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Layer-by-Layer Structured Membranes of Silk Fibroin and Polyethylenimine on Electrospun Silk Fibroin Nanofibers

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Abstract: Self-assembled silk fibroin (SF)-polyethylenimine (PEI) multilayered films were fabricated on ethanol treated electrospun SF nanofibrous substrates *via* the electrostatic layer-by-layer (LBL) adsorption. The film coated membranes were characterized using scanning electron microscopy (SEM), transmission electron microscope (TEM) and X-ray photoelectron spectrophotometer (XPS). The SEM images showed that the multilayers of SF-PEI were formed on the surface of the ethanol treated SF nanofibres. The characteristics such as the fiber shape and porous structure were well maintained as the number of the coated SF-PEI bilayers was less than five. However, obvious adhesive substances and blocked pores were observed on the surface of the fibers as the number of bilayers of SF-PEI increased to six. Furthermore, the obvious core-shell structures were observed by TEM. The thickness of five SF-PEI bilayers was approximately 80nm. Additionally, the XPS results also revealed that the SF-PEI multilayer composite membranes formed. The adsorption mainly depended on a simple electrostatic interaction between the layers of SF and PEI. These SF-PEI multilayer assembled nanofibrous membranes could be a promising material for use as a sensor, gene delivery agent and scaffolds.

Keywords: Electrostatic adsorption, morphology, polyethylenimine, self-assembly, silk fibroin.

1. INTRODUCTION

Ultra-thin nanocomposite films with various inorganic and organic compositions, have gained widespread attention. These new nanocomposite materials offer great potential applications, such as adsorbents, sensors, data-storage devices, microelectronics, memory devices, display technologies and composites [1]. Electrostatic layer-by-layer (LBL) method is one of the most frequently utilized processes for fabrication of functional ultra-thin films. The mechanism is based on alternating deposition of oppositely charged particles or polyelectrolytes (PEL), and electrostatic attraction between opposite charges and electrostatic repulsion between same charges coexist during the process of self-assembly [2]. These forces cannot make the adsorption capacity of each layer increase indefinitely. Therefore, the properties of composite nanofibrous membranes mainly depend on two aspects: the characteristics and the order of each layer [3]. Recently, many researchers have been involved in the study of ultra-thin films with nanofibrous membrane as matrix, mainly due to the characteristics of electrospun nanofibres, such as high porosity, large surface area per unit mass, high permeability and small interfibrous pore size. Furthermore, the thickness, morphology, composition and function of the targeted materials can be controlled by changing the number of PEL adsorption cycles and altering the composition of the layer component [4]. By using the LBL assembly method, large variety of materials involving PEL [4], nanoparticales [5] and protein [6] are able to be assembled onto nanofibrous substrates to form functional nanostructured materials. These functional nanomaterials prepared by the LBL method have been applied in many fields, such as sensors [7], catalysts [8], environmental remediation [5] and biomedical materials [9]. Ding *et al.* fabricated a series of multilayer on electrospun cellulose acetate (CA) nanofibers, including phthalocyanine and polyacrylic acid (PAA) [10], TiO_2 nanoparticales [7] and PAA, polyoxometalate and polyethylenimine (PEI) [11]. Multilayer of Poly (diallyldimethy lammonium chloride) PDADMAC) and PAA on CA nanofibrous membranes were prepared by S Xiao through electrostatic LBL assembly. Furthermore zero-valent iron nanoparticles were fabricated and immobilized onto these polyelectrolyte (PE) multilayer-assembled electrospun CA nanofibers, which is very useful for potential environmental applications [5].

SF is a common natural macromolecule, which is extracted from the *Bombyx mori* silkworm. The electrospun SF nanofibres including many amino acids are an excellent substance as a result of charged surface. Furthermore, SF can

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be easily regenerated and fabricated into nanofibrous membrane *via* electrospinning [12].

PEI is a cationic polyelectrolyte (PEL). The chemical structure of the PEI was shown in Fig. (**1**). The research focused on the field of application, such as immobilization materials for biosensors, sensing material for various gases, gene delivery agent and scaffolds [13-15]. However, it is very difficult to fabricate pure PEI nanofibres by electrospinning directly [16]. Consequently, Water-insoluble SF nanofibres were used as the fiber substrates for the electrostatic LBL adsorption of PEI.

