Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2021

Supporting Information for

Bio-inspired dual-functional phospholipid-poly(acrylic acid) brushes grafted porous poly(vinyl alcohol) beads for selective adsorption of low-density lipoprotein

Yameng Yu [a,1] , Jingzhe Dong [a], Boya Ma [a], Xinbang Jiang [a], Chen Guo [a], Zhuang Liu [a], Yamin Chai [a], Lichun Wang [a], Lisha Sun [c,], Lailiang Ou [a,*] and Wenzhong Li [b,*]*

[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.

[c] General Hospital, Tianjin Medical University, Tianjin 300052, 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

*Lisha Sun - General Hospital, Tianjin Medical University, Tianjin 300052, China; E-mail: misssunlisha@sina.com

Experimental Section

Acid-base titration method for determination of carboxyl groups on PVA-COOH

The content of the carboxyl groups on PVA-COOH was measured by a standard acid-base titration method. 0.2 g of PVA-COOH was fully swelled in deionized water for several hours prior to use. The beads were then loaded into a flask with 25 mL of 0.1 M sodium hydroxide (NaOH) solution and incubated at 25 °C under oscillation. After one hour incubation, the liquid supernatant was separated and collected, and acid-base titration was performed on the supernatant liquid. Generally, 10 mL of the supernatant liquid was added into a conical flask with 90 μL methyl orange indicator, and hydrochloric acid (HCl) solution at about 0.05 M was used to determine molar concentration of the supernatant liquid. The exchange capacities of PVA-COOH were calculated from the titration results by equation (S1):

$$C_{COOH} = (C_{NaOH} - C_{HCl} \times V_{HCl}/V_{NaOH}^{'}) \times V_{NaOH} \times 0.6016/ M_{PVA} \quad (S1)$$

where C_{COOH} is the immobilized carboxyl groups on crosslinked PVA beads (PVA) (mmol/mL), C_{NaOH} is the molar concentration of standard NaOH solution (mmol/mL), C_{HCl} is the molar concentration of HCl solution for titration, V_{HCl} is the volume of HCl solution in titration (mL), $V^{'}_{NaOH}$ is the volume of supernatant liquid after incubation with PVA-COOH (mL), V_{NaOH} is the volume of standard NaOH solution (mL), M_{PVA} is the mass of PVA-COOH (g), 0.6016 is the transfer factor between mass and volume for the prepared beads.

Evaluation for the *in vitro* biocompatibility of PVA@PAA-PE

For routine blood test, fresh blood from healthy donor was collected with Ethylene Diamine Tetraacetic Acid (EDTA) test tube. 2 mL of freshly drawn blood was mixed with the prepared adsorbent and incubated at 37 °C. After one hour incubation, the blood cells were separated, and the red blood cells (RBC), white blood cells (WBC) and platelets (PLT) were examined by Tianjin Medical University General Hospital.

For anticoagulant activity, fresh blood of healthy donor was collected with sodium citrate anticoagulated blood collection tubes. The blood samples were centrifuged at 4000 rpm for 15 min to obtain platelet-poor plasma (PPP). 2 mL of PPP was added to 0.2 g of adsorbents and incubated at 37 °C with gently oscillation. Pure PPP without samples was used as controls. The anticoagulant properties of PVA@PAA-PE were elvaluated by activared partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT) and fibrinogen (FIB). The measurements were performed by a semiautomatic blood coagulation analyzer CA-50 (Sysmex Corporation, Kobe, Japan) by Tianjin Medical University General Hospital.

For hemolysis analysis, fresh blood of healthy rabbits was anticoagulated by adding 2% potassium oxalate and diluted with 0.9% NaCl solution in order to produce the RBC suspension. The RBC suspensions (0.1 mL for each sample) were severally mixed with: (a) 5 mL of 0.9% NaCl solution as a negative control; (b) 5 mL of deionized water as a positive control; (c) 5 mL of 0.9% NaCl solution and 2 g of PVA as control group; (d) 5 mL of 0.9% NaCl solution and 2 g of PVA@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 PVA or PVA@PAA-PE, $OD_{\text{Positive Control}}$ is the supernatant from the blood incubated with deionized water, $OD_{\text{Negative Control}}$ is the supernatant from the blood incubated with normal saline.

