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Anticoagulation polyvinyl chloride extracorporeal circulation catheters for heparin-free treatment

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1.1 Antibacterial experiment

Pristine PVC and PVC@HMP-1 samples cut into 1 cm×1 cm was incubated with 5 mL *E. coli* suspension which concentration is certain, respectively. After incubation at 37 ℃ for 18 h, each sample was gently washed with PBS buffer solution to clean off the unstable bacterial, subsequently, the pieces were treated to be observed with SEM examination.

1.2 Platelet activation

The platelets were received from fresh whole human blood centrifugated at 1000 rpm for 15 min. Each membrane sample pieces to be tested (1 cm×1 cm) were put into 24-well plate and infiltrated with PBS buffer solution likewise we did before. Thereafter, the samples taken from above solution and added 800 mL platelets onto membrane surface. Subsequent incubated 2 h at 37 ℃, the testing membrane was gently rinsed with PBS to clean platelets adsorbed weakly and then fixed with 2.5 wt.% glutaraldehyde for 24 h at 4 ℃. After that, the samples were dehydrated with gradient concentration of alcohol (50 %, 60 %, 70 %, 80 %, 90 %, 100 %) for 15 min each time.

1.3 protein adsorption

Bovine fibrinogen (BFG) was dissolved in normal saline solution at a concentration of 5 $mg \cdot mL^{-1}$. then each membrane sample was fully immersed into it at 37 ℃ for 2 hours. Hereafter, the above membrane was taken from protein solution, subsequently shaking cleaned with 2 wt.% sodium dodecyl sulfate (SDS) solution for 2 hours. Each washing solution was measured by Micro BCA™ protein assay reagent kits according to the operation manual to accurately determined the specific amounts

of adsorbed protein on the corresponding membrane.

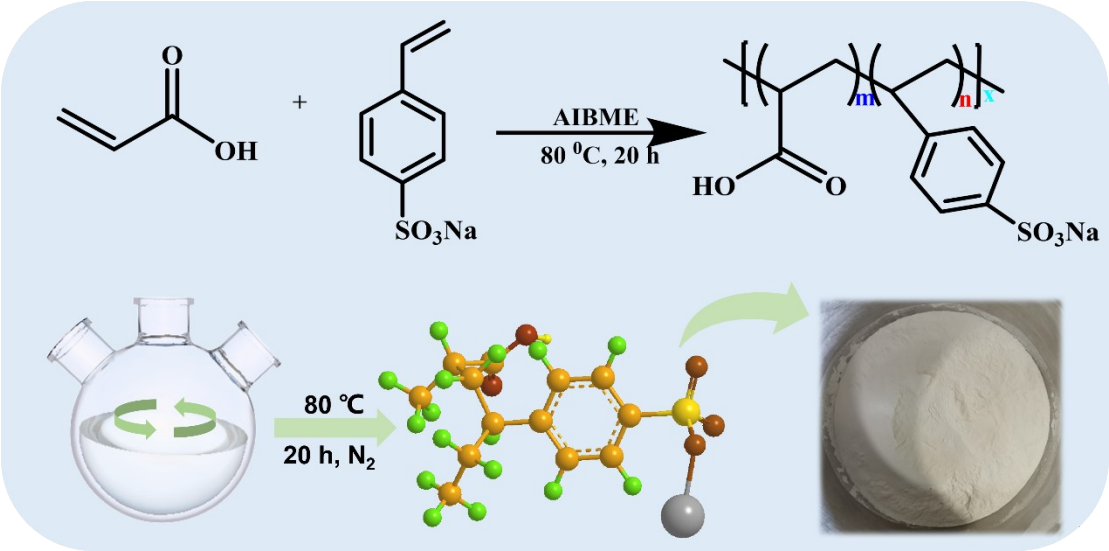

Figure S1. Schematic illustration of reaction equation for heparin-mimicking polymer synthetization and morphology for final product.