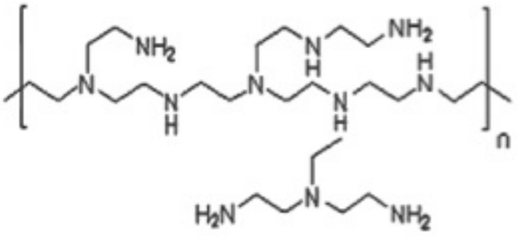

Fig. (1). Chemical structure of PEI.

In the present work, the electrospun SF nanofibres treated with ethanol were employed as the substrate. And SF- PEI multilayered films were coated on these substrates *via* an LBL assembled method. Their morphology and surface chemistry were investigated using SEM, TEM and XPS.

2. EXPERIMENTAL

2.1. Materials

Water-insoluble SF nanofibrous substrates were prepared as reported previously [17]. PEI was used as-received from Xiya Chemical Regent Inc (M_w=80000, China).

2.2. Formation of SF-PEI Films on Nanofibrous Substrates

SF solution used for deposition was prepared by drying the SF dilute solution (dialysis solution). The concentration was 2 g/L with a pH value of 10 (adjusting by 1M acetic acid and 1M sodium hydroxide). PEI was dissolved in deionized water with vigorous stirring. The volume concentration of PEI was fixed as 2 g/L. Prior to deposit PEI film, the ethanol treated SF nanofibrous membranes were first dipped into dilute sodium hydroxide solution for 5 min to endue with negative charges (as shown in Fig. **2**). Followed PEI films were deposited onto negatively charged SF substrates in PEI solution for 15min, and then rinsed in water baths for 2min. The desired numbers of SF-PEI bilayers can be achieved by repeating above steps (as shown in Fig. **3**). The original SF nanofibrous membrane was regarded as a base layer. Each assembly of a PEI layer followed by a SF layer was considered as bilayers. The SF-PEI multilayers thus obtained were represented as $(\text{SF-PEI})_n$.

2.3. Characterization

The ethanol treated SF nanofibrous substrate was observed with field emission scanning electron microscope (FESEM) (JEM-2100F, JEOL Co., Japan). The SF-PEI Layer-by-layer structured membranes with gold coating were characterized using scanning electron microscope (SEM) (JSM-6360LVSEM, JEOL Co., Japan) with an accelerating voltage of 15kV. The fiber diameter distributions were determined by an image measuring software on the FESEM and SEM images. The microstructure of the films coated SF substrate was observed using a transmission electron microscope (Tecnai G2 F20, *FEI Co.,* US). The surface chemistry of the SF-PEI layer-by-layer structured substrates was characterized by an X-ray photoelectron spectrophotometer (Thermo Escalab 250, *Thermo Electron Corporation.*, US).

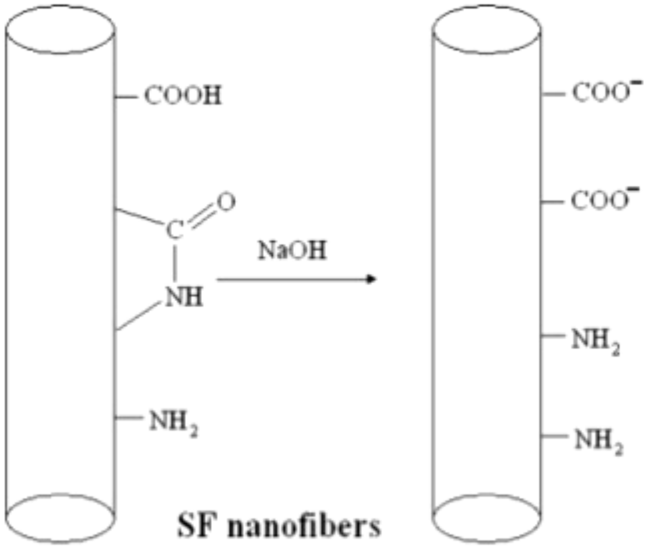

Fig. (2). Schematic diagram of negatively charged SF nanofibres.

