Supporting Information for

Amphiphilic shell nanomagnetic adsorbents for selective and highly efficient capture of low-density lipoprotein from hyperlipidaemia serum

Yameng Yu[a], Boya Ma[a], Xinbang Jiang[a], Chen Guo[a], Zhuang Liu[a], Nan Li[a], Yamin Chai[a], Lichun Wang[a], Yunzheng Du[a], Biao Wang[a], Wenzhong Li[b,], Lailiang Ou[a,*]*

[a]Key Laboratory of Bioactive Materials, Ministry of Education, College of Life Science, Nankai University, Tianjin, 300071, China

[b]School of Ophthalmology & Optometry, Eye Hospital, School of Biomedical Engineering, Wenzhou Medical University, Wenzhou, Zhejiang, 325027, China.

[*]Lailiang Ou - Key Laboratory of Bioactive Materials, Ministry of Education, College of Life Science, Nankai University, Tianjin, 300071, China; E-mail: ouyll@nankai.edu.cn

[*]Wenzhong Li - School of Ophthalmology & Optometry, Eye Hospital, School of Biomedical Engineering, Wenzhou Medical University, Wenzhou, Zhejiang, 325027, China; E-mail: bcrtlwz@gmail.com

Experimental section

Reagents and Materials

Iron trichloride ($FeCl_3 \cdot 6H_2O$), sodium acetate, tetraethyl orthosilicate (TEOS), (3-aminopropyl), triethoxy silane (APTES), N,N'-dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS) and trimethylamine (TEA) were purchased from Aladdin Industrial Corporation (Shanghai, China). Poly(acrylic acid) with average molecular weight about 5000 was purchased from Macklin Reagent (Shanghai, China). Phosphatidyl ethanolamine (PE) was purchased from Mreda technology limited company (Beijing, China). All of the other reagents were purchased from Tianjin Bohai Chemical Industry Group Co., LTD (Tianjin, China) of analytical grade. All human serum samples were provided from HLP patients in Second Hospital Affiliated to Tianjin Medical University and were collected following experimental protocols reviewed and approved by the Ethics Committee of Nankai University (Tianjin, China). The work described here has been carried out in accordance with The Code of Ethics of the World Medical Association, and an informed consent was obtained for experimentation with all donors.

Preparation of magnetic nanoparticles (MNPs)

$FeCl_3 \cdot 6H_2O$ (0.43 g) and sodium acetate (1.2 g) were dissolved into ethylene glycol (14 mL). The mixture was stirred for 30 minutes at room temperature until a uniformly dispersed brown yellow solution was obtained. The mixture solution was then transferred into a Teflon-lined stainless steel autoclave and the reaction was performed at 200 °C for 8 hours. The obtained MNPs were collected and in turn washed with ethanol and distilled water, followed by vacuum drying at 60 °C for 24 hours.

Preparation of core-shell structured MNPs with silica oxide coating ($Fe_3O_4@SiO_2$)

The silica coated magnetic core-shell nanoparticles ($Fe_3O_4@SiO_2$) were synthesized

via the Stöber method. Typically, MNPs (0.5015 g) were dispersed in the ethanol-water solution (500 mL) via ultrasonication. Then ammonia solution (10 mL) and TEOS (1.07 mL) were added consecutively to the reaction mixture, and the reaction was performed at 25 °C for 6 hours. The resulting particles were collected and washed in turn with ethanol, deionized water, followed by vacuum drying at 60 °C for 24 hours.

Preparation of amino functionalized core-shell MNPs (Fe_3O_4@SiO_2-NH_2)

The dried nanoparticles (1 g) were resuspended in dried toluene (131.6 mL) via ultrasonication, followed by addition of APTES (1.55 mL). The reaction mixture was heated to reflux for 12 hours to obtain amino modified particles (Fe_3O_4@SiO_2-NH_2). The resulting nanoparticles were collected and washed with ethanol, followed with vacuum drying at 60 °C for 24 hours.

Sample preparation for water contact angle analysis

The surface hydrophobicity of samples was examined by water contact angle (WCA) analysis. The sample preparation of WCA was shown as follow: nanoparticles (5.00 mg) was added in N, N'-dimethylformamide (0.25 mL), the suspension was sonicated for 30 minutes to obtain a uniformly distributed sample solution. The sample solution was then dropwise added onto a glass slide, followed with air-dry for 12 hours and vacuum drying at 60 °C for 24 hours.

