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Substrate independent ultrathin hydrogel film to enhance polymer membrane performance: permeability, antifouling property, and antibacterial property

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1. Fabrication of the hydrogel film attached membrane

1.1 Preparation of the double bond grafted PES membrane

PES membrane was prepared by phase separation technique. Typically, 9.6 g (16 wt% of the total solution) PES was dissolved in 40 g DMSO to reach a homogeneous solution. After degassing the air, the solution was spin-coated on a glass surface, then immersed into DI water. The thickness of the membrane was controlled around 60 μm. The prepared membrane was washed by DI water for several times and stored in DI water for one week before use.

1.2 Synthesis of the hydrogel precursors

Synthesis of ene-functionalized P(SBMA-co-AA). P(SBMA-co-AA) was firstly synthesized by free radical polymerization initiated by KPS in water at 70 °C. The mole ratio of SBMA to AA was 5:5. For ene-functionalization, 20% AA segments were designed to graft allylamine, and the condensation reaction was carried out in pure water in the presence of EDC/NHS. The mole ratio of allylamine/EDC/NHS was set at 1:1:1. Typically, the copolymers were completely dissolved in pure water; then, weighted EDC/NHS was added to the solution with a pH of 4.5. After stirring for 2 h, aqueous allylamine solution was added to the medium associated with the adjustment of the pH to 8.0 by 0.1 mol/L NaOH solution. After 48 h reaction, the solution was dialyzed in pure water to remove the unreacted EDC/NHS for 4 days. Finally, the functionalized polymers were achieved by freeze-drying.

Synthesis of POEGMS. POEGMS was synthesized via the polycondensation of mercaptosuccinic acid and ethylene glycol.[1] OEG diol (120.0 g), MSA (30.0 g), and $Sc(OTf)_3$ (0.1 g) were added into 250 mL three-necked flast, and preheated to 80 °C under the nitrogen atmosphere with vigorous stirring to esterification until the melt system turned into transparent. Then, the polycondensation reaction was carried out at 90 °C with a pressure below 30 mmHg. After 2 h, the reduced pressure was gradually increased to 0.3-3 mmHg and maintained for 10 h to complete the polycondensation. The crude product was dissolved in methylene chloride and passed through an aluminum column to remove the catalyst. The resulting solution was concentrated and precipitated into cold diethyl ether for three times. The final product was dried in a vacuum oven for 48 h.

2. Characterization of the modified membranes

2.1 Chemical compositions and surface morphology characterizations

The surface morphology of the modified surfaces was observed by a field emission scanning electron microscopy (FE-SEM) (JSM-7500F, JEOL, Japan) with a voltage of 5 KV. AFM was also used to investigate the surface morphology in tapping mode using a tip with a spring constant of 40 N/m and a resonance frequency close to 300 kHz, and the thickness of the hydrogel films were analyzed by the section function of AFM. The chemical compositions of

the modified surfaces were also analyzed by X-ray photoelectron spectroscopy (XPS). XPS measurements were carried out on a Kratos AXIS Ultra spectrometer with a monochromatized Al K^{α} X-ray source (1486.6 eV photons), at a constant dwelling time of 100 ms and a pass energy of 40 eV. The core-level signals were obtained at a photoelectron take-off angle of 90º. The hydrophilicity/hydrophobicity of the modified surface was investigated on the basis of contact angle measurement, using a contact angle goniometer (OCA20, Dataphysics, Germany) equipped with a video capture.

2.2 Total AgNPs content and release behavior tests.

To calculate the total Ag loading amounts, the sample of 1.0 × 1.0 cm^2 sample was digested with HNO_3 (80 %, 5.0 mL) for 1 h, and then the digestate was diluted to a final volume of 50 mL. Determination of Ag was carried out by inductively coupled plasma-mass spectrometry (ICP-MS). Three replicates of each sample were analyzed.

To test the Ag release, a 1.0 × 1.0 cm^2 sample was immersed into 2.0 mL DI water. The solution was taken out and diluted to 20 mL with 5 % HNO_3 at given intervals. The solution was analyzed by ICP-MS to determine the Ag content.

