Preparation of Antibacterial Electrospun PVA/Regenerated Silk Fibroin Nanofibrous Composite Containing Ciprofloxacin Hydrochloride as a Wound Dressing

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Abstract: Electrospinning technique was used for the fabrication of poly (vinyl alcohol) (PVA) / regenerated silk fibroin (SF) composite nanofibers, loaded with ciprofloxacin HCl (CipHCl) as a wound dressing. Electrospun PVA/SF/CipHCl composite nanofibers were stabilized against dissolving in water by heating in an oven at 155 ℃ for 5 min. Incorporation of CipHCl into electrospun nanofibers was confirmed by SEM and FT-IR spectra. Further the mechanical properties test illustrated that the addition of CipHCl enhanced the mechanical properties of PVA and PVA/SF nanofibers. The antibacterial activities against Escherichia coli (*E. coli*) (gram-negative) and Staphylococcus aureus (*S. aureus*) (gram-positive) organisms were evaluated by disk diffusion method; and results suggested that electrospun PVA/CipHCl and PVA/SF/CipHCl composite nanofibers showed a remarkable antibacterial activity.

Key words: *electrospinning*; *poly(vinyl alcohol) (PVA)*; *regenerated silk fibroin (SF)*; *mechanical properties*; *antibacterial activity*; *wound dressing*
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Introduction

Electrospinning technique, which is a highly versatile method offering many advantages due to its good diameter, high surface to volume ratio, highly porous structure of nanofibers, absorbability, hemostatic properties, semi permeable for respiration of cells, and a better conformation to the wound surface.

In order to avoid the harmful effects of organic solvents, many water soluble polymers have been used, such as polyvinyl alcohol (PVA), polyethylene oxide (PEO), silk fibroin (SF), poly acrylic acid (PAA) and collagen to fabricate green and eco-friendly electrospun nanofibers for biomedical applications.

Ideal wound dressings should make the wound free from infection, and have excellent biocompatibility [1]. However, the infection under dressing caused by burns, split skin, pressure, sores, and diabetic ulcers can lead to prolonged healing time or, in serious conditions, death. For that wounds often require treatment with antibiotics [2-3]. Wound dressings containing antibiotics are preferred, especially for chronic wounds to make selective targeting, and reduce the frequency of dressing replacement. On one hand, ideal wound dressing should have wound healing properties as oxygen permeability for the wound respiration, the capability of swelling to absorb excess exudates. SF is an attractive natural fibrous protein material, which has been used for several biomedical applications, such as membranes [4-5], sponges [6-7], gels [6,8], and films [8]. On the other hand it has been investigated also in tissue engineering and drug delivery carrier area due to its biocompatibility, biodegradability, low inflammatory response, good oxygen and water vapor permeability, and good mechanical properties [10-11]. PVA is well-known, biologically friendly polymer due to its favorable properties such as no toxicity, no carcinogenicity, biocompatibility, and good mechanical properties [12-13]. Due to its flexibility and good swelling properties in aqueous mediums, PVA has been investigated as a wound dressing [2]. However, PVA has poor stability in water, which gives some limitations to its application in aqueous mediums, especially for drug delivery systems [14,16]. To solve this problem, PVA was cross-linked to give better characteristics [14,17]. Ciprofloxacin HCl (CipHCl), a fluoroquinolone antibiotic, is one of the most widely used antibiotics for wound healing as it has low minimal inhibitory concentration against both gram-positive and gram-negative bacteria which cause wound infections [18] and the frequency of spontaneous resistance to ciprofloxacin is very low [19].

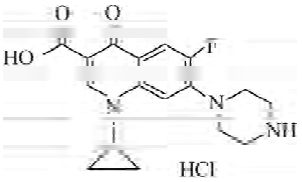

Fig. 1 Chemical structure of CipHCl

Recently, some articles have been investigating the electrospinning of SF blends in biomedical, tissue engineering applications such as SF/PEO [20], SF/chitosan [21]. PVA/SF/$AgNO_3$ composite nanofibers was prepared by electrospinning. After posttreatment of heat treatment in the oven and subsequent UV irradiation, the heat treated PVA/SF/$AgNO_3$ composite fibers showed a very strong antimicrobial activity [22]. The addition of PVA to SF was found to enhance the mechanical properties of SF.

