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Bi-layered disulfiram-loaded fiber membranes with antibacterial properties for wound dressing

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Keywords: Bi-layered disulfiram-loaded fiber membranes; Antibacterial activity; Surface wettability; Electrospinning; Wound dressing

Abstract

In this study, the bi-layered disulfiram-loaded fiber membranes with antibacterial activity and different surface wettabilities are prepared using electrospinning technology. In the application of wound dressing, the hydrophilic surface of fiber membranes is beneficial for the cell adhesion and drug release to heal the wound, meanwhile the hydrophobic outside surface is able to block water penetration to reduce the probability of wound infection. The obtained bi-layered drug-loaded fiber membranes are composed of polyvinylidene fluoride (PVDF) bottom surface and disulfiram (DSF)/polylactic acid (PLA) top surface. To modify the top surface wettability, the oxygen plasma modification of bi-layered membranes was carried out. We analyzed the morphology, wettability and chemical compositions of bi-layered drug-loaded fiber membranes by various techniques. And the bi-layered disulfiram-loaded membranes showed potent antibacterial activity in vitro against both *Escherichia coli*

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(Gram-negative) and *Staphylococcus aureus* (Gram-positive). Thus, the obtained bi-layered disulfiram-loaded fiber membranes are very suitable for wound dressings application.

1. Introduction

Fiber membranes are widely applied in biomedicine used as wound dressings, tissue scaffold and medicine carrier. [1-3] The fiber membranes consist of various fibers with different diameters. In the application of wound dressing, it would avoid physical wound damages and balance wound microenvironments, meanwhile the membranes can serve as the drug carrier to promote the wound healing. [4,5] Therefore, drug-loaded fiber membranes prepared by biocompatible materials have become more and more popular as wound dressings. Among several approaches to prepare composite fiber membranes, electrospinning is considered to be the most practicable and helpful technique. [6] The electrospinning fiber membranes with 3D reticulate structure are very similar to the configuration of natural extracellular matrix (ECM), so it would provide suitable support site for cell adhesion and proliferation. [7] As wound dressings, the high specific surface area of electrospinning fiber membranes is conducive to absorb the wound exudate and release drugs. The small pore formed by interlacing of fibers ensures the gas exchange, meanwhile it would avoid the invasion of external liquids and bacteria effectively to protect the wound from infection. Moreover, electrospinning process is simple, flexible and cost-effective. [8] According to the actual situation of the wound, electrospinning fiber membranes can be incorporated various drugs flexibly, such as antibacterial agents, vitamins and growth factors, to further promote wound healing. [9, 10] Therefore, electrospinning composite membranes have been widely used in wound healing. [11, 12]

In addition, it is noteworthy that the surface wettabilities of wound dressing are vital. [13] The hydrophilic surface of the wound dressing adheres to the wound directly, which is

conducive to the cell adhesion and drug release to treat the wound, meanwhile the hydrophobic surface would block the penetration of water containing microorganisms to protect the wound from infection. [7] Thus, the membranes with different wettability surfaces have great potential in wound dressing applications. Nevertheless, the traditional electrospinning membranes are single layer with the same wettability on both surfaces. Therefore, it is desirable to develop bi-layered fiber membranes with different wettability surface layers to optimize the wound treatment.

Fiber membranes with antibacterial activity have gained much interest as wound dressings. [14] Disulfiram (DSF) is an old oral drug for abstinence from alcohol. [15] Lately, there are several approaches reporting that the DSF possesses the potent antimicrobial activity. [16-18] Notably, the repurposing of DSF is hopeful for reducing the drug-resistance of bacteria. However, DSF would degrade rapidly in the acidic gastric juice and bloodstream. Thus, the poor water solubility and physiological instability of DSF obviously restricts its practical clinical application. [19-22]

In this study, we developed a disulfiram-loaded scaffold using the electrospinning method to enhance the stability of DSF and to facilitate its appropriate distribution. To be specific, the bi-layered drug-loaded fiber membranes were composed of a PVDF bottom layer and a DSF/PLA top layer. The morphology, wettability and chemical components of the obtained membranes were measured by scanning electronic microscope (SEM), drop shape analysis, X-ray diffractometer (XRD) and X-ray photoelectron spectrometer (XPS). The bacteriostatic ability of the bi-layered drug-loaded fiber membranes was verified against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) in vitro. The

results indicate that the bi-layered drug-loaded fiber membranes are very suitable for application in the field of wound dressings.

2. Experimental methods

2.1. Materials

Polyvinylidene fluoride (PVDF, $M_w \approx 543\ 000$ g/mol) powder and disulfiram (DSF) powder were purchased from Sigma-Aldrich. Polylactic acid (PLA, 4032D, $M_w \approx 160\ 000$ g/mol) powder was purchased from NatureWorks LLC. All other analytically pure chemicals were acquired from Beijing Chemical Works. *Escherichia Coli* (*E. Coli*) and *Staphylococcus aureus* (*S. Aureus*) were supplied by the local laboratory.

