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Full Length Article

Layer-by-layer structured polysaccharides-based multilayers on cellulose acetate membrane: Towards better hemocompatibility, antibacterial and antioxidant activities

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ABSTRACT

The development of multifunctional cellulose acetate (CA) membranes with enhanced hemocompatibility and antibacterial and antioxidant activities is extremely important for biomedical applications. In this work, significant improvements in hemocompatibility and antibacterial and antioxidant activities of cellulose acetate (CA) membranes were achieved via layer-by-layer (LBL) deposition of chitosan (CS) and water-soluble heparin-mimicking polysaccharides (i.e., sulfated *Cantharellus cibarius* polysaccharides, SCP) onto their surface. The surface chemical compositions, growth manner, surface morphologies, and wetting ability of CS/SCP multilayer-modified CA membranes were characterized, respectively. The systematical evaluation of hemocompatibility revealed that CS/SCP multilayer-modified CA membranes significantly improved blood compatibility including resistance to non-specific protein adsorption, suppression of platelet adhesion and activation, prolongation of coagulation times, inhibition of complement activation, as well as reduction in blood hemolysis. Meanwhile, CS/SCP multilayer-modified CA membranes exhibited strong growth inhibition against *Escherichia coli* and *Staphylococcus aureus*, as well as high scavenging abilities against superoxide and hydroxyl radicals. In summary, the CS/SCP multilayers could confer CA membranes with integrated hemocompatibility and antibacterial and antioxidant activities, which might have great potential application in the biomedical field.

1. Introduction

Cellulose acetate (CA) membranes have been frequently used in blood purification therapies, such as hemofiltration, hemodialysis, and hemodiafiltration [1,2]. However, one of the most serious problems of CA membranes is material-induced thrombus formation on the blood-contact surface because of their inadequate hemocompatibility, which restricts the practical application of CA membranes in the biomedical field to some extent [3,4]. Thus, the hemocompatibility must be further improved for better use.

Over the past decades, numerous modification strategies have been carried out to improve the hemocompatibility of CA membranes, such as blending [5,6], surface grafting [4], and coating methods [7]. Blending hemocompatible polymers into the CA matrix is considered as the simplest method. Ye et al. have reported that blending with a phospholipid polymer is effective for improving hemocompatibility and reducing protein fouling on CA flat-sheet membranes and hollow fiber membranes [6,8,9]. Senthilkumar et al. blended polyetherimide and polyethylene glycol with a CA matrix and found that the blood compatibility of the obtained CA membrane significantly improved [3]. However, phase separation occurred probably because of the poor miscibility between the polymers and CA, causing a decrease in mechanical stability of the membrane. Surface grafting modification through covalent immobilization of low-molecular-weight compounds [10], hydrophilic polymer [4], and biologically active heparin [11] onto CA membrane surface is another method used to improve the hemocompatibility of CA membranes [12]. The involved covalent grafting methods are various, such as surface-initiated ATRP [13,14] and plasma surface grafting [15,16]. Although covalent grafting makes a stable linkage between the molecules and membranes surfaces for a long time, some inherent limitations might still restrict its wide implementation, such as reduced molecular bioactivities, high cost of treatment equipment, or complicated uncontrollable chemical reactions [17,18].

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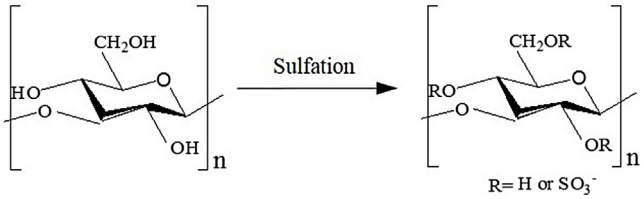
The above-mentioned limitations may be circumvented using the layer-by-layer (LBL) self-assembly technique, which was first introduced by Decher and Hong [19,20]. This technique, which involves the alternate adsorption of oppositely charged species from dilute solution, has proven to be a simple, inexpensive, efficient and versatile surface modification approach by constructing nanoscale/microscale multilayers with predetermined layer composition and tailored properties onto a solid substrate surface [21]. The LBL technique has been used on various substrates with bioactive molecules as building blocks, such as enzyme [22], DNA [23], or protein [24]. Recently, interest has arisen in the use of LBL multilayers to develop biocompatible and bioactive surfaces, and these functionalized surfaces are of great significance for basic and applied studies in the biomaterial field [25–27]. In particular, the polysaccharide-based multilayers have attracted considerable attentions in biomaterials and drug delivery application due to their excellent biocompatibility [28,29]. Considerable research has been conducted to improve the hemocompatibility of biomaterial surfaces using polysaccharide-based multilayers [30,31]. The polysaccharides used in such LBL systems primarily involve the positively charged polyelectrolyte chitosan (CS), a natural biopolymer that has many favorable biological properties, such as biocompatibility, biodegradability, antimicrobial activity, and hemostatic activity [32–34], and has been used for biomaterial applications, such as tissue engineering and wound dressings [35]. However, the literatures indicated that CS can activate both complement and blood coagulation systems [36–38], resulting in surface-induced thrombosis and blood coagulation for blood-contacting applications. The research of Amiji suggested that the thrombogenic property of CS can be effectively reduced by complexation–interpenetration methods using the sulfonate derivative of poly(ethylene glycol) [39], anionic polysaccharides [40]. The choice of negatively charged polysaccharides, including heparin [41], chondroitin sulfate [42], dextran sulfate [32], hyaluronic acid [43], and alginate [33], is diverse. Among of the above-mentioned anionic polysaccharides, heparin is the most commonly used anticoagulant drug in clinical practice and has also been widely used as an anticoagulant coating on the blood-contacting material surfaces [17]. However, the main problems of using heparin are its high cost and potential side effects (internal bleeding, fast biodegradation, and thrombocytopenia), so heparin may be unsuitable for the large-scale biomedical applications [44,45]. Therefore, it is quite necessary to find alternative anticoagulants from natural products with low cost and minimal side effects for modifying biomedical material surfaces.

