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Electronic Supplementary Information (ESI)

Polystyrene-divinylbenzene based nano-$CaCO_3$ composites for the efficient removal of human tumor necrosis factor-α

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Materials and Methods

Nano-$CaCO_3$ particles with spherical morphology, mean diameter of 20 nm, and 99.9% purity were obtained from DK Nano Technology Co. Ltd (Beijing). Styrene and divinylbenzene were purchased from *Aladdin*, and were removed from the polymerization inhibitor for further polymerization reactions. Blood bags were ordered from the Red Cross (Tianjin). The recombinant human cytokine TNF-α and the human TNF-α Quantikine ELISA Kits were obtained from R&D systems Europe Ltd (Abingdon, UK). Inflammatory model plasma was prepared by Lipopolysaccharide (LPS, *Aladdin*) administration at a concentration of 1.0×10^{-4} g/L[1, 2]. All other chemicals were of analytical or higher grade and were used directly, without further purification.

Sample preparation:

A typical polymerization synthesis suspension solution of PS-DVB resins, nano-$CaCO_3$ particles, styrene (monomer), divinylbenzene (crosslinker), an inert solvent of isobutanol (porogen), and tertbutyl peroxybenzoate (initiator), was homogeneously mixed in a beaker with weight ratios (w/w) of 2:20:80:100:1, at 25 ℃ for 30 min, and then the mixture was transferred into a three-necked flask, containing 406 g 1% gelatin solution. Following, the suspension was program-heated under 95 ℃ for 8 h, at 200 rpm. Light microscopy confirmed the incorporation of the 2 wt% nano-$CaCO_3$ particles, which were spherical in shape, with a size range of 0.5-0.8 mm. After polymerization, the adsorbent was hereafter designated as "PS-DVB/Nano-$CaCO_3$", and was purified in a chromatographic column by consecutive washes with water, acetone and ethyl alcohol. Then, the nano-composite were thoroughly washed with distilled water to remove any remaining solvent, and stored in 0.9% saline solution at 4 ℃ for further use. The styrene divinylbenzene co-polymer microspheres in the absence of nano-$CaCO_3$ particles were also prepared following a similar process and were subsequently named "PS-DVB".

Sample characterization:

The X-ray diffraction (XRD) patterns of the as-synthesized samples were recorded on a Bruker AXS D8-FOCUS diffractometer, using monochromatic Cu-Kα radiation. Infrared absorption spectra were measured in the range of 4000–400 cm^{-1} by a fourier transformed infrared (FTIR) spectrometer (Nicolet 6700), at a resolution of 4 cm^{-1}, using pressed KBr pellet samples. Scanning electron microscope (SEM) images were taken on a Hitachi 4800-S with an accelerating voltage of 30 kV. The chemical

composition and the corresponding elemental mapping images of adsorbents were assessed by an energy-dispersive X-ray (EDX) analyzer (Hitachi EDS), which was directly connected to the SEM instrument. The macroscopical morphology of adsorbents was investigated by standard light microscopy (LEICA M165 C). Transmission electron microscopy (TEM) samples were prepared as follows: PS-DVB/Nano-$CaCO_3$ were crushed and homogeneously dispersed in ethanol by ultrasonification (GTSONIC-L6, Guangdong, China), then a copper wire, coated with a thin layer of carbon, was dipped into the solution, and the sample was dried overnight in a desiccator. The dried sample was analyzed with a FEI Tecnai F20 EM, operated at 200 kV. N_2 adsorption/desorption isotherms were measured at liquid nitrogen temperature (77 K) on the Micromeritics ASAP 2460 analyzer. Specific surface areas were calculated by the Brunauer–Emmett–Teller (BET) method using the adsorption isotherms. Pore size distributions (PSDs) were calculated by the Barrett–Joyner–Halenda (BJH) method using the desorption isotherms. The calcium carbonate content in the dried composite resins was measured by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Electron Corporation). The mechanical properties of nano-composite materials were measured by Particle Counter GWF-6JA (Tianjin, China).