2.2. Preparation of bi-layered membranes

The bi-layered drug-loaded fiber membranes composed of the bottom layer PVDF fibers and top layer DSF/PLA fibers were fabricated using ths electrospinning method. In brief, the PVDF powder was added in dimethylformamide (DMF) /acetone (1:1, v/v) solvent, then the obtained PVDF solution with 21 wt% was stirred at 40 ^{o}C for 12 h. The DSF/PLA (1:5, w/w) blended solution was obtained by adding the DSF powder in the DMF/acetone (1:1, v/v) solution of PLA (12.5 wt%) and stirring for 12 h at 40 ^{o}C. During electrospinning, the flow rate of spinning solution was kept on 0.5 ml/h. A 27-gauge steel needle was connected to an positive voltage of 13 kV. A grounded Al foil was applied as the collector. And the distance between the needle and collector was about 14 cm. PVDF fibers were collected on Al foil-covered plate as the bottom layer and the subsequent DSF/PLA fibers were directly deposited on the PVDF layer. The collected bi-layered fiber membranes were completely dried at room

temperature overnight before further use. The obtained bi-layered fiber membranes are denoted as PLA-PVDF and DSF/PLA-PVDF.

2.3. Plasma treatment of bi-layered membranes

The oxygen plasma modification of bi-layered drug-loaded fiber membranes was carried out in a 13.56 MHz RF power generator (Diener Electronic Pico, Germany). The maximum power of the generator is 300 W. The oxygen partial pressure was precisely adjusted to 0.3 mbar. All plasma treatments were performed for 40 s at 30% of the maximum power. The modified sample was denoted as plasma-treated DSF/PLA-PVDF.

2.4. Characterization of bi-layered membranes

The morphological features of bi-layered membranes were examined using SEM (FEI QUANT-250 FEG, USA) at an operating voltage of 7 kV. The elementary compositions of bi-layered membranes were analyzed by an energy dispersive spectroscope (EDS, Oxford Instruments, UK). The degree of crystallinity of samples was measured by X-ray diffraction (XRD) with Cu/K_α radiation at 30 kV and 200 mA.

Water contact angles (WCAs) of the bottom and top surface layers of bi-layered membranes were measured by drop shape analysis (Kruss DAS100, Germany) at room temperature. The WCA value of water drop (5 μl) was tested after the droplet was stable. The chemical compositions of the bi-layered membranes before and after plasma modifications were studied by X-ray photoelectron spectrometer (XPS, Thermo Fisher Scientific, USA).

2.5. Antibacterial activity

The antibiotic ability of the bi-layered membranes was tested based on agar plate spreading susceptibility. The inhibition zones were detected against a representative Gram-

negative bacteria *E. Coli* and a representative Gram-positive bacteria *S. Aureus*. The bi-layered membranes were divided into round plate (diameter = 15 mm) and exposed under UV light for 1 h to sterilize. About 100 μL (10^5 CFU) of each bacteria was cultured on the agar medium. Within each culture-medium, three types of membranes (PLA-PVDF, DSF/PLA-PVDF and plasma-treated DSF/PLA-PVDF) were placed on the surfaces, and then incubated at 37 °C overnight. The bacteriostatic circles around the membranes were measured to evaluate the antibacterial activity against the test organism.

3. Results and discussions

The bi-layered drug-loaded fiber membranes were prepared using two step electrospinning process as stated in Fig. 1. In brief, PVDF membranes were firstly fabricated on an aluminum foil. Then the obtained PVDF membranes with the aluminum foil acted as a collector to collect the drug-loaded PLA fiber membranes. At last, the fabricated bi-layered membranes were modified by oxygen plasma treatment to form the plasma-treated DSF/PLA-PVDF bi-layered membranes.

3.1. Surface morphology of bi-layered membranes

The top (PLA) and bottom (PVDF) surface morphologies of bi-layered membranes were obtained by SEM. As shown in Fig. 2, there were not remarkable differences among the PLA-PVDF, DSF/PLA-PVDF and plasma-treated DSF/PLA-PVDF in the surface morphologies. The bottom surface PVDF fibers mainly ranged from 500 to 980 nm. Furthermore, it can be scanned that the morphologies of PLA fibers or drug-loaded DSF/PLA fibers were relatively smooth without beads, which showed that the DSF was compatible with the PLA polymer. The size distribution of top surface fibers was from 150 to 400 nm, in the range of collagen

fibers (50 to 500 nm) in thenative extracellular matrix. [23] So, the obtained bi-layered membranes were similar to ECM in some structural features. It was beneficial for cell adhesion, proliferation and differentiation to improve the vulnus healing. [24]

3.2. EDS and XRD Analysis

The EDS spectra of the PLA-PVDF, DSF/PLA-PVDF and plasma-treated DSF/PLA-PVDF are shown in Fig. 3(a). As observed in Fig. 3(a), the characteristic peak of sulfur (S) in the EDS spectra of drug-loaded membranes verifies that the drug exists on the surface of the DSF/PLA-PVDF and plasma-treated DSF/PLA-PVDF. It demonstrates that the bi-layered drug-loaded membranes succeed in incorporating DSF using the electrospinning method.

