Supporting Information

Detection of circulating tumor cells based on improved SERS-active magnetic nanoparticles

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Materials

Ethylene glycol ($C_2H_6O_2$), sodium acetate anhydrous ($C_2H_3NaO_2$), sodium borohydride ($NaBH_4$), folic acid ($C_{19}H_{19}N_7O_6$), albumin from bovine serum (BSA), 1-ethyl-3-[3-(dimethyllamino)propyl] carbodiimide hydrochloride (EDC·HCl) as well as N-hydroxysuccinimide (NHS) were ordered from Aladdin Reagent Co. Ltd. (Shanghai, China). Iron chloride hexahydrate ($FeCl_3·6H_2O$) was purchased from Alfa Aesar. Tetrachloroauric(III) acid tetrahydrate ($HAuCl_4·4H_2O$), trisodium citrate dehydrate ($C_6H_5Na_3O_7·2H_2O$) and Hoechst were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). 4-mercaptobenzoic acid (MBA), polyethylenimine (Mw~25000), DMSO and MTT was ordered from Sigma-Aldrich. Lymphocyte isolation was ordered from Slolarbio Life Science Co. Ltd. (Beijing, China). Fetal bovine serum (FBS), incomplete DMEM (high glucose) as well as trypsin-EDTA were ordered from KeyGen BioTech. Anti-CD45 antibody [F10-89-4] (Alexa Fluor® 488) Alexa and Anti-Cytokeratin 8 antibody [EP1628Y] (Alexa Fluor® 647) were purchased from Abcam. (Zhejiang, China).

Instrumentation.

The nanoparticles were characterized by transmission electron microscopy (TEM, JEOL. 2100, Tokyo, Japan), and UV-vis spectroscopy (T10CS, Beijing Purkinje General Instrument Co., Ltd., China). The Raman spectra were observed on a confocal microprobe Raman system (Renishaw inVia Reflex, Wolton-under-Edge, U.K.). The laser wavelength was fixed at 785 nm. The range of the scattering spectra was set from 400 to 1500 cm^{-1}, the time of data acquisition was set to 3.0 s, and the laser power was 280 mW. The SERS spectra were observed from liquid samples with homogeneous SERS hotspot. The diameter of the laser beam was tuned to be ~ 1.0 mm to capture lots of SERS hotspot at the same time. The particle size and size distribution of the nanoparticles were measured at room temperature by dynamic light scattering (DLS) using a zeta particle size analyzer (Malvern, England) with a detection angle of scattered light at 173°. Magnetic properties of the nanoparticles

were characterized using a Quantum Design Model-9 PPMS (Quantum Design, USA) by measuring the applied field dependence of magnetization between -30 and 30 kOe at 300 K.

Table S1. Synthesis conditions and characterization results of SPION-PEI@AuNPs-MBA and SPION-PEI@AuNPs-MBA-rBSA-FA.

Nomenclature	C_{Au} (mg/mL)	C_{Fe} (mg/mL)	C_{MBA} (μM)	$C_{rBSA-FA}$ (μg/mL)	SERS intensity
SPION-PEI@AuNPs-MBA1	0.17	0.15	2.5	-	5890±230
SPION-PEI@AuNPs-MBA2	0.17	0.15	5	-	9220±400
SPION-PEI@AuNPs-MBA3	0.17	0.15	10	-	14690±360
SPION-PEI@AuNPs-MBA4	0.17	0.15	25	-	5480±350
SPION-PEI@AuNPs-MBA3-rBSA-FA1	0.17	0.15	10	11	11440±220
SPION-PEI@AuNPs-MBA3-rBSA-FA2	0.17	0.15	10	25	10190±440
SPION-PEI@AuNPs-MBA3-rBSA-FA3	0.17	0.15	10	45	14760±960
SPION-PEI@AuNPs-MBA3-rBSA-FA4	0.17	0.15	10	60	11880±1010