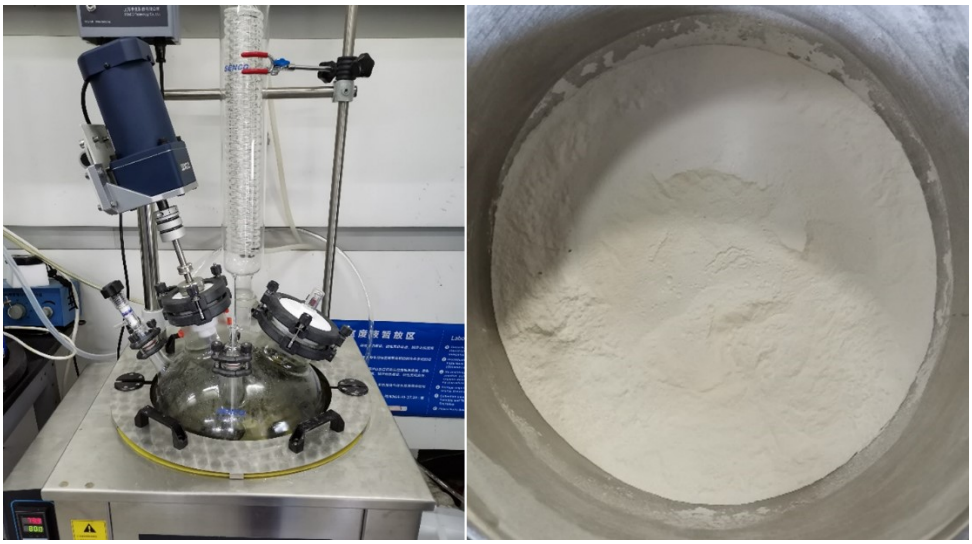

Figure S2. Digital photo of (left) reaction container for pilot-plant-scale experiments and (right) final products of HMP.

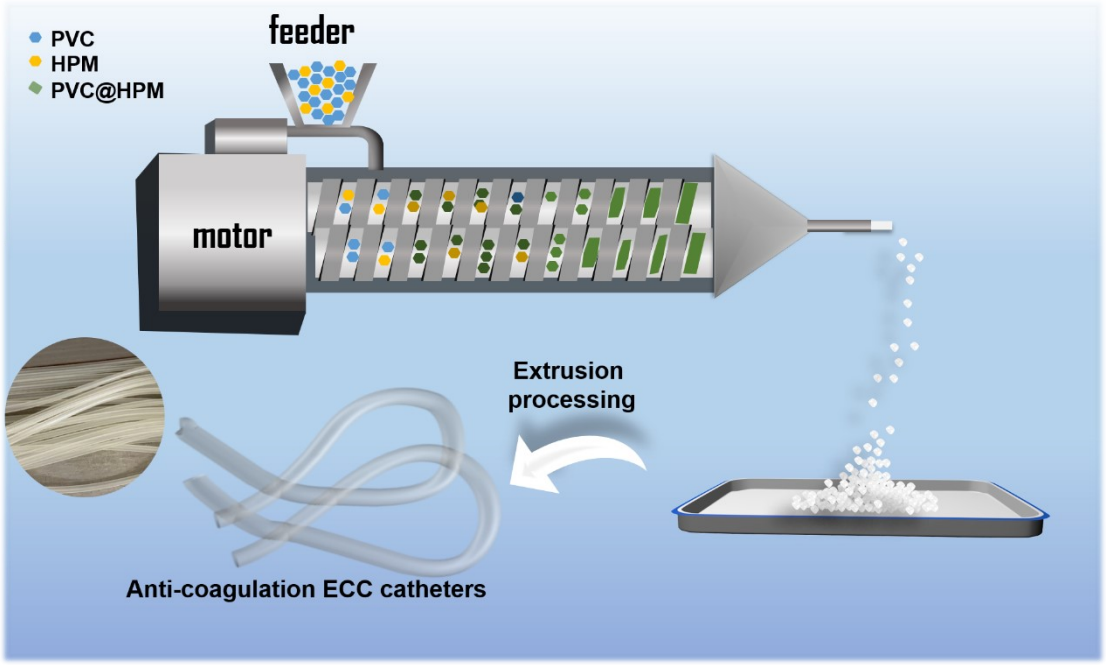

Figure S3. Schematic illustration of fabrication process of anticoagulation PVC ECC catheters.

Figure S4. Digital photos for anticoagulation PVC granular and extrusion end products of blood bags and PVC tubing.