Figs. 3(b) and (c) present the XRD patterns of DSF powders, PVDF fibers, PLA fibers, PLA-PVDF, DSF/PLA-PVDF and plasma-treated DSF/PLA-PVDF. From the PLA fiber pattern in Fig. 3(b), there is a broad diffusion scattering around 20^{o} which indicates that the PLA fibers are in amorphous phase. Amorphous electrospun membranes have higher cell adhesion and proliferation when compared to crystalline material. [25] Hence, the PLA fiber scaffolds as the top surface layer are highly desirable for tissue regeneration. From the patterns of PVDF fibers shown in Fig. 3(b), the reflection sum of (110) α and (200) β at about 20.26 degrees is detected. The results demonstrate that the PVDF electrospinning fibers are mainly in β phase. [26] Notably, as shown in Fig. 3(c), when PLA fibers are covered on PVDF fibers to form bi-layered membranes, the fabricated bi-layered membranes show a characteristic peak of the PVDF electrospinning membranes. Moreover, as exhibited in Fig. 3(c), the XRD patterns of the bi-layered drug-loaded membranes show a very weak

representative peak at 17.38° of the DSF drugs. It proves the incorporation of DSF into the bi-layered membranes.

3.3. Surface properties of bi-layered membranes

The wettabilities of bi-layered membrane surfaces were studied by the measurement of the WCAs of top (PLA) and bottom (PVDF) surfaces. As exhibited in Fig. 4, the WCAs of PVDF and PLA surfaces are around 133.2° and 126.5°, respectively. The water contact angles of bi-layered drug-loaded fiber membranes are around 132.1° and 127.7°, respectively. After plasma modification, the WCAs of PLA surface layer and PVDF layer are at 54.2° and 120.9°, respectively. The results show that PVDF and PLA are both hydrophobic materials, [7] so the untreated bi-layered membrane surfaces are hydrophobic. However, after plasma treatment, the surface of PLA fiber layer on bi-layered drug-loaded membranes is hydrophilic. It is indicated that the two surfaces of bi-layered drug-loaded membranes have different wettabilities. When the bi-layered drug-loaded membranes are applied in vulnus healing, the top hydrophilic surface adheres the wound directly and release antibacterial drugs to sterilize and heal the wound, meanwhile the bottom hydrophobic surface would block the penetration of water containing microorganisms and preserve the wound from infection.[7] Consequently, the obtained bi-layered drug-loaded fiber membranes have a broad application prospect in wound dressing.

The untreated and plasma-treated bi-layered drug-loaded fiber membranes were further analysed by XPS, as shown in Fig. 5, where C 1s peak and O 1s peak are relatively apparent peaks at 284.71 eV and 532.54 eV, respectively. In Figs. 5 (a) and (c), the C 1s content of initial bi-layered drug-loaded membranes was 81.29%, O 1s was 18.71%, and the O/C atomic

ratio was 0.23. After oxygen plasma treatment, the C 1s content of bi-layered membranes was 77.98%, O 1s was 22.02%, and the O/C atomic ratio was increased to 0.28. The changes in C 1s peaks due to the plasma treatment are highlighted in Figs. 5 (b) and (d). The C1s line can be resolved into three peaks. The three peaks are obtained at 284.8eV, 286.4eV and 288.9eV, matching with C-C (nonoxygenated ring carbon), C-O (hydroxyl and epoxy carbon), and C=O (carboxyl), respectively. As shown in Fig. 5 (d), the peak strength of C-O and C=O increases obviously after plasma treatment, which maybe due to the introduction of oxygen functional groups and the increase of carboxyl and hydroxyl groups. Thus, it is also the main reason that the top surface of the bi-layered membranes becomes hydrophilic.

3.4. Antibacterial activity

In order to measure the bacteriostatic ability of the bi-layered drug-loaded fiber membranes, bacteriostatic experiments were performed on *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) in vitro. As exhibited in Fig. 6, there are obvious bacteriostatic circles around the drug-loaded fiber membranes. It suggests that the bi-layered drug-loaded fiber membranes show significant antibiotic ability against both *E. Coli* and *S. Aureus*. The bi-layered drug-loaded fiber membranes would promote the diffusion of drugs in surrounding media to protect the regenerated tissue from infection. Thus, the bi-layered drug-loaded fiber membranes have the potential to be a type of good antibacterial wound dressings.

4. Conclusions

In summary, the bi-layered drug-loaded fiber membranes were successfully prepared by electrospinning and plasma treatment. The top and bottom surfaces of the obtained bi-layered fiber membranes had different wettabilities. The top hydrophilic surface of PLA fiber

membranes would contact the wound directly to promote cell adhesion and proliferation, while the outer (bottom) hydrophobic PVDF surface would prevent the penetration of water containing microorganisms and preserve the wound from infection. XRD and EDS analyses show that DSF is successfully incorporated into the bi-layered drug-loaded fiber membranes. The antibacterial experiments suggest that the bi-layered drug-loaded fiber membranes have good antibacterial effects on *Staphylococcus aureus* and *Escherichia coli*. Therefore, the fabricated bi-layered drug-loaded fiber membranes have a broad application prospect in the field of clinical wound dressing.

