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A novel kind of polysulfone material with excellent biocompatibility modified by the sulfonated hydroxypropyl chitosan

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ABSTRACT

In order to ameliorate the biocompatibility of polysulfone (PSf), sulfonated hydroxypropyl chitosan (SHPCS) was grafted from PSf membrane material by Schiff-Base reaction. The original and modified membranes were characterized by attenuated total reflectance-Fourier transform infrared spectroscope (ATR-FTIR), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), water contact angle (WCA) measurement, tensile strength test and antibacterial test in vitro, and the results indicated that the PSf modified by SHPCS (PSf-SHPCS) was synthesized successfully, the hydrophilicity of PSf-SHPCS membrane was improved to a great extent, all the membranes possessed good stability in physiological condition and the PSf-SHPCS membrane had good antibacterial property. Protein adsorption, platelet adhesion, hemolysis assay, plasma recalcification time, activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT) and whole blood clotting time were executed to evaluate the hemocompatibility of membranes decorated by SHPCS, and the results demonstrated that the modified membrane had fine hemocompatibility.

1. Introduction

Nowadays the kidneys of patients are not working properly because of the emergence of some kidney diseases such as acute renal failure, chronic renal failure and uremia, which brings about the requirements of hemodialysis [1]. Hemodialysis membrane is considered as artificial kidney which can get rid of the waste products of blood and maintain the balance between fluid and solute [2]. The first generation of membrane materials adopted to hemodialysis are cellulose and its ramifications [3]. However, the cellulose-based membranes can make patients suffer from First-use Related Syndrome even death [4]. The second generation of membrane materials applied to hemodialysis are a family of polymers such as polyethersulfone (PES) [5] and polysulfone (PSf) [6], which have higher flux than the first and can improve the clearance for "middle" molecules [7]. Nevertheless, the adverse biocompatibility of these hydrophobic polymeric membranes especially the blood compatibility can lead to thrombogenesis directly [8], so it is necessary to develop the third generation of membrane materials used in hemodialysis.

It is investigated that the biocompatibility of materials mainly embraces the blood compatibility and cytocompatibility, and the former is especially important for the hemodialysis materials [9]. In order to improve the blood compatibility of membrane materials, the bulk modification and surface modification [10] are commonly considered to introduce hydrophilic groups and heparin or heparin-like agents [11,12] onto membrane materials by coating, blending and grafting methods [6,13,14]. The bulk modification is more complex than surface modification although it is a little more effective [15]. Grafting method is more useful and stable compared with coating and blending methods [16]. Recently, heparin is widely used as coating agent of hemodialysis to prevent blood clotting, but heparin is very expensive and can easily cause some complications [17]. Kim et al. [12] synthesized heparin-like surface PES ($-SO_3H$) with chlorosulfonic acid via the bulk modification. The sulfonated PES film with good blood compatibility reduced protein adsorption and platelet attachment, whereas the heparin-like surface did not include the skeleton structure of heparin but just the groups. Thomas Groth et al. [11] prepared PSf membrane modified by heparin by surface grafting modification to enhance the anticoagulant property. Jie Zhao et al. [14] prepared polypropylene nonwoven fabric membrane grafted with zwitterionic polymer of [3-(methacryloylamino)propyl]-dimethyl (3-sulfopropyl) ammonium hydroxide via O_2 plasma pretreatment and UV-irradiated technique to strengthen the protein antifouling property. In our previous study [18], PES blended with polyurethane pre-polymer immobilizing citric acid was fabricated by bulk, blending and grafting modification, followed by grafting with chitosan (CS) through surface grafting modification to improve the hemocompatibility and antibacterial property.

In summary, the focus of researches tends to prepare a polymer "model" having hydrophobic basement membrane and hydrophilic polymer side, then there is the formation of hydration layer on the membrane surface, which is a classical micro phase separation structure

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[19]. The "model" with micro phase separation structure imitates lipid bilayer structure of biomembrane in the nature, which is likely to be the third generation of hemodialysis membrane materials.