Cytotoxicity

Standard Cell Counting Kit-8 (CCK-8) assay was employed to evaluate cytotoxicity. The conditioned media was prepared by incubation Fe_3O_4@SiO_2@PAA-PE or Fe_3O_4 nanoparticles (as control) into DMEM at a concentration of 1 mg/mL for 24 hours at 37 °C, followed by addition of 10% fetal calf serum and 1% of antibiotics-antimycotics. The cell line used in cytotoxicity test was human umbilical vein endothelial cells (Huvec) with a seeding density at about 30,000/mL. Huvec was seeded into a 96-well plate at seeding volume of 100 μL and incubated in cell culture

media in the incubator at 37 °C and 5% CO_2 for 24 hours. The cell culture media was then replaced with conditioned media to evaluate the cytotoxicity of the prepared hemoperfusion adsorbent. After culture for 24 hours, 10 μL of CCK-8 solution was added into each well of the plate, and then the cells were cultured for another three hours. The absorbance of the solution was measured using a multifunctional enzyme marker at a wavelength of 450 nm. The cell viability was calculated by equation (S1):

$$\text{Cell Viability} = {}^{X}/_{X_0} \times 100\% \quad \text{(S1)}$$

where X is the OD value of cells cultured in conditioned media (Fe_3O_4@SiO_2@PAA-PE or Fe_3O_4), X_0 is the OD value of cell incubated in cell culture media. The live/dead staining assay was performed by using a Calcein/PI cell viability and cytotoxicity kit, the cell growth state was obtained via observation by laser scanning confocal microscope.

Serum protein adsorption

The adsorption capacity for serum protein of Fe_3O_4@SiO_2@PAA-PE was measured from serum of patients with HLP. The fresh serum was obtained from HLP patients (20 subjects) by coagulation-promoting tubes (2 mL) with centrifugation at 3000 rpm for 5 minutes. The serum samples were mixed before adsorption experiments. All the serum samples were collected following experimental protocols reviewed and approved by the Ethics Committee of Nankai University (Tianjin, China). Typically, the adsorbents (0.02 g) were dispersed in phosphate buffer saline (PBS) (0.25 mL) by ultrasonication, followed by addition of HLP serum (0.5 mL). The mixture of adsorbents and serum was incubated at 37 °C for 180 minutes under 160 rpm. After adsorption, the adsorbents were magnetically separated and the levels of albumin (ALB), globulin (GLO) and total protein (TP) were measured by the assistance of The Second Hospital of Tianjin Medical University.

Whole blood contact assay

Whole blood contact assay was performed in order to evaluate the effect of the amphiphilic adsorbent on blood cells, using Fe_3O_4 as a control and Fe_3O_4@SiO_2@PAA-PE as the experimental group. All the samples were throughout washed with physiological saline solution before the test. Fresh whole blood from healthy donor (26-year-old female) was collected with Ethylene Diamine Tetraacetic Acid (EDTA) test tube and mixed together for whole blood contact assay. 0.75 mL of freshly drawn blood was mixed with the samples (0.02 g) and incubated at 37 °C for one hour. After the incubation, the blood samples were separated with the samples magnetically, and the blood parameters were examined by The Second Hospital of Tianjin Medical University.

Hemolysis analysis

For hemolysis analysis, PVA was used as a control and PVA@COOH-PE was used as the experimental group. All the samples were throughout washed with physiological saline solution before the test. Fresh blood from healthy donor (26-year-old female) was anticoagulated by adding 2% potassium oxalate and diluted with physiological saline solution (blood sample: physiological saline solution at a ratio of 4:5, v:v) in order to produce the RBC suspension. The RBC suspensions (0.015 mL for each sample) were severally mixed with: (a) 0.75 mL of 0.9% NaCl solution as a negative control; (b) 0.75 mL of deionized water as a positive control; (c) 0.75 mL of 0.9% NaCl solution and 0.02 g of Fe_3O_4 as control group; (d) 0.75 mL of 0.9% NaCl solution and 0.02 g of Fe_3O_4@SiO_2@PAA-PE as experimental group. All the tubes are incubated at 37 °C for 60 min, blood cells were removed by centrifugation (2500 rpm) and the supernatants were evaluated at 545 nm for the release of hemoglobin. The extent of hemolysis was represented as hemolysis ratio (%) which is calculated by equation (S2):

$$\text{Hemolysis ratio}\ (\%) = \frac{OD_{\text{Sample}} - OD_{\text{Negative Control}}}{OD_{\text{Positive Control}} - OD_{\text{Negative Control}}} \times 100 \quad \text{(S2)}$$

where OD_{Sample} is the supernatant from the blood incubated with Fe_3O_4 or Fe_3O_4@SiO_2@PAA-PE, $OD_{Positive\ Control}$ is the supernatant from the blood incubated with deionized water, $OD_{Negative\ Control}$ is the supernatant from the blood incubated with physiological saline solution.