In this study, we record the usage of antibacterial electrospun PVA/regenerated SF composite containing CipHCl as a wound dressing, which has been found to be an attractive and alternative for many antibacterial drugs. Further, stabilization of electrospun nanofibers containing CipHCl against dissolution in water has been achieved by heat treatment in the oven. The properties of electrospun PVA/SF/CipHCl nanofibers, including the morphologies of the composite nanofibers, FT-IR spectra, the mechanical properties and antibacterial activities, showed that the electrospun composite nanofibrous membranes serving as varied wound dressings.

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1 Experimental

1.1 Materials

PVA (88% hydrolyzed, average M_w = 88 000 g/mol) was purchased from Acros Organics Company. Cocoons of *Bombyx Mori* silk worm was supplied by Jiaxing Silk Co., Ltd., China. CipHCl 98% was purchased from Aladdin Industrial Corporation (Shanghai, China). The solvent used in this work is distilled water.

1.2 Preparation of regenerated SF

Raw silk fibers were degummed three times with 0.5% Na_2CO_3 water solution at 100 ℃ for 30 min and then rinsed with distilled water. Degummed silk (SF) was dissolved in a ternary solvent system of $CaCl_2/CH_3CH_2OH/H_2O$ (1/2/8 in mole ratio) at 65 ℃ for 1 h. Then the solution was dialyzed with cellulose tubular membrane (250 -257 μm) in distilled water for 3 d at room temperature (the water was renewed every 4 h), and after filtering the solution, regenerated SF sponge was obtained by freeze drying [21].

1.3 Preparation of spinning solutions

PVA solution was prepared by dissolving in distilled water at 60 ℃ with gentle stirring for 12 h, and the concentration of PVA was 8%. SF solution was prepared by dissolving in distilled water at room temperature with gentle stirring for 12 h, and the concentration of SF was 13%. Both solutions were cooled down to room temperature and then mixed together at ratio 9∶1 with gentle stirring to give a total concentration of polymer solution of 8.5%. Prior to electrospinning experiment, CipHCl was added to polymer solution and stirred for 1 -2 h, and the concentration of CipHCl was 5%.

1.4 Electrospinning of PVA/SF/CipHCl

Electrospinning was performed at 35 ℃ and 45% relative humidity. The solutions were filled into a 2.5 mL plastic syringe with a blunt-ended needle (ID = 0.7 mm). An aluminum foil was used as receiving plate to collect nanofibers. The distance between the needle and the aluminum foil collector was 15 cm. The syringe was located in a syringe pump (789100C, Cole-Parmer, USA) and dispensed at a rate of 0.5 mL/h at an applied voltage of 18 kV using a high voltage power supply (BGG6-358, BMEICO, Ltd., China). The prepared nanofiber mats were placed in a vacuum dryer at room temperature to remove the residual solvent till constant weight.

1.5 Stabilization of electrospun nanofibers containing CipHCl

The PVA/SF nanofiber membranes were stabilized against dissolving in water by heating in an oven at 155 ℃ for 5 min.

1.6 Antimicrobial assessment

Cultures of the following microorganisms were used in the study: escherichia coli (*E. coli*), staphylococcus aureus (*S. aureus*). Bacterial inoculums were precultured in LB-agar medium, which was prepared using 1.0 g of tryptone, 0.5 g of yeast extract, 2 g of agar, and 1 g of NaCl in 100 mL distilled water for 20 -30 min in a rotary shaker. The medium, pH adjusted to 6.8, was autoclaved at 121 ℃ for 20 min, and then was poured into petri dishes. The tested microorganisms were seeded into respective medium by spread plate method 100 μL (108 000 cells/L) in LB-agar plates, incubated at 37 ℃ for 24 h. Antibacterial activity for heat treated electrospun nanofibers was tested by the disc diffusion method. Electrospun nanofibers like discs (5 mm in diameter each) were placed on tested microorganisms and LB-agar medium surface. Antimicrobial activity of the electrospun nanofibers were recorded in terms of inhibition zone diameters (mm) against the tested microorganisms.