According to the market data analyzed by Fresenius medical company in 2010, 71% of hemodialysis membranes were using PSf as materials [20]. PSf is a very popular membrane material due to its various excellent properties especially the high permeability for low-molecular weight proteins [21]. Consequently, researchers have done a lot of work in terms of polysulfone modification to obtain the "model" discussed above by the surface grafting. On one hand, surface grafting modification is not only simple but also effective to covalently immobilize the polymer on the basement membrane. On the other hand, this method does not alter the general nature of the substrate, and the nature of the polymer is added on the surface, this is to say the materials are hydrophilic but water insoluble. Chitosan (CS) [22], one of the most abundant polymer materials in the nature, can be used as a pharmaceutical excipient as a result of its good haemostatic and antibacterial properties [23,24]. Serpil Aksoy et al. [25] prepared polyurethane films modified by covalent immobilization of CS to increase the antibacterial activity. Mingxian Liu et al. [26,27] synthesized chitosan grafted halloysite nanotubes to improve hemocompatibility (decreased the hemolysis ratio). It is worth noting that CS and heparin both have the same skeleton structure of five-carbon sugar containing oxygen. Researchers also do some studies on functional groups decoration of CS accordingly, for example sulfonation of CS. Vieira et al. [28] prepared the polymeric films of CS and κ-carrageenan, and the results showed that the CS chain was modified and confirmed the existence of sulfonate groups, as well as in the κ-carrageenan chain, indicating surfaces with similar chemical properties to those of heparin.

The objective of this paper was to obtain a sort of materials with good biocompatibility through covalently immobilizing sulfonated hydroxypropyl chitosan (SHPCS) on PSf by Schiff-Base reaction, and the relevant work had been rarely reported. The —CH_2Cl groups was introduced to PSf, and then transformed into the PSf-Cl membrane through a phase-inversion technique, followed by immersing in ethylenediamine to introduce the amino so that sulfonated hydroxypropyl chitosan could be grafted from the modified membrane via glutaraldehyde as the "bridge" eventually. The surface performances of modified membrane (cross-section structure, water contact angle (WCA), surface grafting density and tensile strength test) were examined. The blood compatibility and antibacterial property of modified membrane (protein adsorption, platelet adhesion, hemolysis assay, plasma recalcification time, activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), whole blood clotting time (WBCT) and antibacterial test) were also investigated.

2. Materials and methods

2.1. Materials

Polysulfone (average Mn: 22,000) and Tin(IV) chloride were purchased from Sigma, USA. Chitosan (CS) (viscosity: 100–200 mPa.s) with a degrees of deacetylation of about 95%, paraformaldehyde (AR) and isopentyl acetate were obtained from Aladdin Industrial Corporation. Chlorotrimethylsilane (AR), methanol, ethanediamine (EDA; AR), sodium dodecyl sulfate (SDS), sodium hydroxide (NaOH; AR), propylene oxide (AR), formamid, orangeII and glutaraldehyde bovine serum albumin (BSA; fraction V) were purchased from Sinopharm Chemical Reagent Company, China. *N*, *N*-dimethylacetamide (DMAC) was acquired from Guangdong Guanghua Sci-Tech Company, China. Isopropanol (AR), chloroform, glacial acetic acid and acetone were obtained from Chengdu Kelong Chemical Reagent Company, China. Chlorosulfonic acid (CP) was acquired from Beijing Mashi chemicals Company, China. MD44 dialysis bag (diameter: 28 mm; rejection molecular weight: 8000 dal) was obtained from Shanghai Leibusi Company. The *Pseudomonas aeruginosa* strain (ATCC 27853), Nutrient Broth and Agar Powder were purchased from Guangdong Huankai Microbial Sci.&Tech. Company.