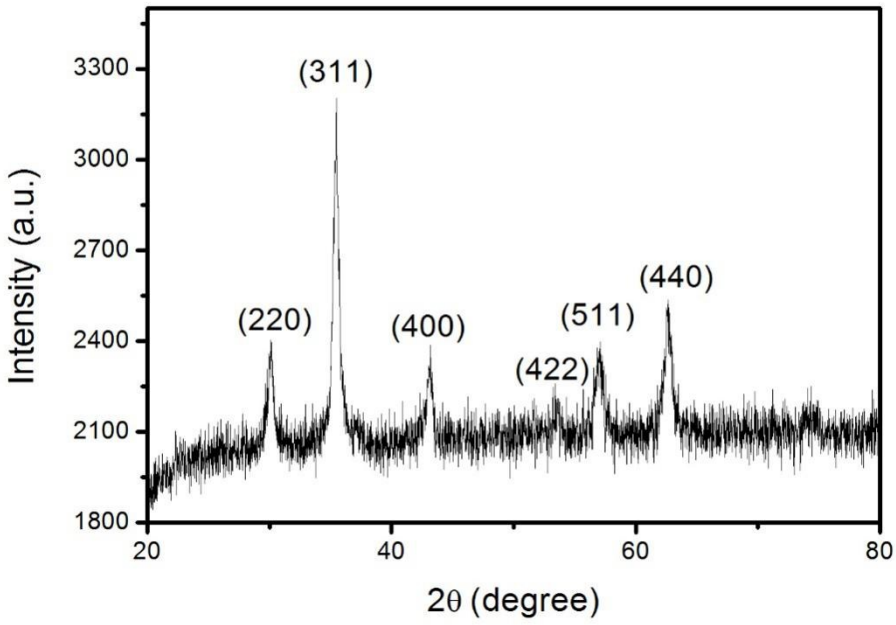

Fig. S1 X-ray diffraction (XRD) spectrum of SPION-PEI.

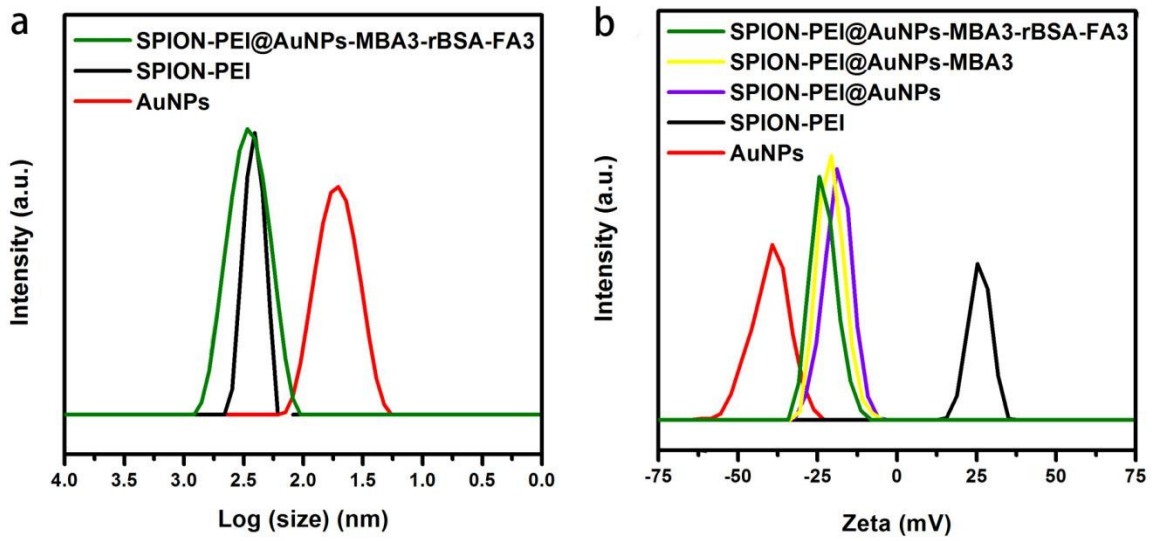

Fig. S2 (a) Size distribution of SPION-PEI@AuNPs-MBA3-rBSA-FA3, SPION-PEI and AuNPs. (b) Zeta potential of SPION-PEI@AuNPs-MBA3-rBSA-FA3, SPION-PEI@AuNPs-MBA3, SPION-PEI@AuNPs, SPION-PEI and AuNPs.