Ethical Approval

All analyses were based on previous published studies, thus no ethical approval and patient consent are required.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

Authors Contributions

C. Xie contributed the central idea, analysed most of the data, and wrote the initial draft of the paper. J. Yan analysed the XPS data. S. Cao performed the antibacterial activity experiment. R. Liu performed the surface wettability measurement. B. Sun analysed the XRD data. Y. Xie assisted the surface wettability measurement. K. Qu provided the materials of antibacterial activity experiment. W. Zhang provided the most of materials, reagents. Z. Weng

contributed to refining the ideas and revised the manuscript. Z. Wang discussed the results and revised the manuscript.

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Competing Interests

The authors declare that they have no competing interests.

Availability of data and material

All data generated or analysed during this study are included in this published article.

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Figures and captions

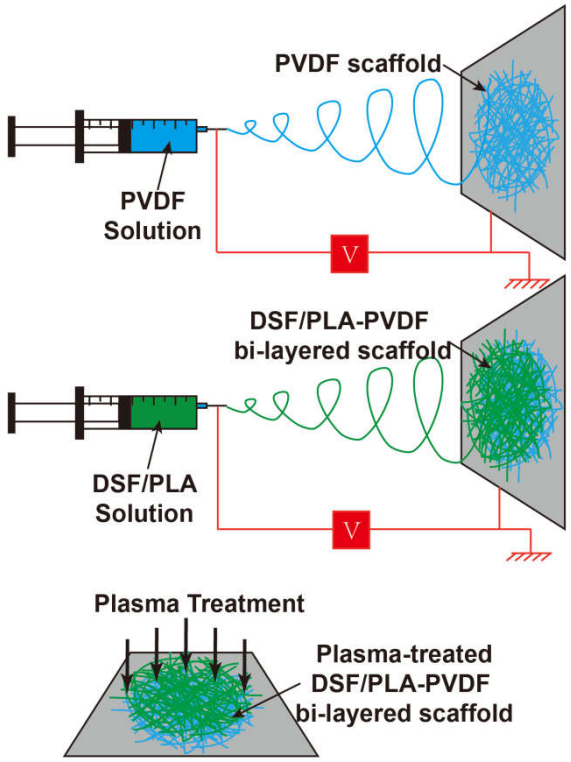

Fig. 1. Schematic diagram showing the preparation of the bi-layered drug-loaded fiber membranes.

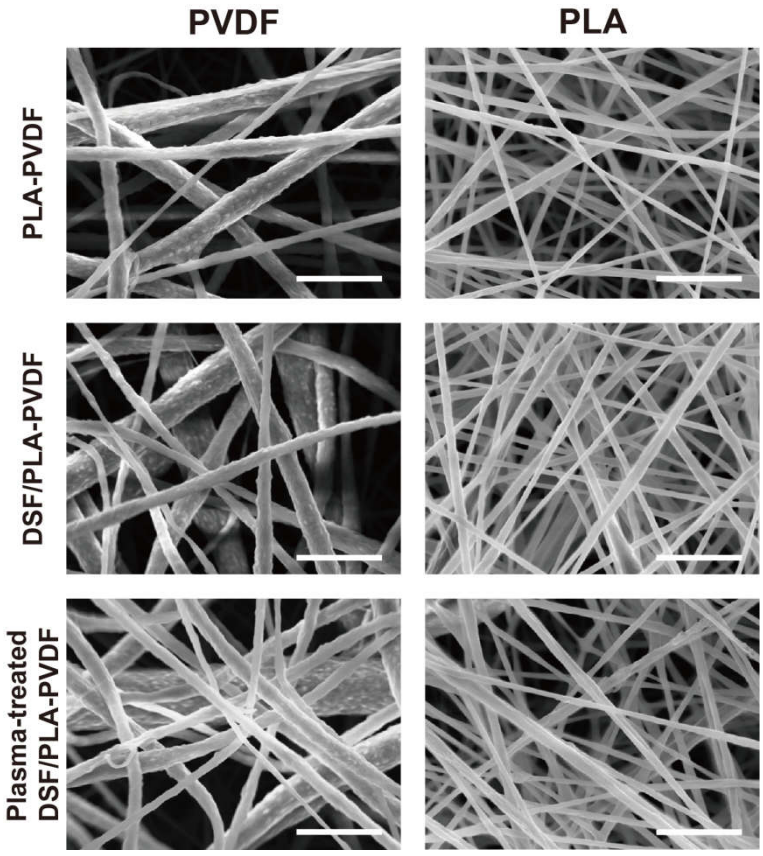

Fig. 2. The top (PLA) and bottom (PVDF) surface SEM images of PLA-PVDF, DSF/PLA-PVDF and plasma-treated DSF/PLA-PVDF. (scale bar, 5 μm)

