A novel electrospun membrane based on moxifloxacin hydrochloride/poly(vinyl alcohol)/sodium alginate for antibacterial wound dressings in practical application

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Abstract
This study reports on the performance of sodium alginate (SA)/poly(vinyl alcohol) (PVA)/moxifloxacin hydrochloride (MH) nanofibrous membranes (NFM) capable of providing antibacterial agent delivery for wound-dressing applications. The aim of this work was to prepare antibacterial NFM with good permeability properties by employing PVA and SA as carriers. A group of 12% PVA/2% SA solutions blended in various ratios (8:2, 7:3, 6:4, 5:5 and 4:6, v/v) and containing 0.5, 1, 2 or 4 wt% MH were studied for electrospinning into nanoscale fiber mats. The optimum ratio found to form smooth fibers with uniform fibrous features was 6:4. The drug release behavior of the electrospun, the antibacterial effects on *Pseudomonas aeruginosa* and *Staphylococcus aureus* and the animal wound dressing capabilities were also investigated. As much as 80% of the MH was released from the electrospun after 10 h of incubation at 37 °C. In addition, the NFM with 0.5 MH exhibited less activity, whereas those with higher concentrations of MH exhibited greater antibacterial effect. Furthermore, the MH-loaded electrospun accelerated the rate of wound dressing compared to other groups. The results of the in vitro and in vivo experiments suggest that MH/PVA/SA nanofibers might be an interesting bioactive wound dressing for clinical applications.

Keywords
Biodegradable polymers, biomaterials, electrospinning, nanotechnology, wound healing

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Introduction
Over the last decade, extensive scientific research into improving wound healing has been conducted (Said et al., 2011). Wound dressings typically have the following requirements: they must control bleeding, prevent infection, absorb secretions, promote wound healing, reduce scarring. And an ideal wound dressing should be non-toxic, have good biocompatibility, permeability, skin flexibility and biodegradability (Francesko & Tzanov, 2010). In the past, traditional dressings such as bandages, cotton wool and gauzes were used to offer protection against bacteria, but limited swelling capacity and moisture vapor permeability restrict their applications. To overcome this issue, modern dressings as vehicles for delivering therapeutic agents to wound sites have been applied in the form of films, sponges, foams, hydrogels (Cui et al., 2011; Balata et al., 2014; Vasile et al., 2014). And recently nanofibrous membranes (NFM) produced via electrospinning are beginning to emerge as wound dressing materials (Sill & von Recum, 2008; Charensriwilaiwat et al., 2012; Ignatova et al., 2013; Mogossanu & Grumezescu, 2014), due to the unique properties of electrospun mats – high specific surface area and small-size pores are very favorable for the adsorption of body fluids and for preventing penetration of bacteria and thus provide good conditions for wound healing (Zahedi et al., 2010; Rieger et al., 2013).

In the electrospinning field, water-soluble synthetic polymers, such as polyethylene oxide (PEO) (Chen et al., 2008) and poly(vinyl alcohol) (PVA) are often used in the preparation of NFM from their blend solutions. Sodium alginate (SA) consists of mannuronate (M) and guluronate (G) arranged in a non-regular varying block-wise pattern and has many potential uses in its bulk form. There has recently been extensive interest in alginate matrices for drug delivery applications because of their biocompatibility, biodegradation and protein-release properties. Therefore, SA has been adopted in the fabrication of hemostatic and liquid-absorbing wound dressings (Stashak et al., 2004; Wang et al., 2011). SA helps in removing the dressing without much trauma, and reduces the pain experienced by the patient during dressing changes (Paul & Sharma, 2004). It provides a moist environment that leads to rapid granulation and reepithelization. However, SA alone did not electrospin (Lu et al., 2006; Alborzi et al., 2010; Shen & Hsieh, 2014). PVA is a non-toxic, hydrophilic polymer with biodegradation and

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adhesive properties that can form solid hydrogen bonds with water (Bolto et al., 2009; Garg et al., 2014). Many researchers have utilized electrospinning to fabricate PVA nanofibrous scaffolds for use as wound healing scaffolds (Draize et al., 1944; Cencetti et al., 2012; Charernsriwilaiwat et al., 2012; Nitanan et al., 2013). A characteristic of materials designed for wound dressing is its antimicrobial effect. Antibiotics are the best bactericides to generate nanofibers with induced antibacterial properties. Moxifloxacin hydrochloride (MH) is a fourth-generation quinolone antibiotic that is widely used for respiratory infections, community-acquired pneumonia, skin and soft-tissue infections. In this study, SA and PVA were employed as carriers, and MH was selected as the drug for the preparation of composite NFM as wound dressings. The properties of the NFM, including its permeability, antibacterial activity and wound healing, skin irritation were investigated.

