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Bionic design for surface optimization combining hydrophilic and negative charged biological macromolecules

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ABSTRACT

While polyethersulfone (PES) membrane represents a promising option for blood purification, the blood compatibility must be dramatically enhanced to meet today's ever-increasing demands for many emerging application. In this study, we report a bionic design for optimization and development of a modified PES membrane combining hydrophilic and negative charged biological macromolecules on its surface. The hydrophilic and ionic charged biological macromolecules sulfonated poly(styrene)-*b*-poly(methyl methacrylate)-*b*-poly-(styrene) (PSSMSS) and poly(vinyl pyrrolidone)-*b*-poly(methyl methacrylate)-*b*-poly-(vinyl pyrrolidone) were synthesized via reversible addition-fragmentation chain transfer polymerization and used together to modify PES membranes by blending method. A hydrophilic membrane surface with negative charged surface coating was obtained, imitating the hydrophilic and negatively charged structure feature of heparin. The modified PES membranes showed suppressed platelet adhesion, and a prolonged blood clotting time, and thereby improved blood compatibility. In addition, the blood clotting time of the modified membranes increased with the blended PSSMSS amounts increment, indicating that both the hydrophilic and negative charged groups play important roles in improving the blood compatibility of PES membranes.

1. Introduction

It is well known that polymeric materials are widely used in the field of blood purification [1–3], but most of them would influence coagulant system by different levels when contact with blood. Poor blood compatibility may result in thrombogenic formation, response of immune system, or other tissue responses [4]. Thus, blood compatibility is one of the most important aspects of the biocompatibility for blood-contacting devices.

In order to substitute for artificial devices in contact with blood, new ways for making blood compatible polymer surface have been proposed with considerably attention to the outer surface of the materials [5–7]. Although materials with truly blood compatibility have not been found, material modification is a very efficient method in improving blood compatibility [8–10]. To improve the blood compatibility of biomaterials, more attention was paid to heparin-like materials [11–15]. Heparin-like material exhibited good blood compatibility like heparin molecules, and the research about the anticoagulant mechanism of heparin-like polymers help to understand the interactive relationship between materials and blood at the molecular level and the relationship between the structure of materials and their anticoagulant activity [16]. Generally, most of these heparin-like materials are composed of ionic polymers containing sulfate, sulfamide and carboxylic acid groups [17–19]. Kima et al. designed a "negative cilia model" with sulfonated PEO (PEO–SO_3), it exhibited heparin-like anticoagulant activity of 14% of free heparin. Then, they grafted PEO–SO_3 with polyurethane to get PU–PEO–SO_3, which exhibited improved biostability and suppressed calcification in addition to the enhanced antithrombogenicity [20].

Furthermore, many reports have demonstrated that the polymers incorporating hydrophilic group also showed good blood compatibility [21–23]. Zhao et al. synthesized an amphiphilic

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hyperbranched-star polymer (HPE-*g*-MPEG) with about 12 hydrophilic arms in each molecule by grafting methoxy poly(ethylene glycol) (MPEG) to the hyperbranched polyester (HPE) molecule and blended with PVDF to fabricate porous membranes via phase inversion process [24]. Wang et al. grafted zwitterionic brush from cellulose membrane (CM) via ARGET–ATRP, and the cellulose membrane had significantly excellent blood compatibility featured on lower platelet adhesion and protein adsorption without causing hemolysis [25].

PES and PES-based membranes show outstanding oxidative, thermal and hydrolytic stability as well as good mechanical and film-forming properties. In recent years, many researchers have focused on improving the blood compatibility of PES membranes. Wang et al. [26] prepared carboxylic poly(ether sulfone) (CPES) membranes by controlling acetylating and surface-oxidating reaction, then grafting bovine serum albumin (BSA) and bovine serum fibrinogen (BFG) onto the membrane surface. The protein-modified membranes showed increased water fluxes and depressed protein fouling abilities. Zhu et al. [27] synthesized copolymers of SMA-*g*-MPEGs, and then used as additives in the preparation of PES membranes via phase inversion process. The surface hydrophilicity and protein adsorption resistance of the modified PES membranes were significantly improved after blending with SMA-*g*-MPEGs. In our previous studies [28,29], poly(styrene-*co*-acrylic acid)-*b*-poly(vinyl pyrrolidone)-*b*-poly(styrene-*co*-acrylic acid) (P(St-*co*-AA)-*b*-PVP-*b*-P(St-*co*-AA)), poly(vinyl pyrrolidone)-*b*-poly(methyl methacrylate)-*b*-poly(vinyl pyrrolidone) (PVP-*b*-PMMA-*b*-PVP) were synthesized by RAFT polymerization and used as additives for the modification of PES membranes to improve blood compatibility. The modified membranes showed lower protein adsorption, suppressed platelet adhesion, and prolonged blood coagulation time, and thereby the blood compatibility was improved. But until now, no negative charged PES membrane surface with adequate hydrophilic structure synchronously was developed for blood purification.

In this study, a hydrophilic and negative charged surface was designed and constructed by blending a synthesized ionic block copolymer of sulfonated poly(styrene)-*b*-poly(methyl methacrylate)-*b*-poly(styrene) (PSSMSS) and an amphiphilic block copolymer PVMV (as shown in Scheme 1) to imitate the heparin structure feature and improve the blood compatibility of PES membranes. Polymethyl methacrylate (PMMA), which is often used as a biomaterial and medical material, was introduced to the block copolymers as a hydrophobic block due to the excellent miscibility of PMMA and PES. It was found that polyvinyl pyrrolidone (PVP) and sulfonated polystyrene (PSS) brushes formed on the modified PES surface and the blood compatibility of the modified membranes was improved. In addition, the effect of negatively charged brushes on the blood compatibility was investigated in detail.