1.7 Characterization

The morphology and structure of pure PVA, PVA/CipHCl, PVA/SF, and PVA/SF/CipHCl nanofibers were analyzed by using SEM (Hitachi TM-1000, Japan) after applying gold coating. Average diameter of nanofibers was determined by analyzing SEM images using visualization software Image-J (National Institutes of Health, USA). The bonding configurations of the samples and the encapsulation of the drug into electrospun nanofibers were characterized by FT-IR 80 Nicolet-670 FTIR spectrometer (Nicolet-Thermo, USA). Mechanical properties testing was performed using a universal materials tester (H5K-S, Hounsfield, UK) at ambient temperature 20 ℃ and 65% relative humidity. Antimicrobial activities of composite nanofibers after heat treatment were tested against *S. aureus* (ATCC 6538) and *E. coli* (ATCC 8099). LB-agar plates containing test samples and control (blank) were incubated at 37 ℃ for 24 h.

1.8 Weight loss and swelling ratio

To evaluate the weight loss and swelling behavior which could originate at the physiological condition, the heat treated CipHCl-loaded electrospun nanofiber mats were put in test tubes containing 10 mL distilled water each and incubated for 12 h at room temperature. Then the water on the specimen surfaces was removed with filter paper and the specimens were weighed in wet condition. The weight loss (W) and swelling ratio (S) of nanofiber mats were calculated as follows, respectively.

$$W/\% = 100 \times (m_d - m_i)/m_d,$$
$$S/\% = 100 \times (m_w - m_d)/m_d,$$

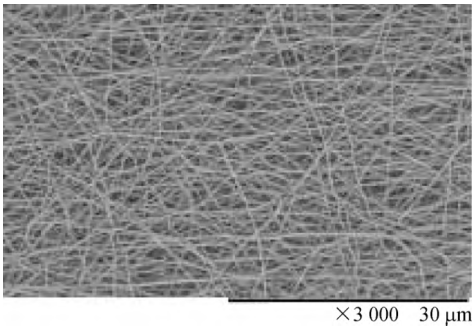
where m_w is the weight of swollen nanofibers sample which is wiped with filter paper, m_d is the initial dry mass of sample, and m_i is the dried mass of immersed sample in buffer medium, measured by drying the swollen nanofiber mats in an oven at 40 ℃ until constant weight.

2 Results and Discussion

Electrospun PVA/SF/CipHCl nanofibers were prepared using simple electrospinning setup in certain parameters and conditions as shown previously, and electrospun nanofibers were stabilized against dissolving in water.

2.1 SEM

SEM images of electrospun nanofibers are shown in Fig. 2.



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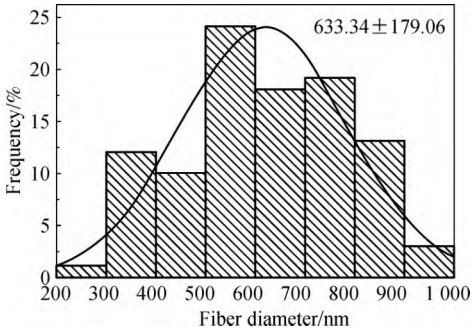

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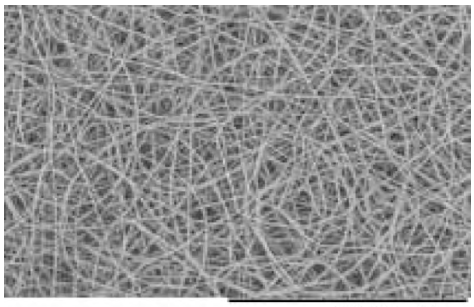