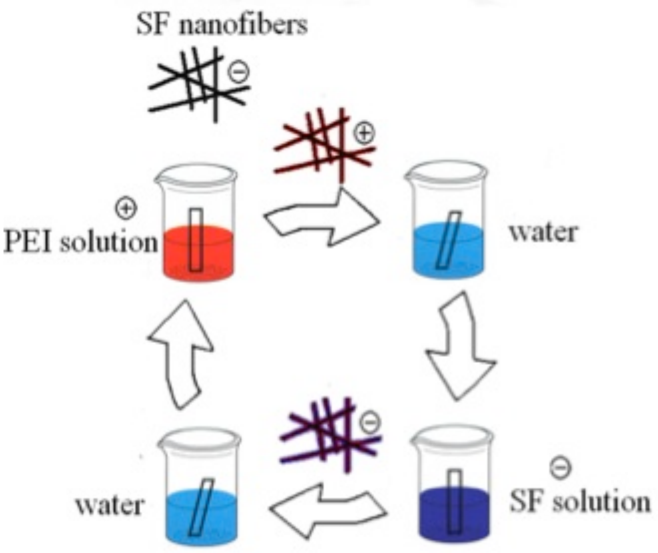

Fig. (3). Flow chart of self-assembly process of SF-PEI.

3. RESULTS AND DISCUSSION

3.1 Morphological Characteristics

In order to obtain good hydrolytic stability, SF nanofibrous substrates were treated with ethanol for 60min to induce the conformation transition [18, 19]. Morphology and diameter distribution histogram of these water-insoluble SF nanofibrous substrates were shown in Fig. (**4**). It could be seen that the ethanol treated electrospun SF substrates exhibited obvious features of electrospun nanofibers, such as thin and uniform fiber shape and porous structure. The average diameter was 382.5 nm.

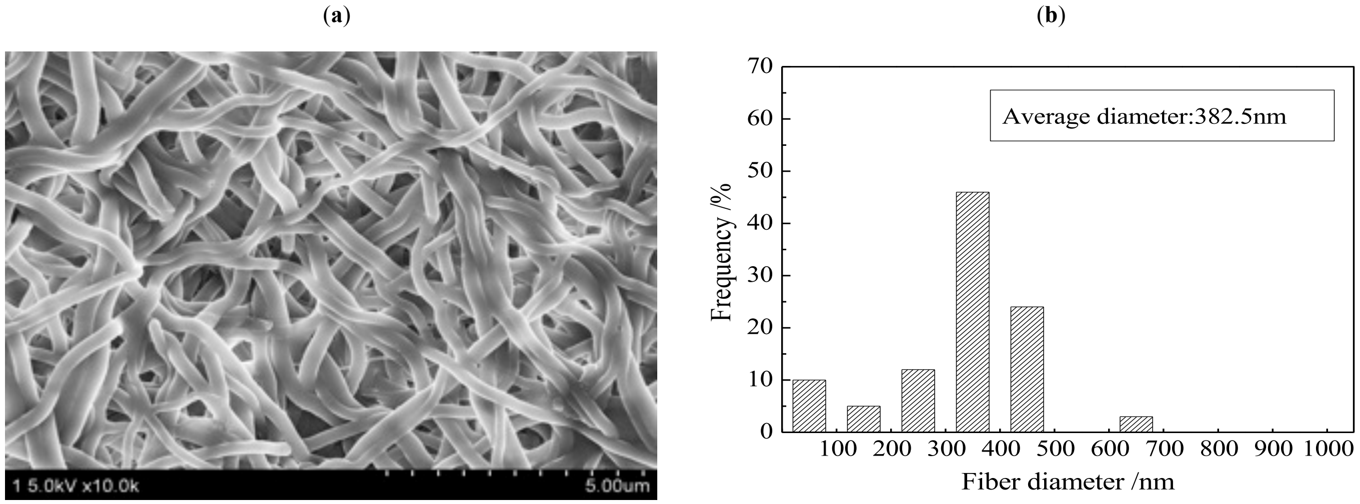
(**a**) (**b**)

Fig. (4). FESEM image (**a**) and diameter distribution (**b**) of SF nanofibres treated with ethanol.