Materials and methods

Materials

SA was purchased from Biological ShengGong Engineering (Shanghai, China). PVA (molecular weight 85 000–12 400) was purchased from Sigma (Sigma-Aldrich, St Louis, MO), and calcium chloride was obtained from Zi Gong Hong He Pharmaceutical (Sichuan, China). Anhydrous alcohol, triethylamine and phosphoric acid were purchased from Chongqing East Sichuan Chemical (Chongqing, China). Boat Brand absorbent gauze was purchased from Chongqing 9th Pharmaceutical (Sichuan, China). Anhydrous alcohol, triethylamine and phosphoric acid were purchased from Chongqing East Sichuan Chemical (Chongqing, China). Boat Brand absorbent gauze was purchased from Chongqing 9th Pharmaceutical Factory (Chongqing, China) and was banded with OYEAH® Sheer Bandage (Hangzhou Outuopu Biological Technology, Zhejiang Province, China). All other chemicals used in this study were of analytical grade.

Preparation of MH/PVA/SA nanofibers

The SA (2%) and PVA (12%) solutions were prepared by dissolving 2 g of SA and 12 g of PVA separately in 100 mL of distilled water (Nie et al., 2009). PVA/SA solutions containing various volume ratios (8:2, 7:3, 6:4, 5:5 and 4:6) of 12% PVA and 2% SA were prepared and the conductivity of the solutions was determined so that the optimal volume ratio could be found. The MH was then dissolved in the mixing solution at a concentration of 0.5, 1, 2 or 4% (w/w, MH to the total SA and PVA quality percentage) (Shalumon et al., 2011). To ensure good dispersion of the MH within the blended solution, the mixture was stirred to complete dissolution and was sonicated prior to electrospinning. The MH/PVA/SA blend solution was transferred to a 5-mL plastic syringe equipped with a flat-end metal needle with an inner diameter of 0.7 mm. A 14 kV voltage was supplied by a direct-current power supply and the feed rate for the polymer solution was adjusted to a constant rate of 0.3 mLh⁻¹. A ground collection plate of aluminum foil was located at a fixed distance of 10 cm from the needle tip. The NFM was carefully detached from the collector and dried under vacuum for 48 h at room temperature (25°C) to completely remove the solvent. The conductivity of the spinning solutions was measured using a conductivity meter (DDS-307A, Shanghai Precision and Scientific Instrument Co, Ltd, Shanghai, China).

Cross-linking

The NFMs were cross-linked in glutaraldehyde vapor at room temperature for 1 h, and heated at 80°C for another 4 h to remove any residual glutaraldehyde.

Characterization of NFM

The diameter and morphologies of the 2% MH/PVA/SA electrospun fibers were observed using a scanning electron microscope (SEM, KYKY Amray 1000B, Beijing, China) operated at an accelerating voltage of 5 kV. Each sample was sputter-coated with platinum prior to analysis. FTIR (Fourier-transform infrared spectroscopy) spectra were recorded on an infrared spectrometer (IR, Spectrum one, Perkin-Elmer, Germany) at 4 cm⁻¹ resolution for 32 scans in the range of 4000–400 cm⁻¹. DSC (differential scanning calorimetry) spectra were recorded under N₂ atmosphere on a Netzsch STA-449 C equipped with Liquid Nitrogen Dewar (CC200 supply system, Netzsch, Germany). Samples were placed in crimped aluminum pans and heated from 0°C to 400°C at a heating rate of 10°C per minute (Meng et al., 2010).

