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Heparin reduced dialysis through a facile anti-coagulant coating on flat and hollow fiber membranes

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ABSTRACT

Heparin free prescription is highly desired for haemodialysis. Versatile strategies have been developed to address the anti-coagulation modification of dialysis membranes. We here develop a facile and effective method to construct an anticoagulant coating on polymeric membranes or a dialyzer through hydrophobic-hydrophobic interaction. An amphiphilic heparin-like copolymer was first synthesized via a free radical polymerization based on alkyl chain lauryl methacrylate (LMA), acrylic acid (AA), and sodium 4-vinylbenze-sulfonate (SSNa) segments. The specific structure of terpolymer was characterized via Fourier transform infrared spectra (FTIR), Proton nuclear magnetic resonance (^{1}H NMR) spectra and size exclusion chromatography (SEC). The amphiphilic copolymer was anchored onto Polylactide (PLA) membrane surface from an aqueous-based solution via hydrophobic-hydrophobic interaction. An Attenuated total reflectance Fourier transform infrared spectra (ATR-FTIR), energy dispersive spectrometer (EDS) and X-ray photoelectron spectroscopy (XPS) confirmed the successful immobilization of heparin-mimicking terpolymers. The modified membranes showed excellent anti-fouling property and hydrophilicity. Moreover, the membranes exhibited outstanding haemocompatibility with suppressed platelet adhesion, low complement activation (C3a and C5a), expanded coagulation time and limited haemolysis ratio. The prolonged activated partial thromboplastin time and thrombin time (APTT>600 s, TT > 140 s) certified the excellent self-anticoagulant performances. To further examine the applicability and effectiveness, a polysulfone dialyzer was modified by this strategy. The ultrafiltration coefficient and clearance performances were examined. Overall, the hydrophobic-hydrophobic interaction strategy simplified the modification procedure of dialysis membrane, implying its great potential in heparin free dialysis application.

1. Introduction

Outstanding blood compatibility and favorable anticoagulation activity is of considerable importance to blood-contacting materials [1,2]. Plasma proteins would first adsorb onto the biomaterials surface implanted in vivo in a few seconds and then activate platelet and coagulation factors on the protein adsorption layer, resulting in platelet thrombosis and fibrin polymerization in 1–2 min [3,4]. Obviously, it is a rapid cascade reaction, thrombus would be formed in a short period time. However, most of polymeric materials employed in clinical demonstrate insufficient blood compatibility, and therefore the high dosage injection of anticoagulant medicine is indispensable during treatment [5–10]. As for dialysis, unfractionated and low-molecular-weight heparin is the most commonly used medicine to inhibit the blood coagulation [11]. Heparin, as a linear, highly sulfated glycosaminoglycan, consists a number of carboxylate and sulfonate groups, which could electrostatically interact with serine protease inhibitor antithrombin III (AT-III), thus stopping the coagulation cascade and promoting anticoagulation [12–15]. However, frequent administration of heparin will bring out substantial potential side effects, such as

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heparin-induced thrombocytopenia (HIT) and catheter-associated sepsis [16].

Therefore, how to enhance the haemocompatibility of artificial polymeric materials and reduce the anticoagulant dosage during dialysis is an urgent problem to be solved. Versatile measures have been employed to enhance the haemocompatibility of biomaterials, e. g. physical blending, surface grafting, surface coating, and surface crosslinking [8,10,17–20]. However, the blending usually sacrifices the physical merit of the bulk matrix owing to the incompatibility between the additive and the matrix [21]. To reserve the merits of bulk membrane, surface modification has been widely adopted. According to our previous research, heparin was anchored onto the target membrane surface via dopamine or 3-aminopropyltriethoxysilane [10,22,23]. However, the direct heparin grafting onto membrane surfaces may deactivate its activity rapidly [24]. Accordingly, synthetic anticoagulant was developed as a replacing solution [25,26]. As we discussed above, the anticlotting property of heparin is mainly due to the presence of carboxylate and sulfonate groups. Thus, the polymer with such functional groups is synthesized to mimic heparin, which is denoted as heparin-like polymer [3]. From our previous work, P(AA-VTES-SSNa) heparin-like copolymer was successfully synthesized to modify the whole dialyzer via surface crosslinking strategy [17]. The modified products showed outstanding haemocompatibility with extended coagulation time (APTT>600 s). However, a large ratio of organic solvent (e. g. 50 wt% Dimethylacetamide) is needed to dissolve the copolymer and swell the membrane surface for the subsequent immobilization. The organic solvent residue in the porous matrix is difficult to be completely washed away from the dialyzer, causing serious haemolysis and endangering the product safety. Moreover, excess organic solvent also influences the morphologies and clearance performances of membranes, which significantly limit the extensive application [27].