TNF-α adsorption assays:

Before all the experiments, the adsorbents used for measurements were fully equilibrated in 0.9% saline solution. For the measurements of static adsorption under different time points, 0.09 g of adsorbents were mixed with 2 mL of 1000 pg

mL^{-1} TNF-α plasma, at 37 ℃. During the process of adsorption, samples were drawn at 10, 30, 60, 90, 120 min. For the measurements of static adsorption under different temperatures, 0.09 g of adsorbents were mixed for 2 h with 2 mL of 1000 pg mL^{-1} TNF-α plasma, at 4, 25 and 37 ℃, respectively. For the measurements of plasma/adsorbents ratios, different volumes of adsorbents were each added into 2 mL of 1000 pg mL^{-1} TNF-α plasma, at 37 ℃, for 2 h. For adsorption isotherms experiments, to determine the plasma/adsorbents ratio, different amounts of adsorbents were weighed and each added into 2 mL of 1000 pg mL^{-1} TNF-α plasma, at 37 ℃, for 2 h. The adsorption performance of TNF-α from plasma was carried out in 5 mL bottles, with constant shaking at 160 rpm, at the given time points and temperatures. Then the samples were centrifuged at 2500 rpm for 5 min and the supernatant collected and stored at -20 ℃, prior to a Quantikine® ELISA kit analysis for the detection of TNF-α in the samples.

The quantities of the adsorbed TNF-α were calculated using the following equation:

$$\text{Adsorption rate (\%)} = (C_A - C_B)/C_A \times 100 \quad (1)$$

$$\text{Adsorption amount (ng } g^{-1}) = (C_A - C_B) \times V/m \quad (2)$$

Where C_A and C_B are the TNF-α concentration in the solution before and after adsorption, respectively, V is the volume of the plasma and m is the weight of adsorbents.

Mechanical strength assays:

To investigate the mechanical properties of nano-composite materials, 5 mL of adsorbent were incubated with 50 mL of purified water, filtered by 0.2 μm

membrane filters for 2 h at 37 ℃, under constant shaking at 300 rpm. Controls solely consisted of purified water. After incubation, the supernatant was collected and the released microparticles were measured by the particle counter.

Blood biochemical analyses: 1 mL of adsorbent was incubated with 6 mL of human plasma, at 37 ℃ for 2 h. 1 mL of physiologic saline was used as a control and was also incubated for comparison. The levels of major serum proteins and ion concentrations in the supernatants were measured by URIT-8260.

***Coagulation assays*:** Fresh blood from healthy rabbits was added into 3.2% sodium citrate coagulation test tube and the supernatant plasma was collected after centrifugation at 3000 rpm for 10 min. Following, 2 mL of freshly drawn plasma, anticoagulated with citrate, were incubated with 0.1 g of the adsorbents at 37 ℃, with gentle shaking for 1 h. Subsequently, the adsorbents were removed by immediate centrifugation (5 min, 4600 g), and the supernatant was directly used for coagulation assays to assess blood compatibility. As a control, 2 mL of fresh rabbit plasma (FRP) was also prepared in a similar manner. The assays were run on a Precil C2000-4 Coagulation System.