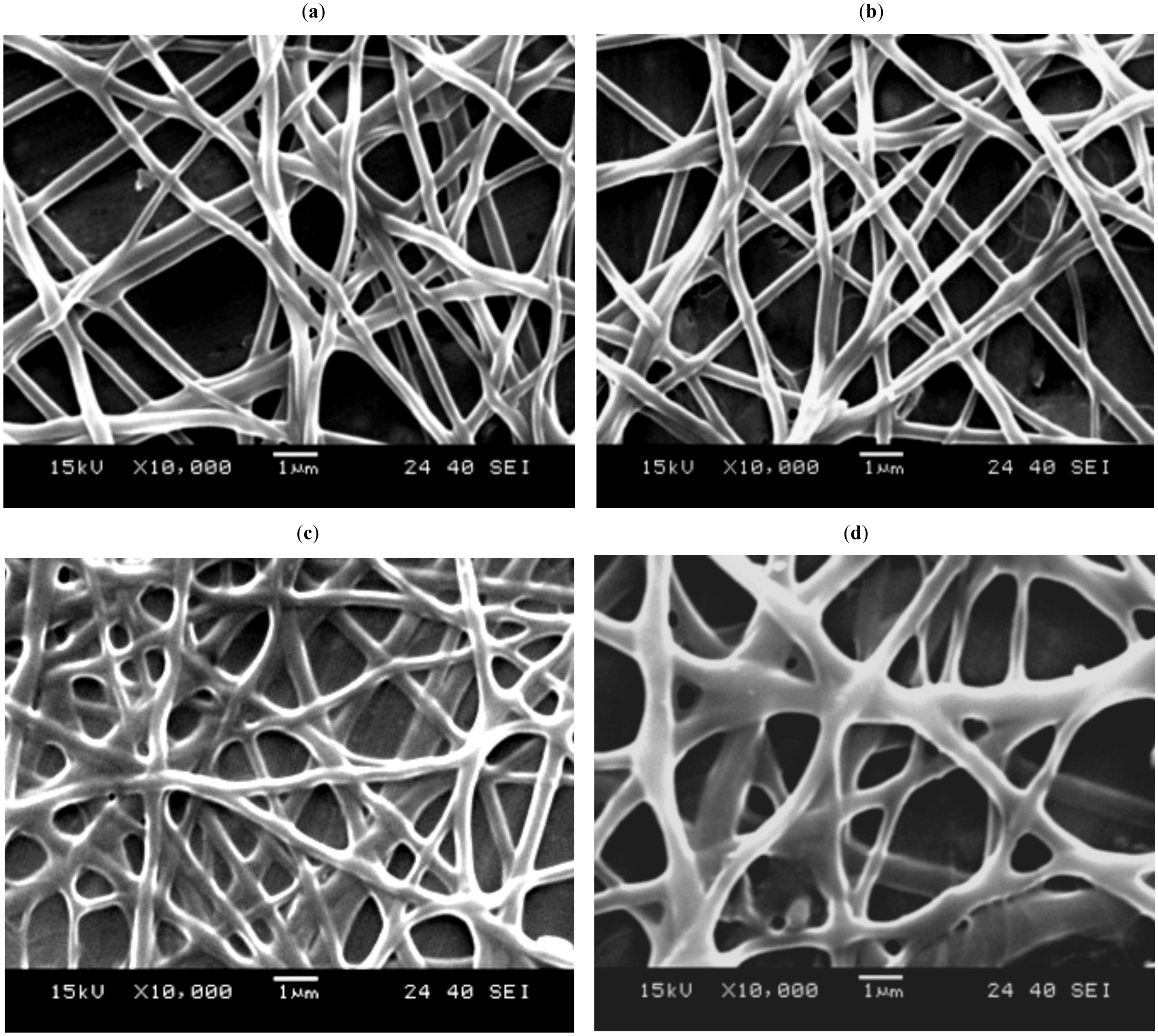
(**a**) (**b**) (**c**) (**d**)

Fig. (5). SEM micrographs of SF nanofibers deposition with (**a**) two, (**b**) four, (**c**) five, (**d**) Six bilayers of SF-PEI.