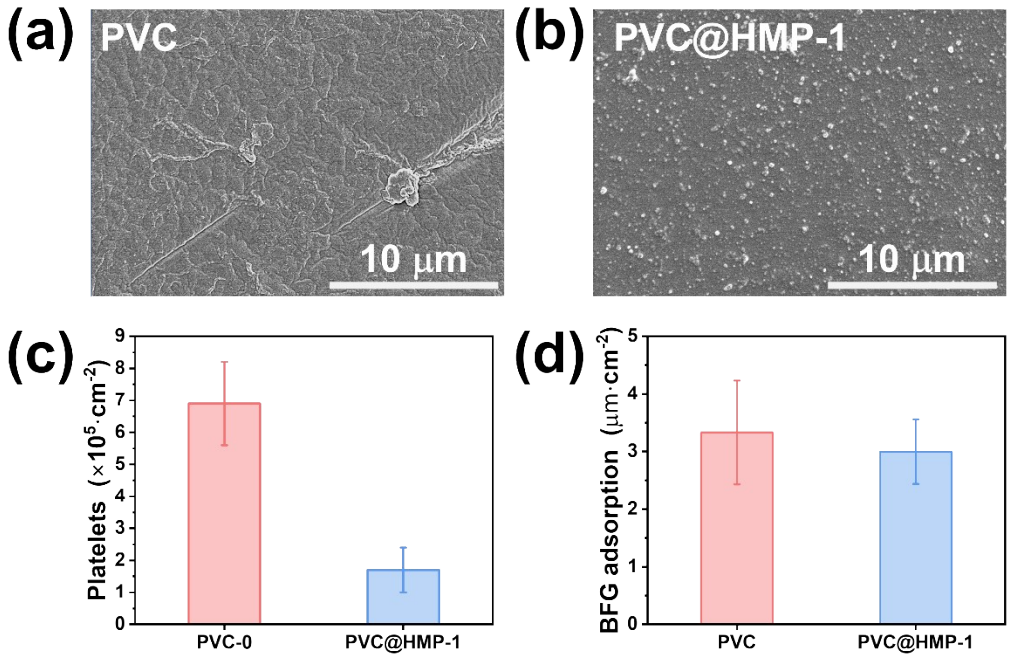

Figure S5. SEM micrographs of platelets on (a) pristine PVC and (b) PVC@HMP-1. (c) The statistical data of adhesion amounts of platelets on pristine PVC and anticoagulation PVC tubing, respectively. (d) Bovine fibrinogen (BFG) adsorption amounts on PVC catheters with or without anticoagulation modification.

As shown in Fig. S5 a, the adhesion amounts of platelets on PVC@HMP-1 significantly reduced in comparison of pristine PVC tubing. In Fig. S5 b, about 6.8◊10^5 platelets displayed on pristine PVC surface per square centimeter, while the PVC@HMP-1 reducing fourfold of platelets adhesion amounts. Platelet adhesion plays a significant role in the coagulation cascade, which could result in a thrombotic complication. Activated platelets would release tissue factor (TF), which is a direct trigger factor for the extrinsic pathway, from alpha granules. The remarkable reduction of platelets indicates PVC@HMP-1 has ability for suppressing activation of coagulation cascade. Moreover, the difference of fibrinogen adsorption amounts on

pristine PVC and PVC@HMP-1 further confirm the anticoagulation properties of the modification catheters in this work. We employed bovine fibrinogen (BFG) as the testing standard as it is the most abundant protein in plasma. In Fig. S5 d, the PVC@HMP-1 decreased about onefold of BFG adsorption amounts in contrast to pristine PVC. Those examinations remarkably demonstrate the anticoagulation PVC catheters could block thrombosis by suppressing the activation of platelets and plasma protein adhesion.