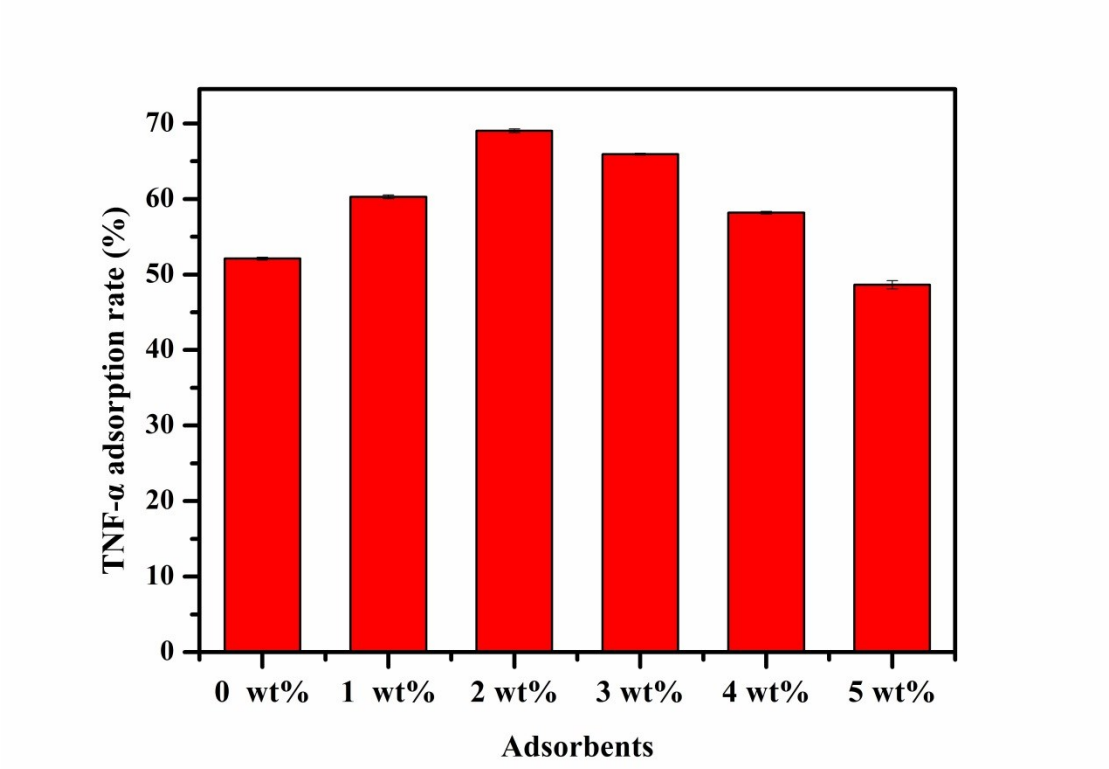

Fig.S1 TNF-α adsorption rates of PS-DVB and PS-DVB/Nano-$CaCO_3$ with different proportion of nano-$CaCO_3$ (T=37 ℃, $C_{TNF-\alpha}$=850.48±4.26 pg mL^{-1}, t=2 h, the plasma to adsorbent ratio was 14).