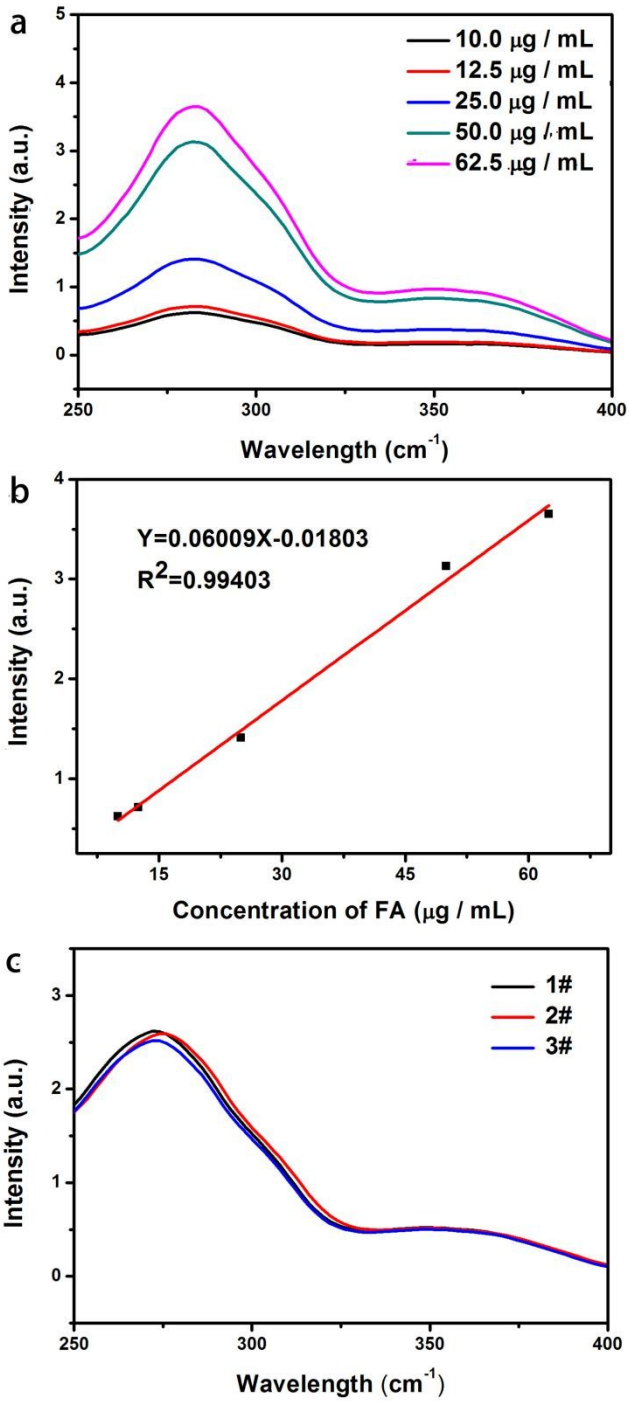

Fig. S3 (a) UV-visible spectroscopy of FA standard solutions with different concentrations. (b) A calibration curve constructed from the spectroscopy of FA standard solutions with different concentrations. (c) UV-visible spectroscopy of the supernatant during the purification of rBSA-FA. FA conjugated content (FCC) = mass of FA conjugated to rBSA / mass of rBSA-FA × 100 %.