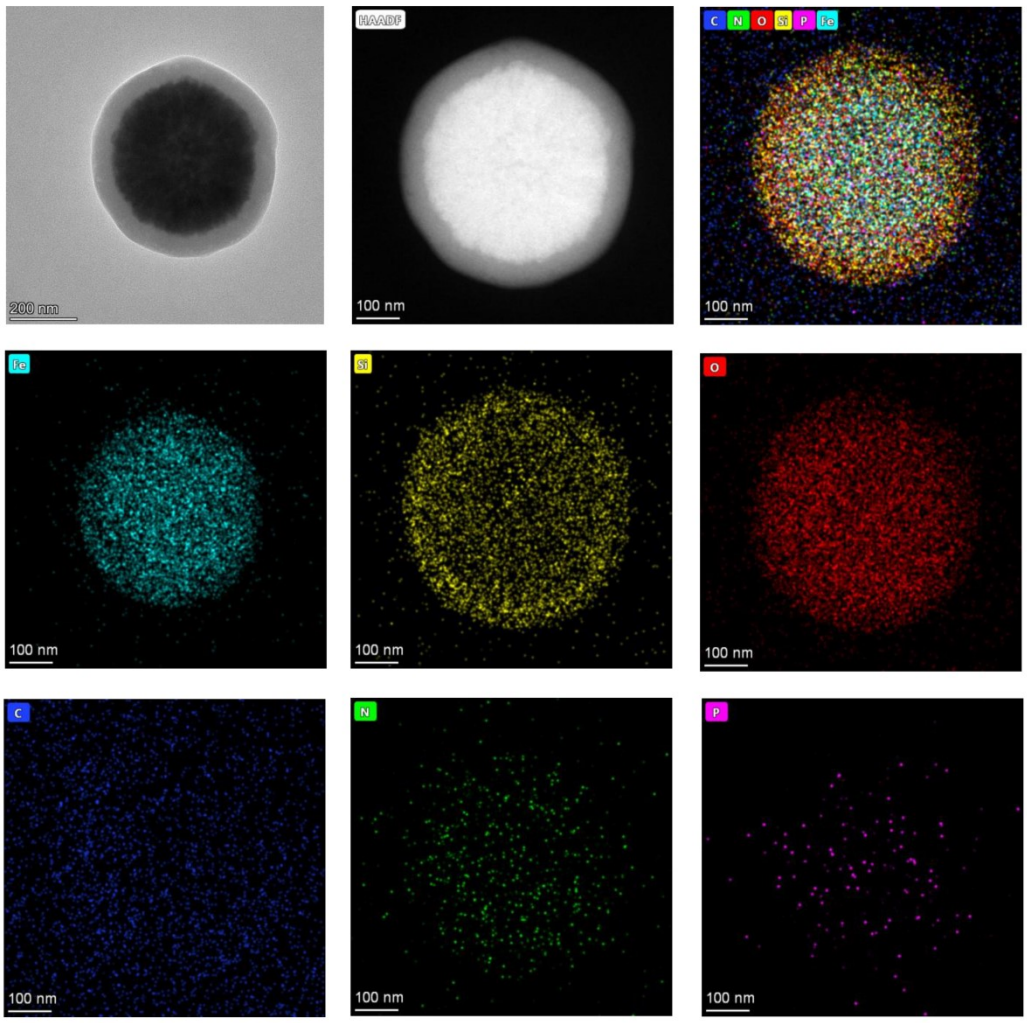

Fig. S1 EDX mapping of Fe_3O_4@SiO_2@PAA-PE.