2.3 Permeability and anti-fouling property tests.

Protein antifouling property, as a key aspect of hemodialysis membrane, should draw our attention. Thus, ultrafiltration of BSA solution through the membrane was carried out to investigate its antifouling property. Firstly, BSA solution was prepared by dissolving BSA in PBS solution with a concentration of 1.0 mg/mL. Then, the membrane was placed in a dead-end ultrafiltration cell with an effective membrane area of 3.9 cm^2. For the test, the membrane was firstly pre-compacted by PBS at apressure of 0.07 MPa for 20 min to reach a steady flux, then the pressure was adjusted to 0.06 Mpa, and the pure water flux within 5 min was measured (this measurement was repeated for 5 times). The pure water flux of the membrane was calculated by using the following equaation:

$$\text{Flux}=\frac{V}{SPT}$$

Where V (mL) is the volume of the permeated solution: S (m^2) is the effective membrane area; P (mmHg) is the pressure applied to the membrane and t (h) is the time for collecting permeated solution.

After the filtration of pure water, the feed solution was switched to 1.0 mL BSA solution, and the operation was the same as that for the pure water ultrafiltration. After the BSA solution filtration, the membrane was immersed in PBS for 1 h, and then the above process was repeated again. The fluxes (Jv) of pure water and BSA solution through the membrane were measured. The protein rejection ratio (R) was defined as the following:

$$R=(1-\frac{C_p}{C_b}) \times 100\%$$

Where C_p and C_b represent the protein concentrations of the permeated and bulk solutions, respectively. The protein concentration was measured by an UV-vis spectrophotometer at the wavelength of 278 nm. After protein filtration, the membrane was cleaned with deionized water. Then, the flux of the cleaned membrane

was measured again and the flux recovery ratio (J_{RR}) was calculated by the following equation:

$$J_{RR}=\frac{Jw2}{Jw1}\times 100\%$$

Where J_{w1} and J_{w2} are the pure water fluxes before and after each proterin ultrafiltration, respectively.

2.4 Antibacterial tests.

Escherichia coli (*E. coli*, gram negative) and Staphylococcus aureus (*S. aureus*, gram positive) bacteria were used as the model bacteria to evaluate the antibacterial property of the Ag nanoparticle loaded hydrogel thin layers. The samples were cut into discs with the diameter of 1 cm; then, the bacterial-resistant property was evaluated by live/dead two color fluorescene method. To investigate the long-term bactericidal efficiency, the samples were immersed into PBS solution for different time intervals, and then the bactericidal efficiency was evaluated by inhibition zone and the optical degree of co-cultured solution. All the glasswares and the samples were sterilized by ultraviolet radiation for 30 min before the microbiological experiment. The detailed operations referred to a previous report.[2]

Firstly, live/dead two-color fluorescence method was used to evaluate the bacterial adhesion characteristics. The membrane discs that co-cultured with *E. coli* or *S. aureu*s suspension at 10^6 cfu mL^{-1} for 12 h were washed with normal saline. Then the membranes were stained with the LIVE/DEAD BacLIGHT Bacterial Viability Kit, and observed under a fluorescent microscopy.

Then, the bacterial inhibition zone method was used to study the antibacterial property. The sterilized discs were placed on *E. coli* or *S. aureus* bacteria agar plate at an inoculum concentration of 10^7 colony forming units per mL (cfu mL^{-1}) and then incubated at 37 °C for 12 h. The presence of the inhibition zone was recorded by a digital camera.

Finally, the antibacterial activity of the membranes to bacteria suspension was investigated. The Ag nanoparticle loaded membrane disc was immersed in 2 mL of E. coli or S. aureus suspensions at 10^6 cfu mL^{-1} and incubated in a shaking incubator at 37 °C for 12 h. Then the optical degree of the bacterial suspension was determined at the wavelength of 500 nm.

2.5 Hemocompatibility tests.

2.5.1 Protein adsorption

BSA and BFG solutions (with a concentration of 1 mg/mL) were selected to conduct protein adsorption experiments. The membranes with the area of 1×1 cm^2 for each were incubated in physiological saline at 4 °C for 24 h and equilibrated at 37 °C for 1 h, and then immersed in the protein solutions at 37 °C for 2 h. Afterwards, the membranes were gently rinsed with PBS and then incubated in 2 wt. % aqueous sodium dodecyl sulfate (SDS) solutions at 37 °C for 1 h under agitation to remove the adsorbed protein. The protein concentrations in the solutions were quantified by Micro BCA™ protein assay reagent kits using an UV–vis spectrophotometer (UV-1750, Shimadzu, Japan) at a wavelength of 562 nm.

