Support information

Experimental

Materials

1-Vinyl-2-pyrrolidone (VP, 99%, with 100 ppm NaOH stabilizer) and acrylic acid (AA, >99.7%, GC), were obtained from Chengdu Kelong Chemical Reagent Company, China. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 98%), N-Hydroxysuccinimide (98%), and 4-Nitrophenyl acetate (98%) were purchased from Aladdin Reagent Co., Ltd. 2,2'-Azobis (2-methylpropionitrile)(AIBN, 99%, recrystallization), N, N'-Methylenebis (acrylamide) (MBA, for molecular biology, ≥99.0%), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (98%), 4-Nitrophenyl acetate (98%), 4-Nitrophenol (AR,≥99.5% (HPLC)), Phosphate Buffered Solution (PBS, 10×,pH 7.2-7.4, sterile, without Ca^{2+}/Mg^{2+}), and MES Buffered Solution (0.05M, pH6.7, containing 0.001M EDTA), were purchased from Aladdin Reagent Co., Ltd., China., The reagent kits of activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) were obtained from SIEMENS Co., Ltd., and fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA, FITC Mw 389.4, BSA Mw ~68,000 with 98% purity) was purchased from Beijing Solarbio Science & Technology Co., Ltd.; N, N'-Dimethylacetamide (DMAc, AR, ≥99.0%) and anhydrous ethanol (AR, ≥99.7%) were obtained from Chengdu Kelong Chemical Reagent Company, China.

Fabrication of PES membranes

Table. S1 Details of all the samples (AA (wt.%) and VP (wt.%) were compared to PES mass. PES(wt.%) was compared to the whole dope solution mass).

Figure	Name	AA(wt.%)	VP(wt.%)	PES(wt.%)	CA(mg/ml)
1	4.5%	4.5	3	20	1
1	6%	6	3	20	1
1	9%	9	3	20	1
1	12%	12	3	20	1
2;4-6	PES	0	0	20	0
2;4-6	m-M	6	3	20	0
2;4-6	CA-M	6	3	20	1
3	CA0.10-M	6	3	20	0.10
3	CA0.25-M	6	3	20	0.25
3	CA0.50-M	6	3	20	0.5
3	CA0.75-M	6	3	20	0.75
3	CA1.00-M	6	3	20	1.00

Blood collection

The blood used in this work was obtained from the West China Medical Center of Sichuan University, by using standardized blood collection tubes with standardized operation. Recombinant hirudin was chosen as the anticoagulant for the tests of platelet activation, contact activation and complement activation. Sodium citrate was chosen as the anticoagulant for the tests of

haemolysis and clotting times. 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). For blood tests, the blood samples from the same person were used, and three replicates were used to reduce errors for the blood tests of each sample. 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 to use the human blood.

Assessment of CA activity

Catalytic bicarbonate removal test

We pumped CO_2 for 2h into 500ml Ultral pure water to obtain saturated CO_2 aqueous solution. Three pieces of 1x1 cm^2 CA-M and free CA with equal activity equivalent were added into 10 ml of saturated CO_2 aqueous solution separately at the room temperature. We monitored the pH value of solution within half an hour by the pH Meter.

An appropriate amount of CO_2 was injected by a pinhole syringe into fresh blood, mixed and stabilized, and incubated with the CA-M samples and free CA with equal activity equivalent. The bicarbonate concentration of blood was detected by i-STAT 1 (MN) 300G analyzer.

Hemocompatibility

Blood routine examination

The blood routine examination was to analyze the effect of the membranes on blood cells. During the test, the 1×1 cm membrane samples were pre-immersed into PBS solution overnight, and then the membranes were incubated in PBS solution at 37℃ for 1 h. Subsequently the PBS was removed and 300 μL whole blood (with ethylene diamine tetraacetic acid as anticoagulant) was introduced for each sample and incubated together for 1 h. Besides, 300 μL blank whole blood anticoagulated by ethylene diamine tetraacetic acid was adopted as control sample, and the control sample was also incubated at 37℃ for 1 h. Finally, the whole blood after the incubation was tested by a hematology system (ADVIA 2120i, SIEMENS) to count blood cells.[1]