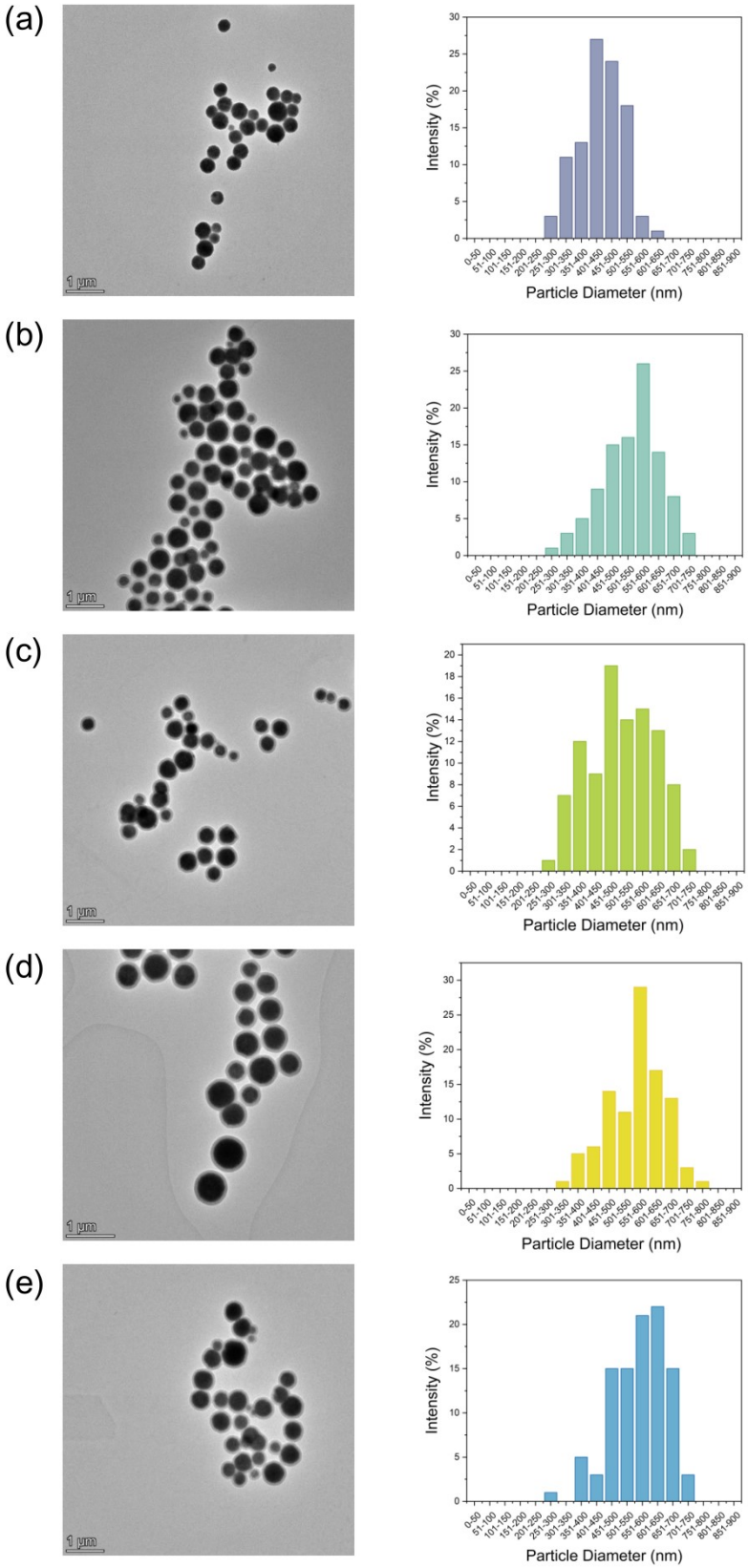

Fig. S2 Particle size distributions of (a) Fe_3O_4, (b) Fe_3O_4@SiO_2, (c) Fe_3O_4@SiO_2-NH_2, (d) Fe_3O_4@SiO_2@PAA and (e) Fe_3O_4@SiO_2@PAA-PE.

