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Supplementary information

Transient blood thinning during extracorporeal blood purification via the inactivation of coagulation factors by hydrogel microspheres

In the format provided by the authors and unedited

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Experimental Section

1. Materials

Polyethersulfone (PES, Ultrason E6020P) was obtained from BASF chemical company. N-vinyl-2-pyrrolidone (VP), acrylic acid (AA), 2-acrylamido-2-methyl-1-prroanesulfonic acid (AMPS, 98%), N,N'-methylene bisacrylamide (MBA), 2,2-azobisisobutyronitrile (AIBN), phosphate buffered saline (PBS), ammonium persulfate (APS), calcium chloride ($CaCl_2$) and sodium hydroxide (NaOH) were purchased from Aladdin Reagent Co. Ltd. N,N'-Dimethylacetamide (DMAc), sodium dodecyl sulfate (SDS), ethanol (C_2H_5OH), normal saline (0.9% NaCl), glutaraldehyde (GA, 50 wt. % in water) were obtained from Chengdu Kelong Chemical Reagent Co. Ltd. Bovine serum albumin (BSA), creatinine, dL-dithiothreitol (DTT), iodoacetamide (IAA), methanol, urea, niacinamide, trichostatin A, PR-619 and trifluoroacetic acid were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA) Trypsin from bovine pancreas was purchased from Promega (Madison, WI, USA). Acetonitrile (ACN) was purchased from Fisher Chemical. Formic acid (FA) was purchased from Fluka. Proteinase inhibitor was purchased from Calbiochem. Micro BCA™ Protein Assay Reagent kit was purchased from PIERCE Inc. Activated partial thromboplastin time (APTT) reagent, prothrombin time (PT) reagent, thrombin time (TT) reagent, coagulation factor VIII deficient plasma, coagulation factor IX deficient plasma, coagulation factor XI deficient plasma, coagulation factor XII deficient plasma and antithrombin III reagent were purchased from SIEMENS Co. Ltd. Human complement fragment 3a (C3a) kit, human complement fragment 5a (C5a) kit, human platelet factor 4 (PF4) kit and human bradykinin (BK) kit were purchased from Cusabio Biotech, China. Thrombin-antithrombin Enzygnost TAT micro kit was purchased from Assay Pro, USA. Fluorescein isothiocyanate-labelled BSA (FITC-BSA) was purchased from Beijing Bersee Technology Co., Ltd., China. Ultrapure water (UP water) and deionized water (DI water) were prepared from a Millipore purification system (Billerica, MA, USA). All the chemicals were used as received.

2. Preparation of the microspheres

2.1 Preparation of the hydrophobic polymer precursor solution

The hydrophobic polymer precursor solution was fabricated by one-pot in-situ cross-linking polymerization. In brief, 4 g PES, 4 g VP, 42 g DMAc and 0.5 g MBA were placed into a 100 mL three-necked round flask. Then 0.02 g AIBN was added after stirring vigorously to obtain a homogeneous solution and bubbling with nitrogen for 30 minutes. The reaction was proceeded at 120 °C under the protection of nitrogen atmosphere with mechanical stirring (600 rpm) for 36 h.

2.2 Preparation of the microspheres

A liquid-liquid phase inversion technique[1] as reported in our previous study was used to prepare the hydrophobic polymeric microspheres (HPMs). The as-prepared hydrophobic polymer precursor solution was loaded into a 10-mL syringe and connected to a 24-gauge stainless steel needle (with an inner diameter of 0.3 mm). Then the solution was dropped into DI water via electrospraying, in which sodium dodecyl sulfate (SDS, 0.5 wt. %) was added to improve the sphericity of the microspheres. The electrospraying process was conducted with a voltage of 8.5 kV and a feeding rate of 1.5 mm/minute at room temperature (about 25 °C). Then the microspheres were filtered and washed in DI water to elute the residual solvent thoroughly.

We chose AA and AMPS to prepare the anticoagulant hydrogel network. To prepare the

reinforced anticoagulant hydrogel microspheres and explore the most optimum ratio of the two polymers in the hydrogel network, various reaction solutions were prepared, and the compositions were shown in **Supplementary Table 1**. The total monomer concentration of the solutions was 30 wt. %, and the mass ratios of the AMPS to AA were 1:4, 2:3, 3:2, and 4:1, respectively. The amounts of MBA and APS in the solutions were 4 wt. % and 1 wt. % to the monomers, respectively. Then the as-prepared hydrophobic polymer precursor solution was dropped into the reaction solutions (0.5 wt. % SDS was also added) directly via electrospraying. The experimental conditions of the electrospraying process were same as the preparation of HPMs. Then the microspheres were kept in the reaction solution for 4 hours to finish the phase inversion. Then the received microspheres were heated and kept at 80 °C for 1 hour to initiate the cross-linking polymerization of anticoagulant monomers. After that, the cross-linked microspheres were immersed in plenty of 0.01 mol/L NaOH solution with magnetic stirring for 24 hours to obtain -COONa and -SO_3Na (sodium salt form). Then the microspheres were washed with DI water to remove the unreacted molecules thoroughly. Finally, the microspheres were kept in phosphate buffered saline (PBS, pH 7.4) until use, and the PBS was refreshed three times a day. The prepared microspheres were named as A_xM_y, where "x:y" means the mass ratios of AA and AMPS in the reaction solution. Additionally, to obtain the non-reinforced hydrogel microspheres (NRHMs), 10 g RAHMs were immersed in 150 mL DMAc with continuously oscillation for 24 hours to dissolve the hydrophobic polymeric skeleton. Then the received NRHMs were washed with DI water to elute the residual solvent and swell fully.

Supplementary Table 1. The compositions of the reaction solutions

Reaction solutions	AMPS		AA		MBA	APS	SDS	DI water
	(g)	(mol)	(g)	(mol)	(g)	(g)	(g)	(g)
A_1M_4	24	0.116	6	0.083	1.2	0.3	0.5	70
A_2M_3	18	0.087	12	0.166	1.2	0.3	0.5	70
A_3M_2	12	0.058	18	0.249	1.2	0.3	0.5	70
A_4M_1	6	0.029	24	0.333	1.2	0.3	0.5	70

3. Characterization of the microspheres

To investigate the compositions of the anticoagulant hydrogel network, the NRHMs with different compositions were completely freeze-dried, and then energy dispersive spectra (EDS) were obtained using a JMS-7500F SEM (JEOL, Japan). Furthermore, freeze-dried hydrogel microspheres were ground into powders. Then elemental analysis (EA) of the powders was obtained through an Elemental Analyzer (Euro EA 3000), while X-ray photoelectron spectroscopy (XPS) of the powders was performed with an XSAM800 electron spectrometer (Kratos Analytical, U.K.).

The optimum composition of the anticoagulant hydrogel network was determined by a series of clotting time experiments. The reinforced anticoagulant hydrogel microspheres with the optimum composition were named as RAHM. The chemical structures of the RAHMs and NRHMs were characterized by Fourier transform infrared spectroscopy (FTIR, Nicolet 560, U.S.A.). The microspheres were completely freeze-dried, and KBr disk method was used to obtain the FTIR spectra between 650 and 4000 cm^{-1}. The hydrophobic polymers (PES and PES-VP) were also characterized by FTIR. The compositions of the RAHMs were also characterized by using a thermogravimetric analyzer (METTLLE TOLEDO, TGA/DSC 3+, Switzerland) under a dry N_2 atmosphere from 25 to 800 °C at a heating speed of 10 °C/ minute. The curves of derivative

thermogravimetric analysis were derived from TGA data. X-ray photoelectron spectroscopy (XPS) of RAHMs was also performed with an XSAM800 electron spectrometer (Kratos Analytical, U.K.).

Furthermore, the surface and cross-sectional micro-morphologies of these microspheres were obtained by scanning electron microscopy (SEM, Phenom Pure, Phenom World, Netherlands). To prepare SEM samples, the microspheres were cut off after being immersed in liquid nitrogen for 1 minute and then completely freeze-dried overnight. Afterwards, the microspheres were attached to a support and then coated with a gold layer under vacuum. Energy dispersive X-ray spectroscopy (EDX mapping) analysis was also used to investigate the distribution of the anticoagulant hydrogel network in RAHM.

The swelling ratios of the RAHMs and NRHMs were analyzed via a gravimetric method.[2] A certain amount of RAHMs and NRHMs were immersed into normal saline, PBS and whole blood, respectively, for 24 hours to be swollen thoroughly. Afterward, the swollen microspheres were weighted after gently removing excess solution with a filter paper. Then the microspheres were dried in an oven at 70 °C to obtain a constant weight. The digital photos of the dried and swollen microspheres were also obtained. The swelling ratios of the microspheres were calculated by using the following equation:[3]

$$\text{Swelling ratio (g/g)} = \frac{W_s - W_d}{W_d} \quad (1)$$

To investigate the mechanical properties of the microspheres, HPM, RAHM and NRHM were thoroughly swollen in PBS and then applied to a universal tensile testing machine (SANS CMT4000) with a constant speed of 2 mm/minute under a 200 kg load mechanical sensor. The digital photos of the RAHM and NRHM before and after compression were also obtained.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) was used to evaluate the stability of RAHM. 20 g RAHM was immersed into 50 mL PBS for 15 days. Then MALDI-TOF analyses were performed on an AXIMA Performance MALDI-TOF mass spectrometer with a SCOUTMTP Ion Source (Shimadzu Biotech MALDI-MS, Japan) equipped with an N_2 laser (337 nm), grid-less ion source, and reflector design. The instrument operated at an acceleration voltage of 25 kV and a reflector voltage of 26.3 kV. DHB (10 mg/mL aqueous solution) and analyst were mixed in a 5:2 ratio, and 0.3−0.5 μL was dropped onto a steel coordinate plate. Data were collected and analyzed with Shimadzu Biotech MALDI-MS software.

4. Evaluation of biocompatibility of the microspheres *in vitro*

4.1. Blood collection

Healthy human fresh blood (from three 24-year-old male donors) was collected using vacuum tubes (5 mL, Jiangsu Kangjian Inc., China) containing sodium citrate (for clotting time tests) or ethylene diamine tetraacetic acid (for evaluation of complement activation, activation of contact activation system and kallikrein/kinin system, platelet activation and blood routine test) as anticoagulant (anticoagulant-to-blood ratio, 1:9 (v/v)). For plasma collection, the blood was centrifuged at 1000 rpm for 15 minutes to obtain platelet-rich plasma (PRP) or at 4000 rpm for 15 minutes to obtain platelet-poor plasma (PPP). The same donor blood samples were used all through the blood tests.

The experiments were approved and performed by West China Hospital, Sichuan University, and all the experiments were performed in compliance with the relevant laws and national guidelines (GB/T 16886.4-2003/ISO 10993-4:2002, General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Standardization Administration of the People's

Republic of China). Informed consent was obtained for any experimentation with human subjects, and all regulations (e.g. IRB) were fulfilled for using human blood.

4.2. Protein adsorption

Protein adsorption experiments were carried out with BSA solution under static condition. Firstly, 1 mg microspheres were pre-immersed in PBS overnight and then incubated at 37 °C for 1 hour. Then the microspheres were immersed in PBS solution, containing BSA with the concentration of 1 mg/mL, and incubated at 37 °C for 1 hour; then the microspheres were rinsed slightly with PBS and DI water. Then the microspheres were placed in a washing solution (2 wt.% sodium dodecyl sulfate (SDS), 0.05 M NaOH) at 37 °C, and shaken for 2 hours to remove the adsorbed protein. The adsorption and desorption times were carefully determined in preliminary experiments. The protein concentration in the washing solution was determined by using the Micro BCA™ Protein Assay Reagent Kit (Pierce). At least 6 parallel samples were applied to get a reliable value, and the results were expressed as mean ± SD (n = 6).

Additionally, the distribution of proteins adsorbed on the surface of microsphere was explored using fluorescein isothiocyanate-labelled BSA (FITC-BSA). First, 5 mg/L FITC-BSA was dissolved in PBS, and then 1 mg microspheres were immersed in the solution at 37 °C for 1 hour. Finally, the microspheres were washed three times with PBS and observed under a fluorescence microscopy (Olympus IX53, Japan).

4.3. Evaluation of platelet activation and platelet adhesion

In order to eliminate the interference of other components in blood, such as erythrocyte and leucocyte, PRP was used for studying platelet adhesion. To study platelet adhesion, the microspheres were pre-immersed into in PBS overnight and equilibrated at 37 °C for 1 hour. Then, the PBS solution (pH=7.4) was removed and 1 mL of fresh PRP was introduced. The microspheres were incubated with PRP at 37 °C for 2 hours. Then, the PRP was decanted off and the microspheres were rinsed three times with PBS solution. Finally, the microspheres were treated with 2.5 wt. % glutaraldehyde in PBS solution at 4 °C for 1 day. The samples were washed with PBS solution, subjected to a drying process by passing them through a series of graded alcohol-PBS solutions (30, 50, 70, 80, 90, 95 and 100%). Platelet adhesion was observed using a FE-SEM (JSM-7500F, JEOL, Japan).

The evaluation of platelet activation was measured via an enzyme-linked immune sorbent assay with a Human Platelet Factor4 (PF4) kit (Cusabio Biotech, China). The microspheres were pre-immersed in PBS in a 24-well cell culture plate overnight. Then, the PBS was removed and 250 μL of human whole blood was introduced. After being incubated at 37 °C for 1 hour, the whole blood was centrifuged for 10 minutes at 2500 g centrifugal force (2-8 °C) to obtain plasma. Then 40 μL of the obtained plasma was diluted for 10 times with PF4-Sample Diluent; then 200 μL of the diluted plasma was added into another Antibody Coated Well (provided by the PF4 kit). Finally, the detections were conducted according to the respective instruction manuals. Whole blood was used as control sample. Three replicates were averaged and the results were expressed as the mean ± SD (n = 3).

4.4. Haemolysis ratio

The haemolysis test was carried out to evaluate the erythrocyte compatibility of the microspheres. 5 mL of whole blood was firstly added to 10 mL of calcium- and magnesium-free PBS solution, and then the red blood cells (RBCs) were isolated from plasma by centrifuging at 2000 rpm for 10 minutes, the centrifugation procedure was repeated at least 5 times. For the haemolysis test, 1 mL of the diluted RBC suspension (approx. 10^8 cells per mL) was added to the microspheres (a certain

number of microspheres was previously immersed in PBS overnight) and incubated at 37 °C for 3 hours. PBS was selected as negative control while deionized water was used as positive control. Then the suspensions were centrifuged at 8000 rpm for 3 minutes, and the absorbance of the released haemoglobin in the suspensions was measured at 540 nm using a UV-vis spectrometer (UV-1750, Shimadzu Co., Ltd, Japan), and then the haemolysis ratios of the microspheres could be calculated by the following formula:

$$Haemolysis\ ratio\ (\%) = \frac{A_s - A_n}{A_p - A_n} \times 100 \qquad (2)$$

where A_s is the absorbance of the suspensions, A_p and A_n are the absorbance the positive control and the negative control, respectively. Replicates were averaged and the results were expressed as the mean ± SD (n = 15).

4.5. Blood count assay *in vitro*

Blood count assays were performed to investigate the effect of the microspheres on blood cells. Firstly, a certain amount of microspheres were pre-immersed in 200 μL of PBS (pH=7.4) in a 48-well culture plate overnight and equilibrated at 37 ˚C for 1 hour. Secondly, 200 μL of fresh whole blood from the volunteer was introduced into each well after the removal of PBS. Thirdly, the microspheres were incubated with whole blood at 37 ˚C for 60 minutes and then the rest of the blood were collected. The whole blood cell differential counts were measured by an automated haematology cell analyzer (BC-5100, Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) under the provided instruction manuals. Six replicates were averaged and the results were expressed as the mean ± SD (n = 6).

4.6. Evaluation of complement activation

Commercial enzyme-linked immune sorbent assays (ELISA) were employed to evaluate the complement activation (Human Complement Fragment 3a (C3a) and Human Complement Fragment 5a (C5a), Cusabio Biotech, China) of the microspheres. The microspheres were pre-immersed in PBS in a 24-well cell culture plate overnight. Then, the PBS was removed and 250 μL of human whole blood was introduced. After being incubated at 37 °C for 1 hour, the whole blood was centrifuged for 10 minutes at 2500 g centrifugal force (2-8 °C) to obtain plasma. For the C3a test, 5 μL of the obtained plasma was diluted for 500 times with C3a-Sample Diluent, and 100 μL of the diluted plasma was added into an Antibody Coated Well (provided by C3a kit). For the C5a test, 10 μL of the obtained plasma was diluted for 10 times with C5a-Sample Diluent, and the diluted plasma was added into another Antibody Coated Well (provided by C5a kit). Finally, the detections were conducted according to the respective instruction manuals. Whole blood was used as control sample. At least 3 parallel sample groups and 2 technical replicates were applied to get a reliable value, and the results were expressed as mean ± SD.

4.7. Evaluation of activation of contact activation system and kallikrein/kinin system

The evaluation of activation level of contact activation system and kallikrein/kinin system for the microspheres were measured via an enzyme-linked immune sorbent assay with a Human Thrombin-antithrombin III complex (TAT) kit (Assay Pro, USA) and a Human Bradykinin (BK) kit (Cusabio Biotech, China). The microspheres were pre-immersed in PBS in a 24-well cell culture plate overnight. Then, the PBS was removed and 250 μL of human whole blood was introduced. After being incubated at 37 °C for 1 hour, the whole blood was centrifuged for 10 minutes at 2500 g centrifugal force (2-8 °C) to obtain plasma. For the TAT test, 50 μL of the obtained plasma was added into an Antibody Coated Well (provided by the TAT kit). For the BK test, 50 μL of the obtained plasma was mixed with 100 μL of horse radish peroxidase (HRP)-labelled antibody

(provided by the BK kit). Finally, the detections were conducted according to the respective instruction manuals. Whole blood was used as control sample. Three replicates and 2 technical replicates were applied and the results were expressed as the mean ± SD.

4.8. Cytocompatibility

Human umbilical vein endothelial cells (HUVECs) were grow in R1640 medium. For the cytotoxicity of the microspheres, 100 μL of the cells was seeded in 24-well tissue culture polystyrene plates (TCPS) at a density of 2.5×10^4 cells per cm^2. After cell culture for 4 hours, 10 mg microspheres, which were dispersed in culture medium and sterilized by γ-irradiation before use, were further added. The plate was incubated at 37 ºC under 5 % CO_2 atmosphere. The medium was changed every two days. At 24, 72 and 120 hours of culture, 100 μL of CCK-8 (final dilution: 1:10), which can react with dehydrogenase in mitochondria to form a water-soluble formazan, was added onto the sample to test the live cells. Then, 100 μL of the supernatant was transferred into a 96-well plate for optical density (O.D.) measurement at 450 nm. The results were expressed as the mean ± SD (n = 15).

4.9. The antibacterial adhesion property of RAHM

To study the antibacterial adhesion property of RAHM, 10 mg HPM and RAHM were incubated with 2 mL bacteria suspensions of *E. coli* or *S. aureus* (10^6 CFU/mL) at 37 ºC for 12 hours. Then the adhered bacterial cells were visualized using SEM (JSM-7500F, Japan) after being fixed by fresh prepared glutaraldehyde (2.5 wt. % in PBS) and dehydrated by a series of graded alcohol-PBS solutions (30, 50, 70, 80, 90, 95 and 100%).