Measurement of water vapor permeability

The water vapor permeability of the NFM was determined as described by Vargas et al. using the ASTM E 96 desiccant method (Vargas et al., 2010). An open cup that contained solid calcium chloride as the desiccant was sealed with the specimen membrane such that the cup mouth was used to mask the non-woven fabric for use as a blank control group (thickness of 0.20 mm), to mask non-woven fabric and MH/PVA/SA electrospun membrane as two stacked layers (thickness of 0.35 mm) for use as the treatment group and to mask non-woven even daub MH/PVA/SA solution after drying of the membrane (thickness of 0.30 mm) for use as a control group. The assembly was then placed in a test chamber at 37°C with a constant relative humidity (RH) of 75% for 24 h. The change in weight of the permeation cup with the specimen was recorded. The water vapor permeation (WVP) was calculated using following equation:

\[ R_{wvp} = W / (A \times \Delta p) \]

where \( R_{wvp} \) is the WVP rate (g h⁻¹ cm⁻² mmHg⁻¹), \( W \) is the amount of water vapor permeating through the film (g h⁻¹), \( A \) is the area of the exposed membrane (11.9 cm²), and \( \Delta p \) is the vapor pressure difference (mmHg). The permeability \( P \) was calculated using following equation:

\[ P = R_{wvp} \times d \]

where \( d \) is the thickness of the membrane.

Degree of swelling and weight loss

Degree of swelling and weight loss of MH/PVA/SA fiber mat was measured after the samples were submerged in distilled water at 37°C for 24 h according to the following equations:

\[ DS = [(W_f - W_d) / W_d] \times 100. \]

\[ Weight\ loss = [(W_0 - W_d)] \times 100. \]
where DS is the degree of swelling, \( W_t \) and \( W_d \) are wet and dry weights of the mat after submersion in the buffer solution for 24 h, respectively, and \( W_0 \) is the initial weight of the sample in its dry state.

**MH release from electrospun nanofibers**

The drug-loaded nanofiber sample was incubated in 50 mL of acetate buffer (pH 5.5) (Schreml et al., 2010) at 37°C. At various times, 1 mL of the release medium was removed and diluted to 10 mL with fresh buffer solution. The amount of released MH was determined using a high-performance liquid chromatography (HPLC) (Kenawy et al., 2002; Charernsriwilaiwat et al., 2012) system equipped with a quaternary gradient pump unit, a variable wavelength detector, an auto-sampler and a thermostated column compartment. Sample analyses were performed on a Diamonsil C18 column (250 mm long × 4.6 mm I.D.; 5 μm) at a temperature of 30°C. Methanol/1% triethylamine (45:55, v/v; pH = 3.0) was used as the mobile phase at a flow rate of 1.0 mL/min, and the effluent was monitored at 280 nm using a UV detector.

**Antibacterial activity test**

*Pseudomonas aeruginosa* and *Staphylococcus aureus* were used as the test organisms and were prepared from fresh colonies on tryptic soy agar (TSA) (Jayakumar et al., 2011). One loopful of the bacteria was inoculated in a test tube. The plates were incubated for 24 h at 37°C. The calculated average diameter of the healing residue area \( HR \) was calculated using the following equation:

\[
HR = \frac{AO - AT}{AO} \times 100
\]

where \( HR \) is the healing rate, \( AO \) is the original incision area (original area of 0.79 cm²), and \( AT \) is the presently measured area.

**Observation and evaluation index**

The wound diameters were measured in directions parallel and perpendicular to the spine 3, 8 and 14 d after injury. The mean erythemal scores were recorded according to the Draize method (Nitanan et al., 2013): (1) erythema and eschar.