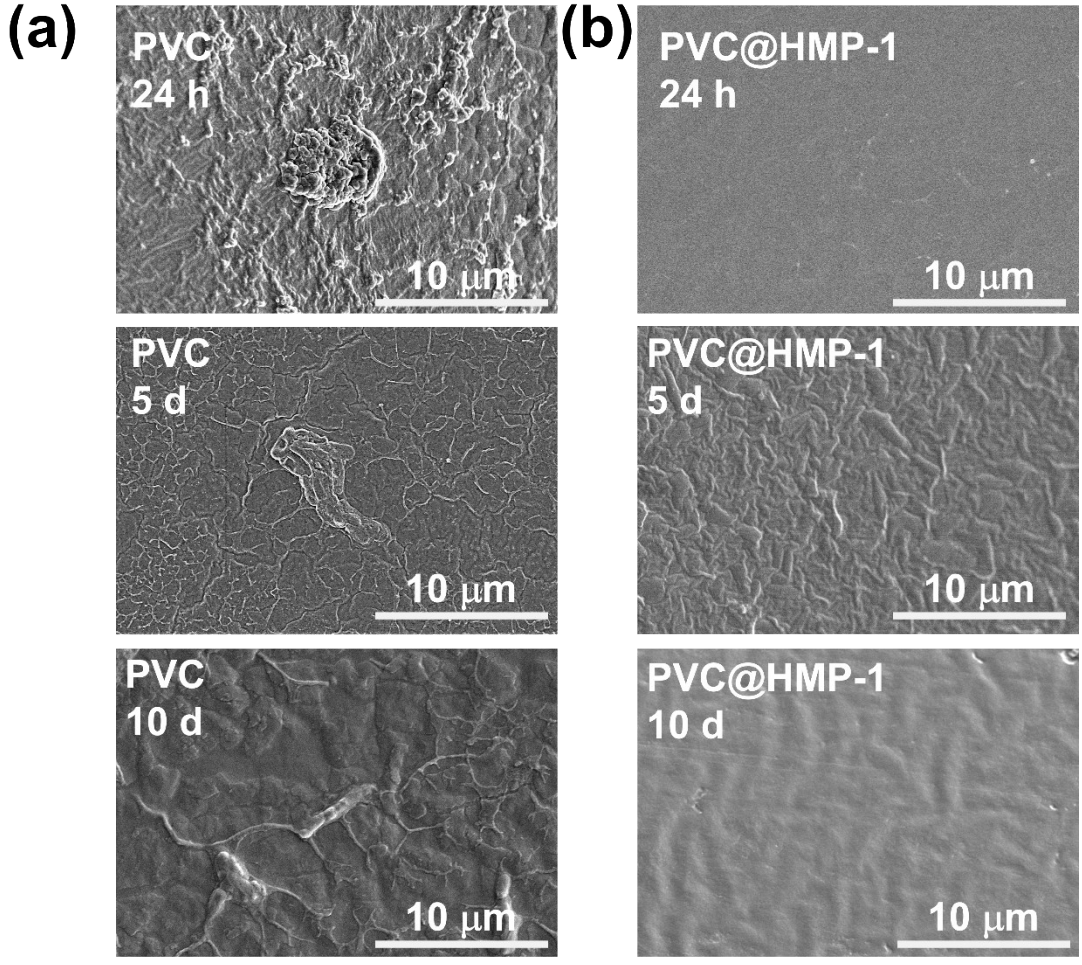

Figure S6. SEM micrographs of *E. coil* bacterial adhesion on (a) pristine PVC and (b) anticoagulation PVC tubing after incubation of 24 hours, 5 days and 10 days,

respectively.

We can clearly observe that the anticoagulation PVC tubing exhibited relatively low amounts of bacterial adhesion in contrast to pristine PVC materials after 24 hours incubation. Thus indicating this anticoagulation PVC tubing has inhibitory effect on biofilm development owing to hydrophilic characteristic contributing a slippery surface preventing the bacterial adhesion. After 5 days, an increasing number of bacterial E. coil gathering on lumen side of pristine PVC, while no obvious bacterial adhered on anticoagulation PVC tubing. The distinctive difference could be observed on catheters after 10 days incubation. The repellence of biofilm development on anticoagulation PVC tubing indicates its great potential for clinical application. In contrast, the heparin-coated catheter in some reports, has limiting evidence on the safety and effectiveness, also may promote biofilm formation.

