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Direct synthesis of heparin-like poly(ether sulfone) polymer and its blood compatibility

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ABSTRACT

In this study, heparin-like poly(ethersulfone) (HLPES) was synthesized by a combination of polycondensation and post-carboxylation methods, and was characterized by Fourier transform infrared spectroscopy, nuclear magnetic resonance hydrogen spectrum and gel permeation chromatography. Owing to the similar backbone structure, the synthesized HLPES could be directly blended with pristine PES at any ratios to prepare PES/HLPES membranes. After the introduction of HLPES, the microscopic structure of the modified PES membranes was changed, while the hydrophilicity was significantly enhanced. Bovine serum albumin and bovine serum fibrinogen adsorption, activated partial thromboplastin time, thromb time and platelet adhesion for the modified PES membranes were investigated. The results indicated that the blood compatibility of the PES/HLPES membranes was significantly improved compared with that of pristine PES membrane. For the PES/HLPES membranes, obvious decreases in platelet activation on PF-4 level, in complement activation on C3a and C5a levels, and in leukocytes activation on CD11b levels were observed compared with those for the pristine PES membrane. The improved blood compatibility of the PES/HLPES membrane might due to the existence of the hydrophilic groups (–SO_3Na, –COONa). Furthermore, the modified PES membranes showed good cytocompatibility. Hepatocytes cultured on the PES/HLPES membranes presented improved growth in terms of SEM observation, MTT assay and confocal laser scanning microscope observation compared with those on the pristine PES membrane. These results indicate that the PES/HLPES membranes present great potential in blood-contact fields such as hemodialysis and bio-artificial liver supports.

1. Introduction

Heparin, a common anticoagulant used to prevent thrombi formation, is a kind of highly sulfated glycosaminoglycan, which has the highest negatively charged density in any known biomacromolecules [1]. Heparin can accelerate the rate of ATIII-mediated inactivation of several clotting enzymes, including thrombin, FXIa, FXa and FIXa, since it leads to a conformational change in the reactive center of AT III and binds to antithrombin III (AT III) via a special pentasaccharide sequence [2]. However, it is difficult to use heparin directly as a blood compatible material because of its water solubility; but many studies have been made on the surface heparinization of hemodialysis membranes. The heparin-immobilized hemodialysis membranes showed improved blood compatibility because of the bioactivity of the grafted heparin. However, the small density of the immobilized heparin was the biggest problem in the earlier studies [3–7]. Although many methods have been developed to enhance the density of the immobilizing sites for heparin and other biological molecules, the use of heparinized surfaces might still be limited owing to the prohibitive cost, the dramatic loss of the bioactivity resulting from the covalent binding or in vivo long-term degradation by virtue of various chemical or biological agents [8–10]. Consequently, research directed at searching for alternative anticoagulant materials has been undertaken based on diverse approaches to modifying hemodialysis membranes. Plenty of investigations on heparin-like polymers have been carried out to develop new materials composed of ionic polymers including sulfate, sulfamide and carboxylate groups, since it was believed that the anticoagulant activity of heparin was caused by the presence of these ionic functional groups [11–13]. Thus, the aim of this study was to prepare heparin-like polymeric hemodialysis membrane by introducing sodium sulfonic (–SO_3Na) and sodium carboxylic (–COONa) groups, which were expected to improve the blood compatibility of the polymeric matrix. Meanwhile, it is very important to find a matrix material

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with good properties, including relatively good biocompatibility and mechanical properties.

In recent decades, the need for clinical hemodialysis and membrane materials has increased dramatically, with the increase in uremic patients. Polyethersulfone (PES), one of the most important polymeric materials because of its good chemical resistance, thermal stability, mechanical and film-forming properties, has already been used in hemodialysis and also in water purification [14–18]. However, heparin was needed to prevent blood clotting, platelet adhesion and aggregation during hemodialysis using PES membranes. In recent years, to improve the blood compatibility of PES hemodialysis membranes, numerous studies have been carried out to modify PES membranes [19,20], such as by blending [21], surface physical treatment [22], surface grafting and coating [23]. Among these, blending of heparin-like polymers was the most convenient way to improve anticoagulant activity. Li et al. modified PES hemodialysis membrane by blending citric acid grafted polyurethane [24]. In a recent study [25], heparin-like macromolecules were synthesized for the modification of PES membranes, and the hemocompatibility of modified PES membranes was improved. The heparin-like macromolecules were synthesized by RAFT polymerization using carboxyl-terminated trithiocarbonate as the RAFT agent, and the monomers used in the polymerization were styrene, *N*-vinylpyrrolidone and acrylic acid. However, when the heparin-like macromolecules were blended with PES, the miscibility was poor; as a result, serious phase separation occurred after blending, and the mechanical property of the PES membranes may also be affected, which may restrict further application for PES-based hemodialysis membranes.