These water-insoluble SF nanofibrous substrates were then LBL-assembled with SF-PEI multilayer to fabricate PEI ultra-thin films coated nanofibrous membranes. In order to maintain the final formed SF-PEI coated SF nanofibrous membranes with porous structure, the number of multilayer deposited onto the ethanol treated SF nanofibrous substrates was optimized. The films coated SF substrates with 2, 4, 5 and 6 bilayers of SF-PEI were fabricated, respectively. Fig. (**5**) showed the morphological structures of SF nanofibres assembled with different layers of SF-PEI. The fiber shape and porous structure were well maintained when the number of SF-PEI bilayers was less than five. Compared with the ethanol treated SF nanofibers (Fig. **4a**), no obvious changes in the morphological characteristics were observed as the number of the coated SF-PEI bilayers was less than four. This observed result indicated the thickness of SF-PEI bilayers was very thin (Fig **5a**, **b**). Increasing the number of bilayers could greatly affect the morphology of LBL-assembled SF nanofibres (Fig. **5c**, **d**). When it increased to five, a rather thick of SF-PEI multilayer was formed with a homogeneous structure on SF nanofibrous substrates (Fig. **5c**). This phenomenon was quite different from that of S Xiao [5], who noted that some pearl necklace-like structures were observed when four or five PAA/PDADMAC multilayers deposited onto CA nanofibres. This difference might be due to the quite long time of LBL-assembly (10min), which leads further to the saturation of deposition of bilayers of SF-PEI. However, the morphology of coated SF nanofibrous membranes deteriorated seriously as the number of SF-PEI bilayers increased to six. As shown in Fig. (5d), an obvious adhesive substance appeared on the surface of the SF-PEI coated SF nanofibres and the pores between the nanofibres were blocked (Fig. **5d**). This might be caused by the 3D growth of SF-PEI films along the ethanol treated SF nanofibrous substrates and the limited space among the adjacent fibers [4]. Therefore, five bilayers of SF-PEI were selected to coat onto the ethanol treated SF substrates.

3.2. Diameter Distributions

Correspondingly, the diameter distribution histograms of SF nanofibers assembled with two, four, five, six bilayers of SF/PEI were shown in Fig. (**6**). The diameter increased with the increasing of the number of the SF-PEI bilayers. When the number of SF/PEI bilayers increased from two to four,