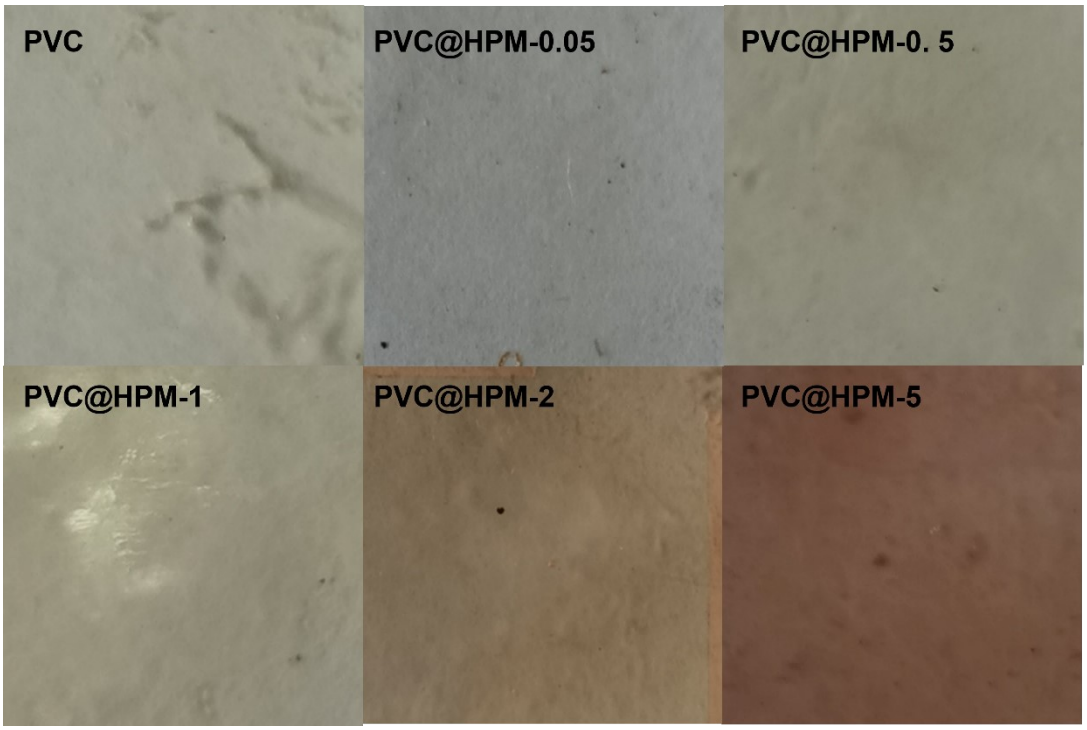

Figure S7. Digital photos for PVC plates with different HMP addition (0.05 wt.%, 0.5 wt.%, 1 wt.%, 2 wt.%, and 5 wt.%).

