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Facile preparation of heparinized polysulfone membrane assisted by polydopamine/polyethyleneimine co-deposition for simultaneous LDL selectivity and biocompatibility

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ABSTRACT

Low-density lipoprotein (LDL) gains worldwide attention for decades as the key risk factor to atherosclerosis that progressively deteriorating into cardiovascular diseases. Until recent years, LDL-apheresis comes to be extensively used as a direct and efficient LDL removal method, with LDL adsorption materials particularly important. In this paper, a new strategy based on the co-deposition of polydopamine (PDA) with polyethylenimine (PEI) onto polysulfone (PSf) membranes, then subsequent heparinization by amino-carbonyl reactions, to achieve LDL selectivity and simultaneous biocompatibility, is proposed. Surface properties of modified PSf membranes are characterized by ATR-FTIR, XPS, FESEM, Zeta potential and WCA measurements. LDL adsorption ability is investigated by ELISA, while blood biocompatibility is evaluated by platelet adhesion experiments. Results suggest that heparin-modified PSf membranes show high selectivity for LDL removal and fine biocompatibility in contact with plasma, as excellent potential materials for LDL-apheresis.

1. Introduction

Cardiovascular diseases, the leading cause of death in recent decades, are caused by atherosclerosis in the majority [1–3], which is commonly initiated by lipids, especially cholesterols, deposited onto inner walls of vessels, finally resulting in blockage and thrombus [4]. Low-density lipoprotein (LDL), the main carrier of cholesterols in plasma, has been regarded as the key risk factor to atherosclerosis over a long time, with an excessive high concentration in human body inevitably promoting the progressions, as is demonstrated by clinical observations [5]. Therefore, how to effectively and specifically reduce LDL level gains worldwide attention for years. Researchers have devoted tremendous efforts to finding out various LDL-lowering methods and well-performed absorbents [6,7]. There are four main methods extensively applied in practical clinic, i.e. diet [8,9], aerobic exercise [10], medication [11,12] and LDL-apheresis (LDL-A) [13–15]. Generally, lifestyle changes and drug therapy are common treatments with poor curative effects, especially when LDL level exceeding 300 mg/dL or for familial hypercholesterolemia (FH) [16]. Then LDL-apheresis turns to be a more direct and efficient choice virtually available, since LDL-A systems are mostly based on plasma exchange and can remove LDL selectively from blood with high-efficiency [17–19]. Among six techniques used currently in LDL-A systems, four of them are based on the principle of adsorption, i.e. immunoadsorption (IMA), dextran sulfate adsorption (DSA), polyacrylate-coated polyacrylamide direct perfusion (DALI), dextran sulfate direct perfusion (Liposorber D) [20,21], suggesting the fact that adsorption material is the critical factor correlating the therapy effect.

Diverse adsorption materials have been employed into LDL-A systems, including immune adsorption materials [22,23], non-ionic adsorption materials [24], hydrophobic adsorption materials [25], anionic adsorption materials [26] and amphiphilic adsorption materials [27,28], etc. Representatively, anionic adsorption materials are most widely adopted as acrylic acid [29], amino acid [30] and heparin [31–33], and for a long time, heparin has been particularly emphasized for its easy attainability, fine biocompatibility and high selectivity by binding with apolipoprotein-B of LDL via electrostatic interactions [31–33]. While, evidence shows that excess amount of heparin residue in plasma can predispose patients to certain degrees of coagulopathy, since heparins act as one kind of blood anti-coagulants [34,35]. Therefore, effective immobilization of heparin onto substrates to avoid leakage is significantly important to ensure the medical safety of LDL-apheresis.

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Multitudinous strategies have been designed to achieve a facile and reliable immobilization of heparin onto biomaterial surfaces. For example, polysulfone membranes were activated by totally chemical methods with three step reactions to form binding sites for subsequent heparin immobilization, and experimental results indicated that heparin-modified PSf membrane exhibited excellent selective affinity for LDL both in single and binary protein solutions [36]. Besides, PAN-based resins [37], Sepharose and magnetic nanocomposites [38] were also heparinized chemically for LDL adsorption with expected performances. Regardless of the enormous variety of heparinization methods as mentioned above, the fact remains that the vast majority of them require substrate surfaces either to possess chemically active groups, or to be functionalized by additional treatments. Therefore, it is indeed necessary to find an effective heparinization method no longer constrained to properties of different substrates.

Recently, a wide range of researchers in chemistry, materialogy, biology or engineering have been gradually concentrating on poly(dopamine) (PDA), which is inspired by polyphenolic proteins in mussels with strong adhesivity to almost any substrate [39]. It provides an universal and powerful approach to surface modification, with those PDA layers formed onto either directly co-deposited with functional molecules, such as PEG, PVA, PSBMA [40,41], or acting as platforms for subsequent reactions to introduce target molecules [42,43]. While, since the process of co-deposition is a disorder in dispersion of dopamine and admixture, certain degrees of embedment occurs inevitably that part of modified polymers were buried under PDA layers and part of them exposed or stretching to outside, as mentioned in our previous work by Liu for dopamine and heparin co-deposition on Au chips [44]. Therefore, a new method is preferred to avoid embedment and loss, especially when target molecules are huge and bioactive (see Fig. S1– S3 for comparison to direct co-depositions in Supplementary materials).