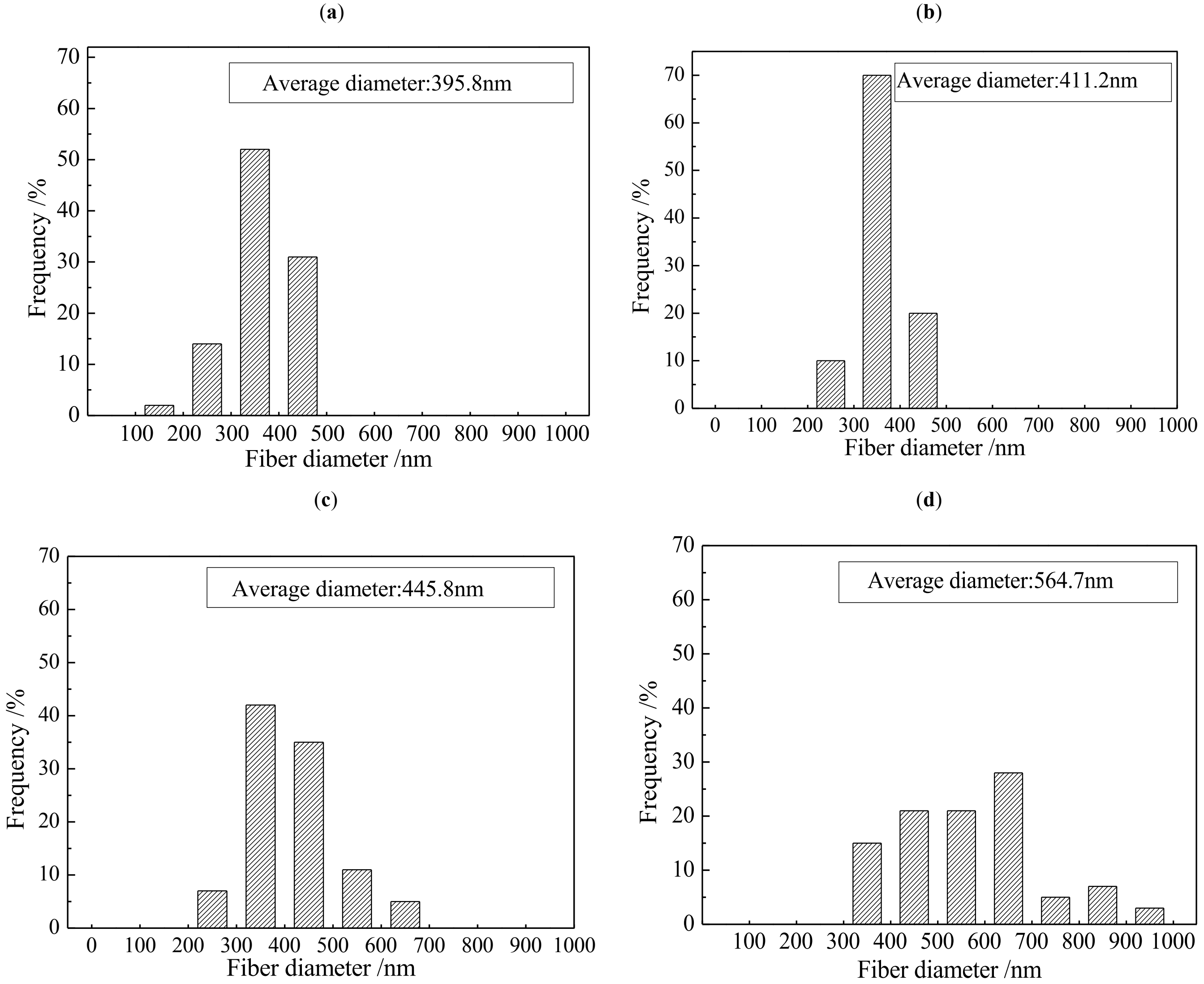

Fig. (6). Diameter distribution histograms of SF nanofibers deposition with (**a**) two, (**b**) four, (**c**) five, (**d**) six bilayers of SF/PEI.

the average diameter of coated SF nanofibers was slightly increased from 395.8 nm to 411.2 nm. Furthermore, the distribution seemed to be concentrated (Fig. **6a**, **b**). The reason might be due to the difference in diameters of the electrospun SF nanofibres, the finer the fiber diameter, the larger the fiber specific surface area [17], which leads further to higher adsorption capacity. The diameter of SF nanofibers assembled with five and six SF-PEI bilayers increased obviously, reaching about 445.8 nm and 564.7 nm, respectively. However, when the number of bilayers increased to six, the distribution deteriorated seriously with the fiber diameter ranging from 300 nm to 1 μm. Therefore, five bilayers of SF-PEI were determined to coat onto the ethanol treated SF nanofibrous substrates for potential applications, such as sensor, gene delivery agent and scaffolds.

3.3. TEM Observation

As shown in Fig. (**7**), the obvious core-shell structures were observed, indicating the multilayers of SF-PEI were coated on the surface of the ethanol treated SF nanofibres, but the layered structure could not be observed. This is due to the LBL structure associated with the interpenetrations among the layers [3], which was hard to distinguish in the layered structure in the TEM image. The image measurement results indicated that the thickness of five bilayers of SF-PEI was about 80 nm. Meanwhile, no visible junctions were found among the adjacent nanofibres, which was consistent with the results of SEM.

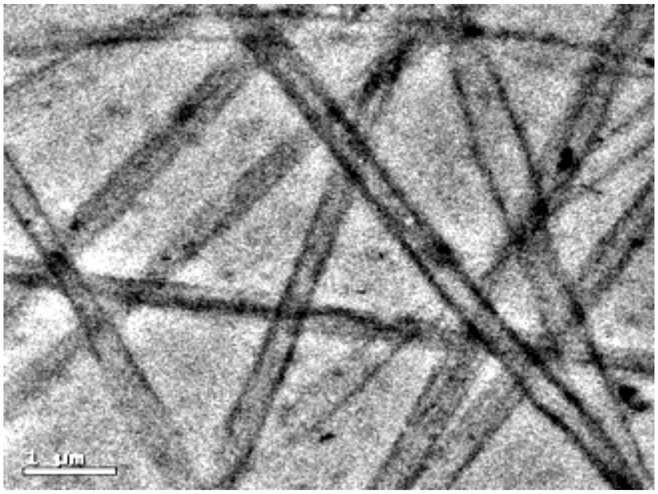

Fig. (7). TEM image of SF nanofibers assembled with five bilayers of SF-PEI.