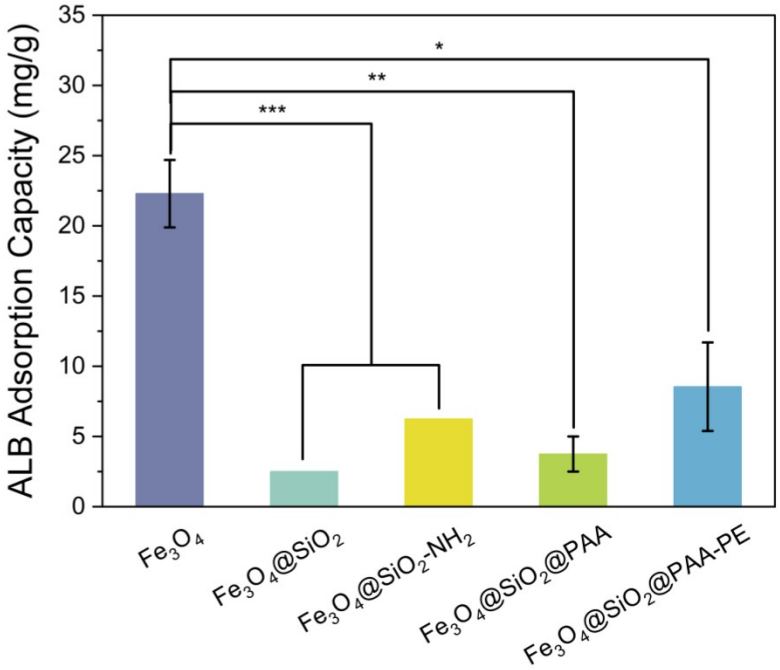

Fig. S3 Serum albumin adsorption from HLP serum.

