Journal of Membrane Science 498 (2016) 135–146

Contents lists available at ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

Heparin-mimicking polyethersulfone membranes – hemocompatibility, cytocompatibility, antifouling and antibacterial properties

Shuang-Si Li [a], Yi Xie [a], Tao Xiang [a], Lang Ma [a], Chao He [a], Shu-dong Sun [a,*], Chang-Sheng Zhao [a,b,**]

[a] College of Polymer Science and Engineering, State Key Laboratory of Polymer Materials Engineering, Sichuan University, Chengdu 610065, People's Republic of China

[b] National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, People's Republic of China

ARTICLE INFO

Article history:
Received 3 August 2015
Received in revised form
18 September 2015
Accepted 20 September 2015
Available online 25 September 2015

Keywords:
In situ cross-linking polymerization
Polyethersulfone membrane
Heparin-mimicking
Biocompatibility
Antibacterial property

ABSTRACT

In this study, a series of heparin-mimicking polyethersulfone (PES) membranes were prepared through a highly efficient, convenient and universal in situ cross-linking polymerization technique coupled with a phase inversion technique. Two kinds of monomers, sodium acrylate (AANa) and sodium styrene sulfonate (SSNa) were used to introduce functional carboxyl and sulfonic groups onto PES membrane surfaces, respectively; and thus to mimic the chemical structure and biological activity of heparin. The heparin-mimicking membranes showed decreased protein adsorption, greatly suppressed platelet adhesion (decreased by more than 93%), and prolonged clotting times (prolonged as much as 60 s for APTTs and 20 s for TTs, respectively) compared to pristine PES membrane, which confirmed the enhanced blood compatibility of the modified membranes. The cell culture and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assays revealed that the heparin-mimicking membranes had a favorable trend in terms of endothelial cell proliferation and cell morphology. Moreover, the membranes showed good antifouling property. These results confirmed that the highly efficient and convenient in-situ polymerization had endowed the heparin-mimicking membranes with excellent biocompatibility, which might have great potential application in blood purification fields. In addition, the membranes were loaded with Ag nanoparticles, for which exhibited significant inhibition capability for *Escherichia coli* and *Staphylococcus aureus*, and thus confirmed the versatility of the protocol.

1. Introduction

Heparin, a linear polysaccharides consisting of repeating disaccharide units of 1, 4-linked uronic acid (D-glucuronic(GlcA) or L-iduronic acid(IdoA)) and D-glucosamine (GlcN), has a higher negative charge density than any other known biological macromolecules due to the presence of negatively charged carboxyl and sulfonic group [1,2]. It is capable of interacting with coagulation factors XIa, IXa, Xa, and IIa (thrombin), and has been widely used as anticoagulant reagent. However, it is difficult to directly use heparin as anticoagulant material to enhance the hemocompatibility of polymeric membranes due to its water solubility; but many studies have been carried out on surface heparinization, such as blending [3], surface grafting and surface coating [4,5], as well as layer-by-layer assembly [6]. These heparin-modified membranes showed improved hemocompatibility due to the bioactivity of heparin. However, as a product derived from animals, direct utilization of heparin for membrane modification does exist some drawbacks, e.g. the high cost of heparin inhibits its large-scale use for membrane surface modification; moreover, a dramatic loss of bioactivity and degradation in vivo will occur in biological systems due to the covalent or noncovalent bindings with blood components etc. which lead to the lack of stability and durability [3,7]. All these may prevent its practical application in biomedical devices. Therefore, it is of great importance to find an alternative to be used for modifying biomedical membranes.

It is considered that the anticoagulant activity of heparin is mainly caused by the existence of the carboxyl and sulfonic groups on the backbone [2]. Accordingly, great efforts had been made to design and synthesize heparin-mimicking polymers, containing sulfate, sulfamide and carboxylate groups [2,8]. The synthetic heparin-mimicking polymers showed some outstanding advantages, such as

* Corresponding author.
** Corresponding author at: College of Polymer Science and Engineering, State Key Laboratory of Polymer Materials Engineering, Sichuan University, Chengdu 610065, People's Republic of China. Fax: +86 28 85405402.
E-mail addresses: zhaochsh70@scu.edu.cn, zhaochsh70@163.com (C.-S. Zhao).

http://dx.doi.org/10.1016/j.memsci.2015.09.054
0376-7388/

anticoagulant ability [9], mediated inflammation [10], and promotion of cell adhesion and proliferation by binding and stabilization of cell growth factors [8,11]. Due to the excellent advantages, the heparin-mimicking polymers were used to design and prepare polymeric membranes to improve the biocompatibility [12–15]. After introducing the functional groups, the membranes showed enhanced biocompatibility. In our recent studies, several heparin-mimicking polymers were synthesized for the modification of polyethersulfone (PES) membranes. However, due to the poor miscibility between the polymers and PES, the blended amounts of the polymers into PES were limited. In order to solve this problem, heparin-mimicking PES was designed for improving the blood compatibility of PES membranes [16–18]. However, the above methods were sometimes limited, since the synthesis of the polymers was a time-consuming complicated process [19–21].