3.4. Surface Chemistry

XPS analysis of the surface of SF nanofibers assembled with zero and five bilayers of SF-PEI was performed to determine the surface elemental composition (atoms or ions and its state). The XPS surveys of the assembled SF nanofibers binding energy ranging from 0 to 700 eV were shown in Fig. (**8**) and the element content were calculated and listed in Table **1**. Fig. (**8a**) showed the XPS survey of ethanol treated SF nanofibrous substrates. Three strong peaks appeared at 289.0 eV, 404.0 eV and 535.8 eV due to "C", "N" and "O", respectively [20]. The five bilayers of SF-PEI assembled sample showed characteristic peaks of C1s, N1s and O1s at 288.2eV, 403.0eV and 535.2eV. Moreover, the content of N increased from 10.69% to 15.10% (Table **1**). The reason was that PEI was rich in amidocyanogen, which further increased the presence of N. This result confirmed the existence of PEI in the LBL films in conjunction with the results of SEM and TEM. In addition, the presence of a single N1s peak in the XPS measurement reflected that the valence state of the nitrogen atoms from PEI did not change after LBL deposition [21]. It indicated that only a simple electrostatic interaction exited between the layers. These findings were consistent with that of B Ding [11].

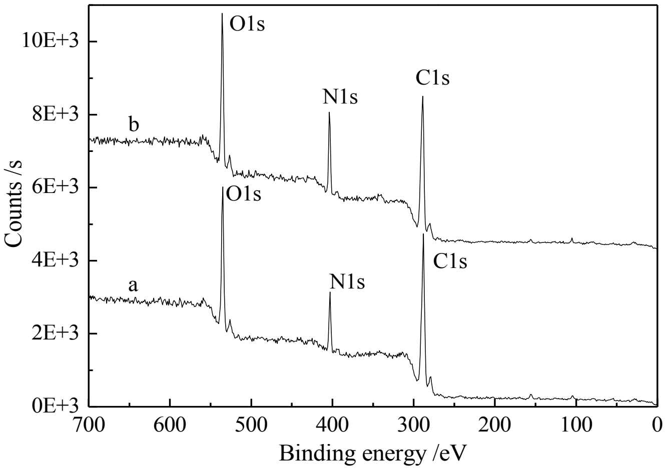

Fig. (8). XPS spectra of SF nanofibrous membranes assembled with (**a**) 0, (**b**) 5 bilayers of SF-PEI.

Table 1. Element content of SF nanofibrous membranes.

At. %	Bilayers of SF-PEI Assembled	
	0	5
C1s	69.59	64.55
O1s	19.72	20.35
N1s	10.69	15.10

4. CONCLUSION

Layer-by-layer structured membranes of SF-PEI on electrospun SF nanofibrous substrates were prepared by an electrostatic LBL self-assembly method. The morphology of LBL films coated SF nanofibrous membranes was greatly influenced by the number of SF-PEI bilayers. The characteristics, such as the fiber shape and porous structure were well maintained as the number of the coated SF-PEI bilayers was less than five. However, obvious adhesive substances and blocked pores were observed on the surface of the fibers as the number of bilayers of SF-PEI increased to six. Furthermore, the obvious core-shell structures were observed by TEM. The thickness of five SF-PEI bilayers was approximately 80 nm. In addition, the presence of a single N1s peak in the XPS measurement showed that the valence state of the nitrogen atoms from PEI did not change after LBL deposition, indicating that only a simple electrostatic interaction existed between the layers. These SF-PEI multilayer assembled nanofibrous membranes can be a promising material for use as sensor, gene delivery agent and scaffolds.

ABBREVIATIONS

LBL = Layer-by-layer
PEI = Polyethylenimine
PEL = Polyelectrolyte
SEM = Scanning electron microscope
SF = Silk fibroin
TEM = Transmission electron microscope
TFA = Trifluoroacetic acid
XPS = X-ray photoelectron spectrophotometer

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

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