4.10. Evaluation of blood thinning property

A semiautomatic blood coagulation analyzer (CA-50, Sysmex Corporation, Japan) was employed to investigate the blood thinning properties of the microspheres. The microspheres were pre-immersed in PBS overnight and then incubated at 37 °C for 1 hour. After that, the PBS was removed, and 300 μL of fresh PPP was introduced. For the activated partial thromboplastin time (APTT) test, 50 μL of the incubated PPP was moved to a test cup and mixed with 50 μL of APTT agent (Dade Actin Activated Cephaloplastin Reagent, Siemens; incubated 10 minutes at 37 °C before use) after incubating at 37 °C for 30 minutes, followed with adding 50 μL of 0.025 M $CaCl_2$ solution, and then the APTT was measured. For the thrombin time (TT) test, 50 μL of the incubated PPP was added in a test cup and mixed with 100 μL of test thrombin agent (Sysmex; incubated 10 minutes at 37 °C before use), and then the TT was measured. For the prothrombin time (PT) test, 50 μL of the incubated PPP was added in a test cup, followed by the addition of 100 mL Thromborel S (Siemens; incubated 10 minutes before use), and incubated at 37 °C for 2 minutes, and then the PT was measured. For the detection of the concentration of fibrinogen (FIB) in plasma, 10 μL of the incubated PPP was added in a test cup and diluted with 50 μL buffer. After incubating at 37 °C for 3 minutes, the complex solution was mixed with 50 μL thrombin reagent (Sysmex; incubated 10 minutes at 37 °C before use), and then the FIB was measured. For the detection of the activities of factor VIII, IX, XI and XII in plasma, 5 μL of the incubated PPP was added in a test cup and diluted with 45 μL buffer solution. After incubating at 37 °C for 30 seconds, the complex solution was mixed with 50 μL corresponding factor deficient plasma (Coagulation Factor VIII deficient plasma, Coagulation Factor IX deficient plasma, Coagulation Factor XI deficient plasma and Coagulation Factor XII deficient plasma; Sysmex; incubated 10 minutes at 37 °C before use). After another 30-second incubation at 37 °C, 50 μL of APTT agent was added, followed with adding 50 μL of 0.025 M $CaCl_2$ solution after incubating at 37 °C for 30 minutes, and then the factor activity was measured. For all experiments, at least 6 parallel samples were applied to get a reliable value, and

the results were expressed as mean ± SD (n = 6).

Plasma recalcification times (PRT) of the microspheres were measured to investigate the blood thinning properties of the microspheres in real blood environment. The testing process was as follows: The microspheres were pre-immersed in PBS overnight and then incubated at 37 °C for 1 hour. After that, the PBS was removed, and 300 μL of fresh PPP was introduced. After incubating at 37 °C for 30 minutes, clotting was initiated by recalcification of PPP with 6 μL 5 wt.% $CaCl_2$ under continuously oscillation. The plasma solution was monitored for clotting by manually dipping of a stainless-steel hook coated with silicone into the solution to detect fibrin threads. Then the PRT was recorded at the first sign of fibrin formation. The plasma was considered as non-coagulation when the PRT was over 180 minutes. Coagulation was analyzed for every 30 seconds between the two readings to increase accuracy and the experiments were repeated six times for each sample.

A simplified blood thinning device was prepared by filling 8 g RAHMs in a 10-mL syringe to validate the pseudo-haemophilia model. Then whole blood without any anticoagulant was collected and passed through the syringe directly. The digital photos of normal whole blood and the pseudo-haemophilia blood were obtained, and the whole blood clotting times were recorded. The normal whole blood and the pseudo-haemophilia blood were then investigated by serum protein electrophoresis, clotting time tests and rapid thromboelastography. After perfusion, the syringe was washed with normal saline for three times. Then digital photos of the syringe before perfusion, after perfusion and after washing with normal saline were obtained.

4.11. Identification of bounded proteins via mass spectrometry-based proteomics study

For the identification of the bounded proteins on the microspheres, a certain amount of HPMs and RAHMs were pre-immersed in PBS overnight and then incubated at 37 °C for 1 hour. Then the PBS was removed and 1 mL of fresh PPP was introduced. After incubating with PPP for 1 hour, the microspheres were removed and rinsed three times with PBS to remove the unbound protein. Then 200 μL of 50 mmol/L NH_4HCO_3 solution was added to the samples. The mixture was centrifuged at 10,000 rcf for 5 min at 4 °C, and the supernatant was discarded. The operation was repeated again. Then 100 μL of 50 mmol/L NH_4HCO_3 solution was added and 10 mmol/L DTT solution was introduced to set the final concentration to 10 mmol/L. The mixture was reduced at 37 °C for 4 hours. After alkylated by 20 mmol/L IAA at room temperature in dark for 60 minutes, the mixture was centrifuged at 10,000 rcf for 5 min at 4 °C and wash once with 200 μL of 50 mmol/L ammonium bicarbonate by centrifugation. Then NH_4HCO_3 solution (100 μL, 50 mmol/L) and trypsin (the mass ratio of trypsin to substrate: 1:100) were added, and the microspheres were digested at 37 °C for 4 hours. Then another trypsin (the mass ratio of trypsin to substrate: 1:100) was introduced again, and the microspheres were digested at 37 °C overnight. After digestion, the peptide was desalted using a desalting column, and the solvent was evaporated in a vacuum centrifuge at 45 °C. The extracted peptides were lyophilized and resuspended in 2-20 μL of 0.1% formic acid before nano LC-MS/MS analysis.

The nano LC-MS/MS analysis was completed on an Ultimate 3000 system coupled with an Orbitrap Elite™ Hybrid Ion Trap-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, USA) with an ESI nanospray source. The detailed parameters were shown as follows:

Nanocolumn：100 μm×10 cm in-house made column packed with a reversed-phase ReproSil-Pur C18-AQ resin (3 μm, 120 Å, Dr. Maisch GmbH, Germany);
Loaded sample volume: 5 μL
Mobile phase: A: 0.1% formic acid in water; B: 0.1% formic acid in acetonitrile.

Total flow rate: 300 nL/min
LC linear gradient:

Time (min)	A	B
0	95%	5%
5	95%	5%
25	50%	50%
30	10%	90%
35	10%	90%
45	95%	5%

The separated peptides were directly entered into the mass spectrometer Orbitrap Elite™ Hybrid Ion Trap-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, USA) for on-line detection. The specific parameters are as follows:
Spray voltage: 2.2 kV
Capillary temperature: 270°C
MS parameters:
MS resolution: 60000 at 400 *m/z*
MS precursor m/z range: 300.0-1600.0
MS/MS parameters:
Product ion scan range: start from m/z 100
Activation Type: CID
Min. Signal Required: 500.0
Isolation Width: 2.00
Normalized Coll. Energy: 35.0
Default Charge State: 2
Activation Q: 0.250
Activation Time: 10.000
Data dependent MS/MS: up to top 20 most intense peptide ions from the preview scan in the Orbitrap

The raw MS files were analyzed and searched against uniprot- homo sapiens protein database based on human blood samples using MaxQuant (1.5.6.5). Only high confident identified peptides were chosen for downstream protein identification analysis and the parameters were set as follows:

Supplementary Table 2. The parameters for protein identification analysis

Parameters	Values
Fixed modifications	Carbamidomethyl (C)
Variable modifications	Oxidation (M)
Enzyme	Trypsin
Maximum Missed Cleavages	2
Peptide Mass Tolerance	10 ppm
Fragment Mass Tolerance	0.6 Da
Mass values	Monoisotopic
Significance threshold	0.05

4.12. Identification of proteins in normal plasma and pseudo-haemophilia plasma via targeted quantitative proteomics technologies

To prove the coagulation factor depletion in plasma on protein level, targeted quantitative proteomics technologies using parallel reaction monitoring models were employed to investigate the relative abundance of coagulation factors in normal plasma and pseudo-haemophilia plasma. A certain amount of RAHMs were pre-immersed in PBS overnight and then incubated at 37 °C for 1 hour. Then the PBS was removed and 1 mL of fresh PPP was introduced. After incubating with PPP for 1 hour, the plasma was collected. The sample was grinded by liquid nitrogen into cell powder and then transferred to a 5-mL centrifuge tube. After that, four volumes of lysis buffer (8 M urea, 1% Triton-100, 10 mM dithiothreitol, and 1% Protease Inhibitor Cocktail) were added to the cell powder, followed by sonication three times on ice using a high intensity ultrasonic processor (Scientz). The remaining debris was removed by centrifugation at 12,000 g at 4 °C for 10 min. Finally, the protein was precipitated with cold 20% TCA for 2 h at -20 °C. After centrifugation at 12,000 g 4 °C for 10 min, the supernatant was discarded. The remaining precipitate was washed with cold acetone for three times and re-dissolved in 8 M urea. High-abundance proteins were removed using Pierce ™ Top 12 Abundant Protein Depletion Spin Columns Kit (Thermo, USA) according to the manufacturer's instructions and the protein concentration was determined with BCA™ Protein Assay Reagent Kit (Pierce).

For digestion, the protein solution was reduced with 5 mM dithiothreitol for 30 min at 56 °C and alkylated with 11 mM iodoacetamide for 15 min at room temperature in darkness. The protein sample was then diluted to urea concentration less than 2 M. Finally, trypsin was added at 1:50 trypsin-to-protein mass ratio for the first digestion at 37 °C overnight and 1:100 trypsin-to-protein mass ratio for a second 4 h-digestion.

The tryptic peptides were dissolved in 0.1% formic acid (solvent A) and directly loaded onto a home-made reversed-phase analytical column. The gradient was comprised of an increase from 4% to 16% solvent B (0.1% formic acid in 90% acetonitrile) over 38 min, 16% to 30% in 14 min and climbing to 80% in 4 min then holding at 80% for the last 4 min, all at a constant flow rate of 500 nL/min on an EASY-nLC 1000 UPLC system. The peptides were subjected to NSI source followed by tandem mass spectrometry (MS/MS) in Q Exactive ™ Plus (Thermo) coupled online to the UPLC. The electrospray voltage applied was 2.0 kV. The m/z scan range was 450 to 1450 for full scan, and intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were then selected for MS/MS using NCE setting as 27 and the fragments were detected in the Orbitrap at a resolution of 17,500. A data-independent procedure that alternated between one MS scan followed by 20 MS/MS scans. Automatic gain control (AGC) was set at 3E6 for full MS and 1E5 for MS/MS. The maximum IT was set at 50 ms for full MS and 140 ms for MS/MS. The isolation window for MS/MS was set at 1.6 m/z.

The resulting MS data were processed using Skyline (v.3.6). Peptide settings: enzyme was set as Trypsin [KR/P] and max missed cleavage was set as 0. The peptide length was set as 7-25. Fixed modification was set as alkylation on cysteine. Transition settings: precursor charges were set as 2 and 3, ion charges were set as 1, and ion types were set as b and y. The product ions were set as from ion 3 to last ion, the ion match tolerance was set as 0.02 Da.

4.13. Mass quantification of individual coagulation factor.

Commercial enzyme-linked immune sorbent assays (ELISA) were employed to evaluate the mass quantification of individual coagulation factor with a Human Coagulation Factor VIII (FVIII) kit (CSB-E13861h, Cusabio Biotech, China), a Human Coagulation Factor IX (FIX) kit (CSB-E08443h, Cusabio Biotech, China), a Human Coagulation Factor XI (FXI) kit (CSB-EL007916HU, Cusabio Biotech, China) and a Human Coagulation Factor XII (FXII) kit

(CSB-EL007918HU, Cusabio Biotech, China). The microspheres were pre-immersed in PBS in a 24-well cell culture plate overnight. Then, the PBS was removed and 1 mL of human whole blood was introduced. After being incubated at 37 °C for 1 hour, the whole blood was centrifuged for 10 minutes at 2500 g centrifugal force (2-8 °C) to obtain plasma. For the FVIII test, 5 μL of the obtained plasma was diluted for 500 times with FVIII-Sample Diluent, and 100 μL of the diluted plasma was added into an Antibody Coated Well (provided by FVIII kit). For the FIX test, 100 μL of the obtained plasma was added into an Antibody Coated Well (provided by the FIX kit). For the FXI test, 2 μL of the obtained plasma was diluted for 2000 times with FXI-Sample Diluent, and 100 μL of the diluted plasma was added into an Antibody Coated Well (provided by FXI kit). For the FXII test, 2 μL of the obtained plasma was diluted for 2000 times with FXII-Sample Diluent, and 100 μL of the diluted plasma was added into an Antibody Coated Well (provided by FXII kit). Finally, the detections were conducted according to the respective instruction manuals. Whole blood was used as control sample. At least 15 parallel sample groups were applied to get a reliable value, and the results were expressed as mean ± SD (n = 15).

4.14. The adsorption experiment for creatinine

To study the adsorption of creatinine, firstly, creatinine was dissolved in phosphate buffered saline (PBS, pH 7.4) and the final creatinine concentration was set at 50 mg/L. 15 mg microspheres were then added to 10 mL creatinine solution and incubated at 37 °C with shaking at 200 rpm. The concentrations of the creatinine solutions were determined by an UV-Vis spectrophotometer (UV-1750, Shimadzu) at 232 nm.

5. Therapeutic efficacy and safety of the pseudo-haemophilia model *in vivo*

5.1. Animal welfare

All procedures involving the use of animals in this study were prospectively reviewed and approved by the Institutional Animal Care and Use Committee. This study was conducted in accordance with the National Institutes of Health Guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). This experiment conformed to the legal requirement in China and was approved by the ethical committee (No. K2016027) of West China Hospital, Sichuan University. During the experimental period, the animals had free access to water and food.

5.2. Animal experiment

To prepare the blood thinning device, 100 mL polypropylene apparatuses were designed and fabricated by 3D-printing. Then 80 g RAHMs were filled into the apparatus and sterilized thoroughly by autoclaving for 1 hour. The sterilized blood thinning device was sealed and reserved until use.

This study was conducted on 15 healthy male beagle dogs aged approximately 2 years and weighing about 15-18 kg. We used a haemodialysis machine (Dialog + haemodialysis machine, B. Braun Melsungen AG, Germany), extracorporeal circulation blood tubes (II-A, Well Lead Medical Co., Ltd., China), 1.8 m^2 haemodialyzers (OCI-HD180, Chengdu OCI Medical Equipment Co., Ltd., China) and 1.2 m^2 haemodialyzers (F12, Shandong WeGo Group Medical Polymer Co., Ltd., China). The extracorporeal volume of the entire extracorporeal circuit was approximately 236 to 275 mL, which included the volumes of extracorporeal circulation blood tube (155 mL), blood-thinning device (15 mL) and the total cell volume of the haemodialyzer (105 mL for 1.8 m^2 dialyzer; 66 mL for 1.2 m^2 dialyzer,). The RAHM group was used as the experimental group while heparin was used as the control group.

Before the initiation of treatment, the dog was firstly weighed and anaesthetized with an

intramuscular injection of 3% pentobarbital sodium (1 mL/kg). Then, the femoral vein was isolated, punctured and fixed with a two-lumen catheter (GDK-812.5P, Gambro Kathetertechnik Hechingen, Germany), connecting to the haemodialysis instrument. Haemodialysis was performed with a blood flow rate of 90 mL/min, which was chosen based on the body weights of the dogs and dialysate flow of 0 mL/min for 2 hours, but without ultrafiltration or normal saline to prefill the blood tube since the main purpose of the experiment was to evaluate the blood thinning properties of the pseudo-haemophilia model. The blood passed through the blood thinning device firstly and then the dialyzer, while no extra anticoagulant medication was used during the treatment. The blood samples were drawn from the dialysis tube at 0, 30, 60 and 120 minutes during the treatment and 120 minutes after the end of the treatment. After the end of the treatment, the blood was returned back to the dog using normal saline to reduce the blood loss of dogs. The dialyzer is properly oscillated, so that the blood cells deposited on the arteriovenous end of the dialyzer float with the rotation of the dialyzer and return to the body with normal saline, but the venous line must not be squeezed. Approximately 100 mL of saline is returned to the body along with blood. After the experiments, the operative wounds of the dogs were carefully sutured, and the dogs were fed for a period of time. The blood count assays, clotting time tests and biological parameters level assays of the collected blood samples were determined by an automated hematology cell analyzer (ADVIA 2120i, SIEMENS AG FWB, Germany), an automatic blood coagulation analyzer (CA-7000, Sysmex Corporation, Japan) and a biochemical analysis instrument (Cabas C311, Roche, Switzerland), respectively. The activities of the coagulation factors of the blood samples were also determined by the same method as described in *Experimental Section 4.2*. The experiments repeated for three times to get a reliable result, and the results were expressed as the mean ± SD (n = 3).

The experimental procedures of the control group were same as the experimental groups except that a loading dose of 0.4 mg/kg heparin was injected into the femoral vein before dialysis, followed by a maintenance dose of 7.5 mg/hour. The blood samples were drawn from the dogs at 0, 30, 60 and 120 minutes during the treatment and 120 minutes after the end of the treatment. The experiments repeated for three times to get reliable results, and the results were expressed as the mean ± SD (n = 3).

Furthermore, real haemodialysis treatment with dialysate flow was also performed to investigate the performance of pseudo-haemophilia model in practical clinical treatment. The experimental procedures were same as above stated except that the dialysate flow was 200 mL/min. The dialysate was proportioned from A and B solutions and highly purified water which was supplied by comprehensive water treatment systems (AquaA, Fresenius Medical Care, Germany). The dialysate quality was consistent with the standard of American Association of Medical Instrument (bacteria < 100 CFU/ml, endotoxin < 0.5 EU/ml, residual chlorine < 0.1 mg/L, water hardness < 17 ppm). Two kinds of haemodialyzers (1.2 m^2 and 1.8 m^2) were used since 1.2 m^2 dialyzer was more suitable for dogs and 1.8 m^2 dialyzer was used to study the blood-thinning property of pseudo-haemophilia model in extracorporeal circulation. During the treatment, the rectal temperature of the dogs was recorded with a calibrated electronic thermometer with resolution of 0.1 °C (Model HI-92740, Hanna Instruments, Bedfordshire, UK) with the probe being inserted to a depth of 3 cm.

In addition, a preliminary animal experiment and an extended animal experiment were also performed. The experimental procedures were same as the experimental groups except that there was no dialyzer in the preliminary animal experiment while a haemoperfusion apparatus (RC180, Chongqing Xierkang Blood Purification Equipment Development Co., Ltd., China) replaced dialyzer in the extended animal experiment.

5.3. Tissue evaluation, whole blood clotting time test (WBCT) and SEM of blood clots

In order to evaluate the systemic toxicity of RAHM, the dogs were sacrificed after the 2-hour dialysis, and the kidney and lung tissues were cut into pieces. Untreated dogs were used as the control group. Then the tissues were fixed in 10% of formalin buffer solution for 48 hours, dehydrated in a graded series (70, 90, 95, and 100%) of ethanol, cleared in xylene and embedded in paraffin. Four slides were prepared from each tissue. Sections of 4–6 μm thicknesses were made by using a rotary microtome and stained with hematoxylin and eosin (H&E). Then a panoramic digital pathological slice scanner (PRECICE 500B, UNIC Technologies INC., China) was used for microscopic observations.