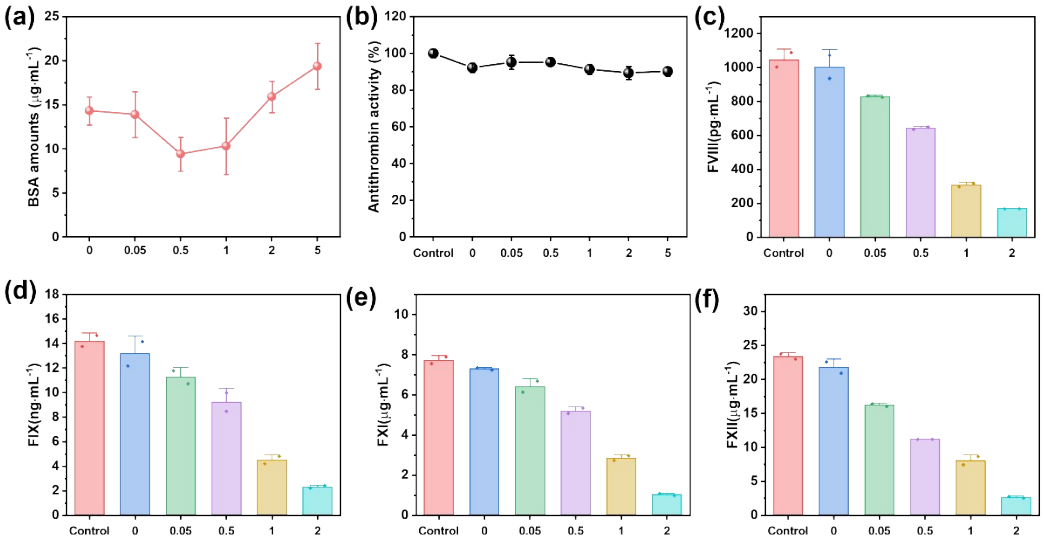

Figure S8. Anticoagulation mechanism exploration. (a) BSA adsorption variation for PVC tubes (PVC with 0 wt.%, 0.05 wt.%, 0.5 wt.%, 1 wt.%, 2 wt.% HMP addition). (b) AT III factors activity and (c) FVIII, (d) FIX, (e) FXI, (f) FXII centration examination for PVC catheters with different HMP addition. All values are expressed as the mean ± s.d.

The investigation of anticoagulation mechanism of HMP provided guidance for the design and development of the modified PVC tubes. As most previous reports revealed, protein adsorption and activation are the root cause for thrombosis and inflammation. When the typical synthetic materials contacted with blood, the non-specific plasma proteins were rapidly adsorbed onto the substrate surface. After the compete adsorption and displacement, the residual protein on surface that bring the glycoprotein receptor (GP IIb/IIIa) would mediate activation of platelets and

recruitment of leukocytes, thus causing activation of coagulation cascade and complement system (**Scheme. 1**). Among numerous plasma proteins, fibrinogen plays a pivotal role in coagulation as its abundance in the plasma, and its ability to promote the platelets activation. Thus, the BSA adsorption test was carried out on PVC@HMP samples (from 0 to 5 wt.%) respectively to explore its initiation on coagulation. As displayed in **Fig. S8 a**, the BSA adsorption amounts on PVC@HMP tubes showed a tendency of decreasing and then increasing with more and more HMP was blended, where 1 wt.% was the turning point. Exceeding HMP addition could promote the BSA adsorption owing to more and more sulfonate groups being introduced to the substrate. Then, the activity of antithrombin III (AT III) was employed on materials to ascertain the difference between HMP and heparin. Heparin, as the most common administrating anticoagulation in clinical, achieves the anti-clotting by promoting the activity of AT III to inactivate the coagulation factors (FXII, FXI, FIX, FX and FII). Differently, the activity of AT III kept a similar tendency for different PVC tubes (**Fig. S8 b**), without any significant variation compared with plasma control, which suggested that the HMP does not affect AT III. Despite that, the concentration of FVIII, FIX, FXI and FXII of plasma incubated with typical PVC tubes (**Fig. S8 c, d, e** and **f**) gradually reduced with the increment of HMP addition (from 0.5 wt.% to 2 wt.%). That powerfully revealed the difference between HMP and heparin, in which the HMP could directly inactivate factors to achieve the anticoagulation effect but not promote AT III.

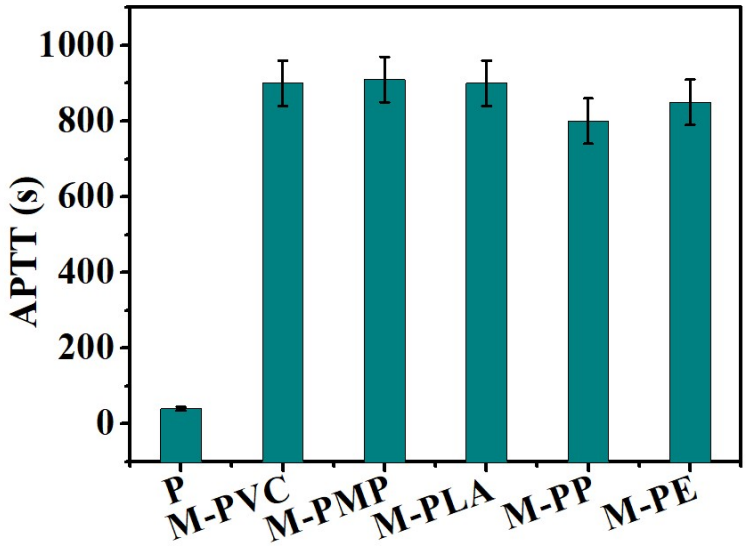

Figure S9. APTT tests for medical-grade materials (PVC, PMP, PLA, PP and PE) with 1 wt.% HMP addition.