2.2. Synthesis of aminated polysulfone (PSf-NH_2) membrane

According to the method described by Ecaterina Avram et al. [29], chloromethylation of PSf was performed to introduce the —CH_2Cl groups. As shown in the scheme 1, PSf (10.0 g, 22.5 mmol) was dissolved in 250 ml of chloroform in a round bottom flask under nitrogen atmosphere. Then, Paraformaldehyde (6.7 g, 225 mmol), $SnCl_4$ (0.26 ml, 2.25 mmol), and $(CH_3)_3SiCl$ (28.5 ml, 225 mmol) were added and the mixture was stirred under reflux for 72 h. Meanwhile, five control groups were reacted at 40 °C, 45 °C, 50 °C, 55 °C, 60 °C, respectively. Next, the solution was precipitated in methanol and washed several times with methanol. Finally, the product was filtered and dried. ^1H NMR measurements were recorded with d-DMSO as internal standard to confirm the compositions of PSf and PSf-Cl by using a 500 MHz BRUKER spectrometer.

18% (wt) PSf-Cl was dissolved in DMAC under constant stirring until a homogeneous solution was obtained. After that, the mixed solution was placed in static for 4–8 h at 25 °C to get rid of bubbles and thus obtained casting solution. The flat polymer membrane was obtained through immersion precipitation phase inversion method. The PSf-Cl membrane was obtained from the flat glass and immersed in ethanol aqueous solution for 24 h at room temperature. The membrane was dried in a vacuum for 12 h, and cut into 2.0 × 2.0 cm^2 pieces.

The PSf-Cl membrane was subsequently incubated into ethylene diamine (EDA) at 25 °C for 20 min to obtain amino groups [11]. The PSf-NH_2 membranes were washed 3 times with ultrapure water and dried for 12 h. The surface amine content of the membrane could be analyzed by ninhydrin colorimetric assay and Orange II staining method. The amino groups on membrane surface could form a compound with Orange II at pH 3, and then the compound dye was desorbed with 1 mM NaOH solution in this paper. The absorbance of the supernatant was determined at 485 nm by UV spectrophotometer.

2.3. Synthesis of sulfonated hydroxypropyl chitosan (SHPCS) polymer

In order to weaken the molecular force in CS, 50 g CS was mixed up with 200 g NaOH and 400 ml ultrapure water in a 500 ml beaker, and then the breaker was placed in a refrigerator to freeze for 10 days. After that, 20 g alkalizing CS, 200 ml isopropanol and 200 ml propylene oxide were added to a round bottom flask and the mixture was stirred under reflux at 45 °C for 4 h. The mixture was filtered and dissolved in appropriate ultrapure water, and then the solution was precipitated in acetone and washed 3 times with methanol. Final hydroxypropyl chitosan (HPCS) was filtered and dried.

In the ice bath below 5 °C, 20 ml formamid was added to a round bottom flask, and then chlorosulfonic acid (six control groups: 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml) was introduced dropwise, followed by adding 2 g HPCS. After stirring at 68 °C for 3 h, the mixture was diluted into 70 ml ultrapure water. The solution was transferred into the dialysis bag to dialyze for 1 day. Next, the dialysis fluid was conducted alkalization deamination and acidification treatment, and then poured into the dialysis bag to dialyze for 2 day again. The resulting SHPCS was obtained by the concentration and drying of the dialysis fluid. Meanwhile, the SHPCS-2 represented the SHPCS obtained by dropping 2 ml chlorosulfonic acid; so did the others.

A Fourier-transform infrared spectroscope (FTIR) (Nicolet 380, USA) was used to detect the functional groups of CS, HPCS and SHPCS-4. The elemental analysis (EA) of SHPCS was investigated by using a Flash2000.

2.4. Synthesis of PSf-NH_2 membrane grafted with SHPCS (PSf-SHPCS)

In a round bottom flask, the PSf-NH_2 membranes were immersed in 30 ml of glutaraldehyde solution (1%, *v*/v) at 25 °C for 8 h with stirring slightly after adding a few drops of glacial acetic acid, and then washed 3 times with ultrapure water. The resulting membranes and a few drops