	16.17±2.13

WBC: white blood cell; RBC: red blood cell; PLT: platelets; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; PDW: platelet distribution width.

Table S2. CTC counting of 1.0 mL whole blood samples collected from healthy donors or breast cancer patients. The purity of CTC was defined as the ratio of captured CTCs against the total number of captured cells.

Blood sample	Healthy/ breast cancer	CTC counting		CTC purity	
		Dynabeads IMNs	IC-MNs	Dynabeads IMNs	IC-MNs
1	Healthy	0	0	/	/
2	Healthy	0	0	/	/
3	Healthy	0	0	/	/
4	Healthy	0	0	/	/
5	Healthy	0	0	/	/
6	Cancer	8	13	72.73%	92.86%
7	Cancer	6	11	66.67%	84.62%
8	Cancer	5	8	55.56%	88.89%
9	Cancer	3	7	60.%	87.5%
10	Cancer	3	5	42.86%	83.33%
11	Cancer	4	9	66.67%	90%
12	Cancer	5	7	62.5%	100%
13	Cancer	5	10	55.56%	90.91%
14	Cancer	2	4	50%	100%
15	Cancer	0	0	/	/
16	Cancer	3	6	50%	100%
17	Cancer	3	4	37.5%	80%
18	Cancer	2	2	40%	66.67%
19	Cancer	4	3	57.14%	75.00%
20	Cancer	7	10	53.85%	83.33%
21	Cancer	5	7	55.56%	87.50%
22	Cancer	0	0	/	/
23	Cancer	3	4	60.00%	80.00%
24	Cancer	4	4	57.14%	100.00%
25	Cancer	5	8	45.45%	80.00%
26	Cancer	4	6	57.14%	85.71%
27	Cancer	6	5	54.55%	83.33%
28	Cancer	2	2	50.00%	100.00%
29	Cancer	0	3	/	60.00%
30	Cancer	2	3	66.67%	100.00%
31	Cancer	3	2	42.86%	50.00%