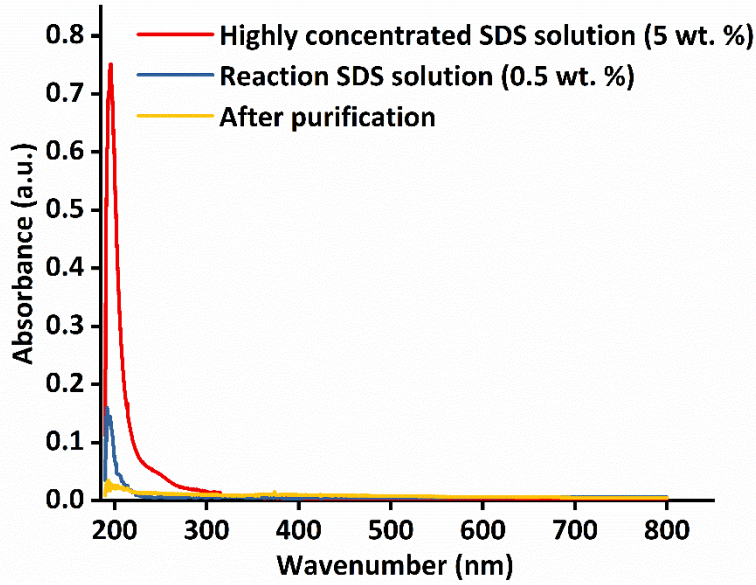
Whole blood samples were collected with vacuum tubes (5 mL, Jiangsu Kangjian Inc., China) without anticoagulant at 0, 30, 60, 120 minutes during the treatment and 120 minutes after the end of the treatment. Each sample was evenly divided into 5 portions and then added into a 48-well tissue culture polystyrene plate one by one. An aliquot of un-coagulated solution was removed and the well was washed twice with PBS. Then the wells with or without the adhered blood clot were photographed. The process was then repeated for 20, 40, 60 and 80 minutes all the way. The blood was considered as non-coagulation when the WBCT was over 80 minutes.

The blood clots before and after the treatment were fixed by fresh prepared glutaraldehyde (2.5 wt. % in PBS) for 4 hours and then washed three times with PBS and dehydrated by a series of graded alcohol-PBS solutions (30, 50, 70, 80, 90, 95 and 100%). Clots were then dried with CO_2 in a critical-point dryer, mounted onto stubs, and gold sputter-coated for SEM using a scanning electron microscope (SEM, Phenom Pure, Phenom World, Netherlands).

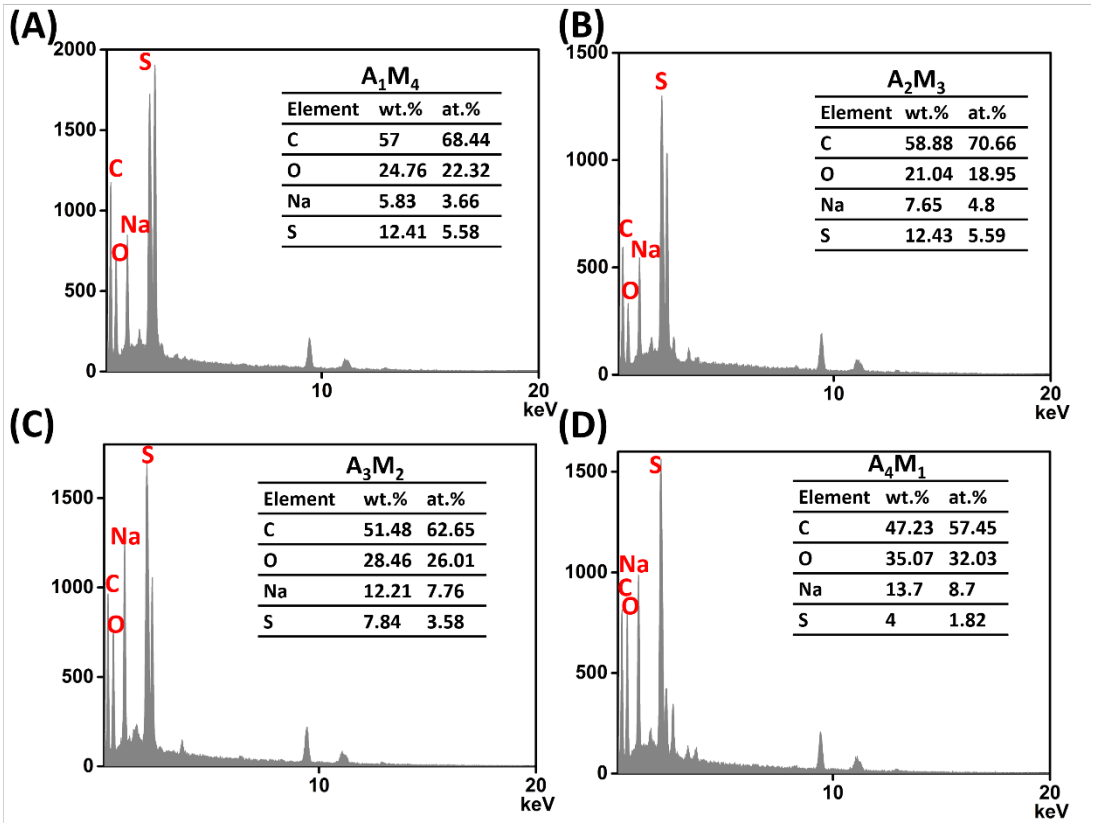
6. Statistical analysis

Each experiment was performed independently in duplicate at least, and quantified with at least triplicates. The results are expressed as the mean ± standard deviation of each sample, as indicated by error bars in all graphs. Statistical analysis was performed using the IBM Statistical Package for the Social Sciences (v.24, SPSS, Chicago, IL, USA). Unpaired Student's t test analysis or one-way analysis of variance (ANOVA) followed by Dunnett's test was used for the statistical analysis of the data. A difference with a p value of less than 0.05 was considered statistically significant.

Characterization of the compositions in the hydrogel network



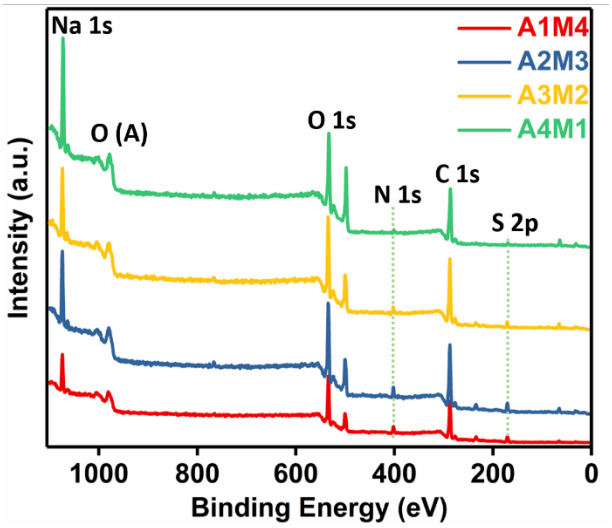
Supplementary Figure 1. The the UV spectra for highly concentrated SDS solution (5 wt. %), reaction SDS solution (0.5 wt. %) and the solution after purification. Highly concentrated SDS solution showed obvious absorbance in the range of 200~300 nm, while the peak decreased dramatically for the reaction SDS solution, demonstrating the low concentration of SDS in the reaction solution. For the solution after purification, the peak of SDS disappeared, indicating that the SDS in the system could be completely removed by washing.



Supplementary Figure 2. The energy dispersive spectra (EDS) of the anticoagulant hydrogel network: (A) A_1M_4, (B) A_2M_3, (C) A_3M_2 and (D) A_4M_1. The mass fractions (wt. %) and atom fractions (at. %) of C, O, Na and S elements in the microspheres were shown in the inserted tables. The existence of sulfur demonstrated the PAMPS was in the hydrogel network. The content of S element decreased significantly with decreasing the content of AMPS in the reaction solution. The repetition times were determined by the scanning count rate of EDS equipment.

Supplementary Table 3. The molar ratios of the AMPS to AA in the anticoagulant hydrogel networks calculated from elemental analysis (EA). The calculated values were almost close to the theoretical values, which further demonstrated the compositions of the hydrogel network.

Reaction solutions	Theoretical value		Calculated value from the composition of S element		Calculated value from the composition of N element	
	AMPS (%)	AA (%)	AMPS (%)	AA (%)	AMPS (%)	AA (%)
A_1M_4	62.14	37.86	57.13	42.87	57.58	42.42
A_2M_3	38.10	61.90	35.82	64.18	33.15	66.85
A_3M_2	21.48	78.52	18.66	81.34	22.68	77.32
A_4M_1	9.31	90.69	14.24	85.76	12.80	87.20

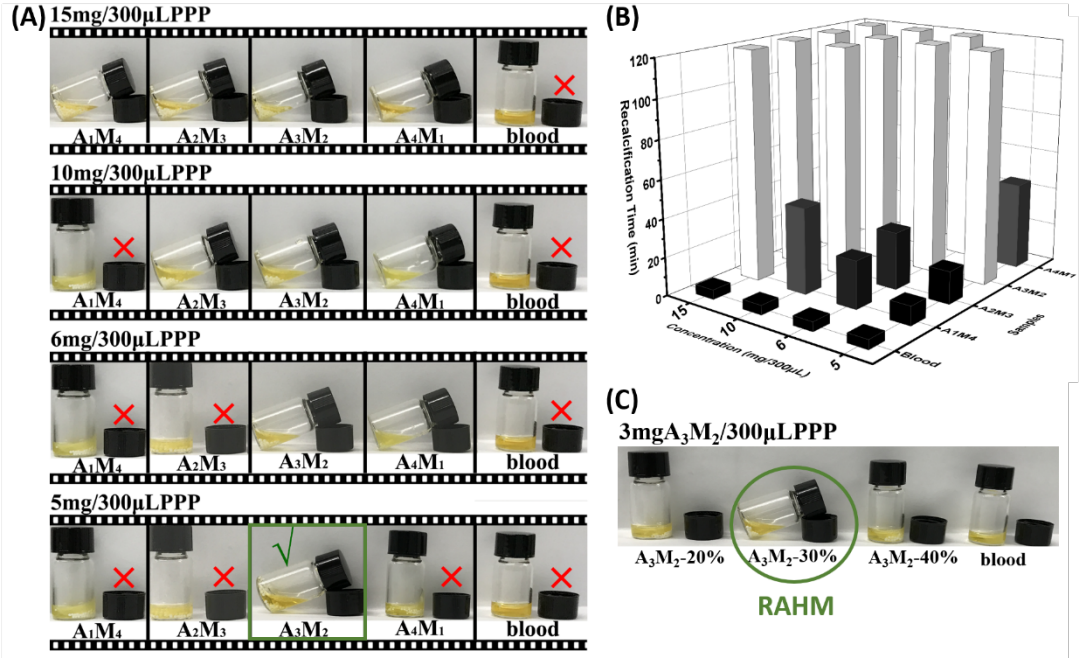


Supplementary Figure 3. The X-ray photoelectron spectroscopy (XPS) wide spectra of the anticoagulant hydrogel network with different compositions. The existence of sulfur and nitrogen demonstrated the PAMPS was in the hydrogel network. The peak intensities of S 2p and N 1s decreased significantly with decreasing the feed ratio of AMPS as expected. The repetition times were determined by the scanning times of XPS equipment.

Supplementary Table 4. The intensities (counts per second (CPS)), sensitivity factors and calculated percentage compositions of C, N, O, Na and S elements in the anticoagulant hydrogel network. Data were obtained from XPS analysis. The percentage compositions of N and S element decreased with decreasing the feed ratio of AMPS as expected.

	Samples	C	N	O	Na	S
	Intensity (CPS)	27446.5	3994.5	34134.5	21359.1	1777.9
A_1M_4	**Sensitivity factor**	0.25	0.42	0.66	2.30	0.54
	Percentage composition	59.80%	5.18%	28.17%	5.06%	1.79%
	Intensity (CPS)	34624.0	4774.2	44986.0	32963.3	3094.7
A_2M_3	**Sensitivity factor**	0.25	0.42	0.66	2.30	0.54
	Percentage composition	58.17%	4.77%	28.63%	6.02%	2.41%
	Intensity (CPS)	33267.3	2531.9	43627.7	31300.0	1166.5
A_3M_2	**Sensitivity factor**	0.25	0.42	0.66	2.30	0.54
	Percentage composition	60.22%	2.73%	29.91%	6.16%	0.98%
	Intensity (CPS)	32248.8	1314.6	48025.8	52507.4	249.6
A_4M_1	**Sensitivity factor**	0.25	0.42	0.66	2.30	0.54
	Percentage composition	56.53%	1.37%	31.89%	10.00%	0.20%

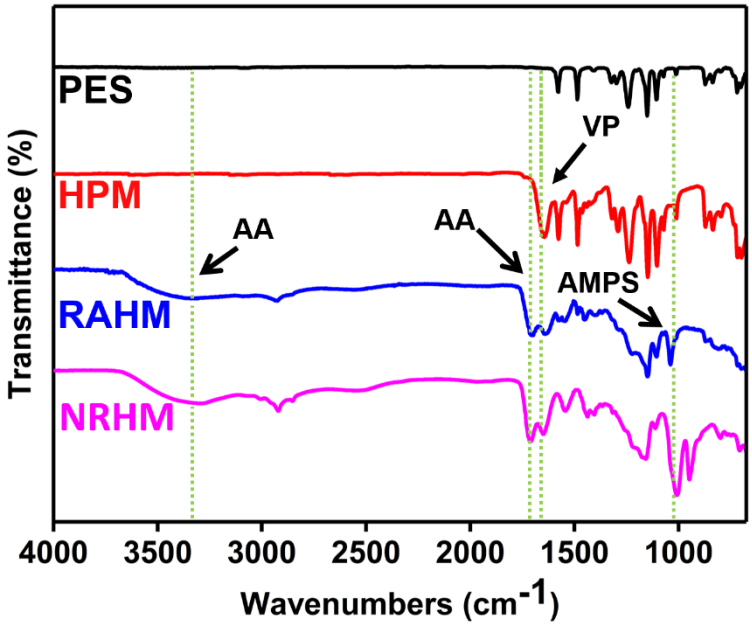
Characterization of the RAHMs



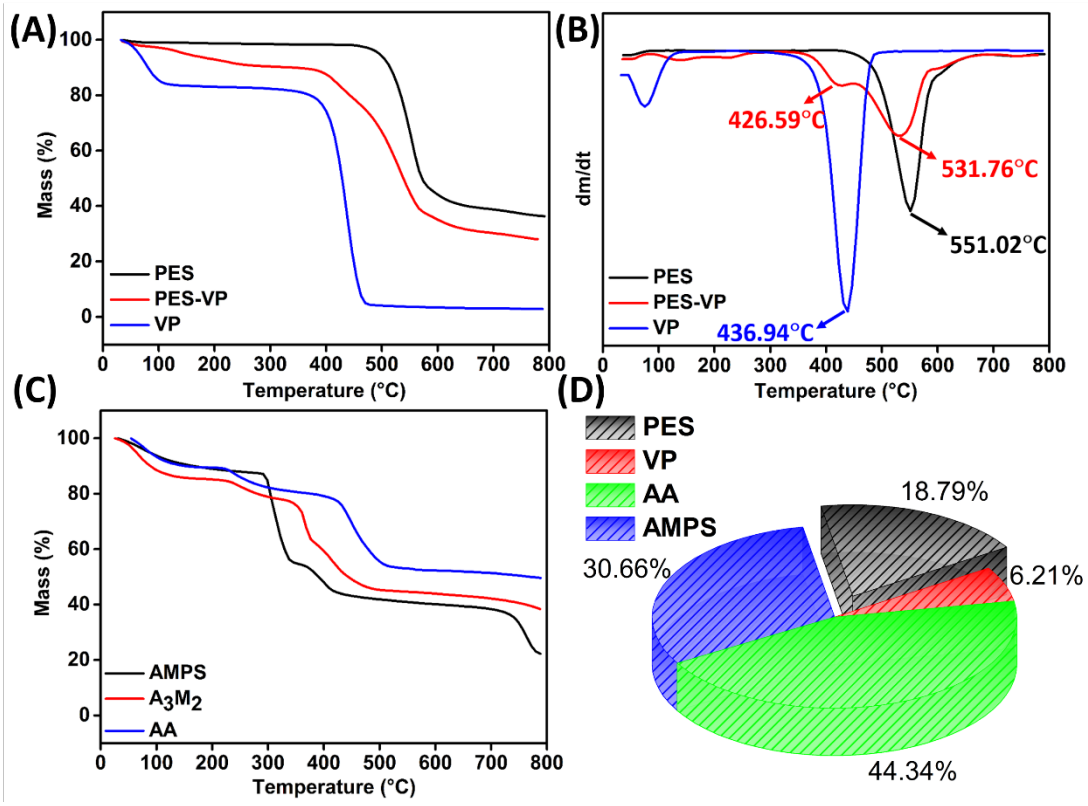
Supplementary Figure 4. A series of recalcification time tests were used to choose the optimum composition of the anticoagulant hydrogel network. (A) Digital photos of the recalcification plasma after incubating with different microspheres. The tilted bottle indicated that the recalcification plasma was non-anticoagulation and maintained fluidity. The upright bottle represented the recalcification plasma was clotted. The concentration of the added microspheres changed from 15 mg/300 μL PPP to 5 mg/300 μL PPP until only one kind of microspheres remained blood thinning capability at low concentration. When the concentration of the microspheres was 15 mg/300 μL PPP, all the microspheres showed excellent blood thinning capability. As the concentration of the microspheres decreased, some kinds of microspheres could not remain their blood thinning ability at high concentration. When the concentration of the microspheres was reduced to 5 mg/300 μL PPP, only A_3M_2 could maintain its blood thinning capability at low concentration. (B) The recalcification times of the microspheres at different concentrations. Three replicates were used to reduce errors and the values were averaged. (C) After verifying the optimum composition, a series of A_3M_2 microspheres were also prepared by changing the mass ratios of monomer in the reaction solutions (from 20% to 40%), and then recalcification time tests were used to choose the optimum microspheres. The A_3M_2 microspheres with 30% monomer mass ratios in the reaction solution showed best blood thinning capability and were named as RAHMs.

Supplementary Table 5. The compositions of the solutions for preparing RAHM.