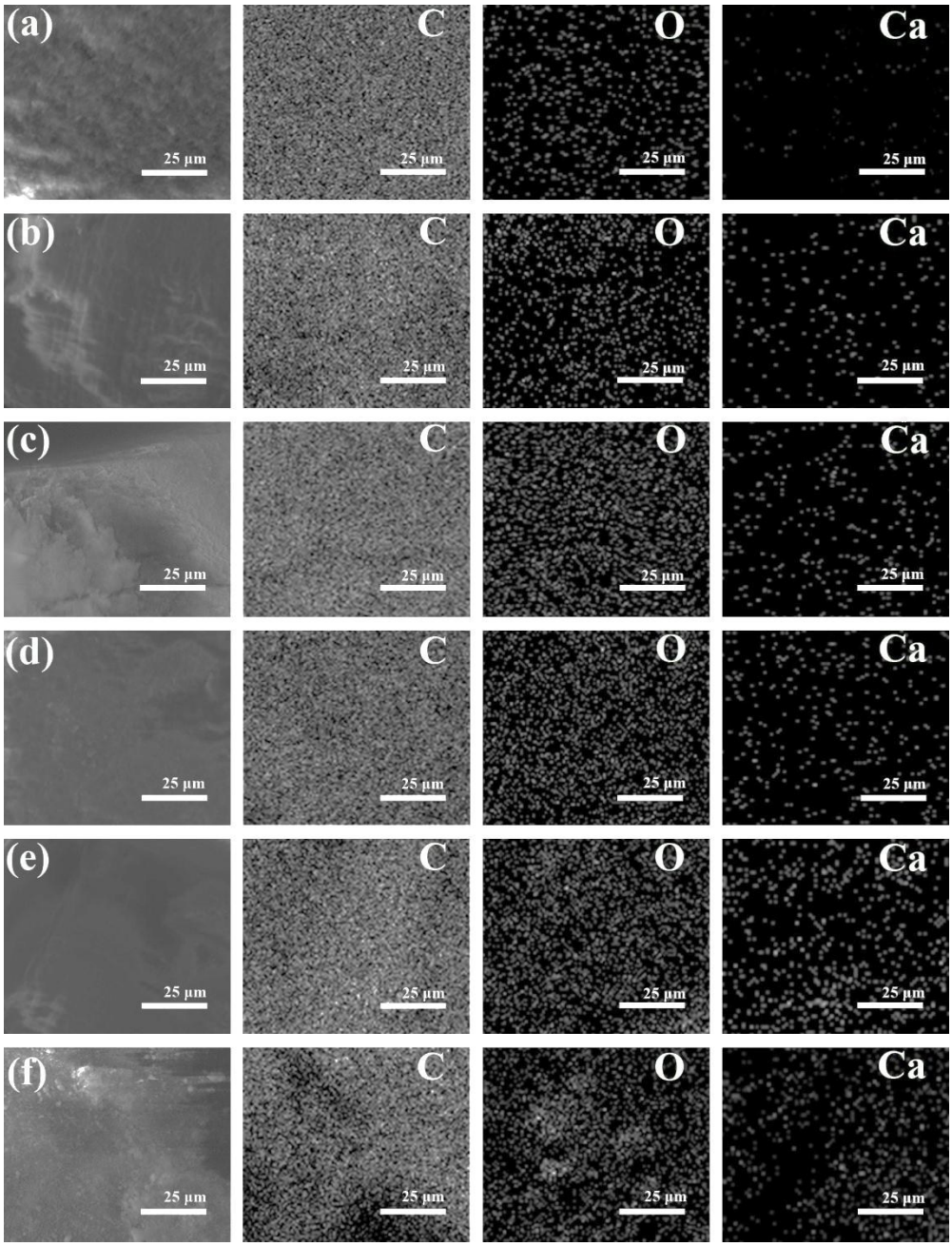

Fig. S2 SEM images of adsorbents: (a) PS-DVB, (b) 1 wt% PS-DVB/Nano-$CaCO_3$, (c) 2 wt% PS-DVB/Nano-$CaCO_3$, (d) 3 wt% PS-DVB/Nano-$CaCO_3$, (e) 4 wt% PS-DVB/Nano-$CaCO_3$ and (f) 5 wt% PS-DVB/Nano-$CaCO_3$, and the corresponding EDX mappings for C, O and Ca atoms.