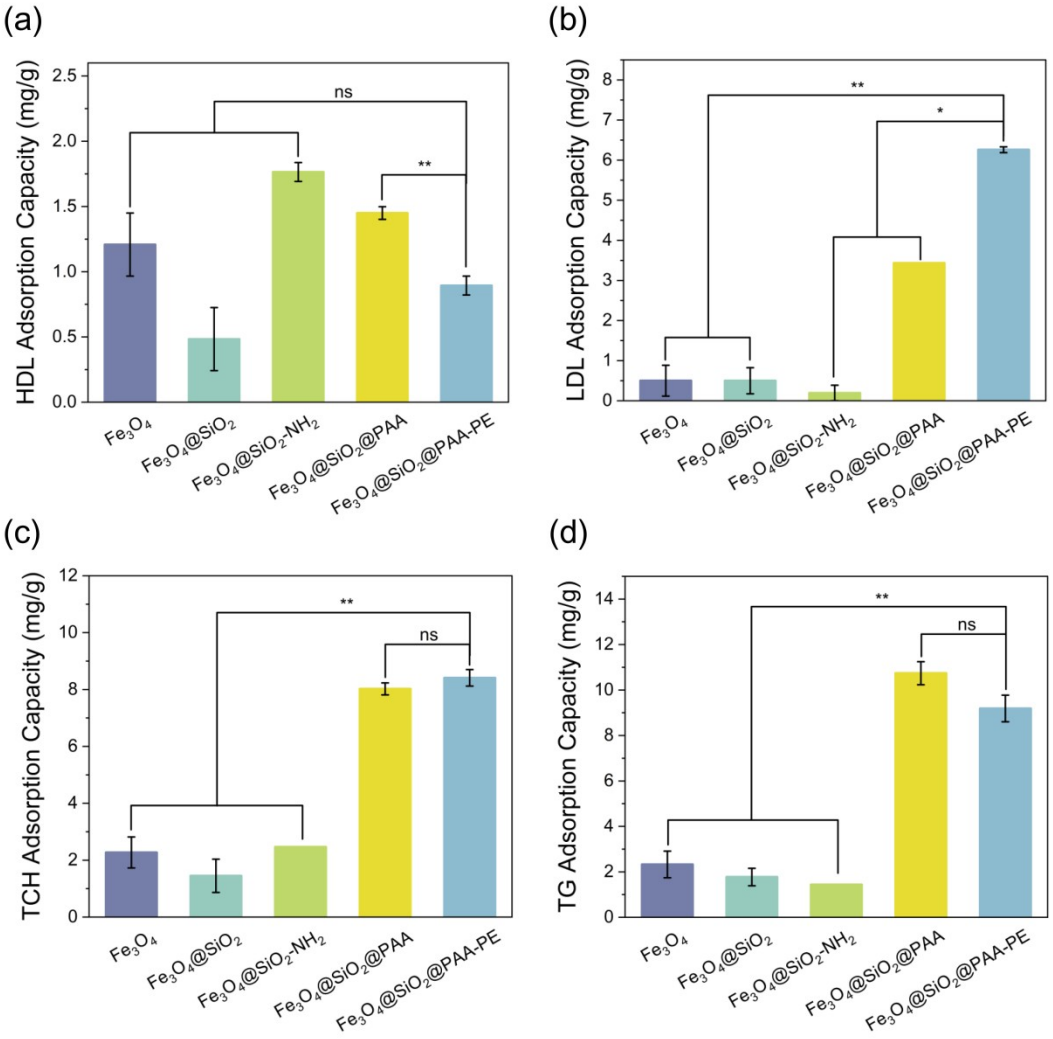

Fig. S4 The adsorptipn capacity for (a) HDL, (b) LDL, (c) TCH and (d) TG from HLP patients' serm on the prepared adsorbents in each synthetic stage.

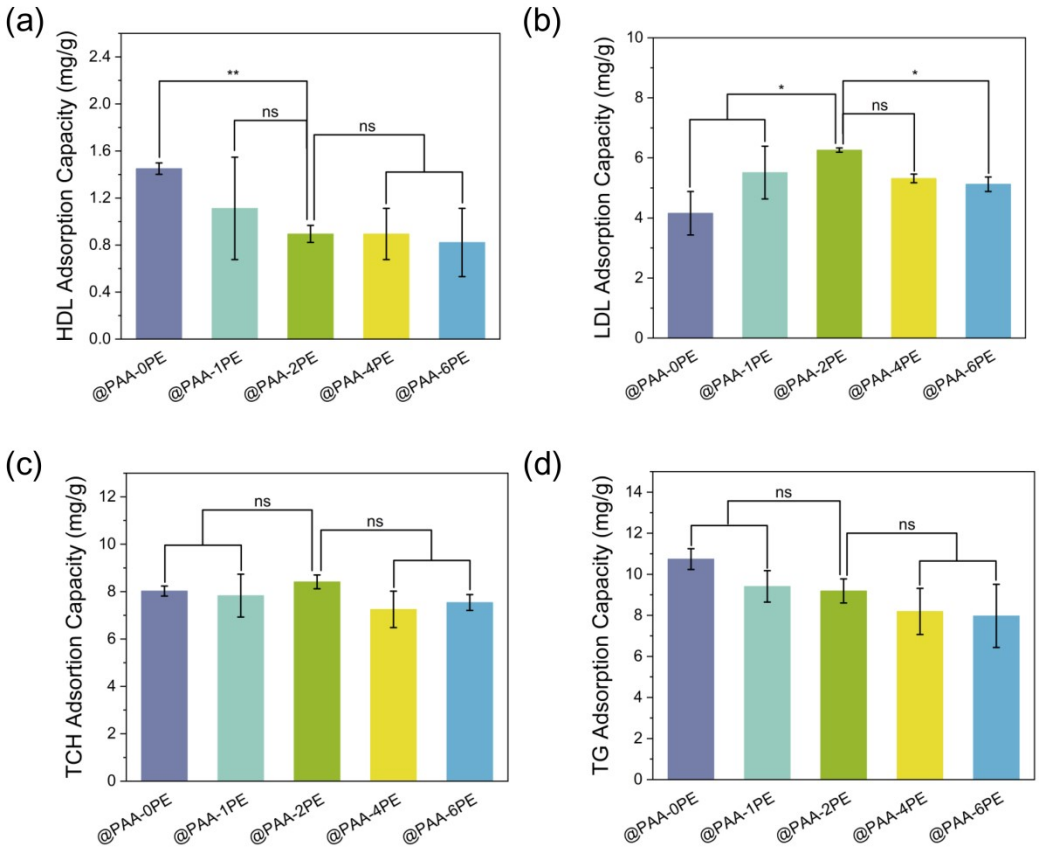

Fig. S5 Adsorption capacity of (a) HDL, (b) LDL, (c) TCH, (d) TG on adsorbent decorated with different PE addition amount (molar ratio).