**Primary skin irritation test**

The samples were assessed for skin irritation using the appropriate ASTM test method (A.S.T.M, 1992, 2005). The research was conducted in accordance with internationally accepted principles for laboratory animal use and care, as found in the NIH Guide for Care and Use of Laboratory Animals. Six healthy New Zealand rabbits (half male and half female) that weighed between 2.0 and 2.5 kg were selected for the skin irritation study. The rabbits, which were identified by ear tag, were individually housed in suspended cages and received rabbit feed on a daily basis; tap water was available ad libitum (Faqi et al., 2011). Animal husbandry was conducted in accordance with the “Guide for the Care and use of Laboratory Animals,” NIH Publication No. 85-23. The fur was removed from the dorsal surface of the rabbits 24 h prior to administration, and the injury model was obtained by scarifying intact skin until capillary hemorrhage with an area of 3 cm × 3 cm on both sides of the spine. The wounds for group I were treated with MH/PVA/SA nanofibers. The wounds for group II received PVA/SA as a blank treatment. The wounds for group III received gauze as a control. The wounds for group IV received no treatment. After 24 h, the experimental area was treated with 0.1 g of MH/PVA/SA nanofiber membranes and was subsequently coated with two layers of gauze (2.5 cm × 2.5 cm) and a layer of cellophane for 5 h. The gauze was then removed, and warm water was used to rinse the residue from the samples. The reaction of the skin, such as the occurrences of erythema and dryness and their extent, was recorded at 1, 24 and 48 h (Kim & Yoo, 2010; Vargas et al., 2010). The mean erythemal and edemal scores were recorded according to the Draize method (Nitanan et al., 2013): (1) erythema and eschar.
formation: no erythema = 0; very slight erythema = 1; well-defined erythema = 2; moderate to severe erythema = 3; severe erythema and slight eschar formation = 4 and (2) edema formation: no edema = 0; very slight edema = 1; slight edema = 2; moderate edema = 3; severe edema = 4.

Cytotoxicity evaluation

The potential cytotoxicity of the nanofibers was evaluated against L929 mouse fibroblasts. The 2% MH/PVA/SA NFMs were sterilized under UV light for 1 h on each side, then immersed in a serum-free medium containing only DMEM and placed in an incubator for 24 h to produce extraction media of 0.2, 0.4, and 0.8 mg/mL. The L929 cells were plated in 90 µL DMEM supplemented with 10% fetal bovine serum at a density of 8000 cells/well in 96-well plates, and the cells were cultured at 37 °C in a wet atmosphere containing 5% CO₂. 24 h after plating, the extraction media containing 10% FBS was replaced, and the cells were incubated for an additional 24 h. Then, the tested extraction solutions were removed. Finally, the cells were incubated in 20 µL MTT-containing medium (5 mg/mL) for 3 h. After the medium was removed, the formazan crystals formed in the living cells were dissolved in 100 µL DMSO. Cell viability (%) was calculated based on the absorbance at 490 nm using a microplate reader (SpectraMax Plus 384, California, USA).

Statistical analysis

The statistical analyses were performed using SPSS version 18.0 (SPSS Inc, Chicago, IL, USA). All data are presented as the mean ± SD. For comparisons among groups, a one-way ANOVA with post-hoc Bonferroni tests was used. Differences were considered statistically significant at p < 0.05.

Results and discussion

Conductivity of MH/PVA/SA solutions

In this study, smooth fibers were obtained through electrospinning by blending 12% PVA and 2% SA such that equilibrium was reached. Table 1 shows the effect of ratios of the PVA and the SA on the conductivity and pH of the electrospinning solutions. The conductivity and pH of the solutions increased with increasing SA content. These increases caused the decrease of electrospinnability, and the morphology of the electrospun fibers to vary from smooth fibers to beaded fibers (images of fibers with different ratio of PVA and SA are given in Supplementary data). To obtain fine uniform fibers, the ratio of SA should be as high as possible for hemostasis and wound healing properties. The optimal ratio found in our study was 12% PVA and 2% SA for a 6:4 ratio.

Table 1. The pH and conductivity for different volume ratios of electrospinning solutions.

| 12%PVA:2%SA(V:V) | pH  | Conductivity (mS/cm) |
|-------------------|-----|----------------------|
| 8:2               | 6.38| 21.0                 |
| 7:3               | 6.45| 26.2                 |
| 6:4               | 6.56| 30.4                 |
| 5:5               | 6.84| 37.0                 |
| 4:6               | 7.15| 42.6                 |

Characterization of electrospun nanofibers

The MH/PVA/SA nanofiber membranes with 0 and 2% MH were characterized using fourier-transform infrared (FTIR) spectroscopy, UV spectroscopy, DSC and SEM.