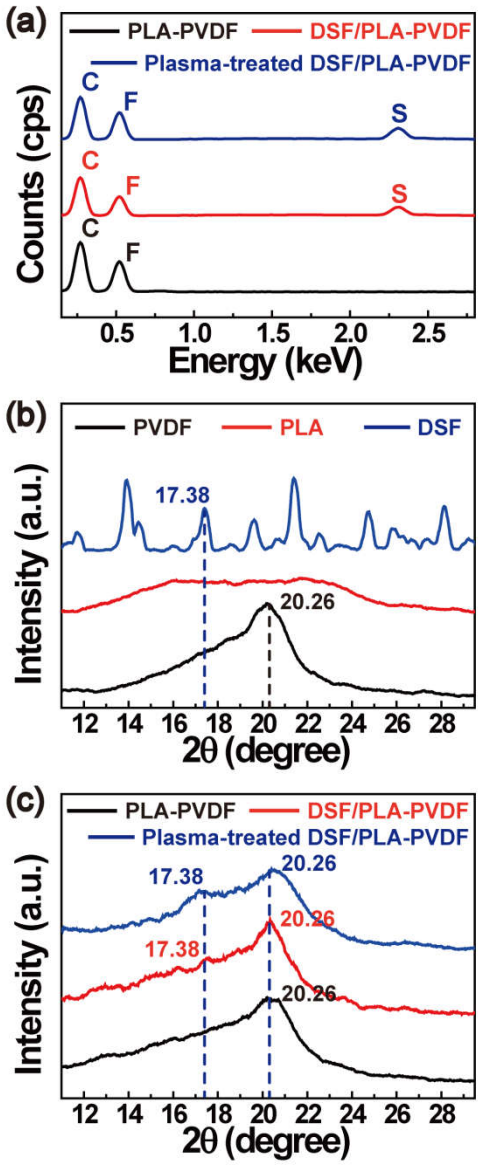

Fig. 3. (a) EDS spectrum of the bi-layered fiber membranes. (b) XRD patterns of DSF powders, PLA fibers and PVDF fibers. (c) XRD patterns of PLA-PVDF, DSF/PLA-PVDF and plasma-treated DSF/PLA-PVDF.

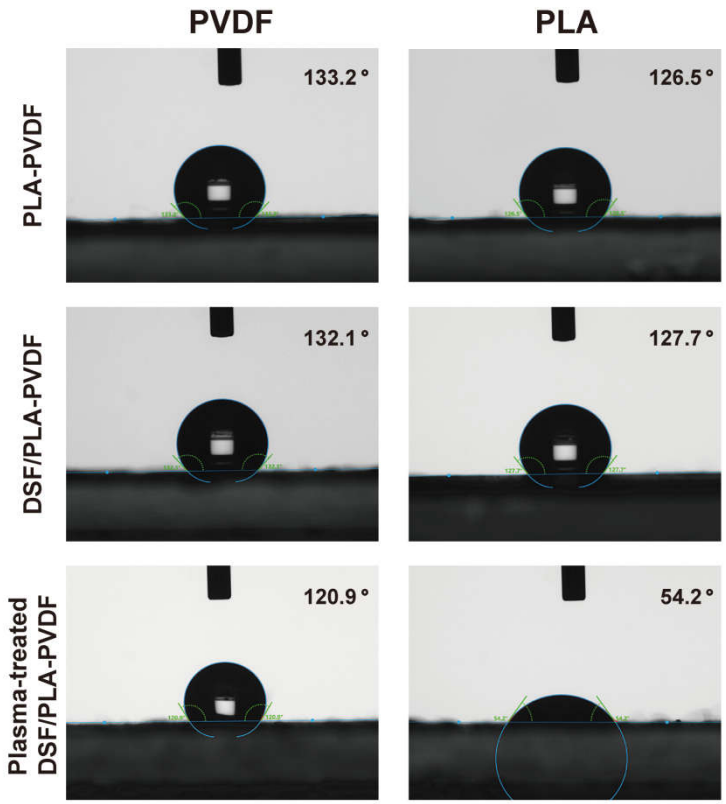

Fig. 4. The top (PLA) and bottom (PVDF) surface water contact angles of PLA-PVDF, DSF/PLA-PVDF and plasma-treated DSF/PLA-PVDF.

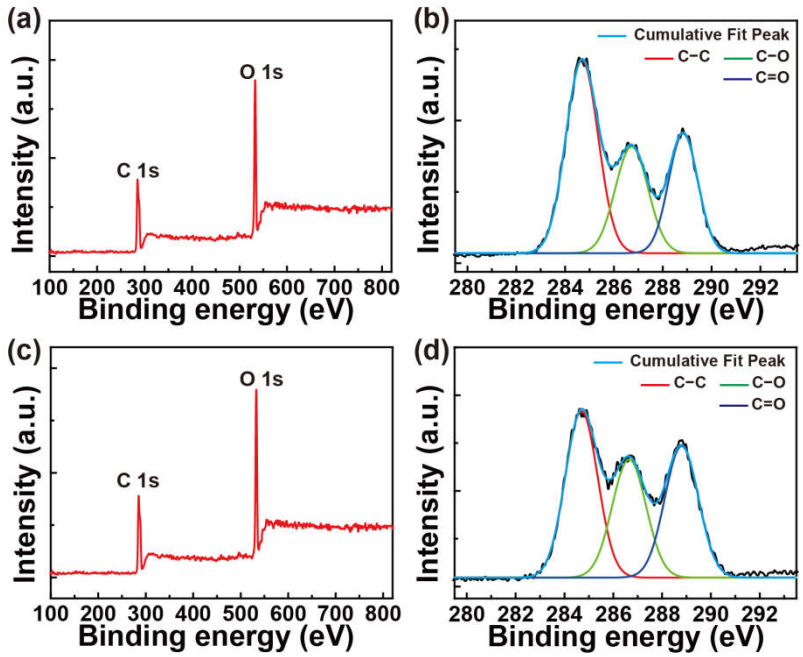

Fig. 5. (a), (b) XPS spectra survey scan and C1s XPS spectra of DSF/PLA-PVDF. (c), (d) The corresponding XPS measurement results of plasma-treated DSF/PLA-PVDF.