2.5.2 Clotting times

Activated partial thromboplastin time (APTT) test was performed as follows. Fresh human blood was centrifuged at 4000 rpm for 15 min to obtain platelet-poor plasma (PPP). The membrane with an area of 1×1 cm^2 was firstly dipped in 0.2 mL PBS (pH 7.4) for 1 h. After removing the PBS, 0.1 mL fresh PPP was introduced and incubated

at 37 °C for 30 min. Then 50 μL the incubated PPP was added into a test-tube, followed by the addition of 50 μL APTT agent (Dade Actin Activated Cephaloplastin Reagent, Siemens;incubated10 min before use), and incubated at 37 °C. Thereafter, 50 μL of 0.025 M $CaCl_2$ solution was added and then the APTT was measured. For thrombin time (TT) test, 50 μL the incubated PPP was mixed well with 100 μL TT agent (Thromborel®S, Siemens; incubated 10 min before use) at 37 °C and the TT was measured.

2.5.3 Hemolysis test

The hymolytic potentials of the modified membranes were evaluated according to an earlier work.[27] 5 mL of whole blood was firstly added to 10 mL of calcium- and magnesium-free PBS solutions, and then the red blood cells (RBCs) were isolated from plasma by centriguging at 500 g for 10 min for 5 repeated times. The obtained RBCs were diluted in PBS to a final volume of 100 mL for further use. For the hemolysis test, 0.2 mL of the diluted RBCs suspension (around 5×10^8 cells per mL) was added to 0.8 mL of hydrogel suspension. RBCs solution dispersed in PBS (pH 7.4) was selected as a negative control and RBCs solution dispersed in DI water was used as a positive control. All the suspensions were centrifuged at 10016 g for 3 min after been incubated in a rocking shaker at 37 °C for 3 h. The absorbance of the released hemoglobin in the suspensions was measured at 540 nm by an UV-vis spectrometer (UV-1750, Shimadzu Co., Ltd, Japan), and then the hemolysis ratio was calculated by the following formula:

$$\text{Hemolysis ratio (\%)} = \left\{\frac{Suspensions_{abs} - Negative\ control_{abs}}{Positive\ control_{abs} - Negative\ control_{abs}}\right\} \times 100\ \%$$

2.5.4 Platelet adhesion experiment

To study platelet adhesion, fresh human blood was centriguged at 1000 rpm for 15 min to obtain platelet-rich plasma (PRP). The membranes with the area of 1×1 cm^2 were dipped in PBS solution at 37 °C for 1 h. After removing the PBS solution, 200 μL of fresh PRP was dropped in each well of the culture plate and then incubated at 37 °C for 2 h. Then the PRP wad decanted off and the membranes were rinsed three times with PBS solution. The adhered platelets on the membrane surfaces were fixed using 2.5 % glutaraldehyde in PBS at 4 °C for 24 h. Finally, the membranes were washed and dehydrated with a series of PBS/ethanol mixtures with increasing ethanol concentration (25, 50, 75 and 100 wt.%). The membranes were dried at room temperature before observed by scanning electron microscopy.

3. Results and discussion

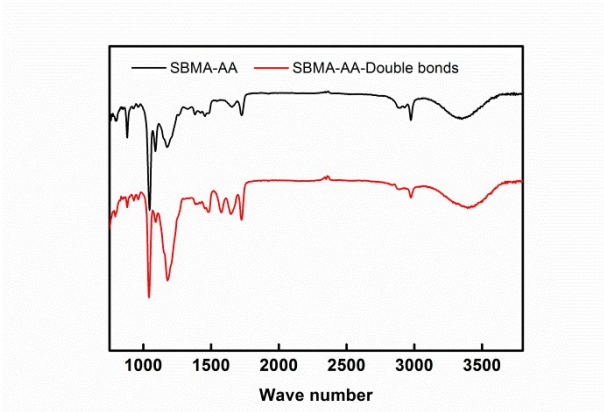

Fig. S1. The FTIR spectrum of the synthesized polymers.

After grafting double bonds, a new peak at 1640 cm^{-1} was observed, which was assigned to the amido bonds. The results indicated that the double bonds were successfully grafted onto the polymer chains.