2. Materials and methods

2.1. Materials

Polyethersulfone (PES, Ultrason E6020P) was obtained from BASF, Germany. Styrene (St; 99.0%) and methyl methacrylate (MMA; 99.0%) were purchased from UNI-CHEM, China. *N*-vinyl pyrrolidone (VP; 99.0%) was purchased from Alfa Aesar, USA, and was pretreated by activated carbons before use. *N*,*N*-dimethylacetamide (DMAC; AR, 99.0%) and *N*,*N*-dimethylformamide (DMF; 99.0%) were purchased from Chengdu Kelong Inc. (Chengdu, China) and used as the solvents. Azo-bis-isobutryonitrile (AIBN) was purchased from Chengdu Kelong Inc. (Chengdu, China), which were used as the initiator. All the other chemicals (analytical grade) were obtained from Chengdu Kelong Inc., China, and were used without further purification.

2.2. Polymer synthesis and characterization

2.2.1. Synthesis and characterization of PSSMSS

The general procedure for polystyrene (PS) synthesis was as follows. St, RAFT agent, AIBN, and DMF were added into a tube. After bubbling for 30 min with nitrogen, the reaction mixture was allowed to warm under a nitrogen atmosphere to 80 °C, and the polymerization lasted for 5 h. After precipitating in ethyl ether, the product was dried under vacuum at 50 °C overnight. To prepare Fourier transform infrared (FTIR) samples, the copolymer was dissolved in DMAC and cast on a potassium bromide (KBr) disc with a thickness of about 0.8 mm. The ^{1}H NMR spectra were recorded on a Varian Unity Plus 300/54 NMR spectrometer using deuterated dimethyl sulfoxide (DMSO-*d*) as the solvent at room temperature. Characterization: FTIR spectra (KBr, cm^{-1}): 2947 (s, $m_{>C-H}$) and 1610 (s, $m_{>Ar}$) for PS; ^{1}H NMR (DMSO-*d*6, ppm from TMS): *d* 6.29–7.52 ppm (s, *H*, Ar—H) for PS.

Copolymerization of MMA with PS was as follows. PS, MMA, RAFT agent, AIBN, and DMF were added into a sealed tube. After bubbling for 30 min with nitrogen, the reaction mixture was allowed to warm under a nitrogen atmosphere to 85 °C, and the polymerization lasted for 10 h. After precipitating in ethyl ether, the product was dried under vacuum at 50 °C overnight. Characterization: FTIR spectra (KBr, cm^{-1}): 1735 (s, $m_{>C=O}$) and 1236 (s, $m_{>C-O-C}$) for PMMA; ^{1}H NMR (DMSO-*d*6, ppm from TMS): *d* 6.29–7.52 ppm (s, *H*, Ar–H) for PS block, and 3.32–3.56 (s, 3*H*, CH_3—O—), 1.76 (s, 2*H*, —CH_2—C—CH_3), 0.73–0.93 (s, 2*H*, CH_3—C—C=O) for the PMMA block.

The fresh prepared St/MMA acid copolymer was sulfonated using concentrated sulfuric as the sulfonating agent. The mass ratio of the copolymer to the acid was 1:3. The copolymer was stirred at room temperature in the acid for 10 h, then the homogeneous solution was poured into ice-cold water, and yellow colored sulfonated polymeric powders of PSSMSS were precipitated. The powders were washed with cold water several times to remove the acid and were dried under vacuum at 50 °C. Characterization: FTIR spectra (KBr, cm^{-1}): 1732 (s, $m_{>C=O}$) and 1209 (s, $m_{>C-O-C}$) for PMMA, 1023 (s, $m_{>}$s=o) for SPS; ^{1}H NMR (DMSO-*d*6, ppm from TMS): *d* 6.81–7.97 ppm (s, 5*H*, SO_3H—Ar—H) for SPS block and 3.32–3.56 (s, 3*H*, CH_3—O—), 1.76 (s, 2*H*, —CH_2—C—CH_3), 0.73–0.93 (s, 2*H*, CH_3—C—C=O) for the PMMA block. The average molecular weights for the PSS and PMMA chains in the block copolymer PSSMSS calculated from ^{1}H NMR were 10.7×10^3 and 23.2×10^3, respectively.

2.2.2. Synthesis and characterization of PVMV

Polymerization of VP was as follows: VP, the RAFT agent, ACVA, and H_2O were added to a sealed tube. After bubbling with nitrogen for 30 min the reaction mixture was allowed to warm to 80 °C under a nitrogen atmosphere, and polymerization was carried out for 5 h. After precipitating in ethyl ether, the product (macro-RAFT agent -PVP) was dried under vacuum at 50 °C overnight. Characterization: FTIR spectra (KBr, cm^{-1}): 1060 (s, $m_{>C=S}$) for the RAFT agent terminated segment, and 1668.2 (s, $m_{>C=O}$) for PVP; ^{1}H NMR (DMSO-*d*6, ppm from TMS): 12.91 (s, 2*H*), 1.59 (s, 12*H*) for the RAFT agent terminated segment, and 3.51 (s, *H*, —CH—N), 3.13 (s, 2*H*, —CH_2—N), 2.20 (s, 2*H*, —CH_2—C=O), 2.04 (s, 2*H*, —CH_2—C—C=O), 1.86 (s, 2*H*, —CH_2—C—N) for PVP.

Copolymerization of MMA with PVP was as follows. MMA, the macro-RAFT agent (-PVP), AIBN, and DMF were added to a sealed tube. After bubbling with nitrogen for 30 min the reaction mixture was allowed to warm to 80 °C under a nitrogen atmosphere and polymerization was carried out for 5 h. After precipitation in ethyl