Preparation and hemocompatibility of electrospun Bacteria Cellulose Sulfate / Polyvinyl Alcohol nanofibrous composite membrane

Zhiming Li, Xueqiong Yin*, Jinming Qin, Li Zhu
Hainan Provincial Fine Chemical Engineering Research Center, Hainan University, Haikou Hainan 570228, People’s Republic of China
Email: yxq88@hotmail.com

Abstract: Bacterial cellulose sulfate (BCS) / poly(vinyl alcohol) (PVA) membrane (BCSm) was prepared through electrospinning BC sulfate together with PVA. To increase the stability of BCSm, BCS/PVA was electrospun together with ethyl cellulose by double nozzles to obtain composite membranes (BCSE). The membranes were characterized with SEM, water contact angles. The results of platelet adhesion experiment showed that the BCSE membrane with only nanofibers had good hemocompatibility.

1. Introduction
Biomaterial is a very important part in medical application, which could be used therapeutically or diagnostically.[1] Hemocompatibility is one of the key biocompatibilities to blood-contacting biomaterials. Heparin is a natural anticoagulants. Biomaterials have been modified to biomimic heparin through chemical grafting, plasma deposition, radiation, self-assembly, etc.[2-3]. The natural blood vessel wall has special topography. Specific chemicals and blood cells are grafted onto material surfaces, and surface microtopography features including roughness, curvature and geometrical figures of the material have also been fabricated to biomimic the structure of blood vessels wall to get good hemocompatibility[4,5]

Nanofibrous mats have been widely researched in the field of biomaterials, owing to their unique structures, such as high porosity, high surface area, similarity with the native extracellular matrix (ECM) [6]. Electrospinning is an effective methodology to prepare nonwoven membrane to mimic the surface of blood vessel wall, including chemical structure and morphology. [7,8] Up to now, electrospun polymer materials have been widely investigated in biomaterials, such as wound healing materials, drug delivery, medical textiles, tissue engineering scaffolds and blood-contacting biomaterials.

BC is an exocellular polysaccharide of microbial fermentation, which has natural nanofibers[9]. BC has been widely used in the functional membrane, food, textile, biomedical, battery, catalyst and other fields [10]. Compared with other polysaccharides, BC has good mechanical properties and biocompatibility. BC could be modified through various chemical modification. -SO_3H groups play an important role in the anticoagulation of heparin and other polysaccharides [11] BC sulfate (BCS) is water-soluble, which could be electrospun in aqueous solution.

In this paper, BC was modified by SO_3H/pyridine (Py) to prepare water soluble BCS. BCS was electrospun together with poly(vinyl alcohol) (PVA) to prepare nanofibrous membranes (BCSm). In order to improve the stability of BCSm in water, BCS/PVA was mixed with ethyl cellulose (EC) by double nozzle electrospinning. The effects of electrospinning conditions were optimized. The
morphology, wettability and hemocompatibility of the membranes were investigated.

2. Experimental

2.1 Materials
BC was purchased from Hainan Million Germany Food Co., Ltd (Mw=4.64×105 g/mol). PVA (99% hydrolyzed, Mw 120,000 g/mol) was purchased from Sigma-Aldrich. N, N-dimethylacetamide (DMAc), Lithium chloride (LiCl), SO$_3$/Py, and KOH were purchased from Sinopharm Group Company Limited (China) and used as received.

2.2 Preparation of BCS
BC gels were purified according to previous research. The obtained BC powders were dissolved in DMAc/LiCl based on the procedure described in our previous paper[12]. SO$_3$/Py (6:1 to the glucose residue of BC) was added to BC solution. The sulfation reaction was carried out for 6 hours. Then, BC solution was poured into 250 ml ethanol. The precipitate was filtered and washed with ethanol for 3 times, and then put into 40 ml 0.5 mol/L NaOH. The obtained solution was dialyzed for 3 days and then lyophilized to obtain BCS powders.

2.3 Electrospinning of BCSm and BCSE membranes
BCS/PVA solution with different mass ratio and concentration was prepared under magnetic stirring for further electrospinning. The electrospinning experiment was carried out on an electrostatic spinning instrument (DT-200, Dalian Dingtong Technology Development Co., Ltd.) at different spin voltage and distance. The electrospun polymer fibers were collected on a collector covered with aluminum foil.