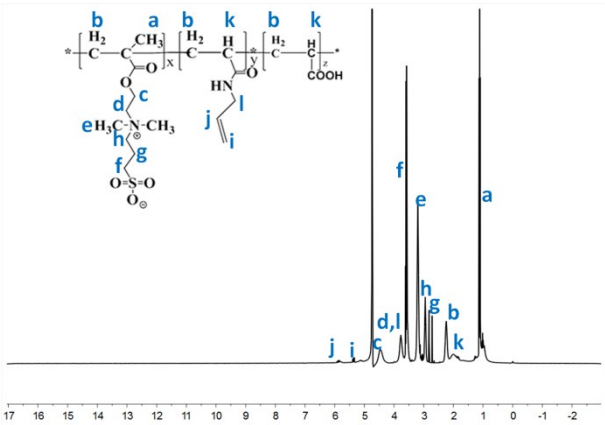

Fig. S2. The ^{1}H NMR spectra of the synthesized polymer containing double bonds.

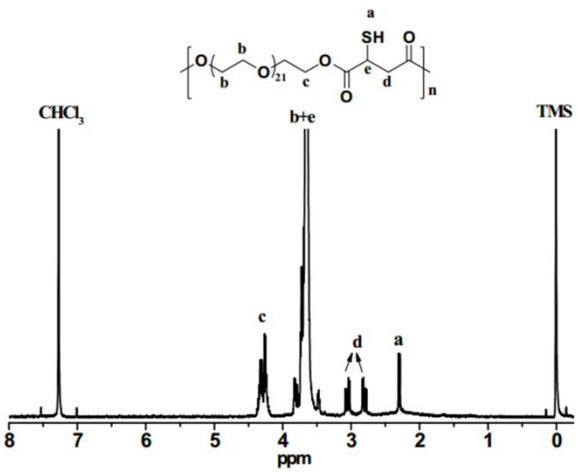

Fig. S3. The ^{1}H NMR spectra of the synthesized polymer containing –SH groups.

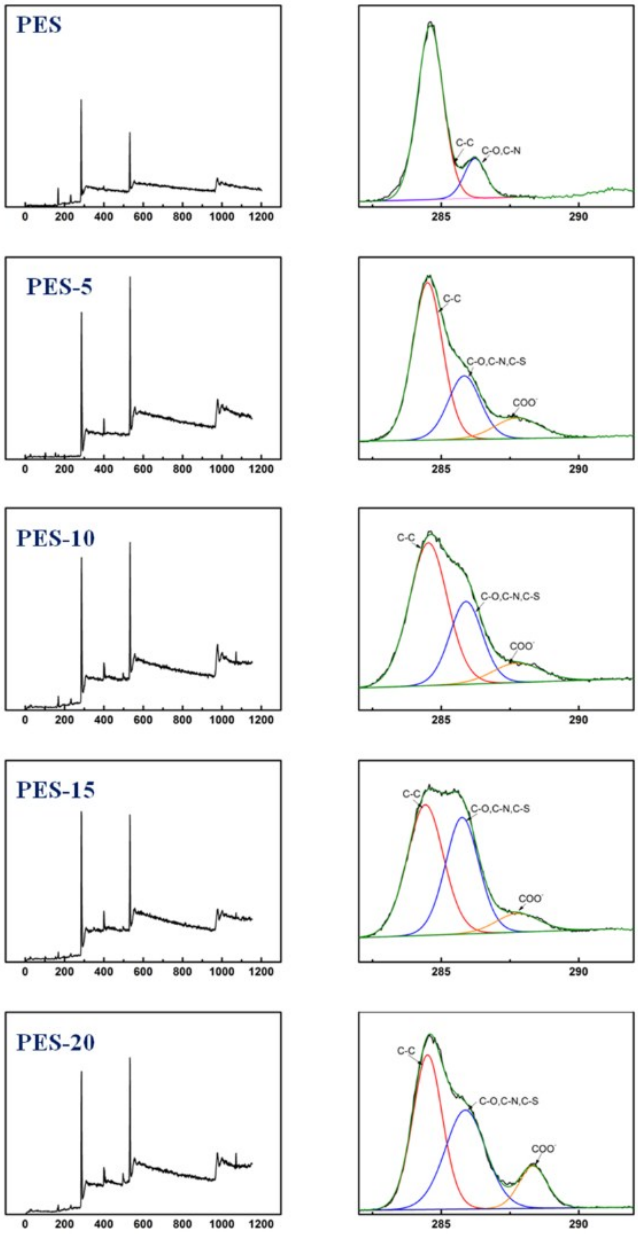

Fig. S4. The XPS spectrum of the modified membranes.