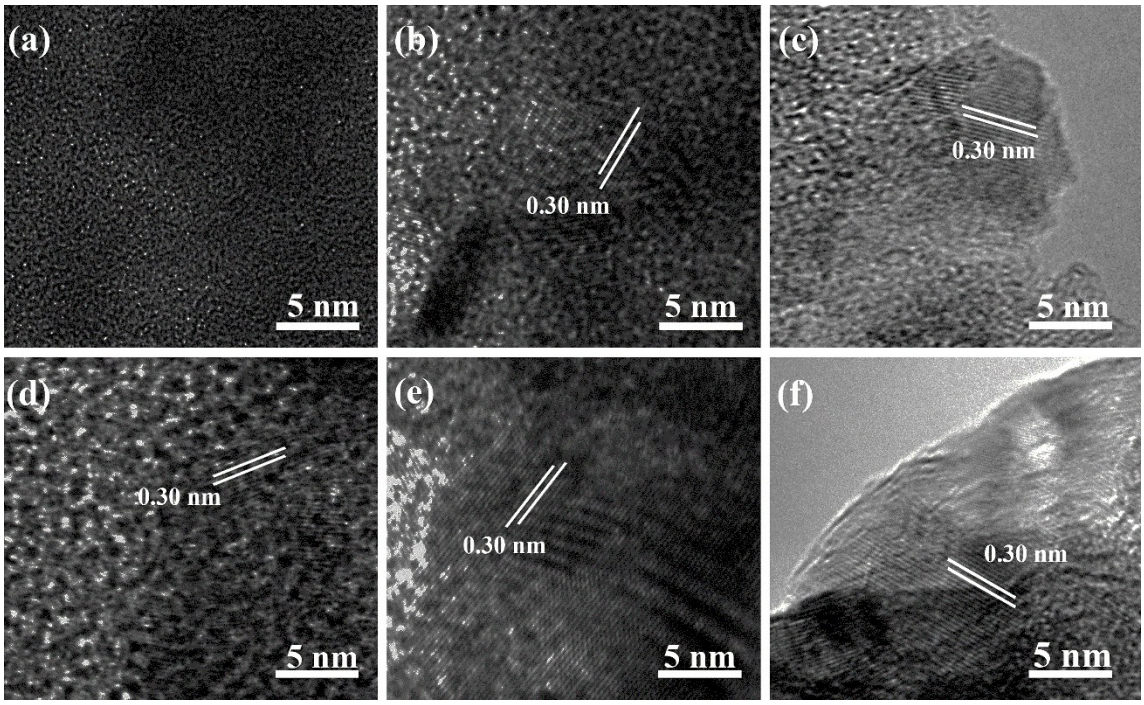

Fig. S3 HRTEM images of adsorbents: (a) PS-DVB, (b) 1 wt% PS-DVB/Nano-$CaCO_3$, (c) 2 wt% PS-DVB/Nano-$CaCO_3$, (d) 3 wt% PS-DVB/Nano-$CaCO_3$, (e) 4 wt% PS-DVB/Nano-$CaCO_3$ and (f) 5 wt% PS-DVB/Nano-$CaCO_3$.

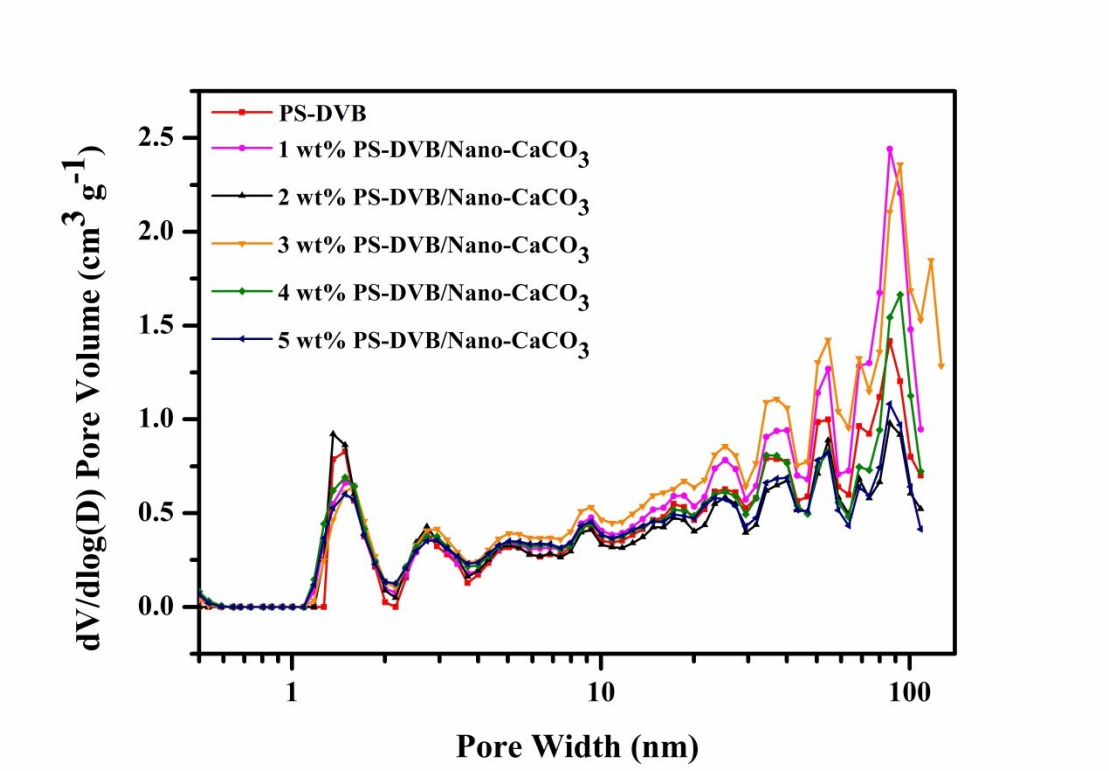

Fig. S4 The PSDs in the range of 0–100 nm, for PS-DVB, 1 wt% PS-DVB/Nano-$CaCO_3$, 2 wt% PS-DVB/Nano-$CaCO_3$, 3 wt% PS-DVB/Nano-$CaCO_3$, 4 wt% PS-DVB/Nano-$CaCO_3$ and 5 wt% PS-DVB/Nano-$CaCO_3$.