To prepare BCSE membranes, BCS/PVA and the EC solution were put into two different syringe and delivered to two nozzle by the syringe pump at a constant feed rate (0.7 mL/h). The distance between the tip of the BCS/PVA nozzle and the electrode was maintained at 20-23 cm, and that of EC nozzle and the electrode was maintained at 23 cm. The electrospun polymer fibers were collected on a collector covered with aluminum foil.

2.4 Characterization of BCSm and BCSE membranes
The surface morphology of BC/PVA and BC-PVA/EC were observed with a S-3000 N scanning electron microscope after gold coating with the voltage of 10 kV. The water contact angle of the membranes was measured on a SL200K contact angle measurement instrument at 20°C. Fourier transform infrared spectroscopy (FTIR) spectra were obtained on a TENSOR 27 spectrometer in the range of 500–4000 cm$^{-1}$.

2.5 Hemocompatibility measurement
Fresh human blood mixed with sodium citrate was centrifuged at 3000 r/min for 10 min to obtain platelet-poor plasma (PPP) and platelet-rich plasma (PRP). To investigate the platelet adhesion of BCSE, 250 uL PRP was poured onto BCSE (0.5 cm × 0.5 cm) and allowed to maintain at 37°C for 2 h. Then the samples were carefully washed with saline to remove the nonadhered blood cells. The adhered blood cells were fixed by glutaraldehyde. The surface of the mats was observed with a JSM-7100F field emission scanning electron microscopy (JOEL, Japan) after gold coating.

3. Results and Discussions

3.1 Preparation and characterization of BCS
The FTIR spectra of BC and BCS were shown in Figure1. For BC (a), the peak at 3300 cm$^{-1}$-3600 cm$^{-1}$ was the stretching vibration peak of –OH, that at 2910cm$^{-1}$ was the stretching vibration peak of C-H, 1160cm$^{-1}$ was the stretching vibration peak of C-O, and 1070cm$^{-1}$ was the stretching vibration
peak of C-O-C. Compared with the spectrum of BC (a), BCS (b) had an obvious peak around 1257cm\(^{-1}\) and 815cm\(^{-1}\), corresponding to stretching vibration peak of O=S=O and the symmetrical stretching vibration peak of C-O-S, respectively. The FTIR spectra verified successful sulfation of BC.

![Figure 1 FTIR spectra of Bacterial Cellulose (a) and Bacterial Cellulose Sulfate (b)]

3.2 Electrospinning and wettability of BCSm

The electrospinning parameters (BCS and PVA amount, electrospinning voltage, and the distance from the nozzle to the collector) were shown in Table 1.

| Sample | BCS (g) | Water (ml) | PVA (ml) | Polymer Conc. (g/ml) | Distance (cm) | Voltage (kv) | NaCl (g) |
|--------|---------|------------|---------|----------------------|---------------|--------------|----------|
| PVA    | 0       | 0          | 15      | 0.08                 | 20            | 20           | 0        |
| BCS1   | 0.47    | 20         | 5       | 0.0235               | 22            | 25           | 0.1      |
| BCS2   | 2       | 40         | 5       | 0.0445               | 20            | 25           | 0.1      |
| BCS3   | 5       | 40         | 5       | 0.11                 | 22            | 25           | 0.1      |
| BCS4   | 6.5     | 40         | 15      | 0.118                | 23            | 35           | 0.1      |
| BCS5   | 6.5     | 40         | 20      | 0.108                | 23            | 25           | 0.2      |

The SEM images of pure PVA and BCS/PVA were shown in Figure 2. As shown in Figure 2, there were fibers and beads in BCS1-BCS3, which was electrospun at similar conditions. And the fibers increased with the increase of polymer concentration, which might be due to polymer chains with low concentration could not conjugate together and the chains were easy to be broken. Further increase the concentration, BCS4 had more fibers while beads were still existed. After sulfation, the molecular weight of BC decreased. Therefore, more PVA is required to assist BCS molecules conjugation. BCS was negatively charged. To prepare BCS5, more salt NaCl and PVA was used to assist BCS electrospin and the voltage was decreased to 25kV to avoid too high stretching force. BCS5 with uniform, smooth, non-bead fibers were obtained by using a polymer solution with a concentration of 0.108 g/ml, containing 6.5g BCS and 20ml PVA, at distance 23 cm and the voltage 25 kv, with the existence of 0.2 g of NaCl.
Table 2 showed the water contact angles (WCA) and surface free energy (SFE) of BCSm membranes. As shown in Table 2 (BCS1-BCS4), WCA decreased with the increase of BCS content due to the presence of -SO₃H and -OH in BCS. BCS5 has only nanofibers and more NaCl was existed in the membrane, which resulted in the highest hydrophilicity and the lowest WCA. The surface free energy and the WCA were opposite to each other. Therefore, the surface free energy increased with the decreasing of WCA.