For cytotoxicity test, standard Cell Counting Kit-8 (CCK-8) assay was employed to evaluate cell viability. The conditioned media was prepared by incubation 4 g of the PVA@PAA-PE or PVA (as control) into 20 mL of cell culture media containing 10% fetal calf serum, 90% of DMEM and 1% of antibiotics-antimycotics at 37 °C for 72 hours. The cell line used in cytotoxicity test was mouse embryonic fibroblasts (NIH3T3) with a seeding density at about 30,000/mL. NIH3T3 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% CO2 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 72 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 (S3):

$$\text{Cell Viability} = {}^{X}/_{X_0} \times 100\% \quad \text{(S3)}$$

where X is the OD value of cells cultured in conditioned media (PVA@PAA-PE or PVA), X_0 is the OD value of cell incubated in cell culture media.

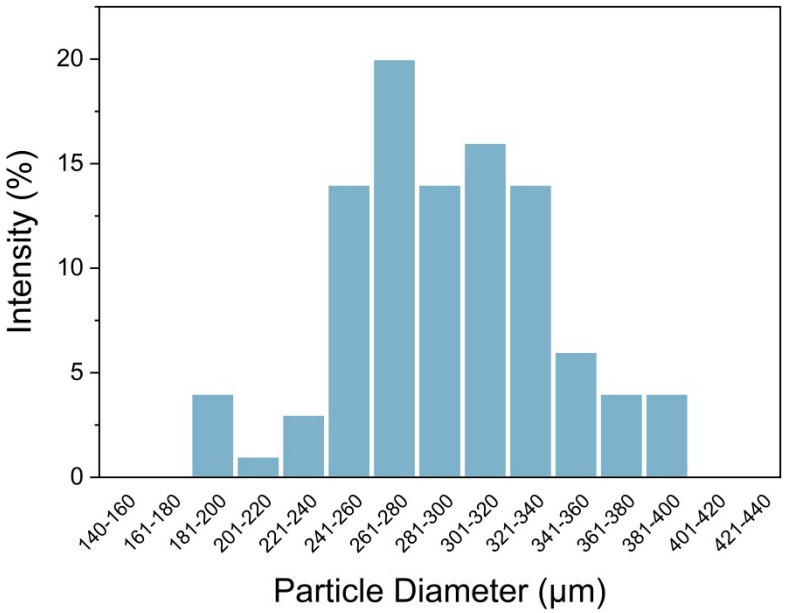

Fig. S1 The particle distribution of PVA@PAA-PE.

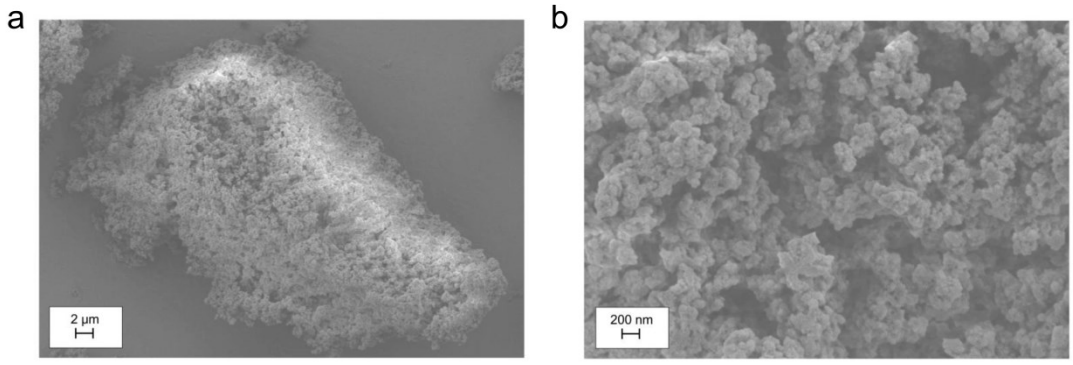

Fig. S2 SEM images of cross section of PVA@PAA-PE at 2 µm scale (a) and 200 nm scale (b).