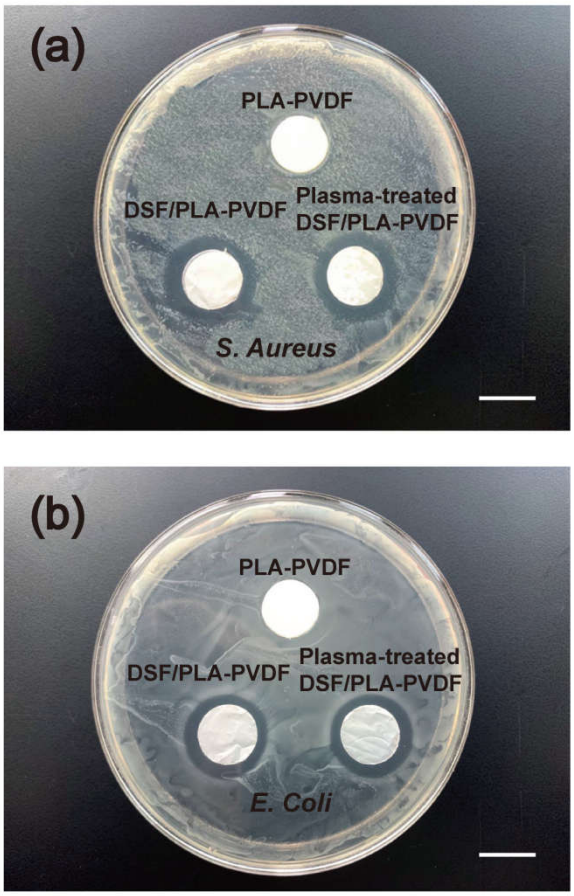

Fig. 6. Inhibition zone of bi-layered fiber membranes against (a) *S. Aureus* and (b) *E. Coli*. (scale bar, 15 mm)

Figures

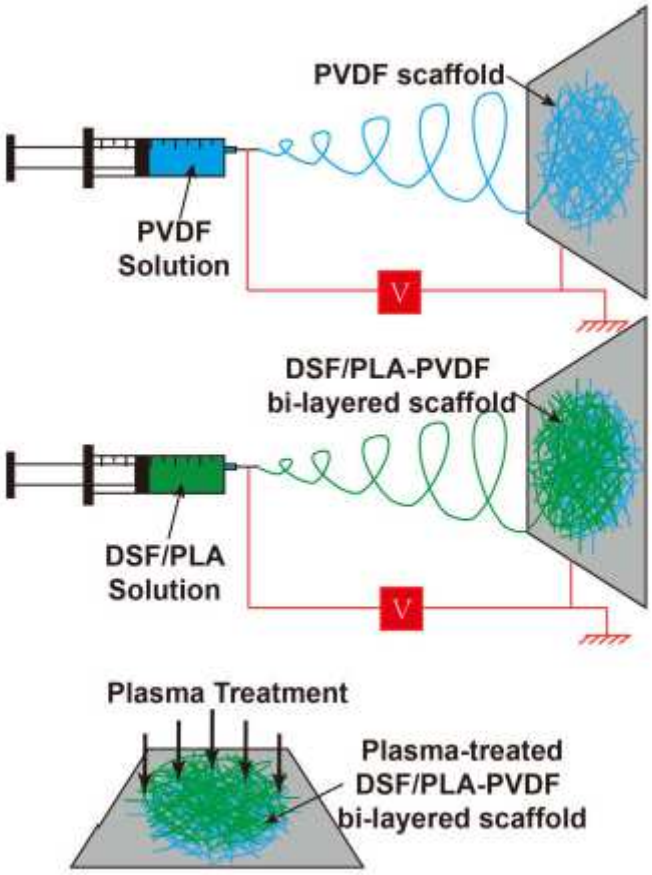

Figure 1

Schematic diagram showing the preparation of the bi-layered drug-loaded fiber membranes.