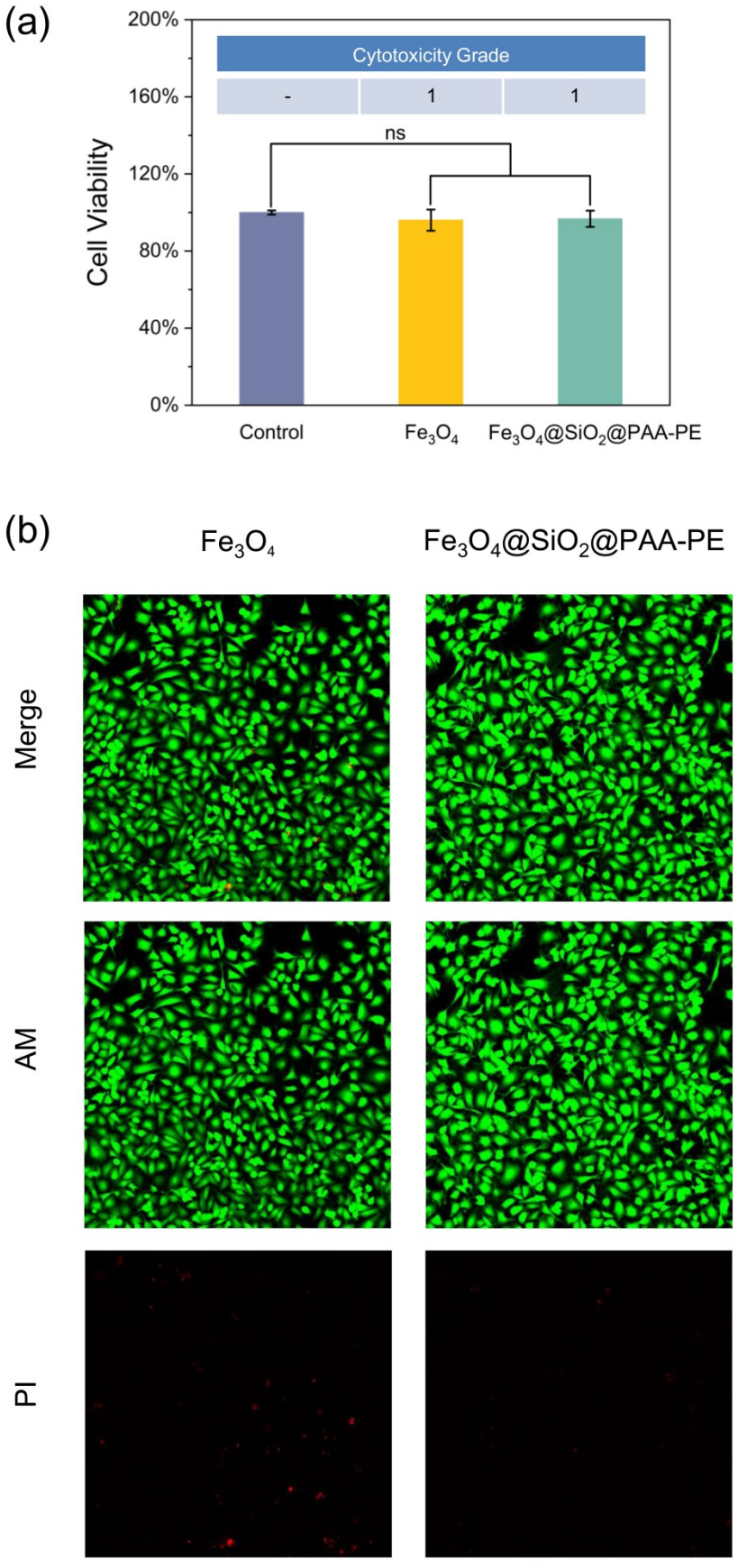

Fig. S6 (a) *In vitro* cytotoxicity. (b) Live/dead staining assay.