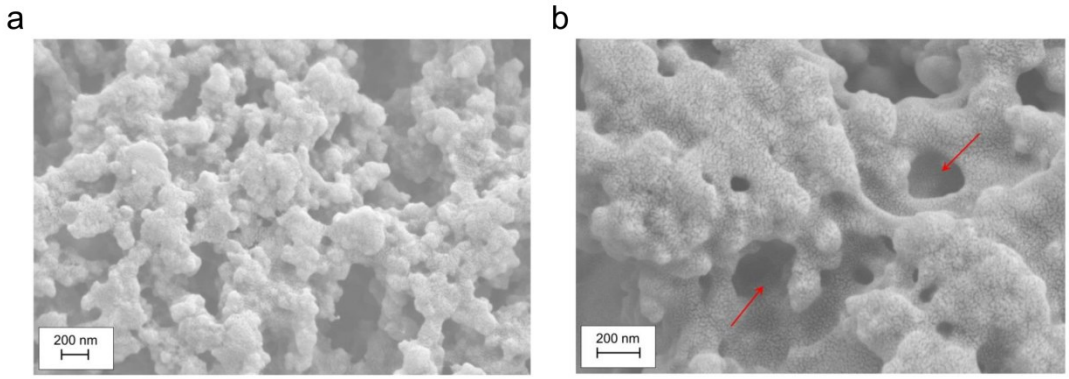

Fig. S3 SEM images of PVA@PAA-PE before and after incubated with LDL solution for 3 hours at 37 °C, 160 rpm. (a) PVA@PAA-PE in PBS at 200 nm scale; (b) PVA@PAA-PE in LDL solution at 200 nm scale.