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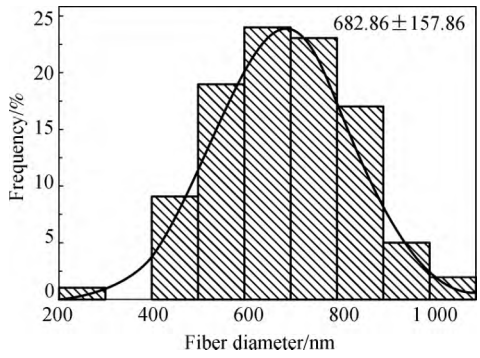

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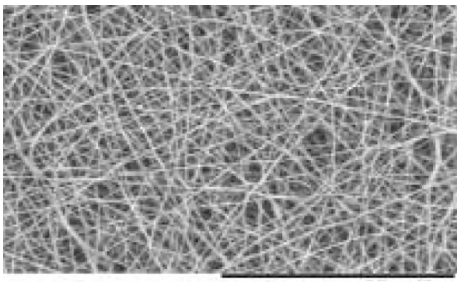

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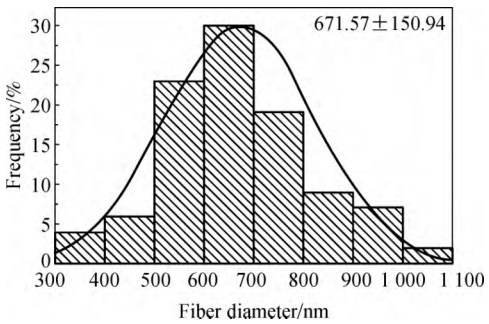

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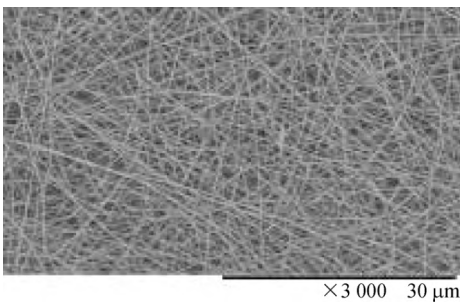

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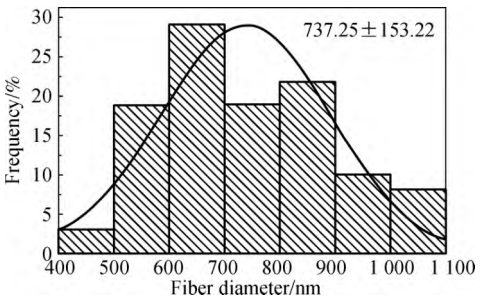

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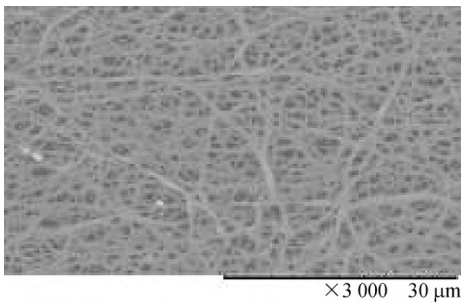

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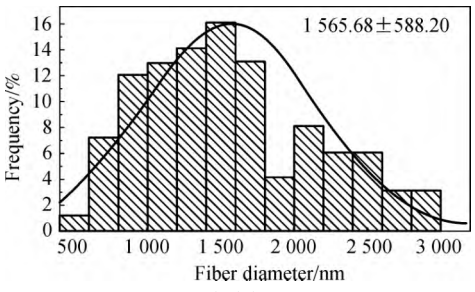