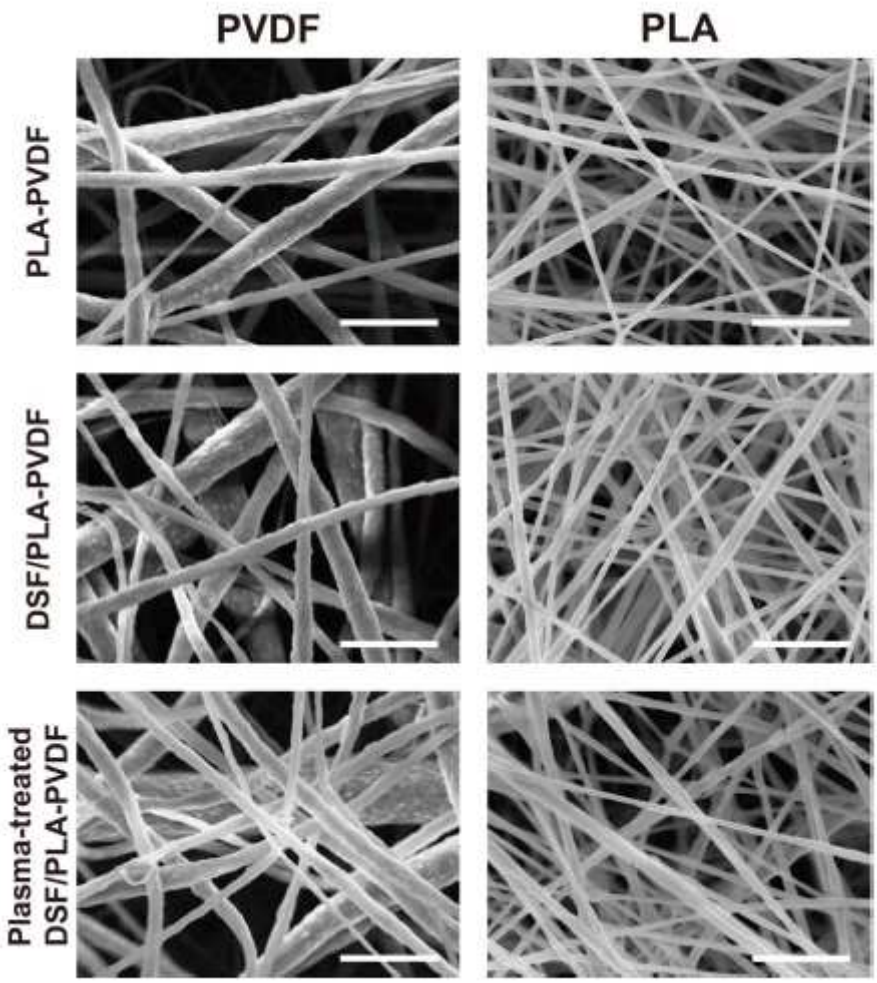

Figure 2

The top (PLA) and bottom (PVDF) surface SEM images of PLA-PVDF, DSF/PLAPVDF and plasma-treated DSF/PLA-PVDF. (scale bar, 5 μm)

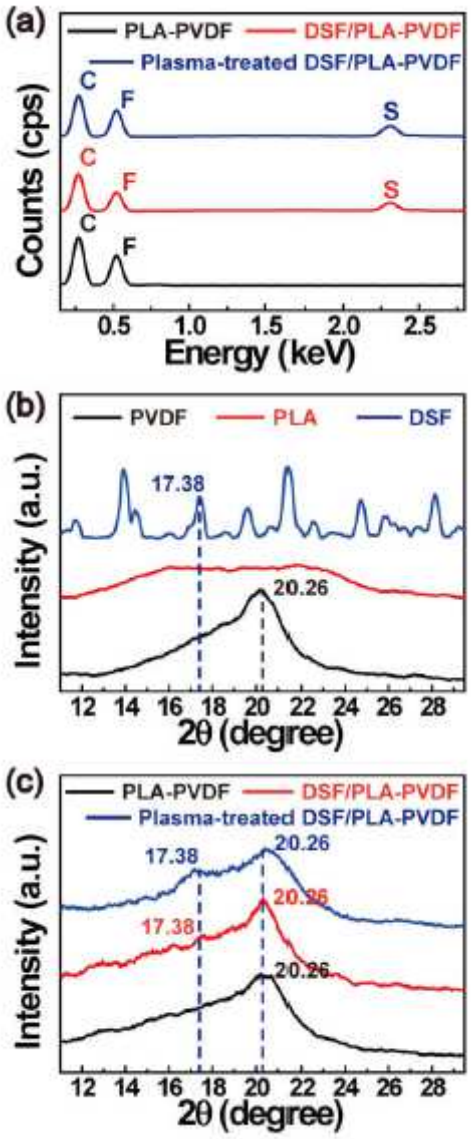

Figure 3

(a) EDS spectrum of the bi-layered fiber membranes. (b) XRD patterns of DSF powders, PLA fibers and PVDF fibers. (c) XRD patterns of PLA-PVDF, DSF/PLAPVDF and plasma-treated DSF/PLA-PVDF.