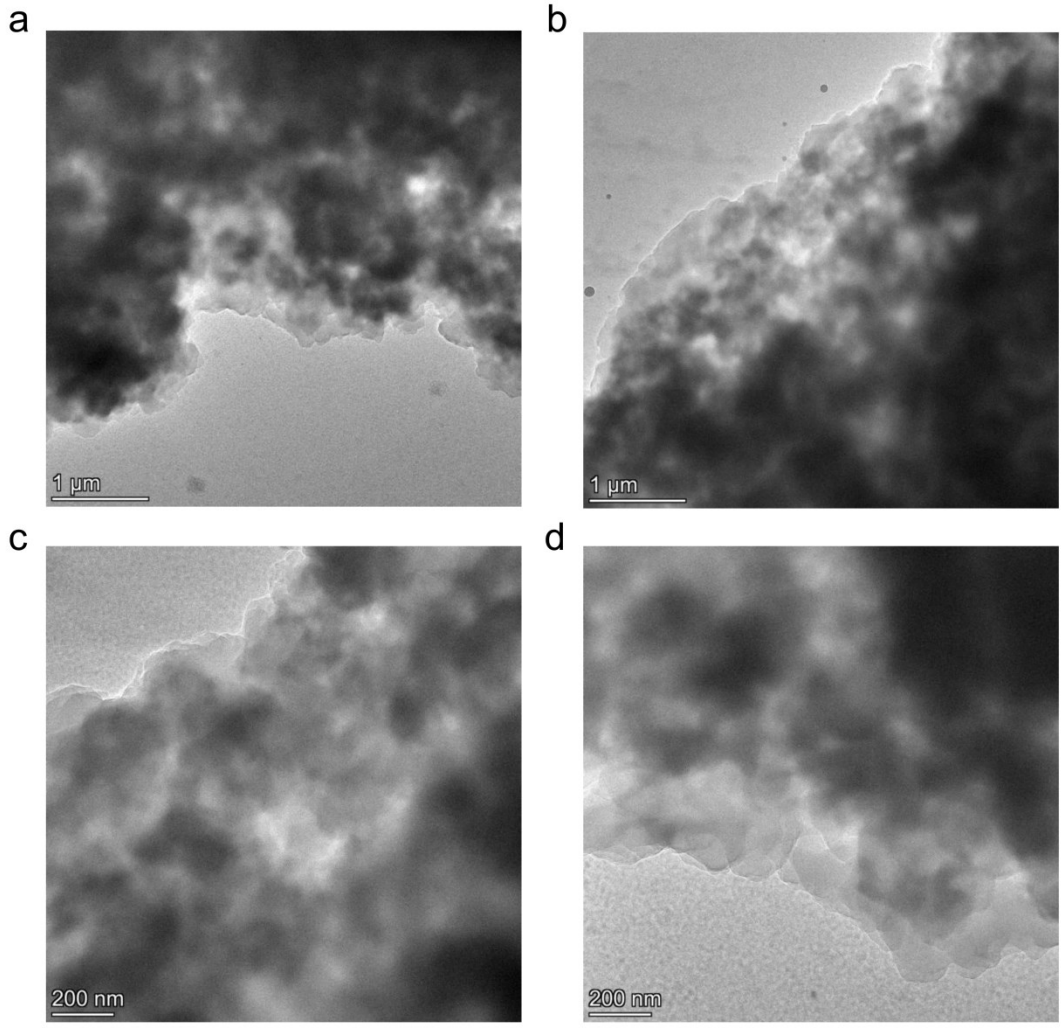

Fig. S4 FETEM images of PVA@PAA-PE at 1 µm scale(a-b) and 200 nm scale (c-d).

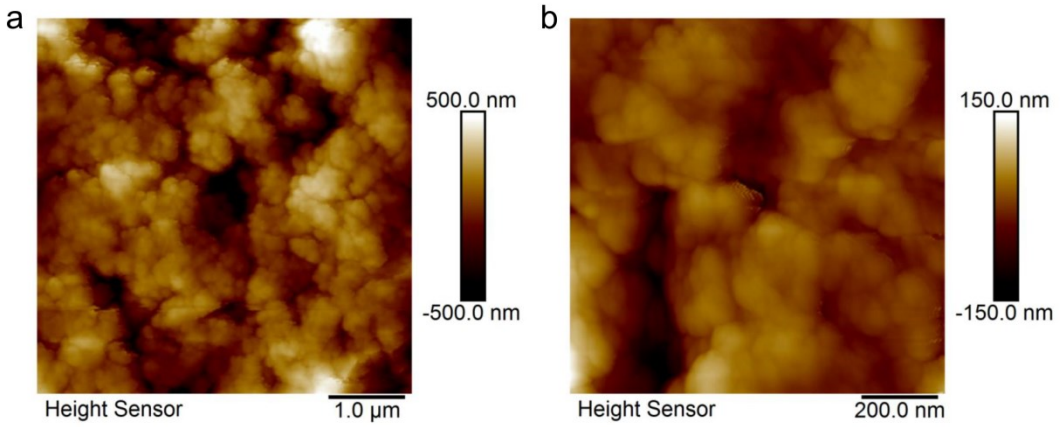

Fig. S5 AFM height images of PVA@PAA-PE surface at 1 µm scale(a) and 200 nm scale (b).