Evaluation of hemolysis

In hemolysis test, the membranes were directly incubated with whole blood that was anticoagulated by EDTA. The hemolysis test was used to examine the effect of various membranes on erythrocytes, for which a ultraviolet and visible (UV–vis) spectrometer (UV-1750, Shimadzu) was utilized.[2] Red blood cells (RBCs) were incubated with a piece of the membrane (1×1 cm^2) at 37 °C for 3 h. To prepare the negative control and positive control, phosphate buffer saline (PBS) solution (pH=7.4) and DI water were incubated with the erythrocyte suspensions, respectively. After the incubation, the erythrocyte suspensions were centrifuged at 8000 rpm for 3 min, and then an UV-vis spectrometer at 540 nm was used to measure the absorbance of the released hemoglobin. The haemolysis ratio was calculated according to the following equation:

$$\text{Haemolysis ratio (\%)} = (A_s - A_n)/(A_p - A_n) \times 100\% \quad (1)$$

where A_s is the absorbance of the suspensions, and A_p and A_n are the absorbance of the positive control and the negative control, respectively.[2]

Protein adhesion test and platelet activation

Fluorescein Isothiocyanate-Bovine Serum Albumin (FITC-BSA) was selected to study the anti-adhesion property against protein. The membrane was immersed in FITC-BSA phosphate buffer saline (PBS) solution and incubated at 37 ◦C for 1h; after being slightly washed by PBS solution, the membrane was observed by laser confocal scanning microscope to verify the surface BSA distribution. Platelet activation was measured via an enzyme-linked immune sorbent assay (ELISA) using a Human PF-4 ELISA Kit (PF4, ThermoFisher).[3]

Platelet activation

PF4 was a marker to characterize the level of platelet activation, and its concentration was measured by a commercial ELISA kit. The membrane was pre-incubated in PBS buffer for overnight. Subsequently, 1×1 cm^2 of the membrane was incubated with 300 μL of fresh blood (anticoagulated with recombinant hirudin, 30 μg/mL) at 37 °C for 1 h. The blood co-cultured with the membrane was extracted to centrifuge at 6500 rpm for 15 min, and the supernatant was diluted into 1/400. 200 μL of the tested samples or standard solutions were introduced into the corresponding ELISA plate well, and the plate was incubated at 20 °C for 1 h. Next, 200 μL of the prepared anti-(h)-PF4-HRP immunoconjugate was added into the wells after washing the plate, and the plate was incubated at 20 °C for another1 h. Then, 200 μL of the TMB substrate was added into the wells after washing the plate, and the

plate was incubated at room temperature for 5 min. The color development was stopped by introducing 50 μL of 0.45 mol/L sulfuric acid. Waiting for 10 min in order to allow the color to stabilize, then the absorbance at 450 nm was measured by a microplate reader (Thermo Scientific™ Multiskan SkyHigh), the concentrations of PF4 were calculated through standard curve. The primary blood without any treatment was used as blank control, and the results were expressed as mean ± SD (n = 3).[4]

Protein adhesion test

Protein adhesion was tested through the observation of fluorescein intensity. The distribution of proteins on membrane surface was observed using fluorescein isothiocyanate-labelled BSA (FITC-BSA). First, 5 mg/L of FITC-BSA dissolved in PBS solution was prepared and then a piece of 1×1 cm membrane was immersed into the solution at 37℃ for 3 h. Finally, the membrane was slightly washed by PBS solution three times and observed with laser scanning confocal microscope (SP8, Leica Microsystems, Germany).

Activation of contact and complement system

ELISA was utilized to assess the complement and contact activation levels of the membranes pre- and post-modification. The kits of Human complement fragment 3a (C3a, Human C3a ELISA Kit, ThermoFisher), C5a (Human C5a ELISA Kit, ThermoFisher) were used in this study [2]. C3a and C5a were used as markers to detect the level of complement activation, and their concentrations were measured by commercial ELISA kits. The membrane was pre-incubated in PBS buffer for 48 h. Subsequently, 1×1 cm^2 of the membrane was incubated with 300 μL of fresh blood (anticoagulated with recombinant hirudin, 30 μg/mL) at 37 °C for 1 h. The blood co-cultured with the membranes was centrifuged at 6500 rpm for 15 min, and the supernatant was diluted (1/1000 for the C3a test, 1/50 for the C5a test). 100 μL of the diluted sample or standard solution was added to the ELISA test well plate coated with specific antibody. The plate was incubated at 20 °C for 2 h, then the liquid in the wells was discarded. The well plate was washed thoroughly by a plate washer (Thermo Scientific™ Wellwash™), then 100 μL of the prepared Biotin-Conjugate was added into each well, and the plate was incubated at 20 °C for another 1 h. Next, 100 μL of the prepared Streptavidin-HPR was added into the wells after washing the plate, and the plate was incubated at 20 °C for 1 h. Finally, 100 μL of 3, 3', 5, 5'-tetramethylbenzidine (TMB) was added for color development, and a terminating agent was added after 30 min. The absorbance at 450 nm was detected by a microplate reader, and the concentrations of C3a or C5a were calculated with reference to the standard curve. The primary blood without any treatment was used as blank control, and the results were expressed as mean ± SD (n=3).[2]