FTIR analysis

The FTIR spectra of the MH, PVA/SA NFM, mixture of MH/PVA/SA and MH/PVA/SA NFM are provided in Figure 1. The PVA/SA NFM blend shows a sharp asymmetric carboxylate band at 1635 cm⁻¹ and a broad hydroxyl band at 3457 cm⁻¹. The characteristic peaks of MH were assigned as follows: 3522 cm⁻¹ (O–H group), 3471 cm⁻¹ (N–H group), 1707 cm⁻¹ (C–O group), 1455 cm⁻¹ (C–C group), 1352 and 1320 cm⁻¹ (C–F group). These characteristic absorption peaks disappeared in the MH/PVA/SA physical mixture and MH/PVA/SA NFM suggests interaction of MH with carrier materials. For the MH/PVA/SA physical mixture and MH/PVA/SA mats, the two spectra were similar with no shifts in any of the peaks, suggesting that the interaction between MH and PVA/SA was not obviously influenced by the electrospinning procedure.

Thermal properties analysis

As shown in Figure 2, the DSC thermograph of the PVA (Figure 2f) shows endothermic peaks at 195 °C and 321 °C, corresponding to the melting and decomposition temperature, respectively. However, for the mixture of SA and PVA (Figure 2d), the mixture of MH/PVA/SA (Figure 2c) and MH/PVA/SA NFM (Figure 2a), the exothermic peak of SA (250 °C) disappears and the decomposition temperature shifts to the lower temperature, confirm the existence of interactions between PVA and SA (Shen & Hsieh, 2014; Yang et al., 2014). It can be drawn a conclusion that the formation of hydrogen bonds between two different macromolecules competes with the formation of hydrogen bonds between molecules of the same polymer (Sionkowska, 2003). Moreover, the characteristic peaks of MH (Figure 2b) disappeared in the MH/PVA/SA physical mixture and MH/ PVA/SA NFM, indicating the amorphous dispersion of MH into the SA/PVA matrix (Ray et al., 2010). And the melting temperatures of the polymers employed in MH-loaded nanofibers were not obviously influenced by the procedure (Figure 2a).

Thermographs are shown in Figure 3. Pure PVA existed one weight-loss step around 300 °C (Figure 3f), which was considered to reflect the decomposition of side chain (T-ds) of PVA. However, for MH/PVA/SA fiber (Figure 3a), two degradation steps could be observed. One weight loss at 20–80 °C was associated with the moisture vaporization, independent of the composition for all samples. The second weight loss at 250–350 °C is due to the thermal degradation of MN/SA/PVA fiber.

SEM analysis

Figures 4(a) and 4(b) show the fibrous morphologies and diameter distributions of the PVA/SA and the 2% MH/PVA/SA electrospun nanofibers, respectively. The SEM images of the electrospun fibers indicate that both the PVA/SA and
Figure 1. IR spectra of different samples: (a) MH; (b) PVA/SA fibers; (c) mixture of MH/PVA/SA; and (d) MH/PVA/SA fibers.
the 2% MH/PVA/SA electrospun nanofibers have uniform fibrous features on the surface. The diameter of the PVA/SA fibers was 148 ± 41 nm, and the diameter of the 2% MH/PVA/SA electrospun nanofiber was 175 ± 75 nm. An increase in the amount of MH did not significantly increase the diameters of fibers.

Permeability and swelling tests
An ideal wound dressing should control the water loss from a wound at an optimal rate (Mishra et al., 2011). The water vapor permeability of a wound dressing should prevent excessive dehydration as well as the build-up of exudates (Balakrishnan et al., 2005). The water-vapor permeabilities of the blank control group (non-woven fabric), the treatment group (non-woven fabric and MH/PVA/SA electrospun membrane as two stacked layers) and the control group (non-woven even daub MH/PVA/SA solution after drying of the membrane) are shown in Table 2. The blank control group and the treatment group show statistically significant differences ($p < 0.05$) in the measured water-vapor permeabilities. The degree of swelling of the MH/PVA/SA fiber
mat was 108 ± 6.45%, meanwhile the percentage of weight loss was low (4.94 ± 1.98%). High swelling ability and low weight loss of nanofiber is important property for wound dressing that used to control wound exudates and keep moist environment on the wound.