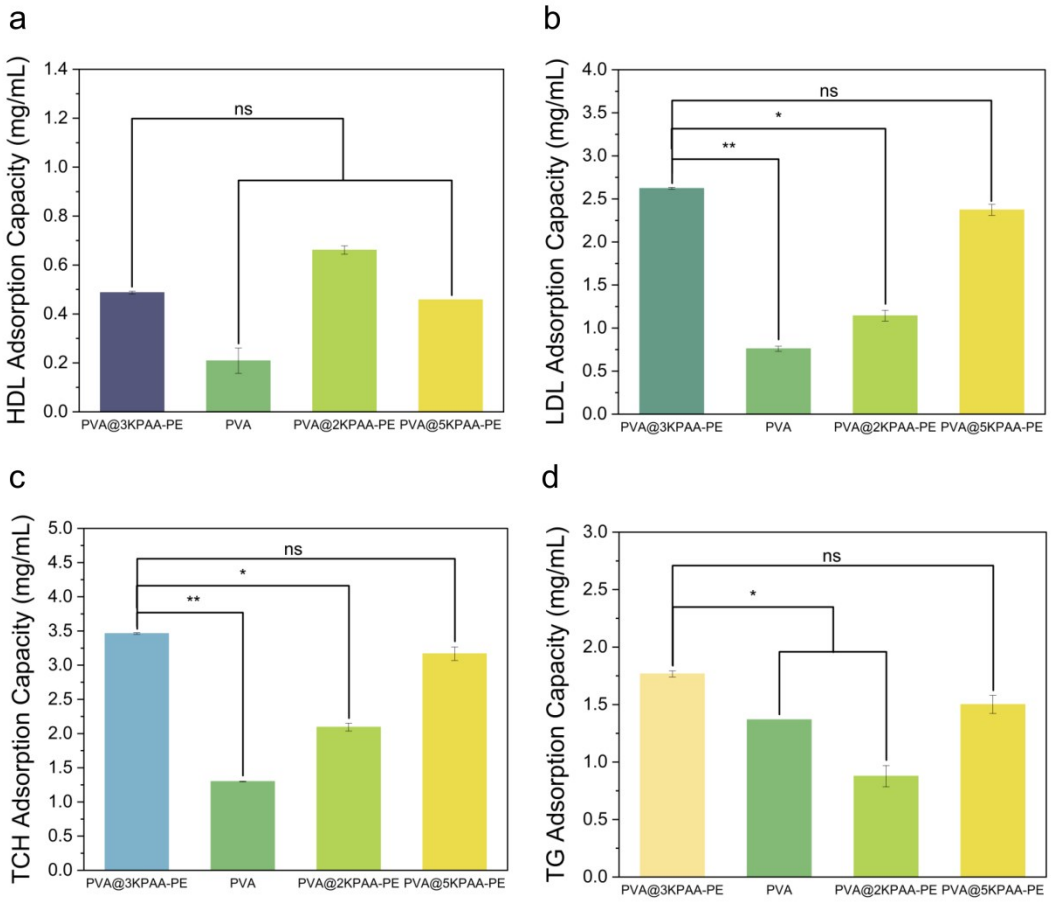

Fig. S6 Optimization of synthesis conditions for PVA@PAA-PE: the effect of PAA molecular weight on the adsorption of HDL (a), LDL (b), TCH (c) and TG (d). (ns= no significance, P*<0.05, P**<0.01, P***<0.001) (1PE – the addition amount of PE to PVA was about 1:1 w/w; 4PE – the addition amount of PE to PVA was about 4:1 w/w; 8PE – the addition amount of PE to PVA was about 8:1 w/w).