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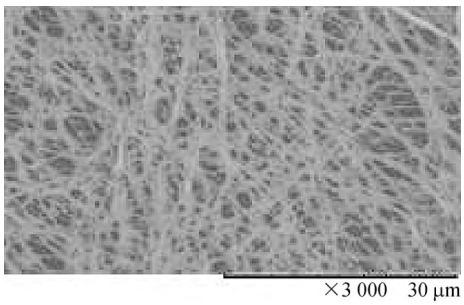

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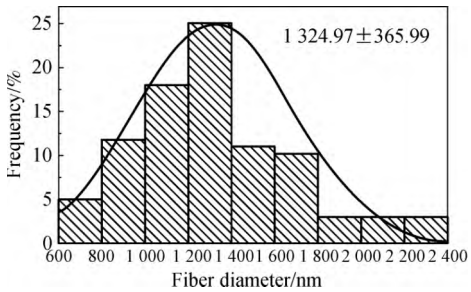

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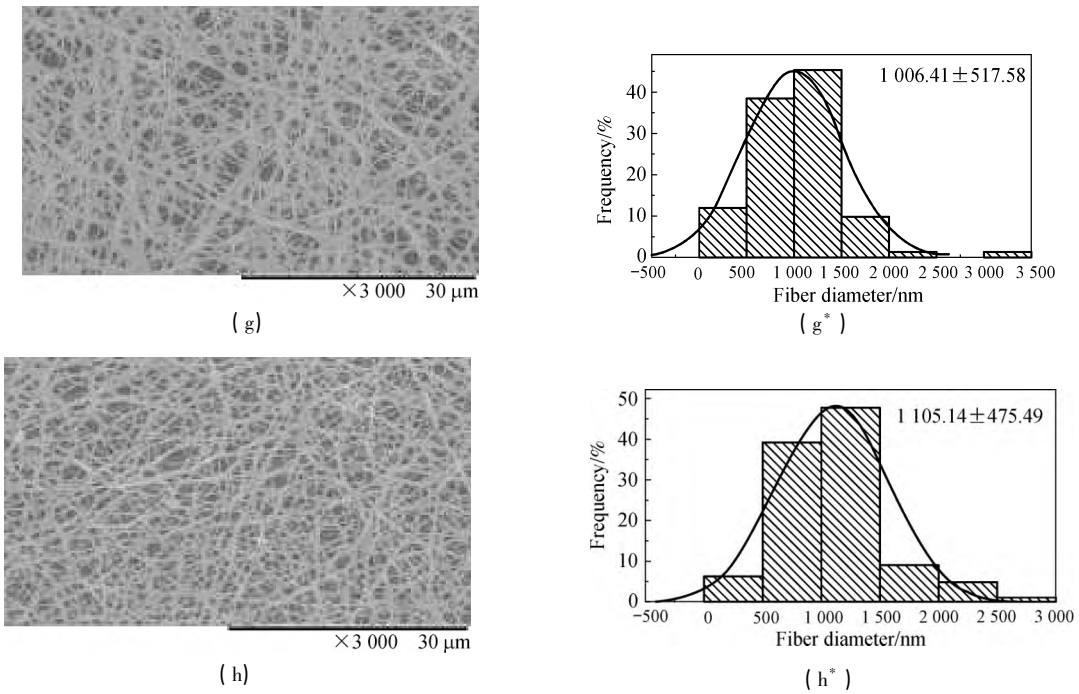


Fig. 2 SEM images of (a) PVA, (b) PVA/CipHCl, (c) PVA/SF, and (d) PVA/SF/CipHCl before heat treatment; (e) PVA, (f) PVA/CipHCl, (g) PVA/SF, and (h) PVA/SF/CipHCl after heat treatment ((a*)-(h*): the corresponding diameter distributions of (a)-(h))

Electrospun nanofibers as shown in Figs. 2(a)-(d) were nanofibers before heat treatment showing a uniform fibers with average diameter (633.34 ± 179.06) nm for PVA, and this value increased after the addition of CipHCl to be (682.86 ± 157.86) nm. The average diameter of PVA/SF nanofibers were (671.57 ± 150.94) nm, which increased after the addition of CipHCl to be (737.25 ± 153.22) nm. The value increased after heat treatment as shown in Figs. 2(e)-(h) due to the removal of residual water within the nanofibers. The removal of residual water allowed PVA-water hydrogen bonding to be replaced by intermolecular polymer hydrogen bonding [23].