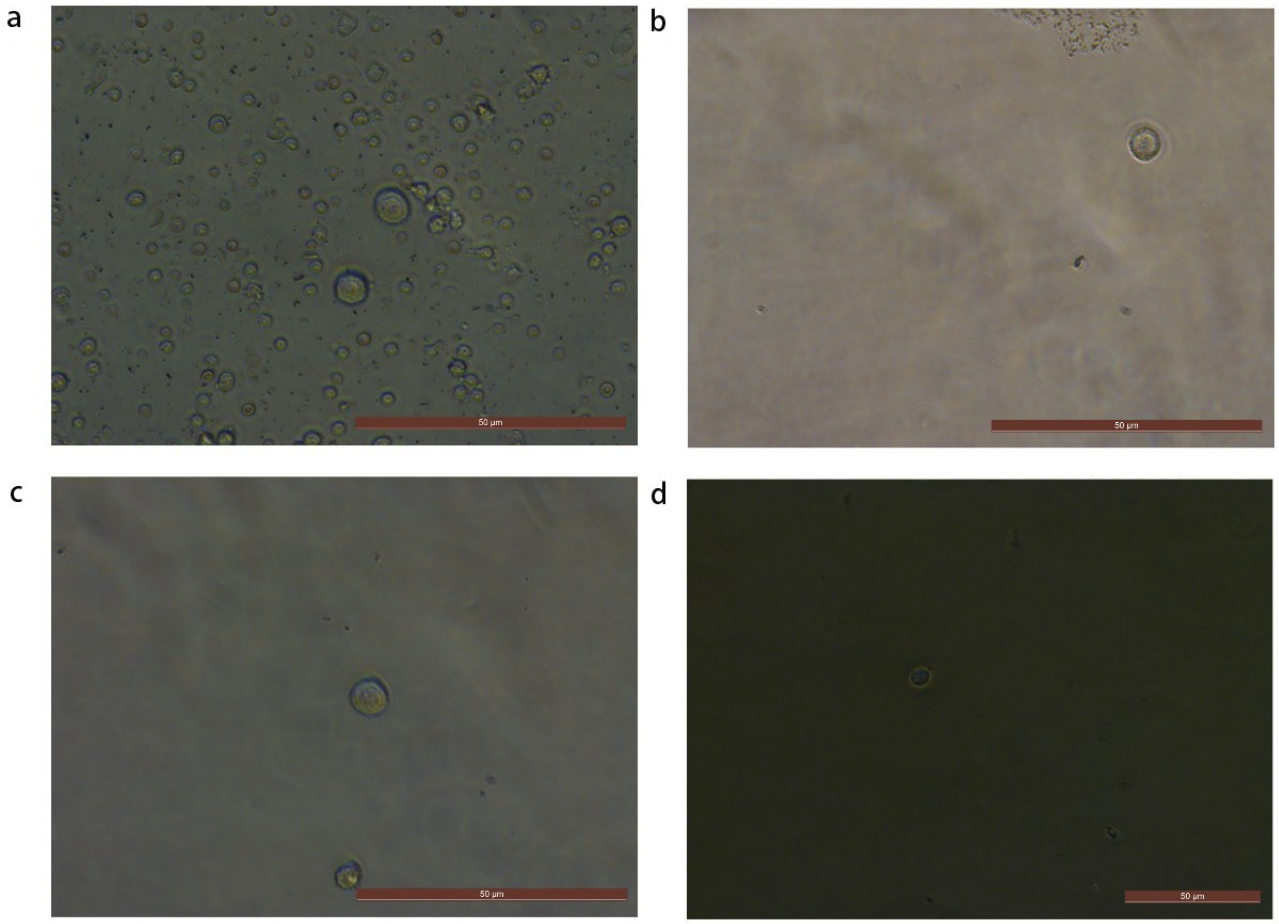

Fig. S4 Microscope images of (a) unseparated HeLa from WBCs, and (b-d) captured HeLa cells from blood.