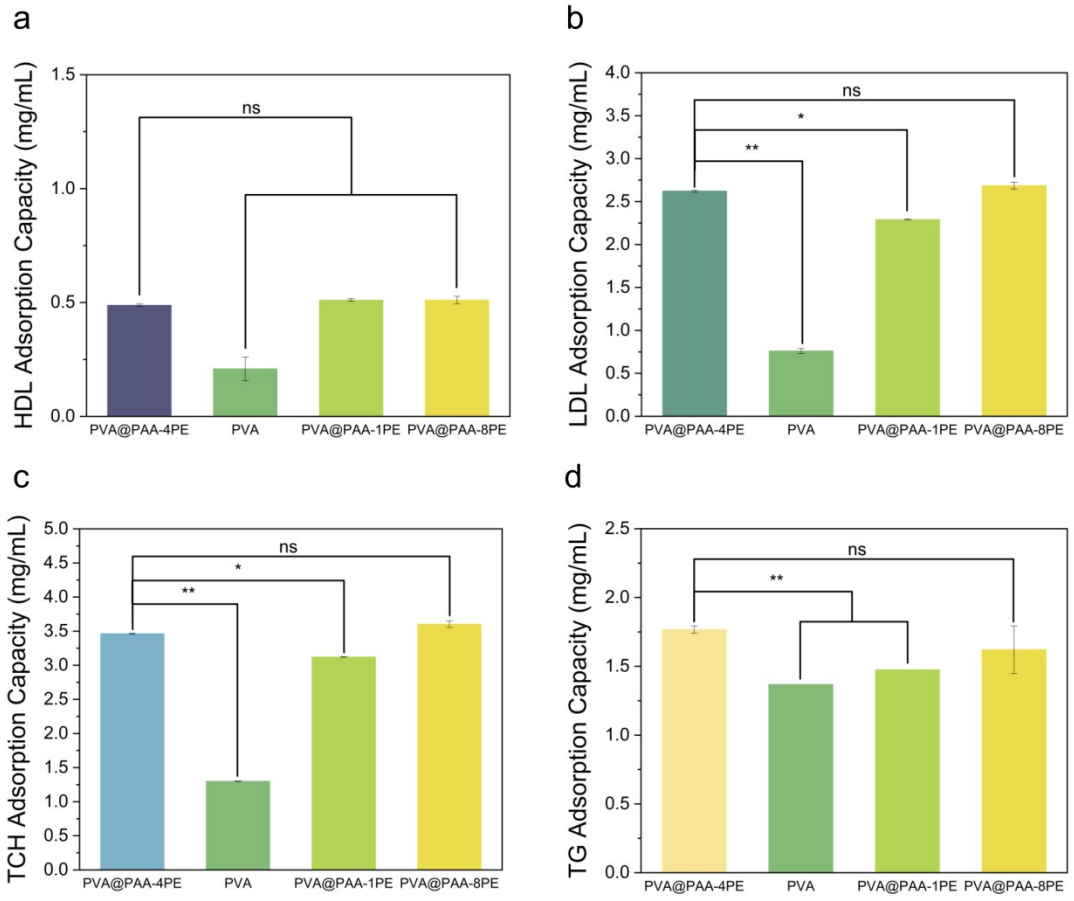

Fig. S7 Optimization of synthesis conditions for PVA@PAA-PE: the effect of addition amount of PE in the synthesis procedure on the adsorption of HDL (a), LDL (b), TCH (c) and TG (d). (1PE – the addition amount of PE to PVA was about 1:1 w/w; 4PE – the addition amount of PE to PVA was about 4:1 w/w; 8PE – the addition amount of PE to PVA was about 8:1 w/w). (ns= no significance, P*<0.05, P**<0.01, P***<0.001)

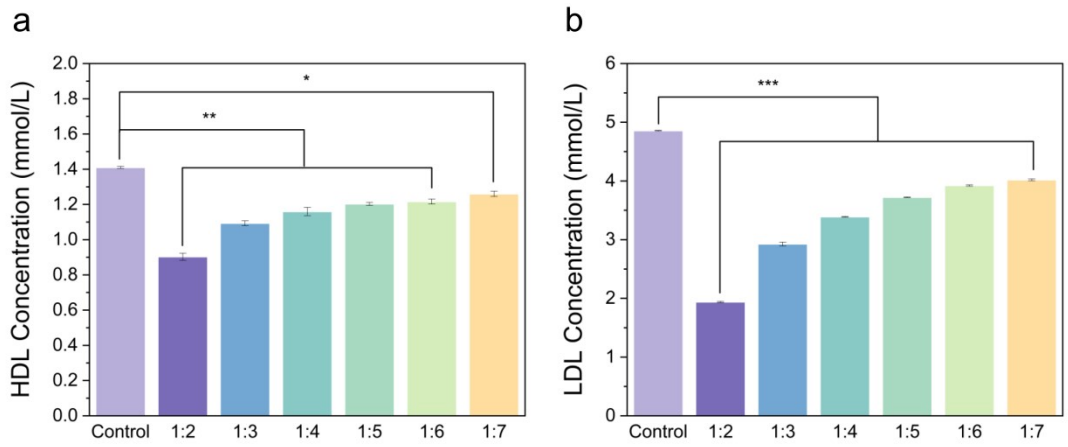

Fig. S8 The effect of adsorption ratio (PVA@PAA-PE to serum, v/v) on the removal of HDL (a) and LDL (b). (ns= no significance, P*<0.05, P**<0.01, P***<0.001)