Anticoagulation tests

Fresh citrate-anticoagulant human platelet-poor plasma (PPP) was utilized to determine the impact of various membranes on blood clotting behaviors. The anticoagulant behaviors of the membranes were determined using the activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT), fibrinogen (Fbg) and thrombin-antithrombin complex (TAT, Thrombin-Antithrombin Complex Human in vitro ELISA kit, Abcam). During the experiments, an automated blood coagulation analyzer (CA-1500, Sysmex) was utilized[5]. Before test, the membranes (1×1 cm^2 for each piece) were pre-incubated in PBS buffer for overnight. After the removal of PBS solution, 300 μL PPP was added and incubated together at 37 ℃ dynamically for 30 min, then the PPP was transferred into a test cup, and the APTT, PT, TT and fibrinogen were measured by a semiautomatic blood coagulation analyser. The control sample was pure PPP.[5]

Thrombin-antithrombin (TAT) complex level assessment

The membranes (1×1 cm^2 for each piece) were immersed into PBS buffer for overnight before the test. Firstly, fresh whole blood anticoagulated with sodium citrate (anticoagulant-to-blood = 1:9 (v/v)) was collected, and the whole blood was centrifuged at 4000 rpm for 15 min to obtain PPP. Secondly, 1×1 cm^2 of the membrane was added into 200 μL of PPP, and 10 μL of $CaCl_2$ solution (100 mM) was added to reactivate the coagulation cascade. The membrane was incubated with recalcified PPP at 37 °C for 30 min. The generated concentrations of TAT were determined by a commercial ELISA kit. Then 50 μL of the sample was added in the 96-well test plate and the plate was incubated at 20 °C for 2 h. Fourthly, after washing the plate, 50 μL of biotinylated human TAT complex antibody was added to each well and the plate was incubated at 20 °C for 1 h. Fifthly, after the plate had been thoroughly washed, 50 μL of SP conjugate was added to each well, and the plate was incubated at 20 °C for 30 min. Finally, 50 μL of chromogenic substrate was added to each well of the plate, and 50 μL of terminating agent was added after 20 min, the absorbance at 450 nm was read by a microplate reader, and then the TAT concentrations were calculated according to the standard curve. The primary PPP and the recalcified PPP were used as controls, and the results were expressed as mean ± SD (n = 3).[6]

Results and discussion

The kinetic parameters

Table. S2 Kinetic parameters of enzyme catalysis

	k_{cat} ($x10^{-7}mM^{-1}$)	V_{max} ($x10^{-4}$ mM/min)	K_m (mM)	k_{cat}/K_m ($x10^{-7}xmM^{-1}min^{-1}$)
CA0.10-M	6.17667	3.08667	2.06433	1.56000
CA0.25-M	5.70700	2.85200	1.32400	2.16543
CA0.50-M	5.67000	2.83333	1.07033	3.39333
CA0.75-M	6.04333	3.02333	0.57390	7.01333
CA1.00-M	6.08933	3.04433	0.28685	7.56667

K_m value of free CA is 1.6990 mM, which is 82.3% of CA0.10-M and 5.9 times of CA1.00-M. The results illustrated that the activity of free CA is within the activity range of CAx-M. For CA1.00-M, the K_m value is lower than that of free CA, which may be attributed to the adsorption and enrichment towards catalyzing substrate (p-NPA) of porous PES support. [7–9] In this study, the k_{cat}/K_m value of free CA was $4.2x10^{-7}(mM^{-1}min^{-1})$, 2.6 times that of CA0.10-M and 55% that of CA1.00-M. The results illustrated that the activity of free CA is within the activity range of CA0.10-M to CA1.00-M.