In this work, we synthesized a heparin-like copolymer, denoted as a poly (LMA-SSNa-AA), with a long alkyl chain lauryl methacrylate (LMA), an acrylic acid (AA), and a sodium 4-vinylbenze-sulfonate (SSNa). The as-synthesized poly (LMA-SSNa-AA) could be effectively adhered to the membrane surface, and form a durable anticoagulant coating [28]. The adhesion mechanism can be explained by the hydrophobic interaction between the long alkyl chain and the hydrophobic surface of the polymeric membrane. The interaction strength is strong enough to acquire a tight bonding according to the classic "Lifshitz theory'' of Van Der Waals forces [29–31]. The mild modification procedure does not need a large amount of organic solvent to swell the membrane surface and therefore the consumption of organic solvent e.g. N, N-Dimethylformamide (DMF) was significantly reduced. Furthermore, the membrane pore structure and the mechanical property were well reserved. It is noted that this amphiphilic random copolymer could be self-assembled to micelles at a certain concentration, which caused great influences on the modification [30,32]. During the micelle producing process, the polymer chains massed together and tangled into a sphere, ultimately leading to less functional groups exposed outside. To optimize the modification effect, the suitable concentration of modified solution was investigated. Then a series of modified membranes were prepared by varying the concentration of terpolymer solution. The surface chemical composition, morphology, hydrophilicity, permeability and antifouling merits of the as-prepared PLA membranes were systemically explored. And the haemocompatibility was evaluated in terms of platelet adhesion, complement activation (C3a and C5a), haemolysis ratio and anticoagulation time (APTT, TT). The optimum concentration of terpolymer solution was determined, and the self-anticoagulant dialyzer was constructed accordingly. The dialyzer related parameters such as ultrafiltration coefficient and toxins clearance rate were investigated from animal dialysis experiments.

2. Experimental section

2.1. Raw materials

Poly (lactic acid) (PLA, 2003D) was supplied by Natural Works, US, and PLA membranes were prepared according to our previous work [10]. Azobisisobutyronitrile (AIBN, AR), Bovine serum albumin (BSA, AR), lauryl methacrylate (LMA, AR), acrylic acid (AA, AR), and sodium 4-vinylbenze-sulfonate (SSNa, AR) were purchased from Aladdin Reagent Co., Ltd., China. The solvent N, N-Dimethylformamide (DMF) obtained from Sino pharm of China. The fresh human whole blood, platelet-poor and platelet-rich plasma were kindly supplied by Ningbo Blood Center (China). APTT and TT reagents were purchased from Shanghai Sun Biotech Co., Ltd. C5a and C3a ELISA kits were provided by CUSBIO Biotech Co., Ltd. Polysulfone dialyzers (LST140-A) were supplied by Lengthen Co., Ltd, Jiangsu, China.

2.2. Synthesis and characterization of heparin-like copolymer

Poly (lauryl methacrylate-co-acrylic acid-co-Sodium 4-vinylbenzene-sulfonate) was synthesized through free radical polymerization. In brief, lauryl methacrylate (LMA, 8.9 g, 3.5 mmol), sodium p-styrene-sulfonate (SSNa, 7.2 g, 3.5 mmol) and acrylic acid (AA, 2.1 g, 3 mmol) were firstly dissolved into 100 ml DMF. The reactant solution was agitated at 25 °C for 60 min under the atmosphere of nitrogen. Then, the initiator AIBN accounting for 1% of monomer total mass was put into the mixture. Subsequently, the polymerization system was heated up to 80 °C under continuous stirring for 24 h. The resulting solution is a white suspension. After exposing the solution to air to stop the polymerization, the suspension was distilled by the rotary vacuum evaporator, and then dried in the vacuum drying oven.

To remove the dimer, small molecular copolymer or unreacted monomer, the evaporation product was re-dissolved in dimethyl sulfoxide (DMSO), and then precipitated in diethyl ether for three times. The specific information of the purified polymer was investigated by FTIR (Nicolet 6700, USA), SEC (Malvern Viscotek TDA305max, UK) employing DMSO as mobile phase and Pullulan as standard, as well as ^{1}H NMR (AVANCE III 400 MHz, Switzerland) using DMSO-d_6 as a solvent.

This amphiphilic random copolymer solution engendered an apparent Tyndall effect upon reaching a certain concentration, demonstrating the micelle was possibly formed. To verify the formation of micelle, OCA 25 was utilized to measure the variation of surface tension with the concentration. The transition point from sharp reduction to a gentle plat can be considered to be critical micelle concentration (CMC). In addition, another certification of micelle produced is the increment of the particle size, measured by the dynamic light scattering particle size analyzer (Zetasizer Nano ZS, Britain). To further certify the formation of the micelle, transmission electron microscope (TEM, JEM2100, Japan) was utilized to specifically observe the shape evolution.

2.3. Fabrication of heparin-like membrane

As discussed above, the polymer chains were subjected to the remarkable conformation change in the modified solution with various concentrations, which could influence the heparinized modification. To determine the most suitable concentration for better anticoagulant property, different amounts of purified copolymer were added into the mixture of DMF and deionized water with a volume ratio of 1:9 respectively to obtain different modified solutions (1.8 wt%, 3.6 wt%, 5.4 wt%, 7.2 wt% and 9 wt%). The amphiphilic terpolymer can be well dissolved in aqueous solution, which therefore substantially reduced the usage of organic solvent DMF.

In addition, the terpolymer chain has both hydrophobic and hydrophilic parts, thus the proportion of organic solvent in the mixed solution might affect the chain conformation. To further discuss the influences of