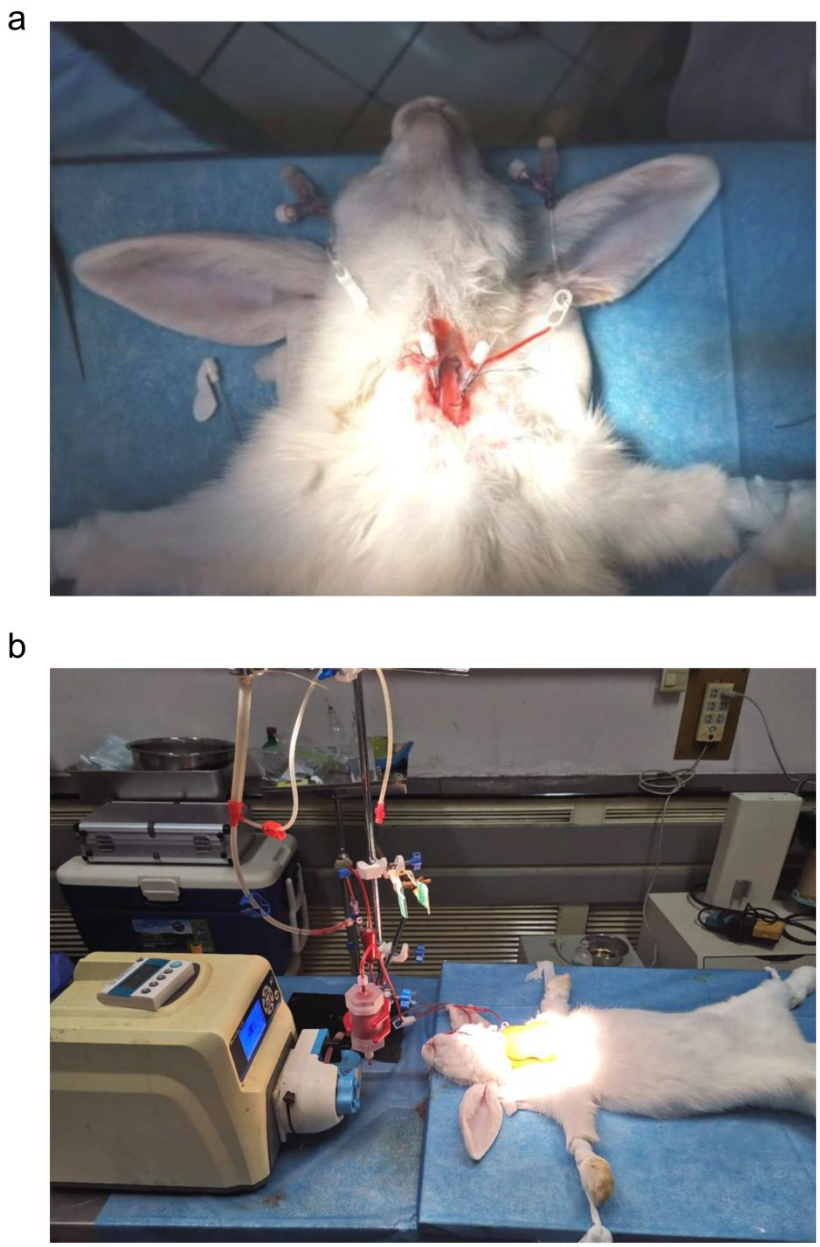

Fig. S9 Digital images for in vivo whole blood perfusion. (a) A skin incision was made from one centimeter below thyroid cartilage to one centimeter above sternum on the left side of neck, the carotid artery and vein were exposed through blunt dissection of mussel layer and fat pad, and an indwelling needle (20G) was interposed into the carotid artery and vein after ligation of proximal part of the vessels, followed by hose fixation and distal end ligation. (b) The whole blood perfusion system.

Table S1 Elemental composition of PVA, PVA-COOH@PE, PVA@2KPAA-PE, PVA@3KPAA-PE and PVA@5KPAA-PE calculated by XPS spectrum.

Atomic %	C	N	O	P
PVA	68.92	5.16	25.92	-
PVA-COOH@PE	71.31	3.27	24.86	0.55
PVA@2KPAA-PE	68.59	5.13	26.23	0.05
PVA@3KPAA-PE	68.42	4.82	26.14	0.30
PVA@5KPAA-PE	67.92	5.06	26.75	0.26

PVA: macroporous crosslinked poly(vinyl alcohol-co-triallyl isocyanurate) beads;

PVA-COOH@PE: grafting of PE to PVA-COOH;

PVA@2KPAA-PE: PE-PAA brushes grafted macroporous crosslinked poly(vinyl alcohol-co-triallyl ospcyanurate) beads, of which the mean molecular weight of PAA was about 2,000;

PVA@3KPAA-PE: PE-PAA brushes grafted macroporous crosslinked poly(vinyl alcohol-co-triallyl ospcyanurate) beads, of which the mean molecular weight of PAA was about 3,000;

PVA@5KPAA-PE: PE-PAA brushes grafted macroporous crosslinked poly(vinyl alcohol-co-triallyl ospcyanurate) beads, of which the mean molecular weight of PAA was about 5,000.