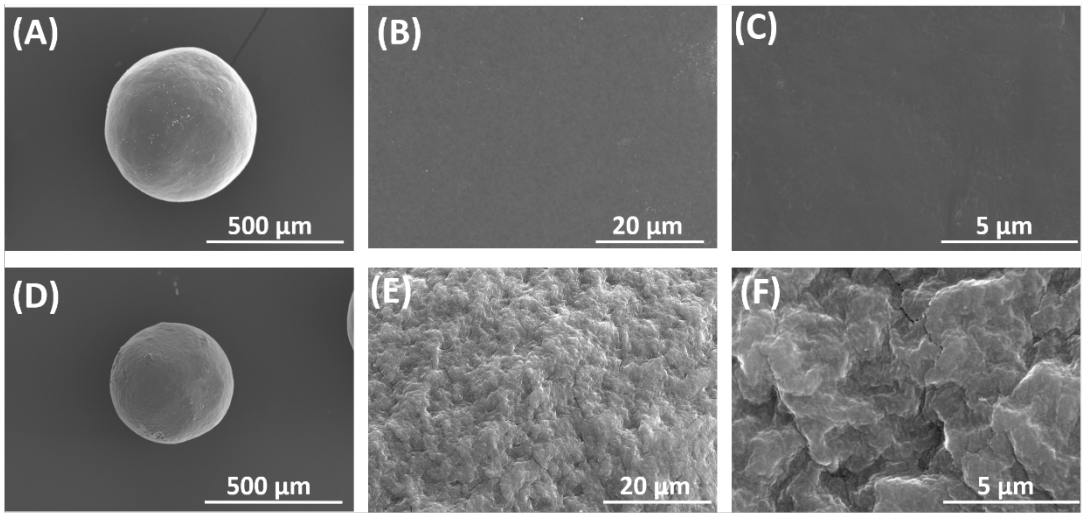
Sample	Reaction solution (g)						Polymer precursor solution (g)				
	AMPS	AA	MBA	APS	SDS	DI	PES	VP	AIBN	MBA	DMAC
RAHM	12	18	1.2	0.3	0.5	70	4	4	0.02	0.5	42



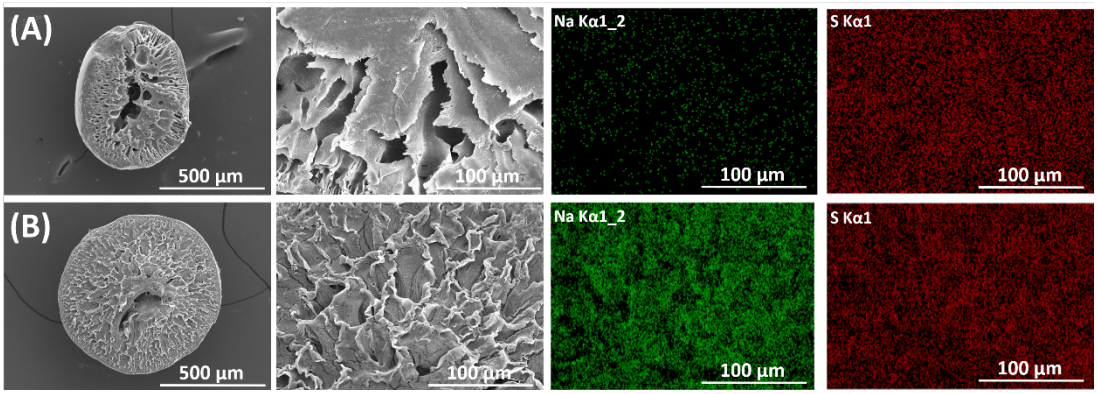
Supplementary Figure 5. The FTIR spectra of PES, HPM, RAHM and NRHM. The peak at 1670 cm^{-1} was attributed to the -C=O in PVP,[4] indicating PVP was successfully introduced into HPM, RAHM and NRHM. The characteristic peaks of AMPS were at 3448 cm^{-1} and 1080 cm^{-1}, which were ascribed to amide N-H stretch and the S-O stretching vibration of $-SO_3$ group, respectively.[5] The new peak appeared at 1735 cm^{-1} was the characteristic vibrations of C=O stretching of −COOH.[6] The results indicated that the anticoagulant hydrogel network was successfully introduced into the hydrophobic polymeric skeleton. The repetition times were determined by the scanning times of FTIR equipment.



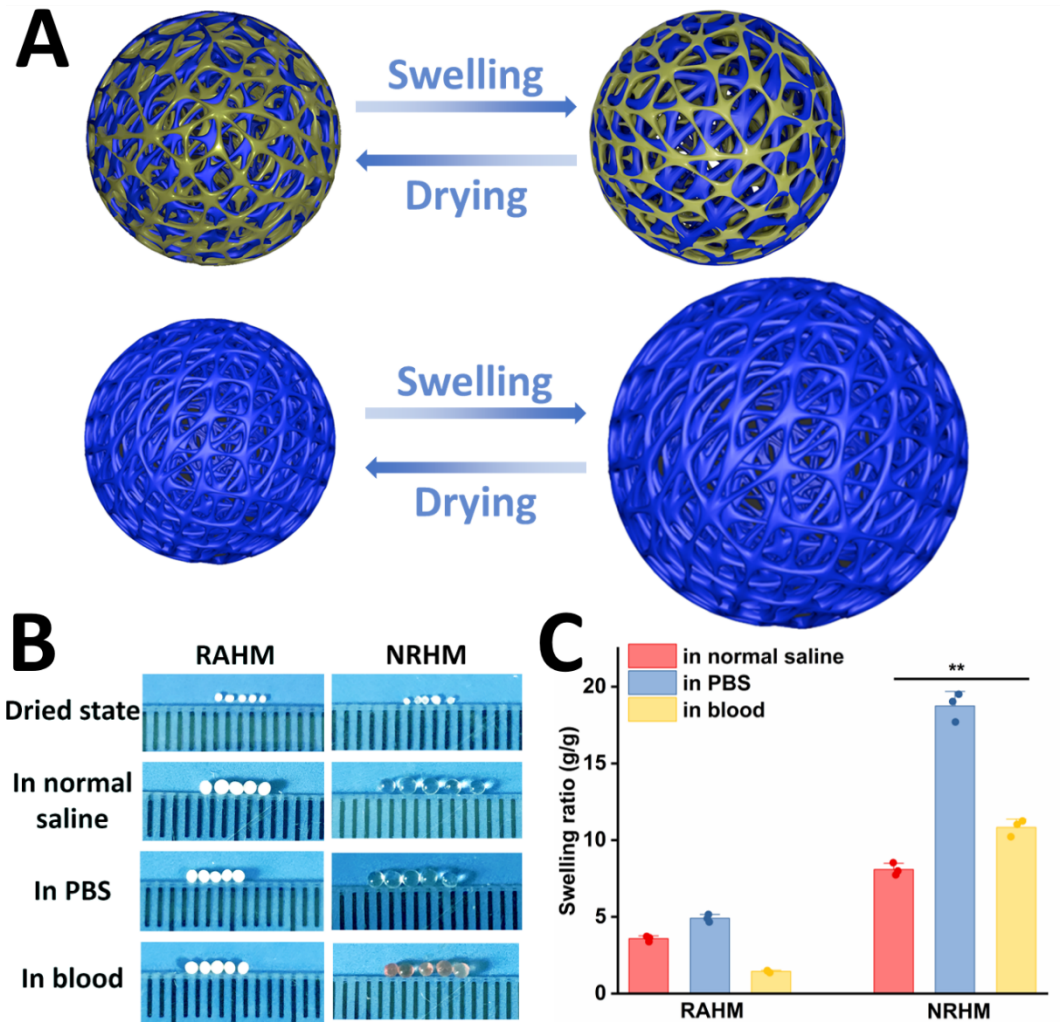
Supplementary Figure 6. (A) The TGA curves of PES, PES-VP and VP. (B) The DTG curves of PES, PES-VP and VP. The DTG curve of PES-VP showed two peaks at 426.59 °C and 531.76 °C, which could be ascribed to the degradation of VP and PES skeleton, respectively, indicating that VP was successfully cross-linking polymerized in PES matrix. (C) The TGA curves of AMPS, A3M2 and AA. (D) The mass fractions of PES, VP, AA and AMPS in the RAHM. The mass fractions of PES and VP in the hydrophobic polymeric skeleton and the mass fractions of AMPS and AA in the anticoagulant hydrogel network were verified by calculating the weight loss of the TGA curves. The mass fractions of the hydrophobic polymeric skeleton and the anticoagulant hydrogel network were verified by dissolving the polymeric skeletons with DMAc fully and calculating the weight loss before and after dissolving. The experiments were performed independently in duplicate with similar results.



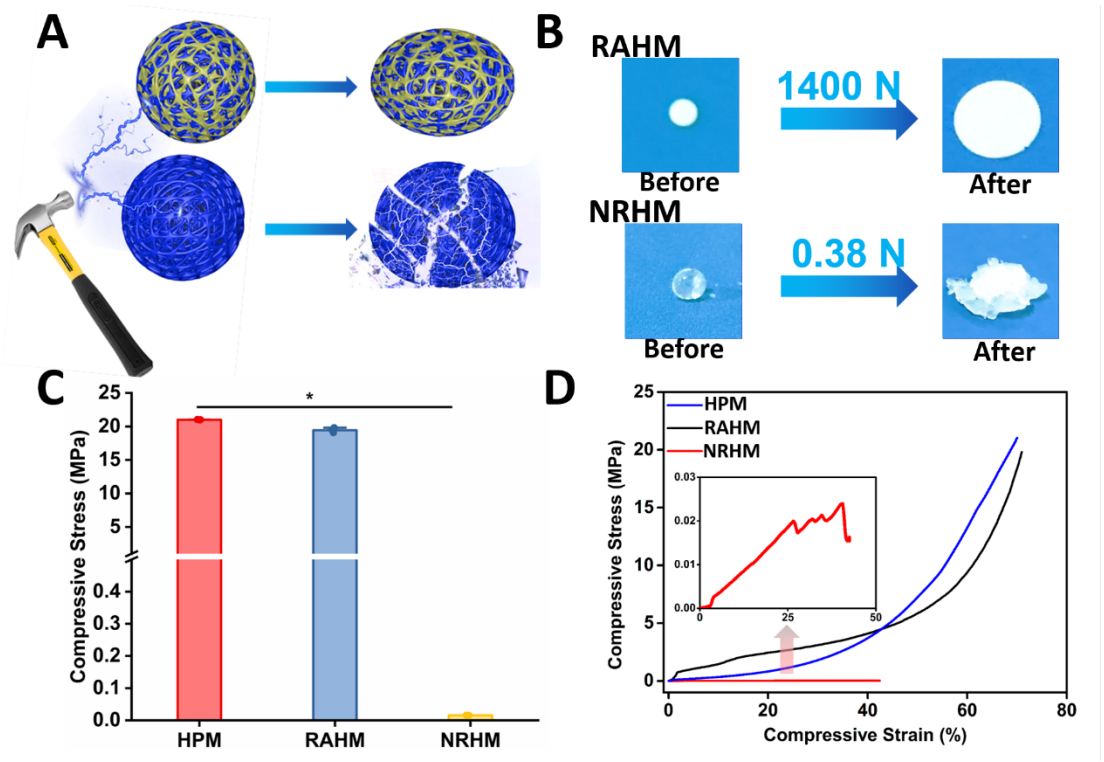
Supplementary Figure 7. The surface micro-morphologies of HPM (A)-(C) and RAHM (D)-(F) at different magnification times. The microspheres maintained good sphericity after cross-linking reaction and the average diameter of the microspheres could be controlled in about 450-550 µm. The surface of HPM was smooth, while after introducing the hydrogel network, the surface became rough and some furrows could be obviously observed, which might be caused by the surface enrichment of the hydrophilic hydrogel. The experiments were performed independently in duplicate with similar results.



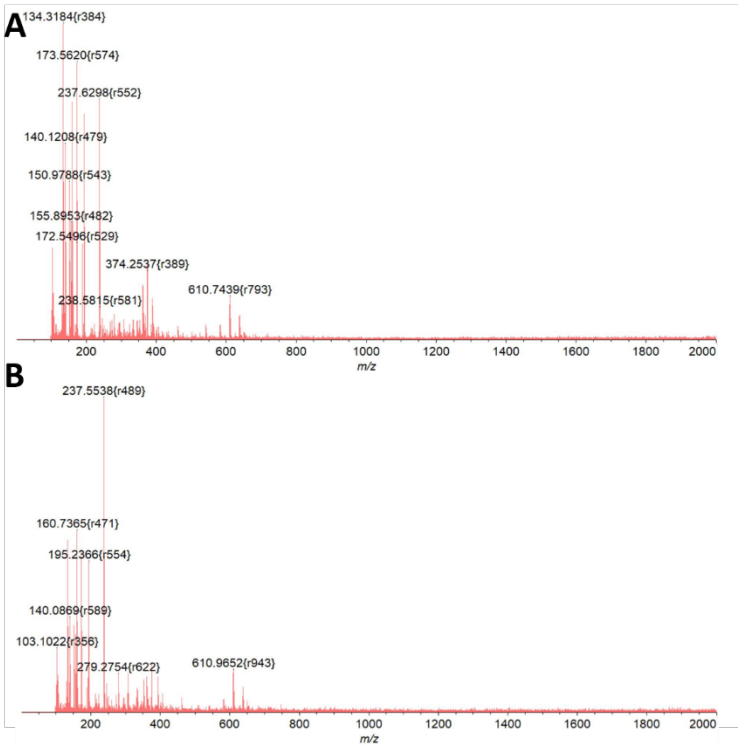
Supplementary Figure 8. The cross-sectional micro-morphologies and EDX mapping of HPM (A) and RAHM (B) at different magnification times. The HPM showed a typical finger-like structure of PES. The HPM showed uniform distribution of S element and rare distribution of Na element. For the cross-sectional of morphology of RAHM, the finger-like structure disappeared and was replaced by typical porous structure. The content of Na element in RAHM was obviously higher than HPM, since the carboxyl and sulfonic groups had been converted into -COONa and -SO_3Na (sodium salt form). The uniform distribution of Na element indicated that the hydrogel network was mixed with the hydrophobic polymeric skeleton uniformly. For SEM, the experiments were performed independently in duplicate with similar results. For EDX mapping, the repetition times were determined by the scanning count rate of EDX equipment.



Supplementary Figure 9. (A) Schematic diagram for the swelling ratios of the RAHM and NRHM. The hydrophobic skeleton in the RAHM would restrict the swelling of hydrogel network, thereby achieving great dimensional stability. (B) Digital photos of the RAHMs and non-reinforced hydrogel microspheres (NRHMs) in dried state, normal saline, phosphate-buffered solution (PBS) and blood, respectively. (C) The swelling ratios of the RAHMs and NRHMs in normal saline, PBS and blood. (All the values are expressed as mean ± SD, n = 3 biologically independent samples, significance determined by an unpaired, two-sided t test, Student's t test, $^{**}P = 0.000$ for normal saline group, $^{**}P = 0.000$ for PBS group, $^{**}P = 0.001$ for blood group).

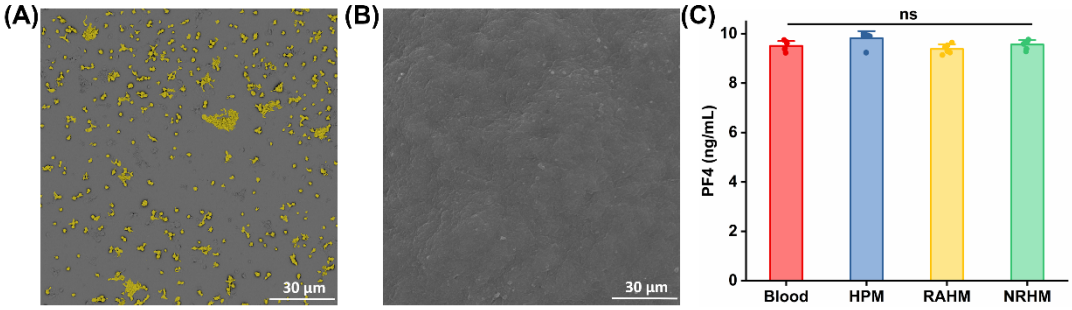


Supplementary Figure 10. (A) Schematic diagram for the reinforced mechanical strength of the RAHMs. (B) Digital photos of the RAHMs and NRHMs before and after the compression. (C) The compressive stress of the HPMs, RAHMs and NRHMs. (All the values are expressed as mean ± SD, n = 3 biologically independent samples, significance determined by one-way ANOVA followed by Dunnett's test, $^{*}P = 0.037$ for HPM vs RAHM, $^{*}P = 0.000$ for HPM vs NRHM, $^{*}P = 0.000$ for RAHM vs NRHM). (D) Typical compressive stress-strain curves of HPM, RAHM and NRHM. The experiments were performed independently in duplicate with similar results.

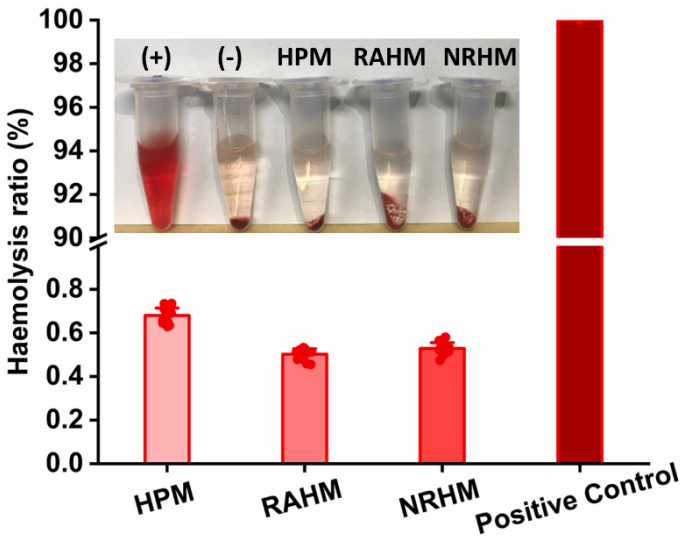


Supplementary Figure 11. The MALDI-TOF-MS spectra of the PBS before (A) and after incubating with RAHM for 15 days (B) using DHB as a matrix in water. No polymer was eluted from RAHM, indicating the good stability of the RAHM. The repetition times were determined by the scanning times of MALDI-TOF-MS equipment.

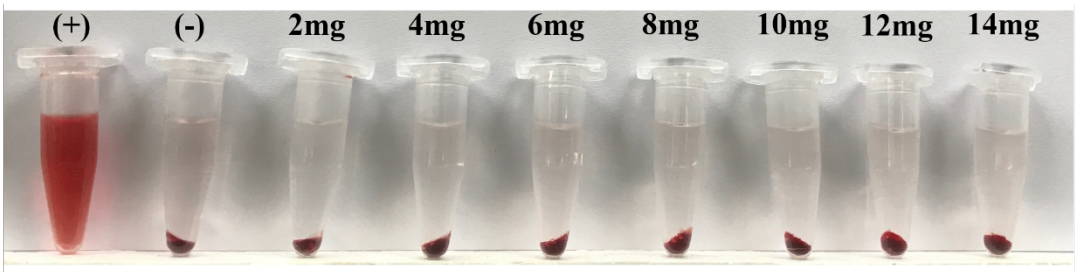
Evaluation of biocompatibility of the microspheres *in vitro*



Supplementary Figure 12. SEM pictures of the platelet adhesion for the (A) HPM and (B) RAHM. The RAHM exhibited few platelet adhesion, pseudopodia and deformation comparing with HPM, indicating the suppressed platelet adhesion of RAHM. The experiments were performed independently in duplicate with similar results. (C) Generated concentrations of platelet factor 4 (PF4) after incubating the HPM, RAHM and NRHM with whole blood. (All the values are expressed as mean ± SD, n = 3 biologically independent samples, 2 technical replicates, significance determined by one-way ANOVA followed by Dunnett's test, P = 0.269 for HPM, P = 0.831 for RAHM, P = 0.997 for NRHM).