Fig. S5 Image containing a few spheres for 5 wt% PS-DVB/Nano-$CaCO_3$ by standard light microscopes.

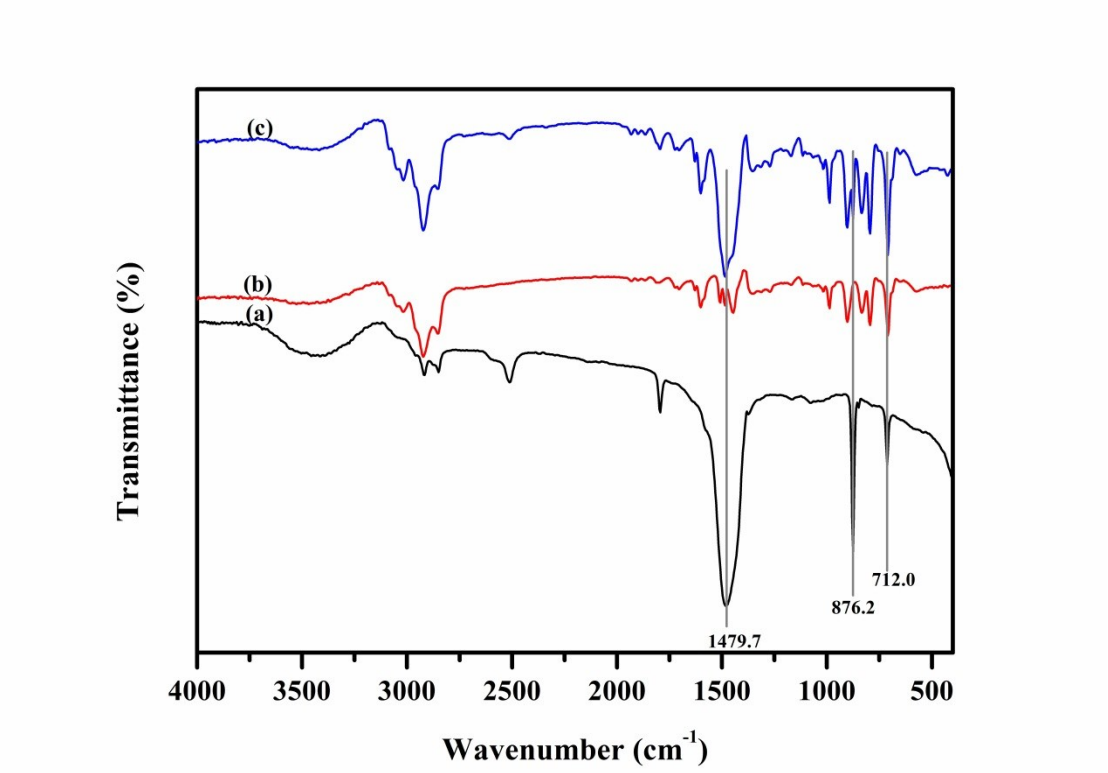

Fig. S6 FTIR spectrum of original nano-$CaCO_3$ (a), PS-DVB (b) and PS-DVB/Nano-$CaCO_3$ (c).

Table S1 The detailed texture parameters of the adsorbents (A: PS-DVB, B: 1 wt% PS-DVB/Nano-$CaCO_3$, C: 2 wt% PS-DVB/Nano-$CaCO_3$, D: 3 wt% PS-DVB/Nano-$CaCO_3$, E: 4 wt% PS-DVB/Nano-$CaCO_3$ and F: 5 wt% PS-DVB/Nano-$CaCO_3$).