To further develop the physical blending method and allow it to be more suitable for industrial applications, we recently provided a method termed “in situ cross-linking polymerization/copolymerization” [22–24]. Due to the semi-interpenetrating network generated during the polymerization/copolymerization, the obtained blending system showed excellent miscibility; and the resulted membranes showed no clear phase separation and displayed not only better blood compatibility but also a good mechanical property [25,26].

In this study, inspired by the above heparin-mimicking concept and in situ cross-linking polymerization method, a simple and convenient method to introduce carboxyl and sulfonic groups into PES membranes by in situ cross-linking polymerization was carried out and the membrane performances were explored. PES was selected as a membrane matrix because of its good oxidative, thermal, and hydrolytic stabilities, as well as good mechanical and film-forming properties; and had been widely applied in the fields of artificial organs and medical devices [27–31]. Sodium acrylate (AANa) and sodium *p*-styrene sulfonate (SSNa) were selected as the functional monomers because of their high reaction activity in free radical polymerization. Then, a series of heparin-mimicking membranes were prepared by a phase inversion technique. Furthermore, the Ag nanoparticles (Ag NPs) were embedded in the membranes to endow with antibacterial property, when considered the membranes for long-time using in future portable hemodialyzer (as shown in Scheme S1). The chemical components, surface and cross-section structure of the membranes were confirmed by ATR-FTIR, element analysis and scanning electron microscopy (SEM). While the hemocompatibility was explored by wettability, static protein adsorption, platelet adhesion, activated partial thromboplastin time (APTT), thrombin time (TT). Furthermore, human vessel endothelial cells (HUVECs) were used as model cells to investigate the cell viability of the membranes. The antibacterial property was tested via bacterial inhibition zone towards *Escherichia coli* and *Staphylococcus aureus*, respectively [32,33].

2. Experimental section

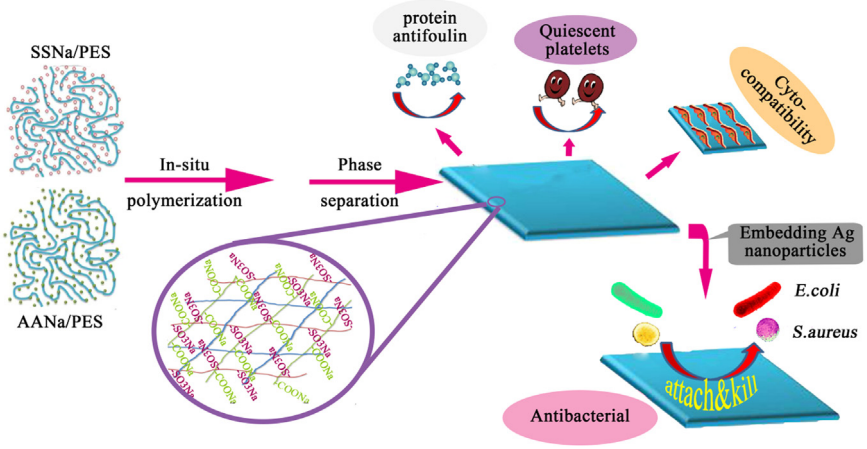
2.1. Materials

Poly (ether sulfone) (PES, Ultrason E 6020P) was purchased from BASF chemical company (Germany). Sodium 4-vinylbenzenssulfonate (SSNa, 90%) and sodium acrylate (AANa) were purchased from Aladdin Reagent Co. Ltd. (China). N, N′-methylene bisacrylamide (MBA, 98%) and azoisobutyronitrile (AIBN, 99%) were obtained from the Chemical Reagent Factory of Kelong, China. The solvent N, N-dimethylacetamide (DMAc) was distilled under reduced pressure with calcium hydride (CaH_2) to remove the water. Bovine serum albumin (BSA) and bovine serum fibrinogen (FBG) were obtained from Sigma Chemical Co. Micro BCA™ protein assay reagent kits were the products of PIERCE. APTT and TT reagent kits were purchased from SIEMENS. All the other chemicals (analytical grade) were obtained from the Chemical Reagent Factory of Kelong, China, and used without further purification. More detailed information for other materials was included in the *Supplementary materials.*

2.2. Preparation of heparin-mimicking membranes

In this paper, two kinds of monomers, AANa and SSNa, were used to introduce carboxyl and sulfonic groups, respectively. To prepare casting solution, PES and the monomer (NaAA or SSNa) were separately dissolved in a 250 mL three-necked round flask with appropriate amount of DMAc until a homogeneous solution was obtained. After pumping and aerating with nitrogen for three times, a mixture of AIBN and MBA was added into the flask (detailed components are presented in Table 1). Then the polymerization was carried out with mechanically stirring (500 rpm) under nitrogen at 75 °C for 24 h, the polymerization was then exposed to air to terminate the reaction. The obtained two kinds of solutions were named APES (for AANa and PES) and SPES (for SSNa and PES), respectively. Finally, different kinds of casting solutions were prepared as shown in Table 1. The membrane preparation process was presented in Scheme 1.

All the membranes were prepared as the following procedures. After 20-min degassing, the casting solution was spin-coated on a glass surface, which was then immersed into deionized water and



Scheme 1. Preparation process and multi-functionality of the heparin-mimicking membranes.