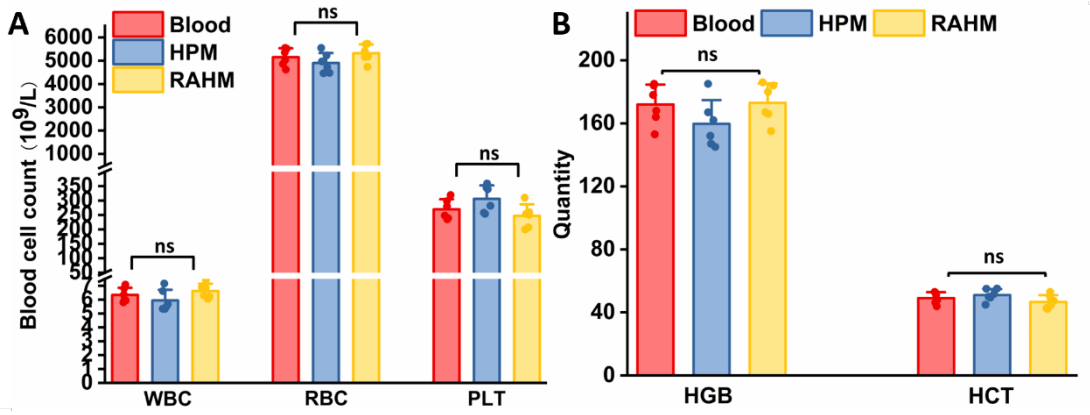
Supplementary Figure 13. The haemolysis ratios of HPM, RAHM and NRHM at the concentration of 14 mg/300 μL PPP. The haemolysis ratios were all lower than 2%, indicating both the hydrophobic polymeric skeleton and the anticoagulant hydrogel network showed great erythrocyte compatibility. (All the values are expressed as mean ± SD, n = 15 biologically independent samples).



Supplementary Figure 14. Digital photos of the RBC suspensions after incubation with different concentrations of RAHM and centrifugation. All the supernatants with different concentration of RAHM were colorless, which indicated that there was no erythrocyte rupture and haemoglobin release. The experiments were performed independently in duplicate with similar results.

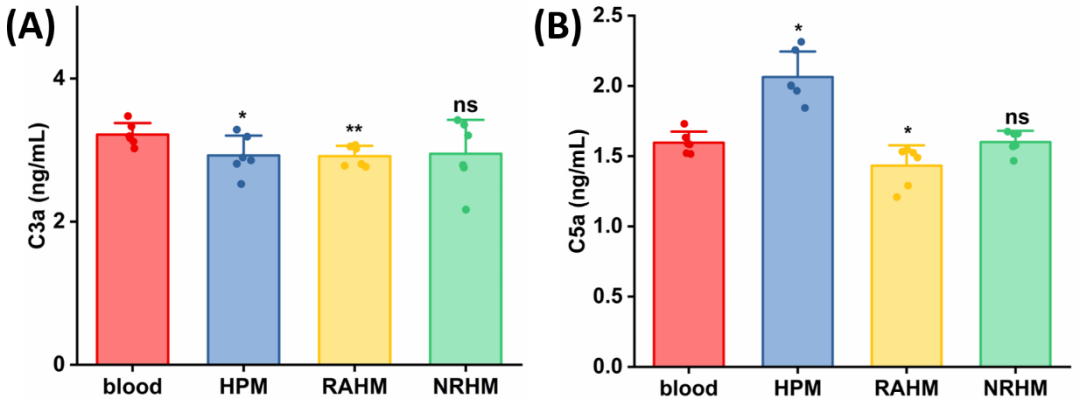
Supplementary Table 6. The results of the blood count assay *in vitro*. (All the values are expressed as mean ± SD, n = 6 biologically independent samples).

Tests	Control	HPM	RAHM
WBC (10^9/L)	6.34 ± 0.52	5.94 ± 0.77	6.63 ± 0.52
%NEUT (%)	59.50 ± 1.10	57.57 ± 1.57	60.33 ± 1.06
%LYMPH (%)	31.87 ± 0.96	32.97 ± 1.68	30.15 ± 1.37
%MONO (%)	4.62 ± 0.26	4.68 ± 0.30	4.53 ± 0.34
%EOS (%)	3.52 ± 1.01	4.37 ± 0.72	4.50 ± 1.28
%BASO (%)	0.50 ± 0.22	0.42 ± 0.08	0.43 ± 0.08
#NEUT (10^9/L)	3.77 ± 0.30	3.76 ± 0.78	4.00 ± 0.34
#LYMPH (10^9/L)	2.02 ± 0.19	1.95 ± 0.17	2.00 ± 0.14
#MONO (10^9/L)	0.29 ± 0.01	0.28 ± 0.06	0.30 ± 0.03
#EOS (10^9/L)	0.22 ± 0.07	0.26 ± 0.04	0.30 ± 0.09
#BASO (10^9/L)	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
%ALY (%)	0.82 ± 0.59	0.47 ± 0.33	0.59 ± 0.26
%LIC (%)	0.03 ± 0.08	0.00 ± 0.00	0.15 ± 0.14
#ALY	0.05 ± 0.04	0.03 ± 0.02	0.03 ± 0.02
#LIC	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
RBC (10^{12}/L)	5.16 ± 0.38	4.90 ± 0.43	5.32 ± 0.38
HGB (g/L)	172.00 ± 12.57	159.67 ± 15.07	173.00 ± 12.23
HCT (%)	49.15 ± 3.70	46.68 ± 4.25	51.00 ± 3.85
MCV (fL)	95.37 ± 0.23	95.32 ± 0.33	95.73 ± 0.67
MCH (pg)	33.33 ± 0.23	32.62 ± 0.50	32.57 ± 0.37
MCHC (g/L)	349.33 ± 2.50	342 ± 5.06	340.17 ± 5.81
RDW-CV (%)	12.05 ± 0.08	12.05 ± 0.08	12.10 ± 0.13
RDW-SD (fL)	48.08 ± 0.40	48.17 ± 0.35	48.52 ± 0.63
PLT (10^9/L)	269.83 ± 34.51	306.00 ± 46.75	247.00 ± 40.03
MPV (fL)	8.63 ± 0.08	8.70 ± 0.14	8.67 ± 0.05
PDW	15.97 ± 0.08	15.82 ± 0.12	15.97 ± 0.18
PCT	0.23 ± 0.03	0.27 ± 0.04	0.21 ± 0.03

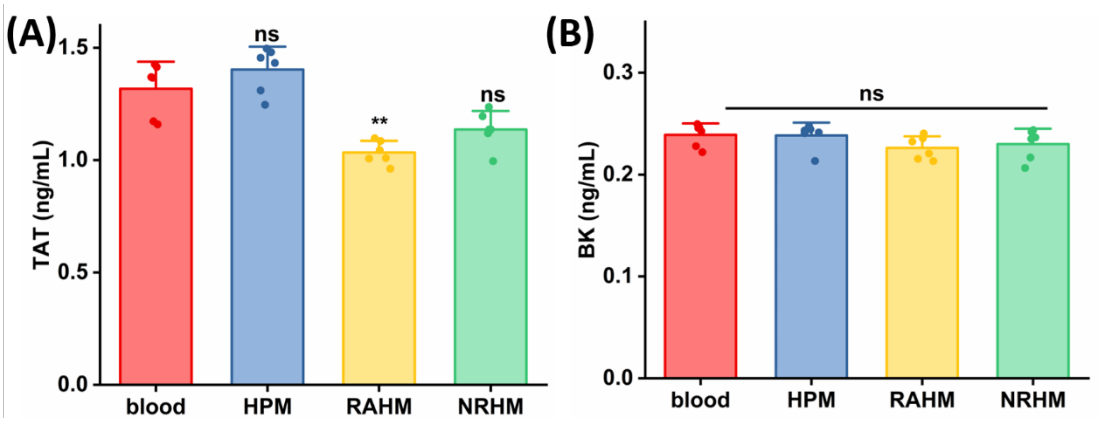


Supplementary Figure 15. Comparison of the blood cell count (A), HGB (g/L) and HCT (%) (B) for blood, blood after incubating with HPMs, and pseudo-haemophilia blood (blood after incubating with RAHMs). (All the values are expressed as mean ± SD, n = 6 biologically independent samples, significance determined by one-way

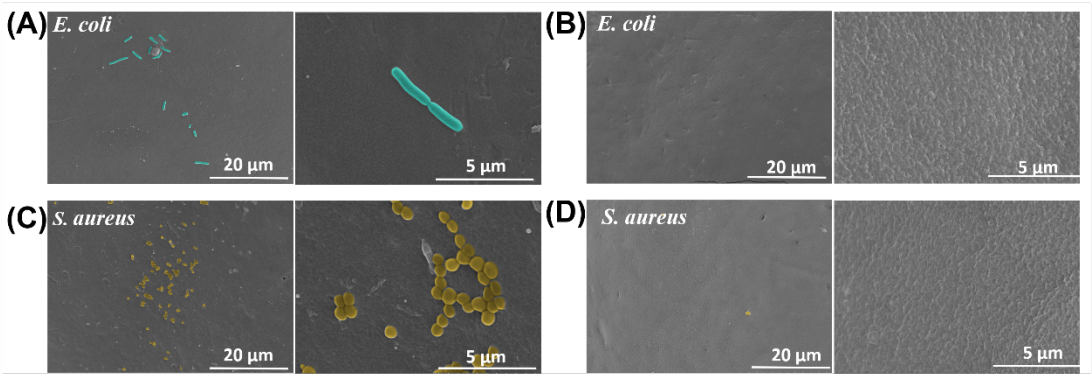
ANOVA followed by Dunnett's test, WBC: P = 0.711 for HPM, P = 0.666 for RAHM. RBC: P = 0.829 for HPM, P = 0.639 for RAHM. PLT: P = 0.655 for HPM, P = 0.386 for RAHM. HGB: P = 0.999 for HPM, P = 0.376 for RAHM. HCT: P = 0.782 for HPM, P = 0.647 for RAHM).



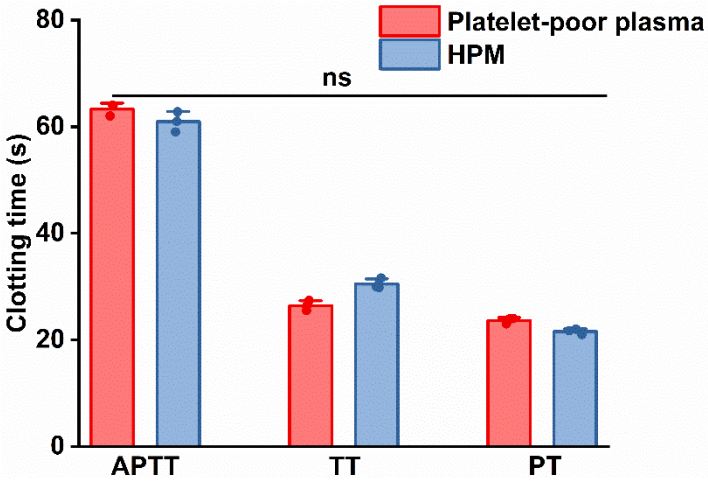
Supplementary Figure 16. Generated concentrations of human complement fragment 3a (C3a) and human complement fragment 5a (C5a) after incubating the HPM, RAHM and NRHM with whole blood. All the values are expressed as mean ± SD, n = 3 biologically independent samples, 2 technical replicates, significance determined by an unpaired, two-sided t test, Student's t test. The RAHM showed obvious suppression for complement activation ($^{**}P = 0.007$ for C3a, $^{*}P = 0.036$ for C5a). The C3a or C5a concentrations of HPM increased a little ($^{*}P = 0.049$ for C3a, $^{*}P = 0.001$ for C5a). While for the NRHM, no significant changes were observed (P = 0.235 for C3a, P = 0.907 for C5a).



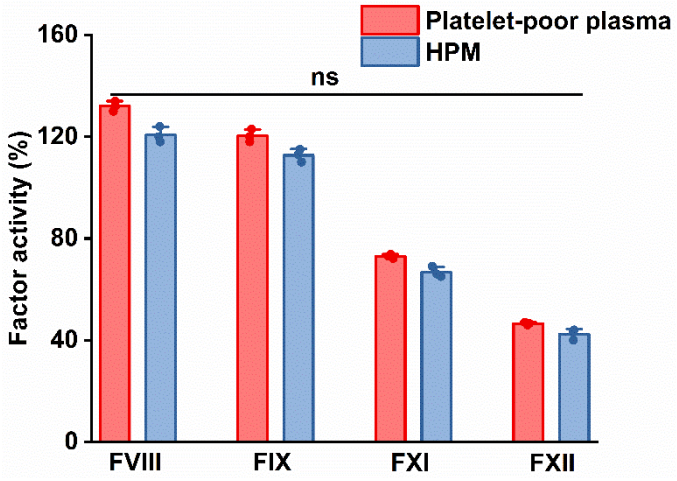
Supplementary Figure 17. Generated concentrations of thrombin-antithrombin (TAT) and bradykinin (BK) after incubating the HPM, RAHM and NRHM with whole blood. (All the values are expressed as mean ± SD, n = 3 biologically independent samples, 2 technical replicates, significance determined by one-way ANOVA followed by Dunnett's test. TAT: P = 0.706 for HPM, **P = 0.006 for RAHM, P = 0.071 for NRHM. BK: P = 1.000 for HPM, P = 0.350 for RAHM, P = 0.801 for NRHM.)



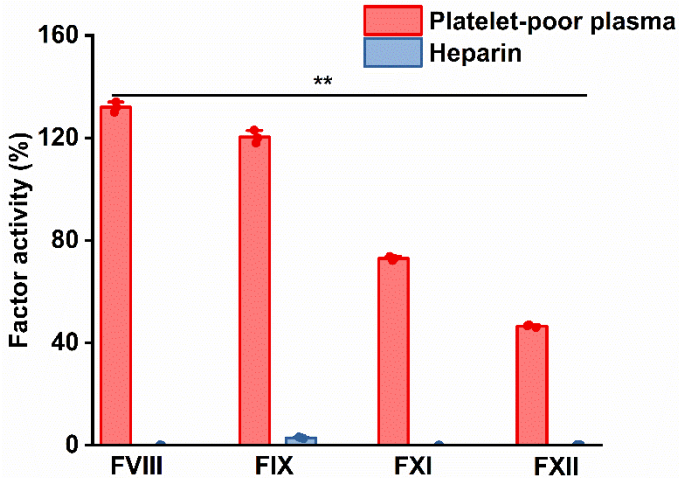
Supplementary Figure 18. SEM images that revealed the bacterial adhesion on the surfaces of the microspheres after incubating with bacteria within 12 h. The escherichia coli (*E. coli*) adhesion on the surface of HPM (A) and RAHM (B). The staphylococcus aureus (*S. aureus*) adhesion on the surface of HPM (C) and RAHM (D). HPM showed obvious bacterial adhesion while there was nearly no bacterial adhesion on the surface of RAHM, which might be due to the increased hydrophilia and the abundant negative charges. The antibacterial adhesion property of the RAHM could contribute to its long-term preservation. The experiments were performed independently in duplicate with similar results.



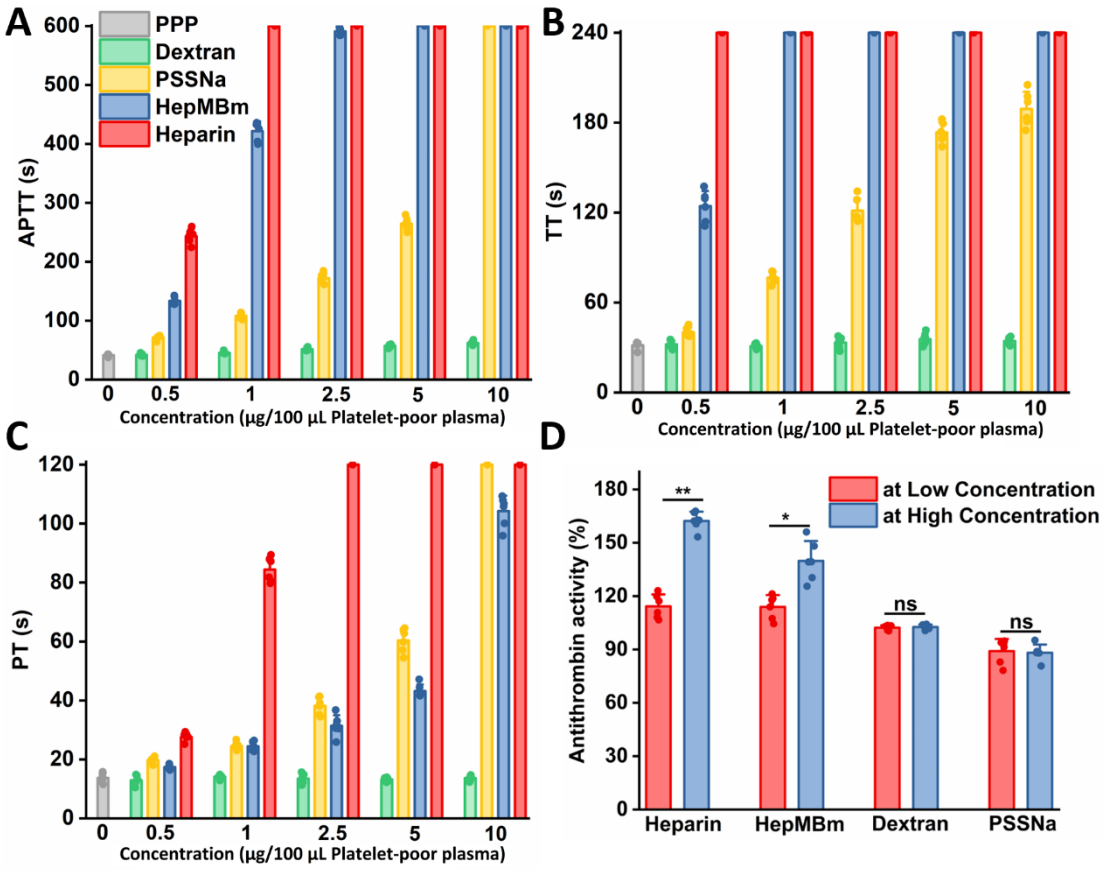
Supplementary Figure 19. The activated partial thromboplastin time (APTT), thrombin time (TT), and prothrombin time (PT) of HPM comparing with platelet-poor plasma (PPP). All the values are expressed as mean ± SD, n = 3 biologically independent samples, significance determined by one-way ANOVA followed by Dunnett's test, P = 0.661 for APTT, P = 0.051 for TT, P = 0.067 for PT. The clotting times of plasma after incubating with HPM were similar to the control group, indicating the blood thinning capability of RAHM was mainly ascribed to the hydrogel network.