In the present study, to address this problem, heparin-like PES containing sodium sulfonic ($-SO_3Na$) and sodium carboxylic (–COONa) groups was synthesized. Many reports have noted that sulfonated polymers show good anticoagulant activity, like heparin, since the negatively charged pendent sodium sulfonic ($-SO_3Na$) groups expel the blood component by electrical repulsion, and the polymers incorporating sodium carboxylic (–COONa) groups also show good blood compatibility [26,27]. Thus, PES with both sodium sulfonic ($-SO_3Na$) groups and sodium carboxylic (–COONa) groups may have heparin-like properties and good blood compatibility. In previous studies, modification of the PES matrix was addressed in several efforts to sulfonate the PES chain. These methods included post-sulfonated [28] or direct synthesis of PES from monomers containing sodium sulfonic ($-SO_3Na$) groups [29,30]. The post-sulfonated method might not only decrease the mechanical and thermal stabilities, but also lack control of the degree of sulfonation and the position of the sulfonic group. Although direct synthesis of PES from monomers containing sodium sulfonic ($-SO_3Na$) groups had more advantages than that of post-sulfonation, the material was incapable of introducing other functional groups, which limited the application of the PES matrix [31,32]. With respect to introducing other functional groups, monomers with amino-substituted groups ($-NH_2$) were combined with the reaction. Zhu et al. reported that several hydrophilic monomers, such as *N*-isopropyl acrylamide and *N*,*N*-dimethylamino-2-ethyl methacrylate, could be successfully introduced into PES using amino-substituted groups ($-NH_2$) [33]. Thereby, the combination of the previous amino-substituted and sodium sulfonic groups and post-carboxylated reaction would be an easy and effective method of preparing heparin-like PES (HLPES). The advantage of the method was the possibility of controlling the degree of sulfonation, the avoidance of cross-linking [34] and the degradation reactions during the post-carboxylation of PES, which might result in a better mechanical property.

Yet, no report has focused on the improvement in blood compatibility of PES via the introduction of both sodium sulfonic ($-SO_3Na$) groups and sodium carboxylic (–COONa) groups through a simple and direct method. The present study presents a method of synthesizing PES containing sodium sulfonic ($-SO_3Na$) groups and amino groups (NNPES) via a condensed polymerization, which would endow the PES with the potential for facile modification via a post-functional method. After post-carboxylation of the synthesized NNPES, the HLPES was prepared. Since the synthesized HLPES has the same backbone as PES polymer, the synthesized HLPES could be directly mixed with pristine PES without phase separation at any ratios. Furthermore, the HLPES may also inherit the excellent cytocompatibility and histocompatibility from PES, which makes it exhibit much more perspective in the modification of PES membranes than the other synthesized heparin-like macromolecules.

2. Experiments

2.1. Materials

The 3,3′-disulfonated-4,4′-difluorophenyl, disodium salt (⩾99%), 4,4′-difluorobiphenyl sulfone (⩾99%), 4,4′-sulfonyldiphenol (⩾99%), 3,3′-diamino-4,4′-dihydroxydiphenyl sulfone (⩾99%) and heparin sodium salt were purchased from Xiya Reagent Corporation (China) and used without purification. Heparin sodium salt is unfractioned heparin: the molecular weight ranges from 8000 to 18,000 Da and the average molecular weight is 12,000 Da. Toluene, *N*,*N*-dimethylacetamide (DMAc) and other solvents (Chemical Reagent Factory of Kelong, China) were dried by stirring with CaH_2 and then distilled before use. Polyethersulfone (PES; Ultrason E6020P) was purchased from BASF (Germany), and dried at 90 °C for 24 h before use. Bovine serum albumin (BSA), bovine serum fibrinogen (FBG), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), fluorescein diacetate (FDA) and propidium iodide (PI) were purchased from Sigma Aldrich (US). All the other chemicals (analytical grade) were obtained from Chemical Reagent Factory of Kelong (China), and were used without further purification.

2.2. Synthesis of SO_3Na-NH_2-PES (NNPES)

In a typical procedure (Scheme 1), 3,3′-disulfonated-4,4′-difluorophenyl sulfone (disodium salt) (0.917 g, 2.0 mmol), 4,4′-difluorobiphenyl sulfone (0.750 g, 3.0 mmol), 3,3′-diamino-4,4′-dihydroxydiphenyl sulfone (0.560 g, 2.0 mmol), 4,4′-sulfonyldiphenol (0.763 g, 3.0 mmol), DMAc (50 ml), toluene (40 ml) and potassium carbonate (K_2CO_3) were introduced to a three-neck round-bottom flask equipped with a Dean–Stark device. After vacuuming and back-filling nitrogen several times, the flask was transferred to an oil bath preheated to 155 °C, and kept for 5 h in a nitrogen atmosphere. Then, the reaction system was heated to 180 °C and kept for 12 h. K_2CO_3 was used as a proton scavenger, and the water formed during the reaction was removed as an azeotrope with toluene by the Dean–Stark device. The reaction mixture was cooled to room temperature and precipitated in ether. After volatilization of the ether, the residual monomers and K_2CO_3 in the product were removed by dispersing the polymer into distilled (DI) water and stirring, followed by centrifugation, and the purification procedure was repeated several times. The resultant polymer (NNPES) was finally collected by filtration and fully dried in a vacuum oven at 60 °C for 12 h.

2.3. Synthesis of HLPES

Typically, NNPES and excess maleic anhydride were put in a round-bottom flask and stirred until fully dissolved in DMAc. The reaction mixture was allowed to transfer to an oil bath preheated to 100 °C and kept for 18 h. Then, the reaction mixture was cooled to room temperature and precipitated into DI water. The residual

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