Adsorbents	Pore volumes ($cm^3\ g^{-1}$)					Specific surface areas ($m^2\ g^{-1}$)					S_{BET} ($m^2\ g^{-1}$)
	< 2 nm	2–10 nm	10–50 nm	> 50 nm	Total	< 2 nm	2–10 nm	10–50 nm	> 50 nm	Total	
0 wt%	0.479	4.858	47.21	70.68	123.3	429.5	860.2	1775	1358	4422.7	628.5
1 wt%	0.323	1.704	24.81	71.41	98.25	469.5	940.4	2078	2164	5651.9	773.5
2 wt%	0.513	4.603	40.39	50.41	95.92	511.2	906.4	1525	995.2	3937.8	837.5
3 wt%	0.317	3.012	32.31	119.6	115.3	425.6	1111	2335	2777	6648.6	801.1
4 wt%	0.365	2.146	24.03	57.36	83.90	519.7	986.7	1758	1455	4719.4	768.8
5 wt%	0.285	2.712	21.57	42.03	66.60	441.7	991.7	1622	1080	4135.4	664.5

Table S2 Mechanical property of adsorbents (mean±SD, n=3).

Size ranges (μm)	The amount of desquamated tiny particles (mL^{-1})	
	PS-DVB/Nano-$CaCO_3$	PS-DVB
< 20	29.20±10.28	40.05±21.70
20-25	120.35±64.41	228.90±54.72
26-50	12.80±6.75	18.45±10.24
51-100	0.50±0.10	0.85±0.16
> 100	0.10±0.00	0.20±0.10

Table S3 Kinetic parameters for the adsorption of TNF-α onto three adsorbents at 37 ℃.

Adsorbents	$Q_{e,exp}$ (ng g^{-1})	Pseudo-first-order			Pseudo-second-order		
		$Q_{e,calc,1}$ (ng g^{-1})	k_1 (min^{-1})	R^2	$Q_{e,calc,2}$ (ng g^{-1})	k_2 (g ng^{-1} min^{-1})	R^2
PS-DVB/Nano-$CaCO_3$	28.57	28.15	0.1267	0.9988	29.99	0.0075	0.9980
PS-DVB	27.46	27.17	0.0655	0.9981	28.94	0.0027	0.9934
Cytosorb	27.54	27.00	0.0529	0.9940	28.46	0.0018	0.9939

Table S4 Blood biochemistry analyses of adsorbents (A: PS-DVB/Nano-$CaCO_3$, B: PS-DVB and C: Cytosorb) (mean±SD, n=3).

Blood biochemical indexes	Normal Value	Before adsorption	After adsorption		
			A	B	C
TP (g L^{-1})	60-80	58±1.41	44.5±0.71	45.5±0.71	45±0.00
ALB (g L^{-1})	35-55	39±0.00	30±1.41	30.5±0.71	30±0.00
GLB (g L^{-1})	5-45	19±1.41	14.5±0.71	15±1.41	15±0.00
Crea (μmol L^{-1})	44-133	61±2.83	48±1.41	50±1.41	49±1.41
UA (mol L^{-1})	90-360	245±2.83	226.5±0.71	230±1.41	227.5±2.12
BUN (mmol L^{-1})	1.79-7.14	3.65±0.21	3.5±0.42	3.7±0.14	3.2±0.00
Cl (mmol L^{-1})	95-105	69±1.41	64.5±0.71	75±1.41	64±0.00
K (mmol L^{-1})	3.3-5	3.75±0.03	3.54±0.00	3.53±0.04	3.55±0.01
Na (mmol L^{-1})	134-143	138.5±0.71	135.5±0.71	136.5±0.71	137±0.00
Ca (mmol L^{-1})	2.25-2.75	2.40±0.007	2.37±0.02	2.27±0.01	2.47±0.07

Table S5 Coagulation assays of adsorbents (mean±SD, n=3).

Adsorbents	PT(s)	APPT(s)	FIB(g L^{-1})
PS-DVB/Nano-$CaCO_3$	6.8±0.1	13.5±0.1	1.45±0.21
PS-DVB	7.1±0.3	12.5±0.2	1.06±0.29
Cytosorb	7.1±0.3	12.6±0.6	1.26±0.085
FRP	6.6±0.2	13.1±0.4	1.39±0.11

Reference:

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