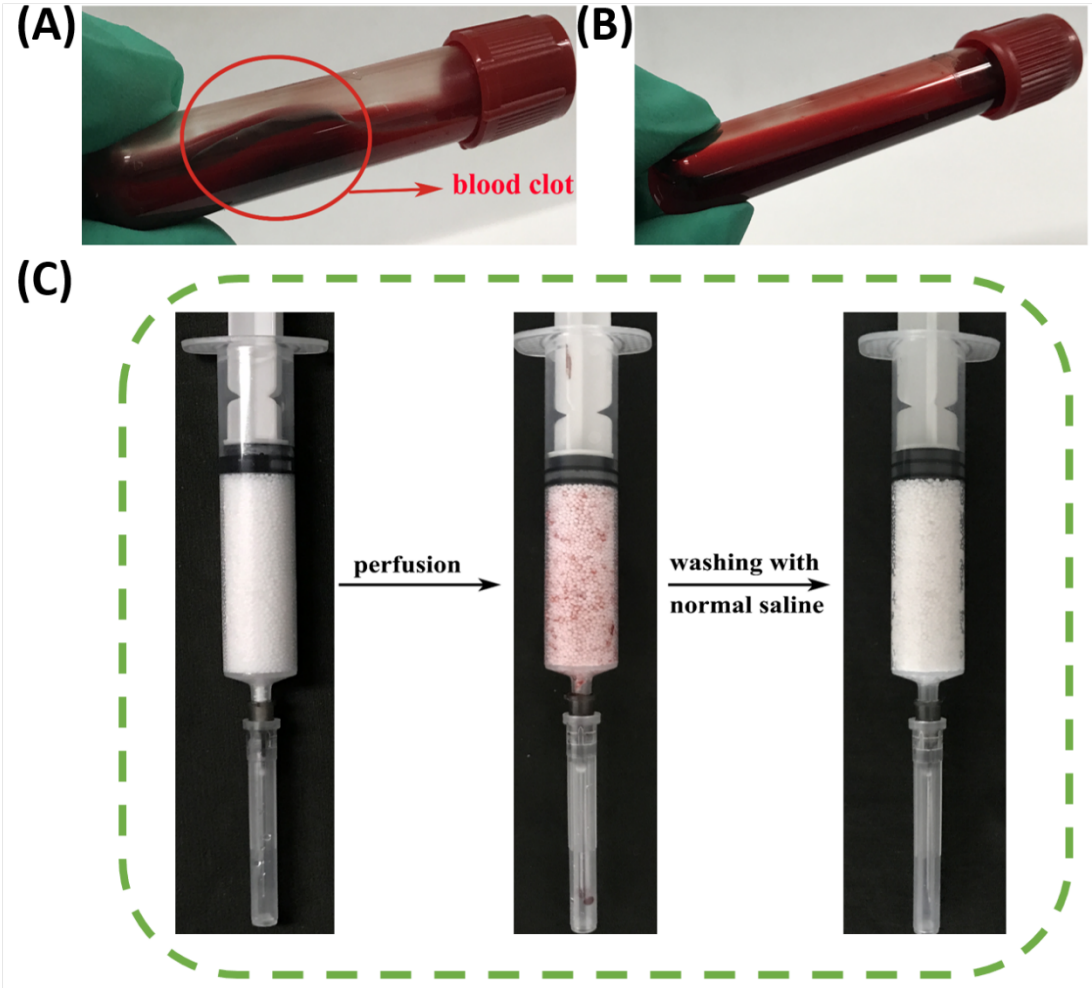
Supplementary Figure 20. The activities of factor VIII, factor IX, factor XI and factor XII of plasma after incubating with the HPMs. Comparing with PPP, the factor activities decreased a little. All the values are expressed as mean ± SD, n = 3 biologically independent samples, significance determined by one-way ANOVA followed by Dunnett's test, no significant changes were observed between the PPP group and HPM group (P = 0.079 for FVIII, P = 0.190 for FIX, P = 0.153 for FXI, P = 0.347 for FXII).



Supplementary Figure 21. The activities of factor VIII, factor IX, factor XI and factor XII of plasma after incubating with heparin (2.5 μg/300 μL PPP). All the values are expressed as mean ± SD, n = 3 biologically independent samples, significance determined by one-way ANOVA followed by Dunnett's test, significant changes were observed between the PPP group and Heparin group ($^{**}P = 0.000$ for FVIII, $^{**}P = 0.001$ for FIX, $^{**}P = 0.000$ for FXI, $^{**}P = 0.000$ for FXII). Heparin showed robust anticoagulant capability and the factor activities were inactivated to almost 0%. The complete inactivation of factor activity might be one of reason for its bleeding risks.



Supplementary Figure 22. (A) APTT, (B) TT and (C) PT values for the plasma after incubating with dextran, poly (sodium 4-styrenesulfonate) (PSSNa), heparin-mimetic biomacromolecule (HepMBm) and heparin at different concentrations. Heparin and HepMBm were biomacromolecules containing both sulfonic groups and uronic backbone, and dextran was uronic macromolecules without sulfonic groups, while PSSNa was macromolecules containing sulfonic groups and alkyl backbone. Poor platelet plasma (PPP) was used as control group. The detector limitations for APTT, TT and PT were 600 s, 240 s and 120 s, respectively. All the values are expressed as mean ± SD, n = 6 biologically independent samples. (D) The antithrombin activity percentage for platelet-poor plasma (PPP) after incubation with dextran, PSSNa, HepMBm and heparin at low (2.5 μg/100 μL PPP) or high concentrations (50 μg/100 μL PPP). All the values are expressed as mean ± SD, n = 6 biologically independent samples. Significance determined by one-way ANOVA followed by Dunnett's test, $^{**}P = 0.000$ for Heparin, $^{*}P = 0.022$ for HepMBm, P = 1.000 for Dextran, P = 1.000 for PSSNa.



Supplementary Figure 23. (A) Digital photo of the whole blood without anticoagulant. Blood clots appeared within a few minutes. (B) Digital photo of the whole blood (pseudo-haemophilia blood) after passing through the simplified blood thinning device. No blood clots were observed and the pseudo-haemophilia blood remained non-coagulation for over 3 hours. (C) Digital photos of the simplified blood thinning device before perfusion, after perfusion and after washing with normal saline. The blood remaining in the syringe could be easily washed away by normal saline. Considering that it's necessary to flush the pipage with normal saline to avoid blood loss after the end of blood purification, the blood thinning device can be introduced into the blood purification system safely.

Supplementary Table 7. The results of serum protein electrophoresis before and after passing the simplified blood thinning device.

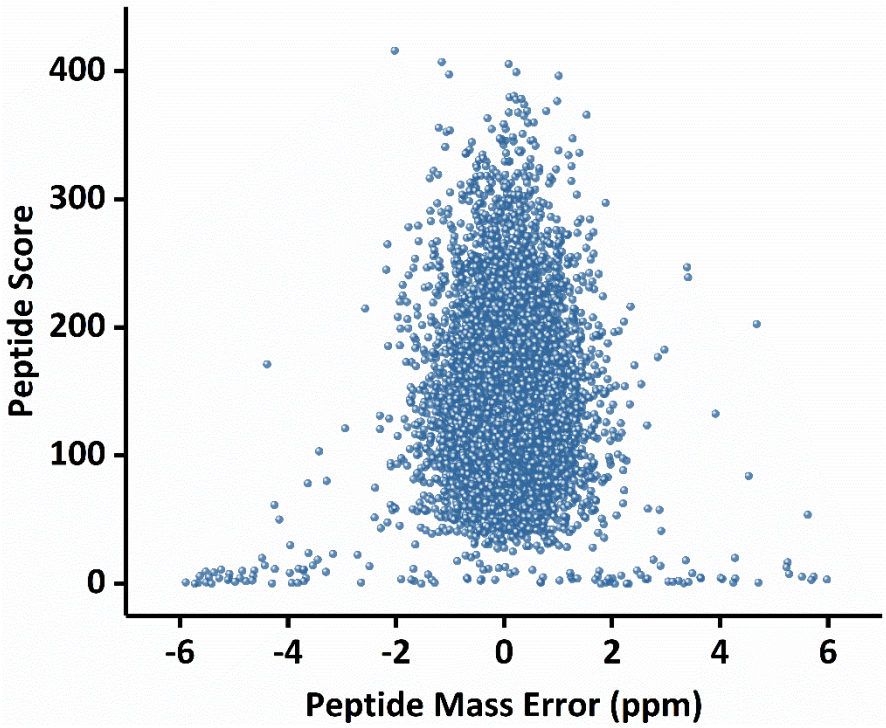
Items	Before treatment	After treatment
albumin	57.9%	57.2%
α_1-globulin	4.5%	4.5%
α_2-globulin	7.9%	9.4%
β_1-globulin	6.4%	6.4%
β_2-globulin	5.6%	5.9%
γ-globulin	17.7%	16.6%

Supplementary Table 8. The results of coagulation function before and after passing the simplified blood thinning device. PT: prothrombin time; INR, international normalized ratio; APTT, activated partial thromboplastin time; APTTR, activated partial thromboplastin time ratio; TT, thrombin time; TTR, thrombin time ratio; FDP, fibrin (-ogen) degradation products; DD, D-dimer.

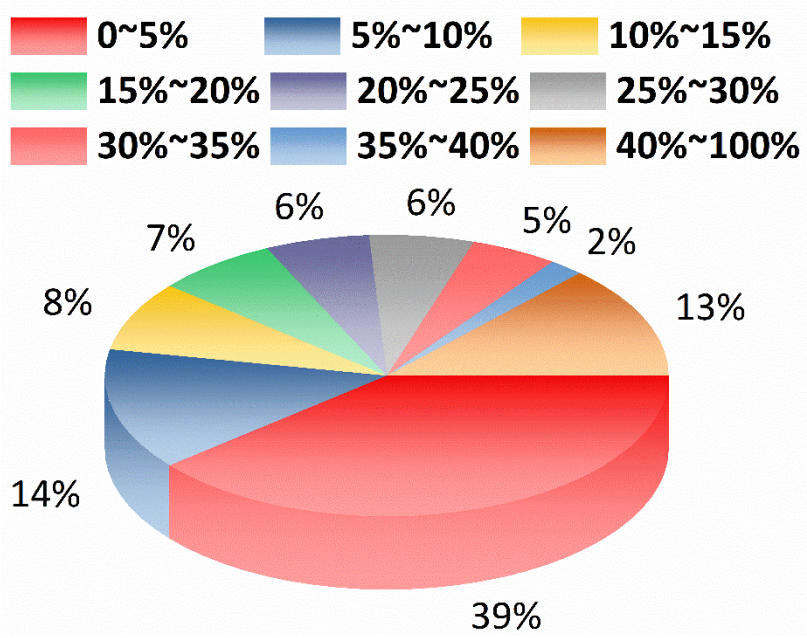
Items	Before treatment	After treatment
PT	9.6 s	14.4 s
INR	0.87	1.32
APTT	32.9 s	122.1 s
APTTR	1.18	4.39
TT	20.0 s	24.0 s
TTR	1.23	1.47
FDP	<2.5 mg/L	<2.5 mg/L
DD	0.15 mg/L FEU	0.12 mg/L FEU

Supplementary Table 9. The results of rapid thromboelastography (rapid TEG) before and after passing the simplified blood thinning device, while heparin was used as the control group. TEG-ACT represents the time until the first evidence of a clot is detected; the K-value is the time from the end of TEG-ACT until the clot reaches 20mm and this represents the speed of clot formation; the angle (α) is the tangent of the curve made as the K is reached and offers similar information to K; the MA is a reflection of clot strength; the EPL is the estimated percent lysis; the LY30 is the percentage of clot which has actually lysed after 30 minutes; the G-value is a log-derivation of the MA and is meant to also represent the clot strength using dynes/sec as its units.

Items	Before treatment	After treatment	Heparin
TEG ACT	97 s	143 s	199 s
K	125 s	245 s	125 s
Angel (α)	67.3 deg	58.5 deg	57.7 deg
MA	58.9 mm	50.0 mm	54.80 mm
EPL	1.4%	0.7%	0.1%
LY30	1.4%	0.7%	0.1%
G	7160.4 d/sc	5009.4 d/sc	6068.8 d/sc



Supplementary Figure 24. Error distribution between the true and theoretical values of the relative molecular mass of all identified peptides.



Supplementary Figure 25. The distribution of sequences coverage of the identified proteins.

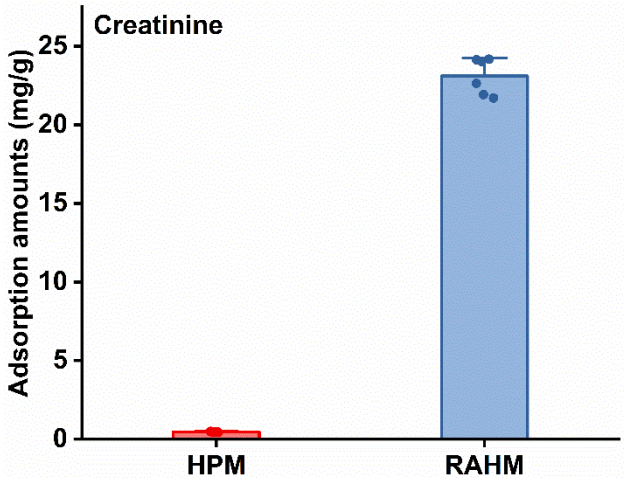
Supplementary Table 10. Relative abundance of representative proteins adsorbed from plasma onto different microspheres. The results are expressed as mean (SD).

Uniprot ID	Protein description	Relative abundance	
		HPM	RAHM
Coagulation Proteins			
P00451	Coagulation factor VIII	0.55 (0.24)	1.90 (0.06)
P00740-2	Coagulation factor IX	0.36 (0.11)	1.85 (0.48)
P03951	Coagulation factor XI	0.02 (0.03)	2.02 (0.24)
P00748	Coagulation factor XII	0.36 (0.25)	1.59 (0.51)
P01008	Antithrombin-III	1.01 (0.34)	0.00 (0.00)
P02679	Fibrinogen gamma chain	2.49 (1.49)	0.09 (0.02)
P02671	Fibrinogen alpha chain	1.87 (0.06)	0.33 (0.05)
P07225	Vitamin K-dependent protein S	1.11 (0.06)	0.00 (0.00)
P04070	Vitamin K-dependent protein C	2.22 (1.02)	0.27 (0.11)
P03952	Plasma kallikrein	0.26 (0.02)	0.70 (0.60)
P01042	Kininogen-1	1.65 (0.82)	0.28 (0.09)
P07355	Annexin A2	1.73 (0.26)	0.44 (0.17)
Complement Proteins			
P13671	Complement component C6	1.30 (0.55)	0.68 (0.01)
P07358	Complement component C8 beta chain	1.75 (0.29)	0.37 (0.03)
P00751	Complement factor B	1.55 (0.62)	0.41 (0.14)
P08603	Complement factor H	1.06 (0.28)	0.88 (0.53)
Immunoglobulin proteins			
A0A0A0MRZ8	Immunoglobulin kappa variable 3D-11	1.46	0.44

		(0.89)	(0.06)
A0A0A0MS15	Immunoglobulin heavy variable 3-4	1.46 (0.24)	0.61 (0.10)
A0A0A0MT36	Immunoglobulin kappa variable 6D-21	1.79 (0.27)	0.10 (0.11)
A0A0B4J1V0	Immunoglobulin heavy variable 3-15	1.66 (0.31)	0.13 (0.12)
A0A0B4J1X5	Immunoglobulin heavy variable 3-74	1.12 (0.22)	0.66 (0.58)
A0A0B4J1X8	Immunoglobulin heavy variable 3-43	0.61 (0.54)	0.00 (0.00)
A0A0B4J1Y9	Immunoglobulin heavy variable 3-72	1.85 (1.16)	0.57 (0.31)
A0A0C4DH25	Immunoglobulin kappa variable 3D-20	1.20 (0.49)	0.77 (0.14)
A0A0C4DH31	Immunoglobulin heavy variable 1-18	1.96 (0.32)	0.14 (0.03)
A0A0C4DH42	Immunoglobulin heavy variable 3-66	0.25 (0.43)	0.00 (0.00)
A0A0C4DH55	Immunoglobulin kappa variable 3D-7	1.36 (0.79)	0.56 (0.03)
A0A0C4DH68	Immunoglobulin kappa variable 2-24	1.33 (0.16)	0.38 (0.38)
A0A0J9YXX1	Immunoglobulin heavy variable 5-10-1	1.36 (0.34)	0.02 (0.04)
A2NJV5	Immunoglobulin kappa variable 2-29	1.67 (0.29)	0.40 (0.11)
P01619	Immunoglobulin kappa variable 3-20	1.31 (0.76)	0.62 (0.05)
P01700	Immunoglobulin lambda variable 1-47	1.12 (0.09)	0.00 (0.00)
P01714	Immunoglobulin lambda variable 3-19	0.87 (0.82)	0.00 (0.00)
P01766	Immunoglobulin heavy variable 3-13	1.05 (0.17)	0.00 (0.00)
P01859	Immunoglobulin heavy constant gamma 2	2.00 (0.23)	0.21 (0.11)
P01860	Immunoglobulin heavy constant gamma 3	1.83 (0.14)	0.31 (0.07)
P01861	Immunoglobulin heavy constant gamma 4	1.07 (0.37)	0.00 (0.00)
P0DOX2	Immunoglobulin alpha-2 heavy chain	1.90 (0.19)	0.32 (0.04)
P0DOX3	Immunoglobulin delta heavy chain	1.21	0.00

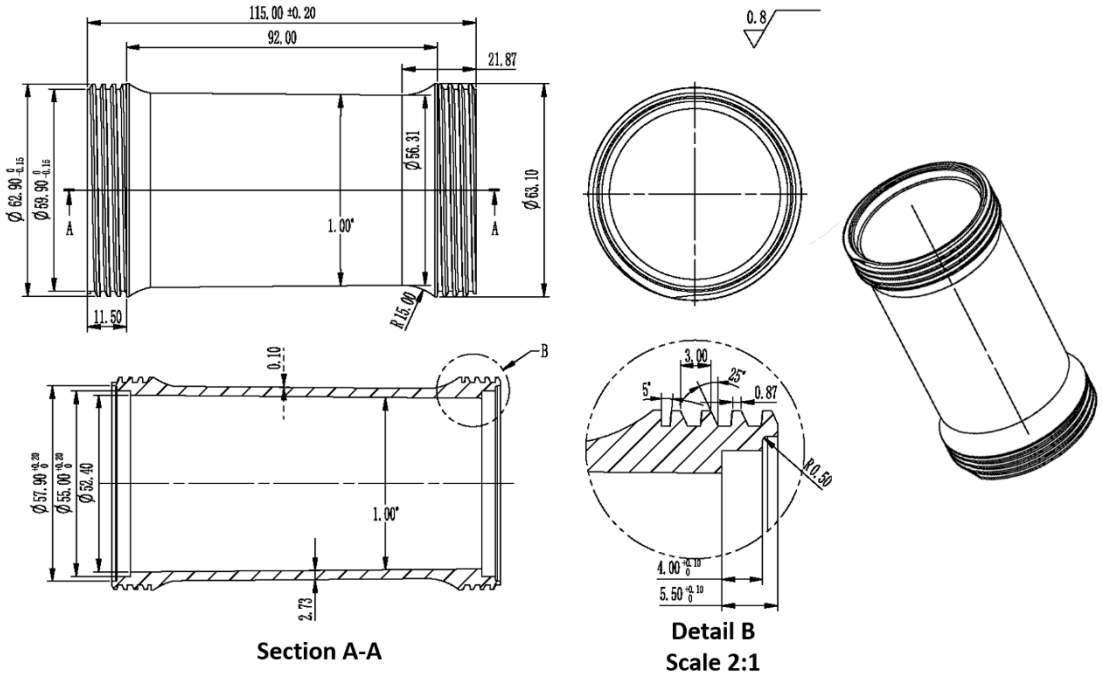
		(0.42)	(0.00)
P0DOX5	Immunoglobulin gamma-1 heavy chain	1.82 (0.46)	0.22 (0.05)
P0DOX7	Immunoglobulin kappa light chain	1.80 (0.37)	0.43 (0.21)
P0DOY3	Immunoglobulin lambda constant 3	2.14 (0.41)	0.16 (0.02)
P0DP01	Immunoglobulin heavy variable 1-8	1.88 (0.30)	0.21 (0.06)
P0DP03	Immunoglobulin heavy variable 3-30-5	1.09 (0.93)	0.87 (0.04)
P0DP04	Immunoglobulin heavy variable 3-43D	1.38 (0.28)	0.71 (0.08)
Apolipoproteins			
O14791	Apolipoprotein L1	1.58 (0.14)	0.61 (0.09)
O95236	Apolipoprotein L3	0.98 (0.35)	0.84 (0.73)
P02647	Apolipoprotein A-I	1.82 (0.48)	0.25 (0.12)
P02652	Apolipoprotein A-II	1.98 (2.67)	0.64 (0.23)
P02654	Apolipoprotein C-I	0.84 (0.67)	0.79 (0.02)
P02655	Apolipoprotein C-II	1.82 (1.58)	0.87 (0.64)
P02656	Apolipoprotein C-III	1.51 (0.37)	0.51 (0.44)
P04114	Apolipoprotein B-100	1.66 (0.87)	0.26 (0.18)
P05090	Apolipoprotein D	1.59 (0.32)	0.69 (0.19)
P06727	Apolipoprotein A-IV	1.35 (0.47)	0.91 (0.09)
P08519	Apolipoprotein (a)	1.70 (1.00)	0.32 (0.34)
Cell adhesion and related proteins			
O00187	Mannan-binding lectin serine protease 2	1.52 (0.44)	0.75 (0.11)
P15924	Desmoplakin	1.53 (0.20)	0.67 (0.12)
Q13835	Plakophilin-1	1.67 (0.37)	0.42 (0.16)