Table S3 K_m values of free CA in previous studies

Samples	K_m(mM)	References
Free HCA	0.48	[10]
CA	1.252	[11]
Free CA	1.594	[12]
Free CA	9.54	[13]
Free CA	6.091	[14]
Free HCA	13.07	[15]
HCA	27.29	[16]

Blood compability

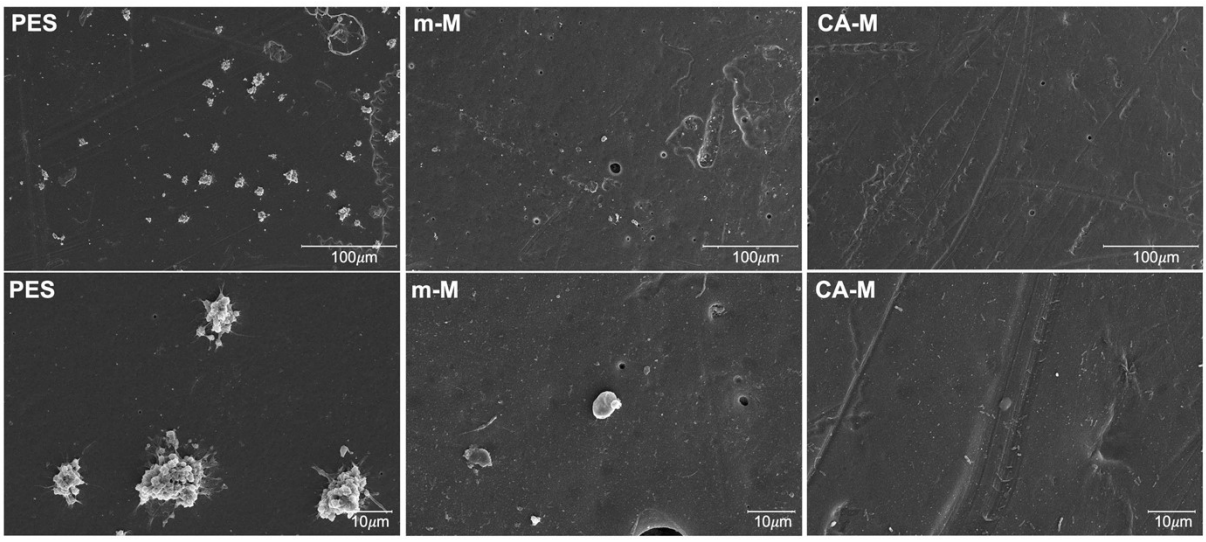

Fig. S1 The adhesion of platelets on the surfaces of PES, m-M, CA-M

We calculated the fluorescence intensity and obtained the following results (**Fig. S2**).

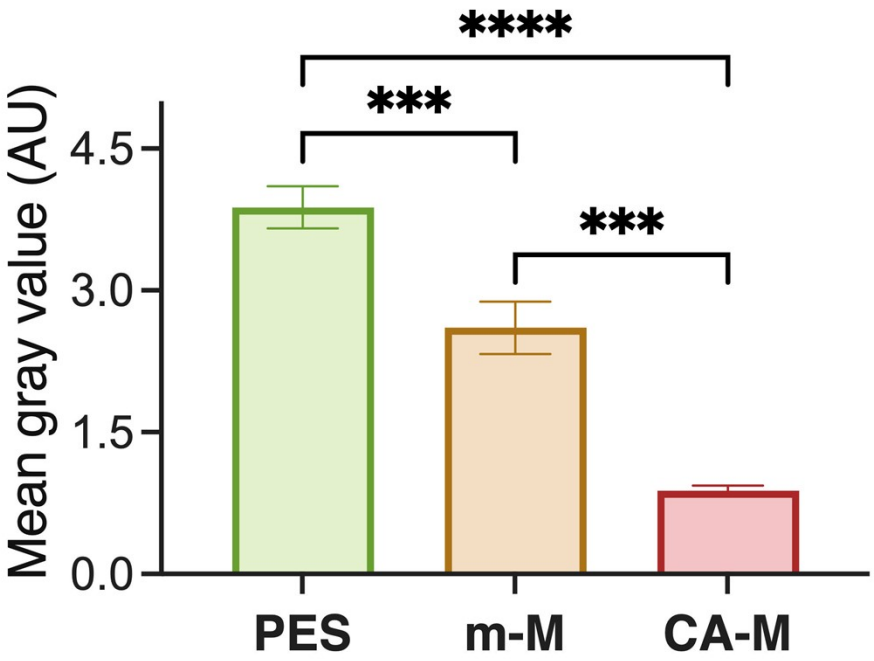

Fig. S2 Mean fluorescence intensity of PES; m-M; CA-M

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