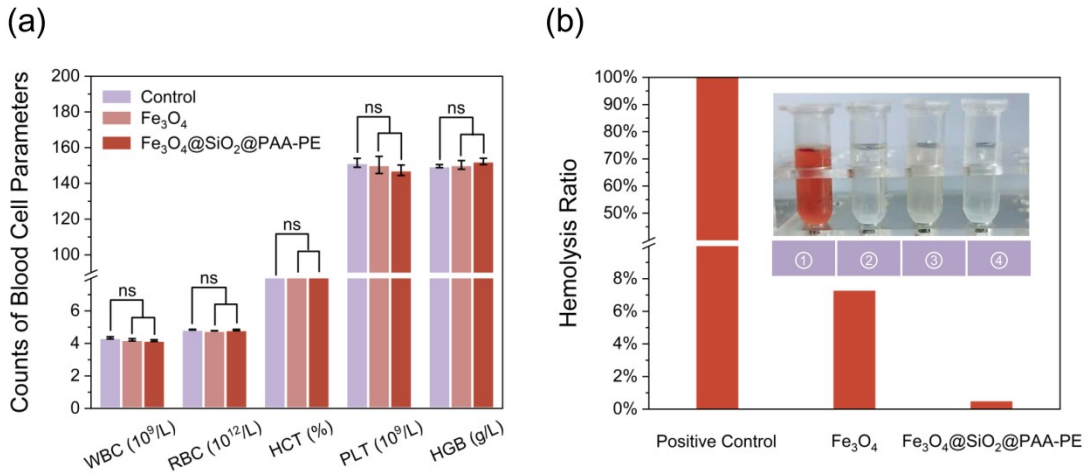

Fig. S7 Blood compatibility evaluated of Fe_3O_4@SiO_2@PAA-PE by whole blood contact assay and hemolysis analysis. (a) Blood cell counts of the fresh blood samples after incubation with Fe_3O_4 nanoparticles and Fe_3O_4@SiO_2@PAA-PE. (WBC: white blood cells; RBC: red blood cells; HCT: hematocrit; PLT: platelets; HGB: hemoglobins) (b) Hemolysis ratios of Fe_3O_4 nanoparticles and Fe_3O_4@SiO_2@PAA-PE. (①:positive control; ②: negative control; ③: Fe_3O_4 nanoparticles; ④: Fe_3O_4@SiO_2@PAA-PE)

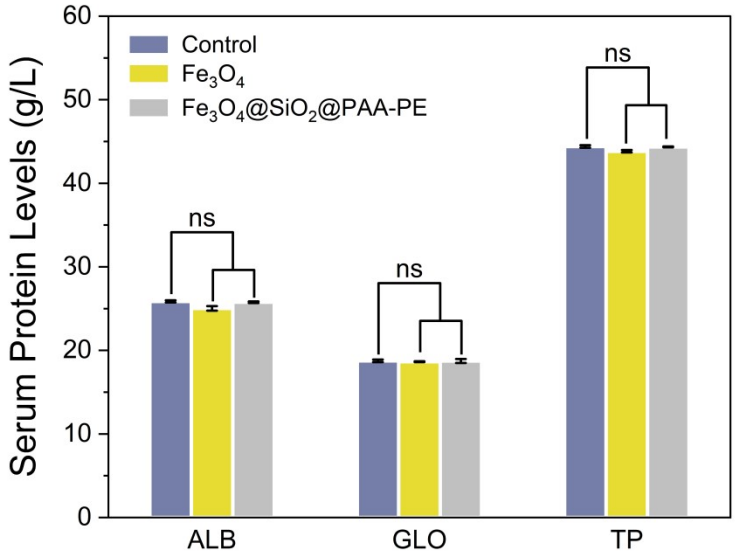

Fig. S8 Serum protein adsorption. (ALB: albumin; GLO: globulin; TP: total protein)

Table S1 The comparison between adsorbent prepared in this study with other adsorbents from previous publications regarding adsorption capacity for HDL, LDL, TCH and TG

Adsorbent	HDL	LDL	TCH	TG
PVA with phosphate groups [1]	0.61 mg/mL	1.70 mg/mL	2.82 mg/mL	2.25 mg/mL
Amphiphilic Dextran [2]	20 %	1.92 mg/mL	2.13 mg/mL	1.35 mg/mL
PVA with carboxyl groups [3]	0.66 mg/g	2.04 mg/g	2.70 mg/g	-
Heparin-chitosan MNPs [4]	-	1.04 mg/g	-	-
Our previous Work [5]	0.38 mg/mL	2.76 mg/mL	3.53 mg/mL	2.18 mg/mL
Adsorbent prepared in this study	0.89 mg/g	6.26 mg/g	7.03 mg/g	9.45 mg/g

Table S2 Content of phospholipid and dichloromethane in adsorbent synthetic procedure

Sample	PE:PAA (n/n)	PE (g)	Dichloromethane (mL)
@PAA-0PE	0	-	-
@PAA-1PE	1:1	0.95	4.75
@PAA-2PE	2:1	1.90	9.50
@PAA-4PE	4:1	3.80	19.00
@PAA-6PE	6:1	5.70	28.50

References:

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