As suggested above, a strategy based on the co-deposition of polydopamine (PDA) with polyethylenimine (PEI), then subsequent heparinization by amino-carbonyl reactions for LDL selectivity and simultaneous biocompatibility, was proposed in this paper. Firstly, PDA and PEI were co-deposited onto polysulfone (PSf) membranes at slightly basic pH to form a PDA/PEI modified layer. Then secondly the amino-carbonyl reaction was applied to combine heparin molecules onto aminated membrane surfaces with the help of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and *N*-Hydroxysuccinimide (NHS), to achieve the heparinization procedure. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), X-ray photoelectron spectroscopy (XPS) were used to determine whether dopamine or heparin had been immobilized onto the membrane surface successfully. Hydrophilicity, as well as biocompatibility of modified membranes were studied by static water contact angle measurement and platelet adhesion experiment, respectively. An Enzyme-linked immunosorption assay (ELISA) was employed to investigate the adsorption of LDL from single and binary protein solution.

2. Experimental

2.1. Materials

Pure polysulfone granules were supplied by Solvay Co., Ltd. (Belgium). Polyethylenimine (PEI, Mw = 70,000 Da, 50 wt% aqueous solution) and heparin (Mw = 6000–20,000, 185 USP units/mg) were obtained from Aladdin Industrial Corporation (Shanghai, China). Low-density lipoprotein (LDL) was provided by Millipore (Massachusetts, USA). Dopamine (DA) hydrochloride, primary antibody anti-β-lipoprotein, secondary antibody anti-chicken IgY (IgG) and human serum albumin (HSA) were purchased from Sigama-Aldrich. Tris(hydroxymethyl)aminomethane were acquired from Amresco (Ohio, USA). 1-Ethyl-3-(aminopropyl)carbodiimide (EDC) and *N*-hydeoxysuccinimide (NHS) were obtained from Shanghai Medpep Co., Ltd. Platelet rich plasma was supplied by the Second Affiliated Hospital of Zhejiang University School of Medicine. Not-fat milk powder was commercial product. All other reagents were obtained from Sinopharm Chemical reagent, Shanghai, China and used as received. Ultrapure water (18.2 MΩ) was self-made with a Hi-tech Laboratory Water Purification system.

2.2. Fabrication and heparinization of PSf membranes

To fabricate dense PSf membranes, pure PSf granules were dissolved into NMP solvent with concentration of 16 wt%, and then constantly stirred at 70 °C for 12 h. After air bubbles were removed from the solution, the homogeneous solution was cast onto a clean glass plate using a casting knife with thickness of 150 nm. Subsequently, the glass plate was put into a vacuum oven at 60 °C overnight to extract NMP solvent and then immersed into ultrapure water for 24 h, to form homogeneous PSf membranes onto. Finally, after peeling off from the glass plate, PSf membranes were dried for another 24 h at 40 °C under vacuum before surface modification was conducted.

The as-prepared PSf membranes were soaked into freshly prepared PDA/PEI mixture solutions in an open vessel, with the PDA concentration of 2.0 mg/ml while the PEI concentration of 1.0 mg/ml. After continuously stirring at 25 °C for 4 h, the resultant modified membranes were taken out and washed with deionized water to remove non-firmly adsorbed PDA and PEI molecules.

As was mentioned above, heparin was immobilized onto membrane surfaces by the amino-carbonyl reaction between amino groups introduced by co-deposited PEI and carbonyl groups from heparin molecules, with the help of EDC and NHS. Thus operationally, the PDA/PEI modified membranes were incubated into heparin and EDC/NHS mixture solutions (5.0 mg/ml of heparin and EDC in citrate buffer solution: 0.2 M Na_2HPO_4 and 0.1 M citric acid, adjusted to pH 4.7 with 1 M NaOH, molar ratio of EDC to NHS = 1:1) at 25 °C for 24 h for covalent immobilization of heparin. Then the immobilized membranes were washed with phosphate-buffered saline (PBS, 0.03 M Na_2HPO_4, 0.02 M K_2PO_4, and 0.137 M NaCl, adjusted to pH 7.0 with 1 M NaOH) to remove physically adsorbed heparin molecules. Finally, heparin-modified PSf membranes were dried at 40 °C overnight under vacuum before subsequent characterizations.

2.3. Characterization

2.3.1. Characterization of surface composition and morphology

Surface functional groups were characterized by Attenuated total reflectance FTIR spectroscopy (ATR-FTIR, Nicolet 6700, Nicolet. Co., USA). Spectra were collected from 400 to 4000 cm^{-1} by calculating 32 scans at a resolution of 4 cm^{-1}. More details in chemical composition were obtained using X-ray photoelectron spectrometer (XPS) on an RBD upgraded pH I-5000C ESCA system (Perkin Elmer, Massachusetts, USA) with Al Kα excitation radiation ($h\nu$ = 1486.6 eV). The whole and narrow scan spectra of all elements were both recorded while the binding energies were calibrated by using the containment carbon (C 1s = 284.8 eV). Surface morphologies were observed with field emission scanning electron microscopy (FESEM, Hitachi, S4800, Japan). An injector was used to coat gold onto the surface so that the membrane can transfer electrons well.

2.3.2. Zeta potential and static water contact angle measurement

Electro kineticanalyzer (Anton Paar, SurPASS, Austria) was utilized to measure the surface charge on the surface membrane with