After grafting the hydrogel layers, the characteristic of $–COO^-$ was observed, and the intensity of the peak increased with the increase of the bilayer numbers, indicating that more functional groups were introduced onto the membrane surface.

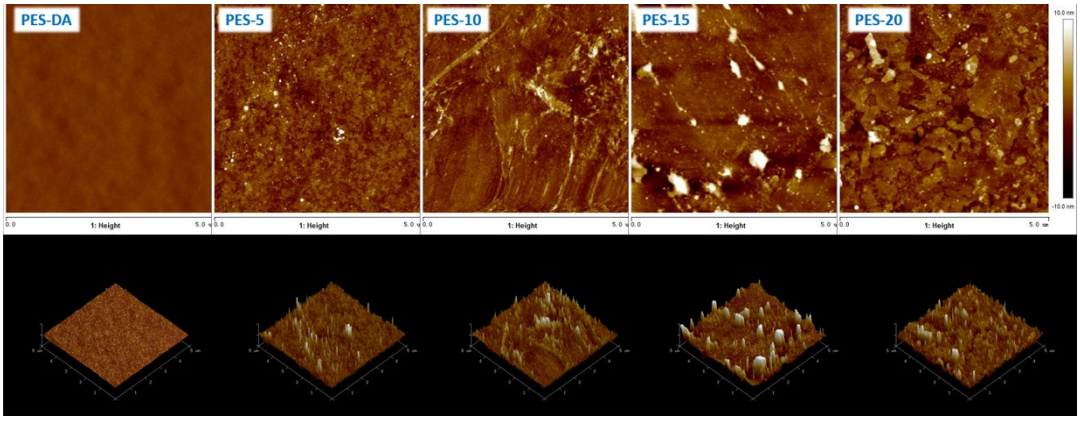

Fig. S5. The AFM images of the modified membranes and the cross-section analysis of the modified membranes.

The surface morphology of the modified membranes were also detected by AFM, as shown in Fig. S5. After grafting the hydrogel layers, the surface became rough, and the roughness increased with the increase of the bilayer numbers.

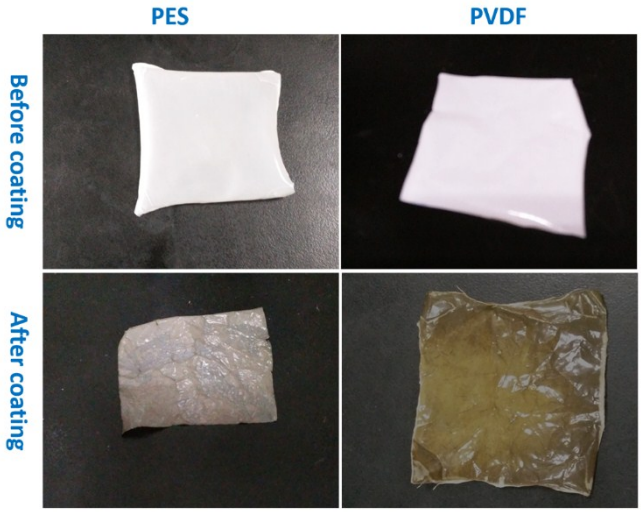

Fig. S6. The color changes of PES and PVDF membrane before and after coating with ene-functionalized dopamine.

To demonstrated its versatility, polyvinylidene fluoride (PVDF) membrane was also selected to coated with the ene-functionalized dopamine, the membrane color became from white to dark brown, which visually demonstrated its versatility for different polymer membranes.

[1] Zhu W, Xiong L, Wang H, Zha G, Du H, Li X, et al. Sustained drug release from an ultrathin hydrogel film. Polymer Chemistry 2015;6:7097-9.

[2] Wang R, Xiang T, Zhao W-F, Zhao C-S. A facile approach toward multi-functional polyurethane/polyethersulfone composite membranes for versatile applications. Materials Science & Engineering C-Materials for Biological Applications 2016;59:556-64.