**MH release from electrospun nanofibers**

The release rate of MH from the electrospun fibers at a pH of 5.5 at a temperature of 37 °C was studied. Figure 5 shows that the release of 2% MH from the PVA/SA fibers exhibited a burst release stage during the first 8 h, followed by a gradual increase in the cumulative release until a plateau was reached at 26 h. Additionally, in the preliminary experiments, it was found that the release curve is similar with the increase of MH (Data not shown). The mechanism by which the MH is released after the initial burst is by diffusion through swelling fibers to reach the medium. The burst phenomenon may be partly due to the formation of pores and to the migration of drugs toward the sample surface with medium convection during the drying and storage processes (Mallapragada et al., 1997; Cui et al., 2011). The mechanism of drug release in the first 8 h was examined on the basis of the first-order, zero-order and Higuchi square root of time models summarized in Table 3 together with goodness of fit results for MH/SA/PVA fibers. Higuchi square root of time model showed acceptable fit to the in-vitro release data ($R = 0.996$), indicating that drug release was controlled by a combination of drug diffusion through the gel barrier formed by the hydrated polymer, and

![Figure 4. Characterization of electrospun nanofibers by SEM imaging: (a) PVA/SA electrospun nanofibers; (b) 2% MH/PVA/SA electrospun nanofibers.](image)

![Figure 5. In vitro drug release profile of the MH from the MH/PVA/SA nanofibers ($n = 3$).](image)

| Groups                | n   | Permeability          |
|-----------------------|-----|-----------------------|
| Blank control groups  | 9   | (7.75 ± 1.80) × 10^{-6} |
| Control groups        | 9   | (6.23 ± 1.03) × 10^{-6} |
| Treatment groups      | 9   | (11.85 ± 3.01) × 10^{-6} |

*p < 0.05

Table 2. Water-vapor permeability test results ($\bar{x} \pm s$).
matrix erosion. Once the PVA/SA matrix is exposed in liquid medium, it begins to swell, its molecular chains are solvated (Jannesari et al., 2011). Therefore, the high degree of swelling along with the dissolution of the PVA/SA nanofibers causes the release of MH.

**Antibacterial studies**

Figure 6 shows the antibacterial activity studies of the PVA/SA fibers, the 0.5%, 2%, 4% MH/PVA/SA fibers against *P. aeruginosa* and *S. aureus*. It can be seen that as the MH concentration was increased, the zone of inhibition increased. Compared with *P. aeruginosa* (Figure 6c), *S. aureus* shows a slightly smaller inhibition zone (Figure 6a). These results show that MH exhibits toxicity for common Gram-positive and Gram-negative bacteria. Table 4 shows the zone of inhibition for all the samples for both strains. There is no

| Classification | Group name                   | Inhibition zone diameter for *S. aureus* (mm) | Inhibition zone diameter for *P. aeruginosa* (mm) |
|----------------|------------------------------|-----------------------------------------------|--------------------------------------------------|
| 0h-cross-linked| PVA/SA                       | /                                             | /                                                |
|                | 0.5%MH/SA/PVA                | 17                                            | 23                                               |
|                | 2%MH/SA/PVA                  | 18                                            | 33                                               |
|                | 4%MH/SA/PVA                  | 22                                            | 38                                               |
| 1h-cross-linked| PVA/SA                       | 17                                            | 25                                               |
|                | 0.5%MH/SA/PVA                | 19                                            | 34                                               |
|                | 2%MH/SA/PVA                  | 23                                            | 38                                               |

Table 4. Bacterial inhibition zones for the PVA/SA and the MH/SA/PVA fibers against *S. aureus* and *P. aeruginosa*.

Figure 6. Antibacterial activity results of the MH/SA/PVA nanofibers against *S. aureus* (a and b) and *P. aeruginosa* (c and d). a and c: 1 h-cross-linked; b and d: 0 h-cross-linked; (1) PVA/SA nanofibers; (2) 0.5% MH/PVA/SA nanofibers; (3) 2% MH/PVA/SA nanofibers and (4) 4% MH/PVA/SA nanofibers.
significant difference between cross-linked and non-cross-linked group indicating that process of cross-linking do not affect the antibacterial effect of NFM.