It is considered that the anticoagulant activity of heparin is mainly caused by the negatively charged groups (carboxyl and sulfonic groups) on the backbone [46]. Antithrombin III (AT) changes its conformation when it binds to heparin through electrostatic interaction between these negatively charged groups and positively charged lysine residues on antithrombin, this alteration exposes the active loop, which is taken up by the active sites of coagulation factors Xa and IIa (also called thrombin), and promotes the inactivation of thrombin by formation the thrombin–antithrombin III (TAT) complex [47]. Accordingly, considerable efforts have been made to design and synthesize heparin-mimicking polymers containing sulfate, sulfamide, and carboxylate groups [44,48–50]. Compared with heparin, the synthetic heparin-mimicking polymers exhibit lower cost, better defined chemical structures, and some specific bioactivities, such as anti-coagulation [51], anti-inflammation [52], anti-tumor [53], and promotion of cell attachment and growth [54]. Zhao et al. [44,48,51,54,70] synthesized a series of heparin-mimicking polymers [e.g., heparin-mimicking polyurethane, sulfonic poly(ether sulfide), and poly(styrenesulfonate-*co*-sodium acrylate), etc.] to improve the hemocompatibility of membranes for medical use.



Scheme 1. Chemical reaction formula of SCP production.

However, these reported synthetic processes are relatively complicated, time consuming, or costly. Over the past decades, sulfated polysaccharides as anionic polymers possess similar chemical structures and biocompatibility to heparin and have attracted great research attention because of their excellent anticoagulant activity [55]. Sulfated polysaccharides, such as sulfated CS, sodium alginate, and hyaluronic acid, have been regarded as heparin-mimicking macromolecules with simple and inexpensive synthetic process and lower potential of biological side effects [55]. In this study, we used fungi polysaccharide as a model biomacromolecule to design sulfated polysaccharide as an anticoagulant reagent for developing a new and green potential alternative to heparin. Many studies have confirmed that the sulfated fungi polysaccharides exerte potent biological properties compared with non-sulfated fungi polysaccharides, such as anticoagulant, antivirus, and antibacterial activities [56]. Almost no reports are available on the use of bioactive sulfated fungi polysaccharides as an LBL building block to improve the surface biological properties of the CA membrane. In this study, we extracted polysaccharides from fruiting bodies of *Cantharellus cibarius*, which is an edible mushroom that mainly consists of polysaccharides, vitamins, minerals, volatile oils, polypeptides, and amino acids, and is widely distributed in Asia, America, and some European countries. Polysaccharide is reportedly one of the major active components of this fungus [57]. The extracted polysaccharides were further modified by concentrated sulfuric acid method to obtain water-soluble sulfated *Cantharellus cibarius* polysaccharides (SCP). SCP exhibited an anionic polyelectrolyte character due to the introduction of sulfonic groups.

In this study, we modified the CA membrane surfaces through the LBL deposition of CS and SCP. The chemical compositions, growth manner, surface morphologies, and wetting properties of CS/SCP multilayers were studied in detail. The hemocompatibility of the modified CA membranes were systematically evaluated by protein adsorption, platelet adhesion and activation, blood coagulation times, coagulation activation, blood-related complement activation, as well as blood cell hemolysis. Moreover, the antibacterial activities of the modified CA membranes were examined against *Escherichia coli* and *Staphylococcus aureus*. The antioxidant activities of the modified CA membranes were studied using the free radical scavenging assay.

2. Materials and methods

2.1. Materials

Commercial cellulose acetate (CA) membranes were purchased from Safelab Technology Co. Ltd. (Beijing, China) and cut into small pieces ($1 \times 1\ cm^2$). The CA membranes were immersed into 2% v/v acetic acid for 1 h before use, and they carried negative charges because of the partial hydrolysis of surface ester groups. The water-soluble polysaccharides were isolated from fruiting bodies of *Cantharellus cibarius* using ultrasound-assisted enzymatic extraction and further modified according to the method described by Zhu et al. [56] to obtain SCP. The chemical reaction formula of SCP production is described in Scheme 1. The hydroxyl groups at