P14923	Junction plakoglobin	1.62 (0.47)	0.65 (0.15)
P07355	Annexin A2	1.73 (0.26)	0.44 (0.17)
Q02413	Desmoglein-1	1.60 (0.12)	0.24 (0.20)
Inflammatory response related proteins			
P00738	Haptoglobin	1.66 (1.67)	0.06 (0.02)
P01011	Alpha-1-antichymotrypsin	2.08 (0.08)	0.11 (0.05)
P02743	Serum amyloid P-component	2.15 (0.31)	0.04 (0.00)
P02751	Fibronectin	1.58 (0.14)	0.53 (0.19)
P02763	Alpha-1-acid glycoprotein	1.49 (1.15)	0.01 (0.00)
Q14624	Inter-alpha-trypsin inhibitor heavy chain	1.65 (0.34)	0.60 (0.03)
P01009	Alpha-1-antitrypsin	2.22 (0.54)	0.12 (0.06)
Q8NE71	ATP-binding cassette sub-family F member 1	2.18 (0.08)	0.04 (0.02)
P61626	Lysozyme C	1.60 (0.68)	0.35 (0.15)

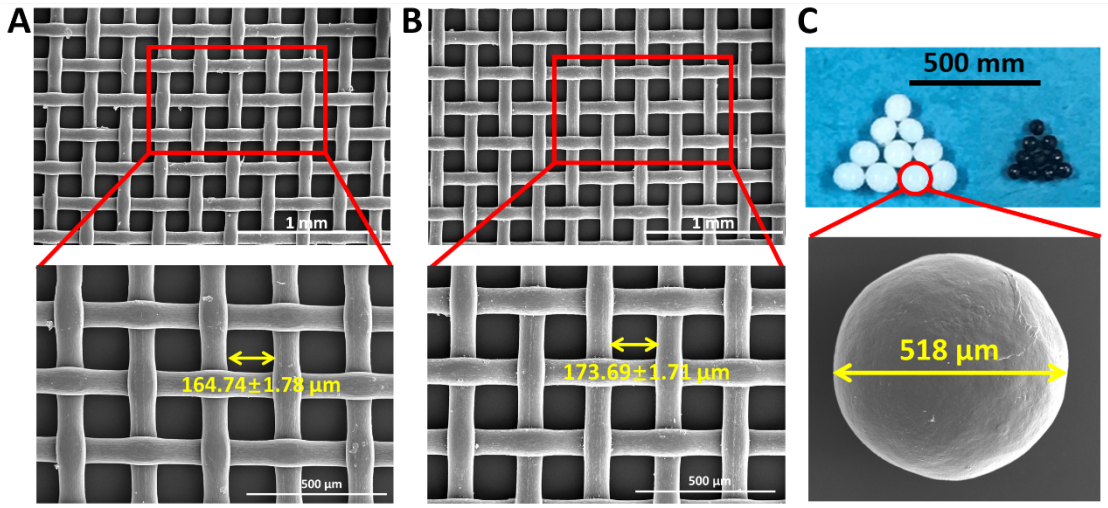


Supplementary Figure 26. The adsorption amounts for creatinine of HPM and RAHM. All the values are expressed as mean ± SD, n = 6 biologically independent samples. The adsorption amount of the RAHM (23.102 mg/g) was obviously higher than that of the HPM (0.450 mg/g) due to the abundant carboxyl and sulfonic groups in the RAHM, which could adsorb creatinine via electrostatic interaction. The ability of RAHM for toxin removal showed some auxiliary help for blood purification since creatinine is one of the endogenous toxins to be removed by blood purification.

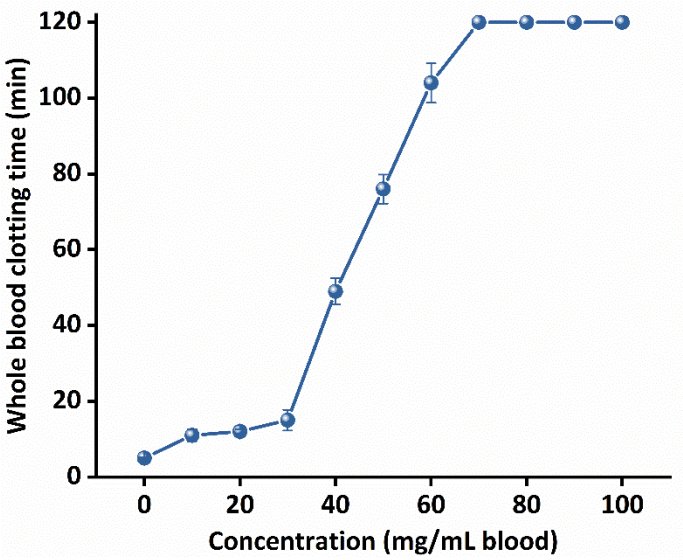
Therapeutic efficacy and safety of the pseudo-haemophilia model *in vivo*



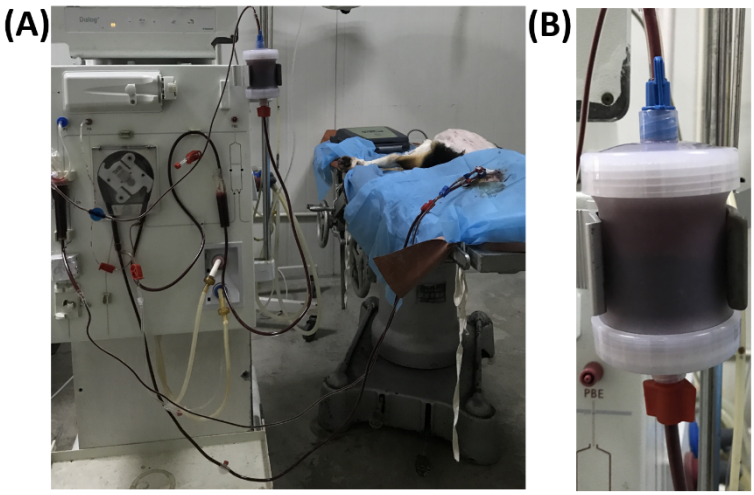
Supplementary Figure 27. The drawings of the originally designed blood-thinning device for establishment of extracorporeal pseudo-haemophilia model. The unmarked geometric tolerances are in accordance with GB/T 1184-H. The general tolerances are in accordance with GB/T 1804-M. The threaded ends of the device are single-start thread and are cooperated together with the end caps. The device is colourless, translucent and made by 3D-printing with a glossy surface using medical-grade polypropylene. No yellowing, dullness, processing defects such as burrs and bubbles can be observed with naked eyes on the inside and outside.



Supplementary Figure 28. The SEM images (×50, up; ×100, down) for the endplate of commercially available haemoperfusion apparatus (A) and blood-thinning device (B). (C) Digital photos of the RAHMs and activated carbon particles. (up) SEM image (×100) for the RAHM. (down)



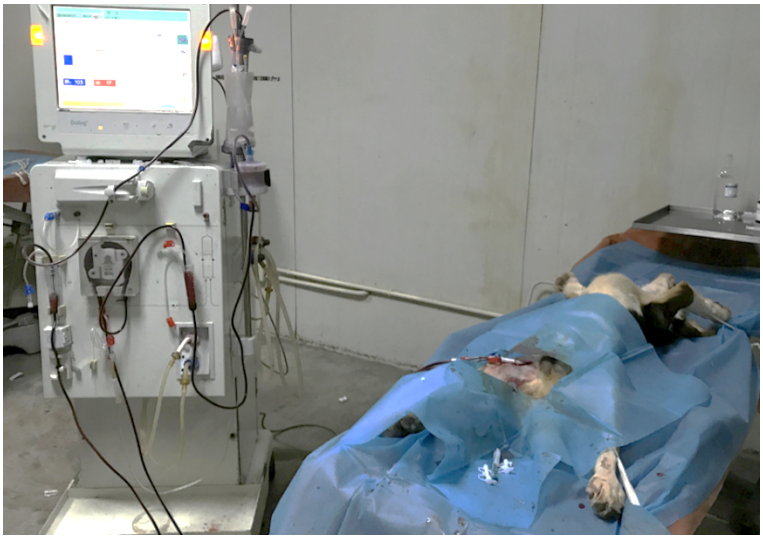
Supplementary Figure 29. The whole blood clotting time test of the RAHMs at different concentrations. Whole blood was collected directly from dogs via vacuum tubes without anticoagulant. After adding the RAHMs at a concentration of more than 70 mg/mL blood, the blood could remain in fluid state, and no clot formation was observed for more than 120 minutes. All the values are expressed as mean ± SD, n = 3 biologically independent samples.



Supplementary Figure 30. Digital photos of the preliminary animal experiment: (A) the blood purification extracorporeal circuit and (B) a blood thinning device filled with 80 g RAHMs. For dogs weighting 15-18 kg, the total blood volume is approximately 1290-1548 mL and the maximum volume of the entire extracorporeal circuit is approximately 525-720 mL. Thus, it's estimated the capacity of RAHMs in the device should be in the range of 50.4-108.36 g. Thus, three kinds of blood-thinning devices, containing 60 g, 80 g and 100 g spheres, were prepared for preliminary experiment. The preliminary canine experiments were successfully performed for over 2 hours and no obvious blood clot was found during the treatment with 100 g or 80 g spheres, while circuit failure was observed at 70-75 min with 60 g spheres. Thus, the blood-thinning device containing 80 g spheres was chosen for further experiments.

Supplementary Table 11. The body weights of the dogs in the animal experiment.

Dog	Body weight (kg)
Dog 1	18.4
Dog 2	16.6
Dog 3	17.7
Dog 4	18.0
Dog 5	18.5
Dog 6	17.4
Dog 7	17.0
Dog 8	17.8
Dog 9	17.5
Dog 10	15.9
Dog 11	16.3
Dog 12	16.0
Dog 13	17.0
Dog 14	16.4
Dog 15	15.7



Supplementary Figure 31. Digital photo of the haemodialysis extracorporeal circuit with the blood thinning device.

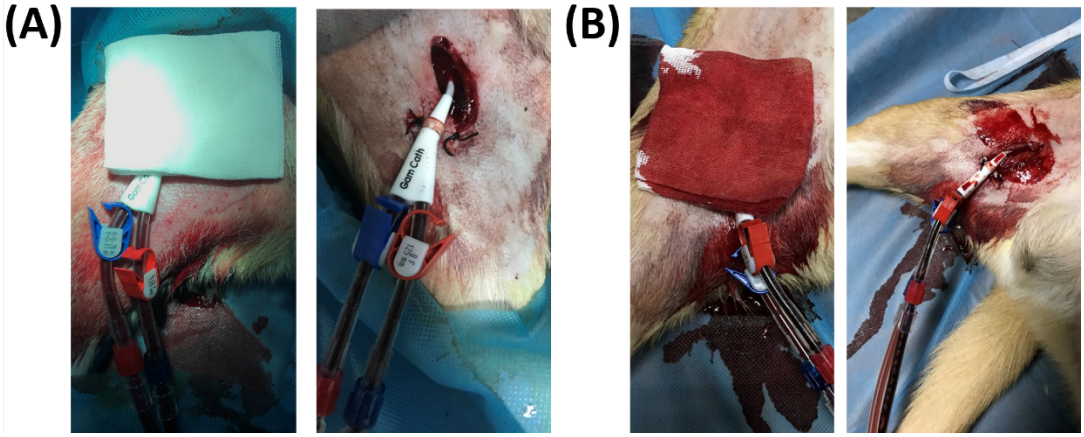
Supplementary Figure 32. Digital photos of the haemodialyzer (OCI-HD180) after blood purification. No obvious blood clot was observed in the haemodialyzer.

Supplementary Table 12. Complete blood count assay for the transfused dog treated with RAHM in different time intervals. *WBC, white blood cell count; #NEUT, neutrophil count; #LYMPH, lymphocyte count; #MONO, monocyte count; #EOS eosinophil count; #BASO, basophil count; %NEUT, neutrophil ratio; %LYMPH, lymphocyte ratio; %MONO, monocyte ratio; %EOS, eosinophil ratio; %BASO, basophil ratio; RBC, red blood cell count; HGB, haemoglobin; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelet.

Tests *	0 min	30 min	60 min	120 min	120 min after HD
WBC (10^9/L)	3.67	3.28	2.9	2.84	3.59
#NEUT (10^9/L)	2.3	2.1	1.92	1.91	2.06
#LYMPH (10^9/L)	1.06	0.86	0.7	0.65	1.17
#MONO (10^9/L)	0.2	0.19	0.2	0.19	0.26
#EOS (10^9/L)	0.08	0.11	0.08	0.08	0.07
#BASO (10^9/L)	0	0	0	0	0
%NEUT (%)	62.7	63.9	66.2	67.1	57.4
%LYMPH (%)	29	26.3	24.2	23.1	32.5
%MONO (%)	5.4	5.8	6.8	6.7	7.2
%EOS (%)	2.2	3.5	2.6	2.7	1.9
%BASO (%)	0.1	0.1	0	0	0.1
RBC (10^{12}/L)	5	5.11	5.37	5.27	5.51
HGB (g/L)	117	120	127	127	132
HCT (%)	37.5	23.4	22.1	27.4	41.3
MCV (fL)	75.1	74.8	75.3	75.1	75.1
MCH (pg)	23.4	23.5	23.6	24	24
MCHC (g/L)	312	314	313	320	319
PLT (10^9/L)	123	132	124	125	134

Supplementary Table 13. Complete blood count assay for the transfused dog treated with heparin in different time intervals. *WBC, white blood cell count; #NEUT, neutrophil count; #LYMPH, lymphocyte count; #MONO, monocyte count; #EOS eosinophil count; #BASO, basophil count; %NEUT, neutrophil ratio; %LYMPH, lymphocyte ratio; %MONO, monocyte ratio; %EOS, eosinophil ratio; %BASO, basophil ratio; RBC, red blood cell count; HGB, haemoglobin; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelet.

Tests *	0 min	30 min	60 min	120 min	120 min after HD
WBC (10^9/L)	2.88	3.39	2.74	3.15	2.44
#NEUT (10^9/L)	1.88	2.19	1.77	1.74	1.77
#LYMPH (10^9/L)	0.78	0.88	0.79	0.98	0.47
#MONO (10^9/L)	0.13	0.21	0.09	0.33	0.13
#EOS (10^9/L)	0.07	0.09	0.07	0.1	0.05
#BASO (10^9/L)	0	0.01	0.01	0.01	0
%NEUT (%)	65.2	64.4	64.6	55.3	72.7
%LYMPH (%)	27.1	25.8	28.8	30.9	19.3
%MONO (%)	4.6	6.2	3.2	10.4	5.2
%EOS (%)	2.4	2.8	2.7	3.1	2.2
%BASO (%)	0.1	0.2	0.3	0.2	0.1
RBC (10^{12}/L)	3.04	3.15	4.6	4.46	5.4
HGB (g/L)	93	95	109	106	133
HCT (%)	38.2	40.3	35	33.7	43.2
MCV (fL)	77	77.2	76.1	75.6	80
MCH (pg)	24	23.9	23.8	23.7	24.6
MCHC (g/L)	312	309	313	314	308
PLT (10^9/L)	138	116	133	104	124



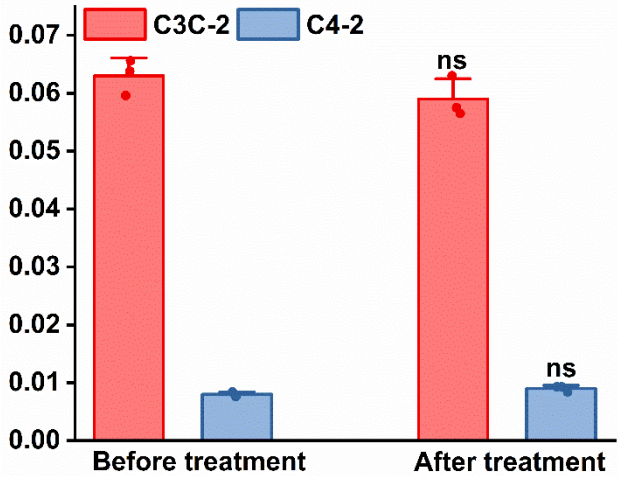
Supplementary Figure 33. (A) Digital photos of the process of blood purification treated with RAHM. (B) Digital photos of an active bleeding event during the process of blood purification treated with heparin, which might be caused by heparin. Simultaneous extracorporeal anticoagulation and intracorporeal safety could be achieved by the pseudo-haemophilia model.

Supplementary Table 14. Biological parameters levels for the transfused dog treated with RAHM in different time intervals. *ALP2L, alkaline phosphatase; ALTL, alanine aminotransferase; ASTL, aspartate aminotransferase; UREA, urea; CREA, serum creatinine; K, serum potassium; Na, serum sodium; Cl, serum chlorine; BILT3, total bilirubin; Ca, serum calcium; BILD2, direct bilirubin; BUN, blood urea nitrogen.