Table S2 Blood lipids adsorption capacity of PVA@PAA-PE at different contact time. (n=3)

	Adsorption Capacity (mg/mL)			
	HDL	LDL	TCH	TG
0 min	0	0	0	0
15 min	0.22±0.02	1.05±0.02	1.41±0.02	0.76±0.03
30 min	0.28±0.01	1.32±0.01	1.76±0.01	0.96±0.02
60 min	0.29±0.03	1.65±0.03	2.17±0.01	1.17±0.02
90 min	0.36±0.01	2.00±0.03	2.59±0.02	1.36±0.02
120 min	0.34±0.00	1.86±0.09	2.47±0.18	1.34±0.06
180 min	0.37±0.00	2.14±0.04	2.74±0.02	1.42±0.02
240 min	0.37±0.01	2.22±0.01	2.90±0.02	1.52±0.04

Table S3 Nonlinear fitting of experimental data with the relative parameters calculated from Pseudo-first-order and Pseudo-second-order models for PVA@PAA-PE. (n=3)

Blood Lipids	Pesudo-first-order			Pesudo-second-order		
	k_1	R^2	Q_e	k_2	R^2	Q_e
HDL	0.0547	0.9638	0.3521	0.2058	0.9856	0.3881
LDL	0.0357	0.9599	2.0590	0.0195	0.9848	2.3555
TCH	0.0380	0.9620	2.6725	0.0164	0.9877	3.0393
TG	0.0410	0.9692	1.4015	0.0347	0.9926	1.5828

k_1: equilibrium rate constants of pseudo-first-order model;

k_2: equilibrium rate constants of pseudo-second-order model;

R^2: correlation coefficient;

Q_e: equilibrium adsorption capacity (mg/mL).

Table S4 Equilibrium concentration verses adsorption capacity of PVA@PAA-PE.

(n=3)

Adsorption Capacity							
HDL		LDL		TG		TG	
C_e	Q_e	C_e	Q_e	C_e	Q_e	C_e	Q_e
0.21±0.01	0.03±0.01	0.17±0.01	0.67±0.01	0.54±0.01	0.88±0.01	0.25 ±0.00	0.48±0.01
0.43±0.00	0.09±0.00	0.56±0.01	1.13±0.01	1.29±0.01	1.45±0.01	0.54±0.01	0.82±0.02
0.62±0.00	0.15±0.00	1.09±0.02	1.47±0.02	2.16±0.01	1.86±0.01	0.85 ±0.00	1.10±0.01
0.81±0.01	0.25±0.01	1.67±0.01	1.75±0.01	3.08±0.01	2.21±0.01	1.16 ±0.01	1.21±0.02
1.00±0.01	0.31±0.01	2.38±0.03	1.93±0.03	4.07±0.02	2.50±0.02	1.47 ±0.01	1.37±0.02
1.19±0.02	0.40±0.03	3.11±0.02	2.16±0.03	5.09±0.02	2.78±0.02	1.85 ±0.01	1.39±0.02

C_e: equilibrium concentration (mmol/L);

Q_e: adsorption amount at equilibrium (mg/mL).

Table S5 Nonlinear fitting of experimental data with the relative parameters calculated from Langmuir and Freundlich models for PVA@PAA-PE. (n=3)

Blood Lipids	Langmuir Model			Freundlich Model		
	Qm	R^2	KL	KF	R^2	n
HDL	7.30E+12	0.9236	2.39E+13	0.3145	0.9946	0.6912
LDL	2.4834	0.9627	0.6453	1.4016	0.9960	2.5891
TCH	3.8480	0.9879	2.1479	1.2566	0.9974	2.0269
TG	2.0129	0.9884	0.7571	1.1001	0.9472	2.0925

Q_m: maximum adsorption capacity (mg/mL);

R^2: correlation coefficient;

KL: constants;

N: constants.