**Wound healing percentage**

In the wound healing test, four full-thickness round wounds with a surface area of 0.79 cm² were created on the back of each rat. Figure 7(a) shows the representative images of wound healing at 1, 3, 8, 11 and 14 d after treatment for each group. Wound closure in all treatments was observed within 14 d. At 3 d, wound closure in the treatment group was similar to the negative control group. In contrast, at 8 and 14 d after surgery, the healing in the treatment group was visibly faster than those in the negative control group, the control group and the blank control group. Moreover, swelling and inflammation were observed in the wounds of the blank control group and the control group on day 8, which continued to day 14. Figure 7(b) shows the changes in the wound healing percentage at different healing times. During the first 3 and 8 d, the MH/PVA/SA nanofiber mats enabled better wound healing than the blank group ($p<0.05$). However, the difference between the commercial woundplast and the blank group was not statistically significant. The wound healing percentage for all treatments between day 11 and recovery were similar.

**Skin HE staining**

Normally, dermal recovery is assessed based on three stages: proliferation, remodeling, and maturation (Wang et al., 2011). Re-epithelialization on the granulation tissue in open wound, forming a barrier between the wound and the environment, is one of the most important indicators for skin healing (Yang et al., 2011). The HE staining of the wound areas in different groups is shown in Figure 8. On day 8, formation of granulation tissue, characterized by the accumulation of non-specific inflammatory cells, was observed in the PVA/SA group (Figure 8e) and the no-treatment group (Figure 8h). In contrast, the granulated tissue in the 2% MH/PVA/SA and commercial woundplast groups (Figure 8f and g) disappeared without capillary hyperplasia. The survival of the wound crust was considered as an indicator of starting of re-epithelization under the sides of the wound. On day 14, it was observed that epithelization was completed after treatment with 2% MH/PVA/SA NFM (Figure 8b) and commercial woundplast (Figure 8c), hair follicles and sebaceous gland formed in the dermis, in almost normal count and characteristics, and the connective tissue of dermis returned to normal. The epidermis in PVA/SA nanofiber and control groups was still partially unclosed (Figure 8a and d) and the skin appendages were still barely visible in these groups. About 2% MH/PVA/SA-treated wound exhibited advancement in all dermal recovery stages than the PVA/SA group and the control group.

**Primary skin irritation test**

The primary skin irritation test was conducted to evaluate the irritancy of the MH/PVA/SA and the PVA/SA NFM through contact with abraded and intact skin of rabbits. The primary irritation index (PII) was calculated for each rabbit as the difference between the sum of the score for erythema...
and edema in the treated group, the blank-treated group, the control group and the blank group. According to the intensity criteria for skin irritation, scores below 0.5 were regarded as no irritation. As shown in Table 5, all of the three groups (the blank group, PVA/SA and MH/PVA/SA) caused no irritation to normal skin, indicating that the bioactive membrane is suitable for use in wound dressings.

### Cytotoxicity evaluation

The cytotoxicity of various concentrations of the extraction medium from the 2% MH/PVA/SA NFM is shown in Figure 9. Cell viability increased significantly when L929 cells were incubated with lower concentrations (0.2–0.4 mg/mL) when compared with the control \( (p < 0.05) \) and no significant cytotoxicity was observed at 0.8 mg/mL of the extraction medium. It can be concluded that the MH/PVA/SA NFM has excellent in vitro biocompatibility at the concentrations tested (0.2–0.8 mg/mL).

![Figure 9.](image)

Figure 9. Cytotoxicity tests from the MTT assays of cell viability. The absorbance was normalized to that of the negative control at each time interval, which was considered 100%. *\( p < 0.05 \) compared with negative control. The data are presented as the mean ± SD \( (n = 5) \).

### Conclusions

An ideal wound healing system must maintain a balance between antibacterial efficacy, cytotoxicity and a moist environment. Our results with electrospun NFM based on SA/PVA demonstrate that SA membranes containing 2%
MH show promise for increasing the effectiveness of wound dressings. They showed a good balance of antibacterial efficacy, cytotoxicity and moist environment. The water-vapor permeability of the NFM samples was measured, and the results showed evidence of good permeability. Furthermore, the MH/PVA/SA NFMs showed good potential for wound healing and antimicrobial activity against strains of *S. aureus* and *P. aeruginosa*.

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**Declaration of interest**

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