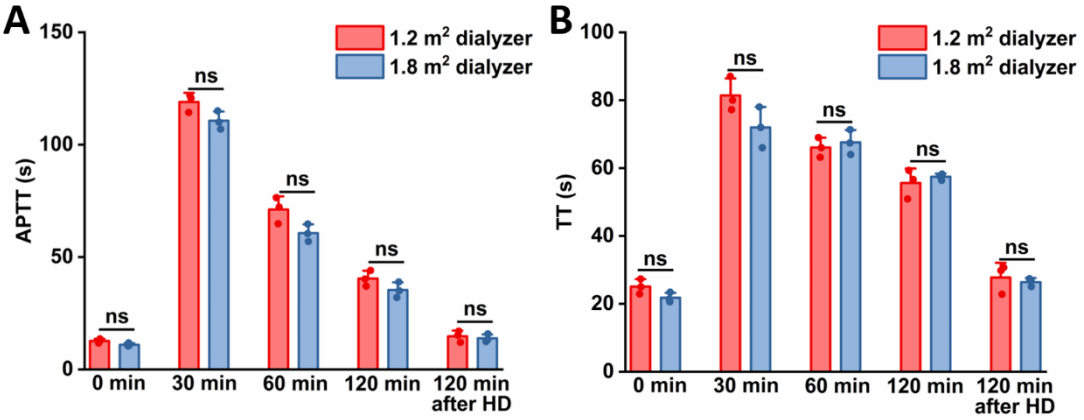
Tests *	0 min	30 min	60 min	120 min	120 min after HD
ALP2L (U/L)	43.5	40	43	41	42.1
ALTL (U/L)	35	29	26	29	40
ASTL (U/L)	33	32	29	25	43
UREA (mmol/L)	4.65	3.83	4.12	3.86	4.32
CREA (µmol/L)	64.3	30.6	22.1	20	37
K (mmol /L)	4.17	4.49	4.84	4.58	4.42
Na (mmol /L)	152	154	152	144	143
Cl (mmol /L)	112.4	119.7	117.9	112	116.5
BILT3 (µmmol/L)	1	1.1	2.3	1.3	1.7
Ca (mmol /L)	1.61	1.00	0.95	0.92	1.69
BILD2 (µmmol/L)	0.3	0	0	0	0.3
BUN (mg/dL)	13	11	12	11	12

Supplementary Table 15. Biological parameters level for the transfused dog treated with heparin in different time intervals. *ALP2L, alkaline phosphatase; ALTL, alanine aminotransferase; ASTL, aspartate aminotransferase; UREA, urea; CREA, serum creatinine; K, serum potassium; Na, serum sodium; Cl, serum chlorine; BILT3, total bilirubin; Ca, serum calcium; BILD2, direct bilirubin; BUN, blood urea nitrogen.

Tests *	0 min	30 min	60 min	120 min	120 min after HD
ALP2L (U/L)	63.2	51.5	42	43	55
ALTL (U/L)	36	22	24	25	27
ASTL (U/L)	30	22	26	29	31
UREA (mmol/L)	4.55	3.28	4.07	4.37	4.9
CREA (µmol/L)	64.3	30.6	42.5	44.8	38.9
K (mmol /L)	4.32	3.33	3.85	4.36	4.9
Na (mmol /L)	151	155	152	152	152
Cl (mmol /L)	114	122	119.1	118	119.2
BILT3 (µmmol/L)	1.2	0.7	1.4	1.2	1.6
Ca (mmol /L)	1.66	1.24	1.35	1.45	1.43
BILD2 (µmmol/L)	0.3	0.2	0.1	0	0.2
BUN (mg/dL)	13	11	12	11	12



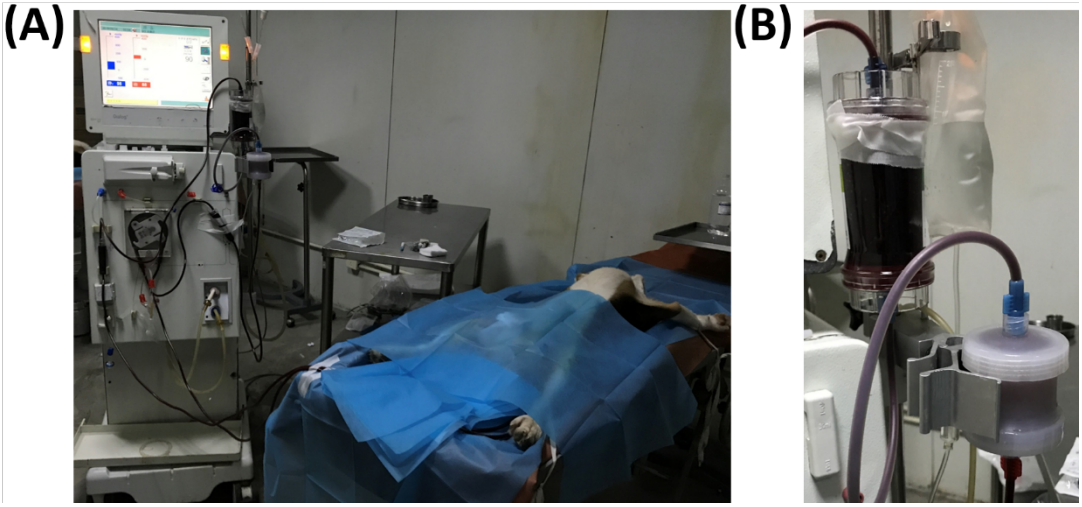
Supplementary Figure 34. Complement levels of the dog treated with RAHM before and after treatment. All the values are expressed as mean ± SD, n = 3 biologically independent samples, significance determined by an unpaired, two-sided t test, Student's t test. No significant changes were observed (P = 0.211 for C3C-2, P = 0.057 for C4-2). The complement levels before and after treatment remained unchanged, indicating no acute inflammation occurred after the treatment.



Supplementary Figure 35. Comparison of APTT (A) and TT (B) *in vivo* for the haemodialysis treatment with 1.2 m^2 dialyzer or 1.8 m^2 dialyzer at different time intervals. All the values are expressed as mean ± SD, n = 3 biologically independent samples, significance determined by an unpaired, two-sided t test, Student's t test. APTT: P = 0.073 for 0 min, P = 0.067 for 30 min, P = 0.062 for 60 min, P = 0.147 for 120 min, P = 0.640 for 120 min after HD. TT: P = 0.098 for 0 min, P = 0.106 for 30 min, P = 0.607 for 60 min, P = 0.509 for 120 min, P = 0.625 for 120 min after HD.

Supplementary Table 16. The rectal temperature of the dogs during the treatment. The results were expressed as the means ± SD. n = 3 biologically independent experiments.

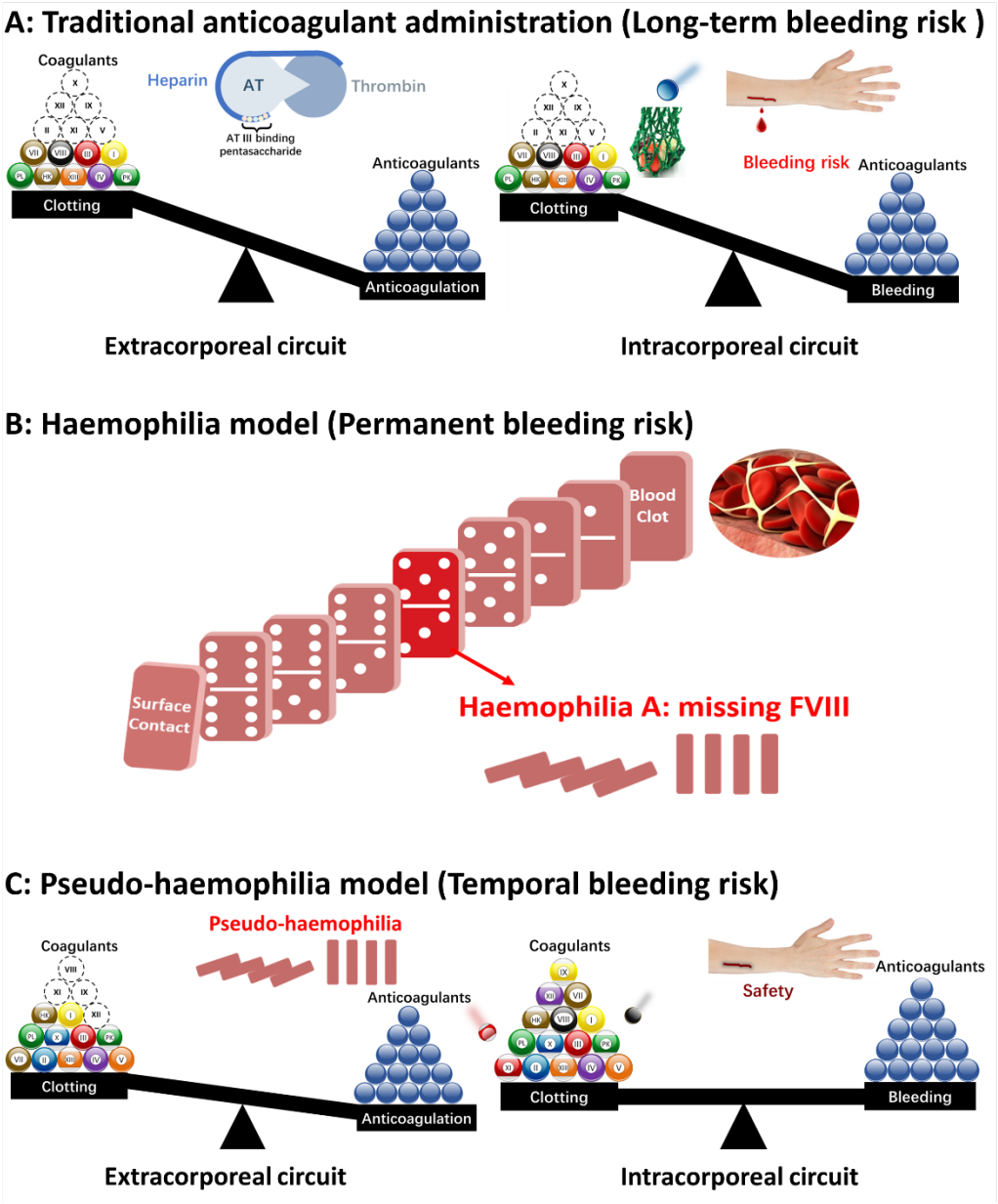
	0 min	30 min	60 min	120 min	120 min after HD
Dog 10	38.38 ± 0.17	38.26 ± 0.18	38.20 ± 0.09	38.14 ± 0.15	38.15 ± 0.05
Dog 11	38.18 ± 0.04	38.05 ± 0.05	38.33 ± 0.06	38.48 ± 0.12	38.38 ± 0.04
Dog 12	38.33 ± 0.06	38.23 ± 0.08	38.22 ± 0.08	38.17 ± 0.06	38.13 ± 0.06
Dog 13	38.43 ± 0.15	38.37 ± 0.06	38.37 ± 0.15	38.40 ± 0.17	38.30 ± 0.26
Dog 14	38.27 ± 0.15	38.50 ± 0.10	38.43 ± 0.05	38.33 ± 0.12	38.33 ± 0.25
Dog 15	38.23 ± 0.25	38.30 ± 0.17	38.27 ± 0.06	38.27 ± 0.21	38.20 ± 0.27



Supplementary Figure 36. Digital photos of the extended animal experiment: (A) the haemoperfusion extracorporeal circuit and (B) the combination of a blood thinning device filled with RAHMs and a haemoperfusion apparatus**.** The extended animal experiment was successfully performed for over 2 hours, while no obvious blood clot was found during this process.

Supplementary Table 17. Systematical comparison of the anticoagulant strategies for blood purification

Anticoagulant strategy	Dose of anticoagulant medications	Limitation
Unfractionated heparin (UFH)	Loading dose: 0.3~0.5 mg/kg Maintenance dose: 5~10 mg/h	Short half-life and continuous administration; heparin-induced thrombocytopenia[8]; excessive bleeding[9]; osteoporosis[10]; alopecia[11]
Low molecular weight heparins (LMWHs)	60~80 IU/kg	Long half-life and high bleeding risks after treatment; heparin-induced thrombocytopenia[12]; skin lesions[13]; elevation of liver enzymes[14]; no effective antidotes
Sodium citrate	180 mL/h (4% sodium citrate solution)	Fetal acidosis[15]; Hypocalcemia[16]; complicated operation and monitoring serum calcium and blood gas[17]
Argatroban anhydrous	Loading dose: 250 μg/kg Maintenance dose: 2 μg/ (kg min)	Anaphylactic reaction and even anaphylactic shock[18]; high cost[19]; not suitable for patients with liver dysfunction[20]
Prostacyclin	Loading dose: 5 ng/ (kg min) Maintenance dose:1 ng/ (kg min)	Hypotension due to vasodilatation[21]; cannot be reversed; not suitable for patients with thrombocytopenia
Nafamostat mesilate	0.5 mg/ (kg h)	Hyperkalemia[22]; hypotension; high cost; abdominal pain[23] and other anaphylaxis[24];
Heparin-free haemodialysis	4 mg/dL heparin saline prefill and soak the tube for 20 minutes	High blood speed (>300 mL/min)[25]; intermittent flushing of the extra-corporeal circuit with saline (100–200 ml every 15–30 min)[25]; high risks of thrombosis[26]
Pseudo-haemophilia model	0	unknown



Scheme 1. (A) Traditional heparin administration during haemodialysis: A dose of heparin is injected directly into the bloodstream and returned to the body. Heparin binds to antithrombin III (ATIII) via the pentasaccharide unit and forms a complex. Then, the activities of factors XIa, XIIa, IXa, Xa and IIa are inhibited to achieve complete anticoagulation. In intracorporeal circuits, systemic heparinization leads to the delayed recovery of haemostasis function and increased bleeding risk. (B) Schematic diagram of haemophilia A: haemophilia A is caused by missing factor VIII. (C) Pseudo-haemophilia strategy during haemodialysis: Blood is introduced into the anticoagulative device and returns to the body without any anticoagulant medications. Then, the pseudo-haemophilia model is established to achieve total regional anticoagulation in the extracorporeal circuit and avoid bleeding risks in the intracorporeal circuit.

References

1 Song Xin, W. R., Zhao Wei-feng, Sun Shu-dong, Zhao Chang-sheng. A facile approach towards amino-coated polyethersulfone particles for the removal of toxins. *J. Colloid Interface Sci.* **485**, 39-50, doi:10.1016/j.jcis.2016.09.025 (2017).

2 Song, X. *et al.* Design of carrageenan-based heparin-mimetic gel beads as self-anticoagulant hemoperfusion adsorbents. *Biomacromolecules* **19**, 1966-1978, doi:10.1021/acs.biomac.7b01724 (2018).

3 Zhao, W., Odelius, K., Edlund, U., Zhao, C. & Albertsson, A.-C. In situ synthesis of magnetic field-responsive hemicellulose hydrogels for drug delivery. *Biomacromolecules* **16**, 2522-2528, doi:10.1021/acs.biomac.5b00801 (2015).

4 Zhao, C., Xue, J., Ran, F. & Sun, S. Modification of polyethersulfone membranes - A review of methods. *Progress In Materials Science* **58**, 76-150, doi:10.1016/j.pmatsci.2012.07.002 (2013).

5 Yadav, M. & Rhee, K. Y. Superabsorbent nanocomposite (alginate-g-PAMPS/MMT): Synthesis, characterization and swelling behavior. *Carbohydrate Polymers* **90**, 165-173, doi:10.1016/j.carbpol.2012.05.010 (2012).

6 Gao, S. *et al.* A Robust Polyionized Hydrogel with an Unprecedented Underwater Anti-Crude-Oil-Adhesion Property. *Advanced Materials* **28**, 5307-+, doi:10.1002/adma.201600417 (2016).

7 Seo, J.-H., Matsuno, R., Konno, T., Takai, M. & Ishihara, K. Surface tethering of phosphorylcholine groups onto poly(dimethylsiloxane) through swelling-deswelling methods with phospholipids moiety containing ABA-type block copolymers. *Biomaterials* **29**, 1367-1376, doi:10.1016/j.biomaterials.2007.11.039 (2008).

8 Greinacher, A. Heparin-Induced Thrombocytopenia. *New England Journal Of Medicine* **373**, 252-261, doi:10.1056/NEJMcp1411910 (2015).

9 Villa, G., Ricci, Z. & Ronco, C. Renal Replacement Therapy. *Critical Care Clinics* **31**, 839-+, doi:10.1016/j.ccc.2015.06.015 (2015).

10 Le Templier, G. & Rodger, M. A. Heparin-induced osteoporosis and pregnancy. *Current Opinion In Pulmonary Medicine* **14**, 403-407, doi:10.1097/MCP.0b013e3283061191 (2008).

11 Hirschboeck, J. S., Madison, F. W. & Pisciotta, A. V. ALOPECIA AND OTHER TOXIC EFFECTS OF HEPARIN AND SYNTHETIC HEPARINOIDS. *American Journal Of the Medical Sciences* **227**, 279-282, doi:10.1097/00000441-195422730-00007 (1954).

12 Xu, Y. *et al.* Homogeneous low-molecular-weight heparins with reversible anticoagulant activity. *Nature chemical biology* **10**, 248-250, doi:10.1038/nchembio.1459 (2014).

13 Hirsh, J. *et al.* Heparin and low-molecular-weight heparin - Mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy, and safety. *Chest* **119**, 64S-94S, doi:10.1378/chest.119.1_suppl.64S (2001).

14 Arora, N. & Goldhaber, S. Z. Anticoagulants and transaminase elevation. *Circulation* **113**, E698-E702, doi:10.1161/circulationaha.105.603100 (2006).

15 Ronco, C. *et al.* Renal replacement therapy in acute kidney injury: controversy and consensus. *Crit. Care* **19**, doi:10.1186/s13054-015-0850-8 (2015).

16 Kreuzer, M. *et al.* Regional citrate anticoagulation is safe in intermittent high-flux haemodialysis treatment of children and adolescents with an increased risk of bleeding.

Nephrology Dialysis Transplantation **25**, 3337-3342, doi:10.1093/ndt/gfq225 (2010).

17 Evenepoel, P., Maes, B. & Vanwalleghem, J. Regional citrate anticoagulation for hemodialysis using a conventional calcium-containing dialysate. *Am. J. Kidney Dis.* **39**, 315-323, doi:10.1053/ajkd.2002.30551 (2002).

18 Barreto, A. D. & Grotta, J. C. Response by Barreto and Grotta to Letter Regarding Article, "Randomized, Multicenter Trial of ARTSS-2 (Argatroban With Recombinant Tissue Plasminogen Activator for Acute Stroke)". *Stroke* **48**, E259-E259, doi:10.1161/strokeaha.117.018294 (2017).

19 Reddy, B. V. Argatroban use in dialysis patients. *Seminars In Dialysis* **17**, 73-73, doi:10.1111/j.1525-139X.2004.17123.x (2004).

20 LaMonte, M. P., Brown, P. M. & Hursting, M. J. Alternative parenteral anticoagulation with argatroban, a direct thrombin inhibitor. *Expert review of cardiovascular therapy* **3**, 31-41, doi:10.1586/14779072.3.1.31 (2005).

21 Deep, A., Zoha, M. & Kukreja, P. D. Prostacyclin as an Anticoagulant for Continuous Renal Replacement Therapy in Children. *Blood Purif.* **43**, 279-289, doi:10.1159/000452754 (2017).

22 Muto, S., Imai, M. & Asano, Y. Mechanisms of hyperkalemia caused by nafamostat mesilate. *General Pharmacology* **26**, 1627-1632, doi:10.1016/0306-3623(95)00072-0 (1995).

23 Yamazato, M. *et al.* Severe abdominal pain associated with allergic reaction to nafamostat mesilate in a chronic hemodialysis patient. *Internal Medicine* **41**, 864-866, doi:10.2169/internalmedicine.41.864 (2002).

24 Maruyama, H., Miyakawa, Y., Gejyo, F. & Arakawa, M. Anaphylactoid reaction induced by nafamostat mesilate in a hemodialysis patient. *Nephron* **74**, 468-469, doi:10.1159/000189371 (1996).

25 Naumnik, B. & Mysliwiec, M. Heparin-free haemodialysis-use and outcomes. *Nat. Rev. Nephrol.* **9**, 381-382, doi:10.1038/nrneph.2013.106 (2013).

26 Chang, T. I., Winkelmayer, W. C., Shen, J. I. & Mitani, A. A. Use and safety of heparin-free maintenance hemodialysis in the USA. *Nephrology Dialysis Transplantation* **28**, 1589-1602, doi:10.1093/ndt/gft067 (2013).