In order to avoid dissolving in water, PVA nanofibers were heat treated at 155 ℃ for 5 min. After that treatment the PVA nanofibers are no longer dissolved in water and it can keep nanofiber structure in water [24], which is one of the important conditions to wound dressing. The advantage of using heat treatment is that no chemicals or harmful materials are used in cross linking. The heat treated electrospun nanofibers were relatively highly stable.

2.2 FT-IR analysis

The chemical structures of CipHCl and different electrospun nanofibers were investigated by FT-IR spectra as shown in Fig. 3. The FT-IR spectra showed characteristic peaks of PVA, a broad band observed from 3 200-3 500 cm^{-1} (O—H stretching) due to the strong hydrogen bonding intramolecular and intermolecular types. A strong band at 2 870-2 950 cm^{-1} was due to alkyl stretching mode (C—H stretching). The bands ranging from 1 710-1 750 and 1 000-1 320 cm^{-1} appeared due to the stretching vibrations of the carbonyl group (C═O) and ester, respectively, from the vinyl acetate group found in partially hydrolyzed PVA polymer. The band ranging from 820-850 cm^{-1} appeared due to presence of alkyl chain back bone. The FT-IR spectrum of SF showed absorption bands at 1 652 cm^{-1} due to the presence of amide I. The bands appearing at 1 543 and 1 260 cm^{-1} were due to the presences of amide II and amide V respectively. Moreover, after the addition of CipHCl, the width of (O—H stretching) absorption at 3 200-3 500 cm^{-1} increased due to intermolecular interactions between hydroxyl groups of PVA, SF, and CipHCl. The band ranging from 1 600-1 650 cm^{-1} in PVA/CipHCl was due to the presence of quinolones groups (N—H bending vibration) of amine. The strong absorption band ranging from 1 000-1 050 cm^{-1} was due to stretching vibration of C—F group.

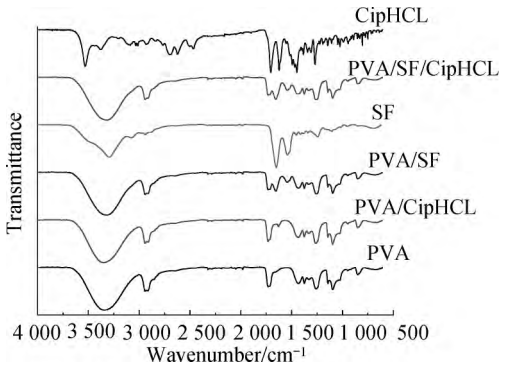


Fig. 3 FT-IR spectra of electrospun PVA, PVA/CipHCl, PVA/SF, SF and PVA/SF/CipHCl

2.3 Mechanical properties

The tensile stress-strain curves of heat treated electrospun nanofibers were shown in Fig. 4. It was found that the addition of CipHCl enhanced mechanical properties of samples due to some interactions between CipHCl with PVA and PVA/SF. The stress and strain of the heat-treated electrospun nanofibers at break were summarized in Table 1. The elongation at break of the heat treated nanofibrous membranes increased by the additions of SF and CipHCl, but some cases did not correspond, such as PVA or PVA/SF after adding CipHCl its elongation at break was declining. The tensile stress of the heat treated nanofibrous membranes increased by the additions of SF and CipHCl. These results suggested that the addition of CipHCl enhanced the mechanical properties of PVA and PVA/SF.