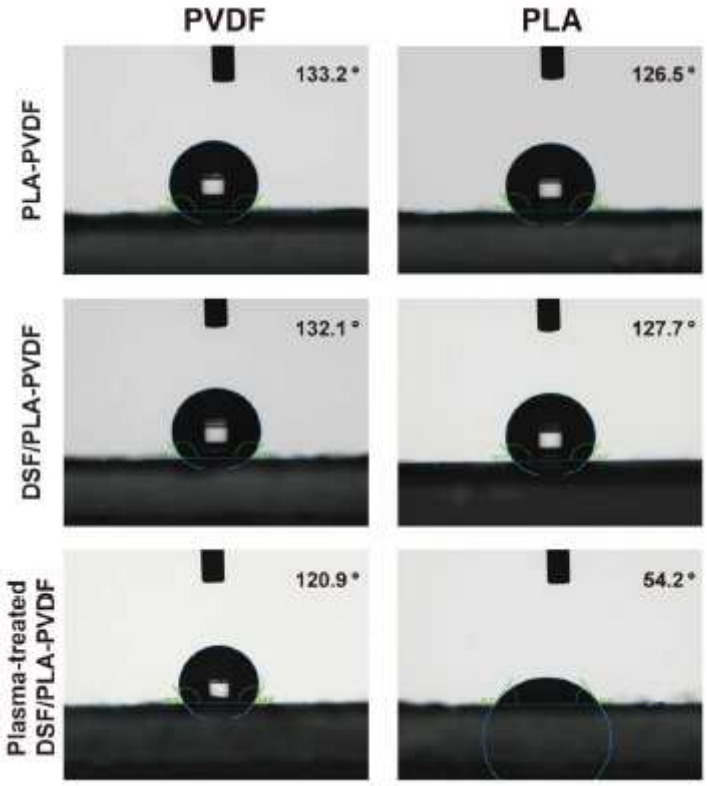

Figure 4

The top (PLA) and bottom (PVDF) surface water contact angles of PLA-PVDF, DSF/PLA-PVDF and plasma-treated DSF/PLA-PVDF.

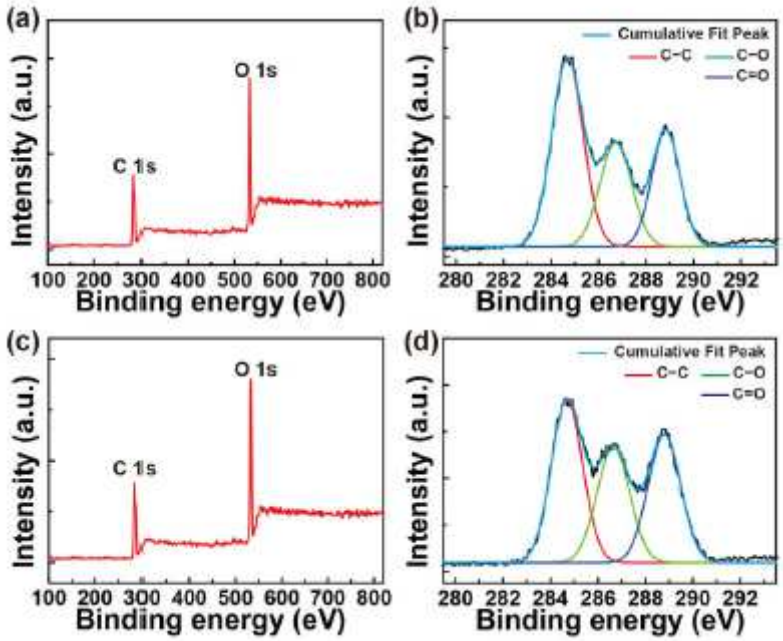

Figure 5

(a), (b) XPS spectra survey scan and C1s XPS spectra of DSF/PLA-PVDF. (c), (d) The corresponding XPS measurement results of plasma-treated DSF/PLA-PVDF.

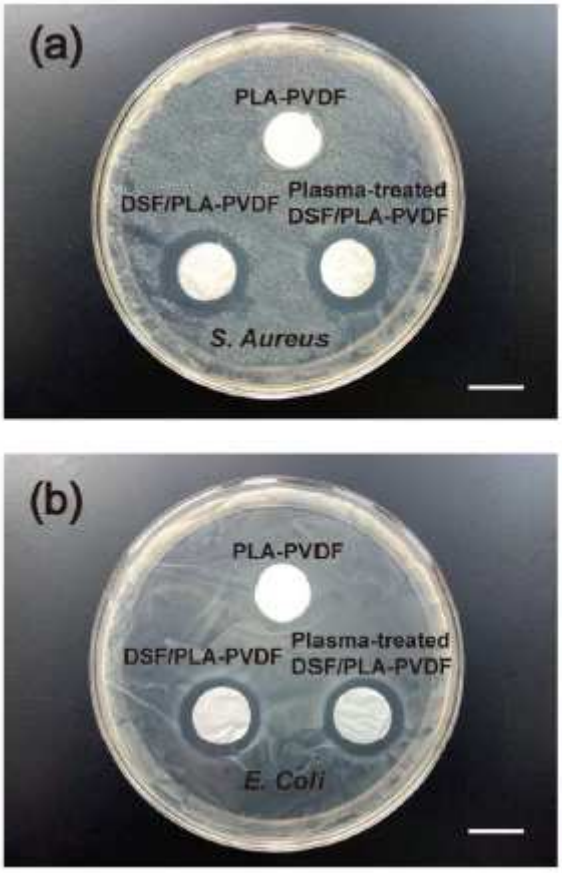

Figure 6

Inhibition zone of bi-layered fiber membranes against (a) S. Aureus and (b) E. Coli. (scale bar, 15 mm)