| WCA (°)  | BCS-1 | BCS-2 | BCS-3 | BCS-4 | BCS-5 |
|----------|-------|-------|-------|-------|-------|
| WCA      | 87.4  | 54.1  | 41.4  | 29.8  | 27.5  |
| SFE (J)  | 316.73| 400.54| 452.2 | 614.54| 634.55|

### 3.3 Preparation and wettability of BCSE membranes

BCSE membranes were prepared by BCS and EC through electrospinning with double nozzles at the same conditions of BCS membranes accordingly. The distance between the two nozzles was kept at 4.5 cm. As shown in Fig. 4, though BCSE1-BCSE3 had nanofibers and beads, similar with that of BCS1-BCS3 membranes, there were more fibers in BCSE membranes. Unlike BCS4, BCSE4 had almost no beads. BCSE5 had similar morphology with BCS5. The differences between BCS membranes and BCSE membranes indicated that the electrospinning with two nozzles is easier than with one nozzle, which might be due to the static interaction between the two nozzles increased the stretching force.

As shown in Table 3, compared with BCS membrane (in Table 2), the WCA of BCSE-3—BCSE-5 were increased. The membrane prepared from 5% EC had a WCA of 95°, indicating hydrophobicity. Therefore, the existence of EC in BCSE membrane increased the hydrophobicity of BCSE-4, BCSE-5. BCSE1 and BCSE2 had low WCA than BCS1 and BCS2, which might be due to the differences of
morphology of the two kinds of membranes. BCSE membranes could be stable in water, which enables a successful detection of anticoagulant experiments in aqueous environment.

| Table 3 WCA and surface free energy of BCSE membranes |
|------------------------------------------------------|
| WCA (°) | 5% EC | BCSE1 | BCSE2 | BCSE3 | BCSE4 | BCSE5 |
|---------|--------|--------|--------|--------|--------|--------|
| SFE (J) |        | 285.2  | 380.65 | 444.4  | 465    | 412.3  | 412.3  |

### 3.4 Hemocompatibility of BCS-PVA/EC

![Figure 4 SEM images of BCSE membranes after contacting with PRP](image)

Low platelet adhesion and activation reveals good hemocompatibility.[13] Therefore, platelet adhesion experiment was carried out to measure the hemocompatibility of BCSE membranes. The SEM images of BCSE after contacting with PRP for 2 h were shown in Figure 4. As shown in Figure 4, the morphology of BCSE1-BCSE3 had been changed after contacting with PRP. The fibers and beads of the membranes became unclear, which might be due to substances such as protein in PRP or disrupted platelets. However, there was no platelets adsorbed on BCSE5 having only nanofibers. BCSE membrane had –SO3H, OH groups, similar with heparin. The nanofibers (diameters 50-100 nm) of BCSE5 are similar with ECM (diameters 50-500 nm). Moreover, the pores in BCSE5 form channels which are similar with the micro-grooves on natural blood vessel surface. The chemical and morphology structure characteristics of BCSE5 enable its good hemocompatibility. Therefore, no platelets were adsorbed on the surface of BCSE5.

### 4. Conclusions

BC was modified by SO3/pyridine to prepare water soluble BC sulfate. BCS membranes with nanofibers were successfully prepared by using a polymer solution with a concentration of 0.108 g/ml, containing 6.5g BCS and 20ml PVA, at distance 23 cm and the voltage 25 kv. BCSE membranes prepared through electrospinning BCS/PVA and EC with double nozzles had better hydrophobicity. BCSE membrane with only nanofibers had no platelet adhesion after contacting with PRP, indicating good hemocompatibility and potent for application in blood-contacting materials.

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