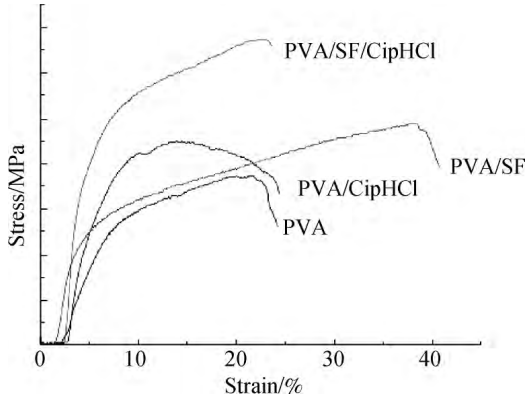


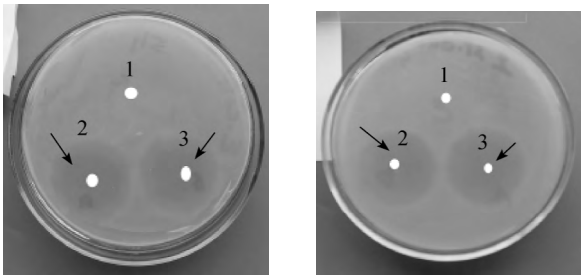
Fig. 4 Tensile stress-strain curves of heat treated nanofiber membranes PVA, PVA/CipHCl, PVA/SF, and PVA/SF/CipHCl

Table 1 Mechanical properties of heat treated nanofiber membranes PVA, PVA/CipHCl, PVA/SF, and PVA/SF/CipHCl

Samples	PVA	PVA/SF	PVA/CipHCl	PVA/SF/CipHCl
Tensile stress /MPa	3.71 ± 0.24	4.87 ± 0.13	4.47 ± 0.22	6.71 ± 0.21
Ultimate strain /%	21.56 ± 0.22	38.42 ± 0.21	14.12 ± 0.21	23.08 ± 0.20

2.4 Antibacterial assessments

Antibacterial activity testing on heat treated nanofibers of PVA (control), PVA/CipHCl, PVA/SF/CipHCl in culture medium was carried out by using disc diffusion method against *E. coli* and *S. aureus* bacterial strains as a model of gram-negative and gram-positive bacteria. The heat treated electrospun nanofibers discs were disinfected by using UV for 2 h then cultured separately in LB-agar medium plates and incubated at 37 ℃ for 16 to 18 h. The samples placed in plates killed all the bacteria over and around them as shown in Fig. 5 and inhibition zone in mm shown in Table 2. Electrospun nanofiber heat treated disc of PVA (control) showed no activity against both organisms. Electrospun nanofiber heat treated discs of PVA/CipHCl exhibited remarkable activity against *E. coli* and *S. aureus* gave an inhibition zone of 30 mm. The electrospun nanofiber heat treated discs of PVA/SF/CipHCl showed a potential activity against *E. coli* gave an inhibition zone of 28 mm, and an inhibition zone of 27 mm against *S. aureus*.



(a) *E. coli* (b) *S. aureus*

Fig. 5 Antibacterial activities of heat treated (1) PVA (control), (2) PVA/CipHCl, (3) PVA/SF/CipHCl (inhibition zone is indicated by arrows)

Table 2 Inhibition zone diameters of electrospun PVA, PVA/CipHCl, and PVA/SF/CipHCl composite nanofibers/mm

Samples	*E. coli*	*S. aureus*
PVA	0	0
PVA/CipHCl	30	30
PVA/SF/CipHCl	28	27

3 Conclusions

Electrospun PVA/SF/CipHCl composite nanofibers as a wound dressing were successfully prepared. They were no longer dissolved in water and were keeping their web structure in water by using the heat treatment technique in an oven at 155 ℃ for 5 min. The incorporation of CipHCl into electrospun nanofibers made some interaction between PVA/CipHCl and PVA/SF/CipHCl, which enhanced mechanical properties of PVA and PVA/SF. Heat treated electrospun PVA/CipHCl and PVA/SF/CipHCl composite nanofibers showed remarkable antibacterial activity against gram-positive and gram-negative bacteria.

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