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Co-electrospun Nanofibrous Membranes of Collagen and Zein for Wound Healing

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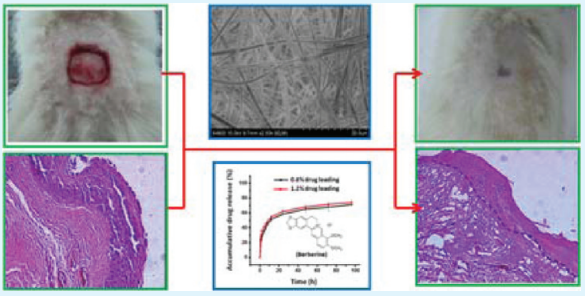
ABSTRACT: To develop biocompatible nanofibrous membranes for wound healing, we investigated the coelectrospinning of two proteins (collagen and zein) in aqueous acetic acid solution. It was found that the combination of zein could improve the electrospinnability of collagen. For the resultant electrospun membrane, its fiber diameter, surface wettability, mechanical, and in vitro degradable properties as well as cell adhesive ability could be modulated by the change of collagen/zein blending ratio. Moreover, berberine drug could be incorporated in situ into the electrospun nanofibrous membrane for its controlled release and antibacterial activity. The addition of berberine showed little effects on the fiber morphology and cell viability. In addition, the wound healing performance of the as-obtained nanofibrous membranes was examined in vivo by using female Sprague–Dawley rats and histological observation.



KEYWORDS: *electrospun nanofibers, collagen, zein, berberine, wound dressing, tissue regeneration*

INTRODUCTION

In recent years, electrospun polymeric nanofibers have been shown great potential as dressing materials for wound healing.[1−4] Typically, such materials have high porosity with excellent pore-interconnectivity, which is particularly important for exuding fluid from the wound. The inherent small pores and high specific surface area enable them to inhibit the invasion of exogenous microorganisms and assist the control of fluid drainage.[5] In addition, some antibacterial and therapeutic agents can be incorporated easily during the electrospinning in order to obtain multifunctional nanofibrous membranes.[6,7] Up to now, various biopolymers and their derivatives have been used widely for this purpose because of their biological origin, nontoxicity, hydrophilicity, biocompatibility, biodegradability, and low cost.[8−16] However, single-component biopolymer solution is often insufficient for good electrospinnability or could not result in good fiber properties.[17−19] To overcome these limitations, recent effort has been given to combine biopolymer with other polymers for the preparation of electrospun polymeric nanofibers.[18−27] For example, Pakravan et al.[18] electrospun a highly deacetylated chitosan in 50% acetic acid in the presence of polyethylene oxide and then obtained beadless nanofibers of 60−80 nm in diameter; Jeong et al.[19] mixed alginate with polyethylene oxide in aqueous solution for the preparation of uniform nanofibers for cell adhesion; Detta et al.[20] coelectrospun gelatin and polyurethane for the fabrication of vascular grafts; Nagarajan et al.[21] prepared the electrospun nanofibers of genetically engineered silk-elastin biopolymer by the in situ incorporation of polymer-micelle complexes; Recently, Tong et al.[22] obtained the core−shell structured nanofibrous scaffolds by the coaxial electrospinning of chitosan and poly(hydroxybutyrate-co-hydroxyvalerate).

In this work, we attempt to develop biocompatible electrospun nanofibers for wound healing by coelectrospinning of collagen and zein in aqueous acetic acid solution. Collagen is one of the main proteins in the connective tissues and has been shown to have many advantageous features including biodegradability, weak antigenecity, and superior biocompatibility in medical applications.[28] However, individual collagen was not used for electrospinning except for the use of potentially toxic and precious 1,1,1,3,3,3-hexafluoro-2-propanol as the solvent,[29] and is often coelectrospun with other synthetic polymers such as polyurethane,[30] poly(3-hydroxybutyrate-co-3-hydroxyvalerate),[31] poly(ethylene oxide)[32,33] or poly(vinyl alcohol).[34] Zein is one of the main plant proteins and has been used to develop biodegradable films and other substrates for tissue engineering and other medical applications.[35] Recent studies[36,37] have shown that it is easy to be electrospun into fibers. Moreover, zein has better biocompatibility and good degradability when compared to those synthetic polymers coelectrospun with collagen. Therefore, the coelectrospinning of collagen and zein may improve the electrospinnability of collagen and result in biocompatible nanofibrous membranes with enhanced properties. To our knowledge, however, no work has been done for such an attempt except for our present study. To optimize collagen/zein combination for the coelectrospinning, we studied various

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blending ratios of these two proteins with respect to their effects on electrospinnability and resultant fiber properties such as surface wettability, mechanical and in vitro degradable properties as well as cell adhesive ability. To provide the electrospun membrane with antibacterial activity, the in situ incorporation of berberine drug during the electrospinning and its effects on the fiber structure and properties were also studied. For the as-obtained nanofibrous membranes, their wound healing performance was examined in vivo by using female Sprague−Dawley rats and histological observation.

EXPERIMENTAL SECTION

Materials. Collagen from bovine achilles tendon and zein from corn were purchased from Guangzhou Qiyun Chemical Factory in China. Berberine was supplied from Aladdin Chemical Company in China. Glacial acetic acid, glutaraldehyde, and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich. The mice skin fibroblast (L929) cells were provided by Guangdong Medical College (China).

Electrospinning of Collagen and Zein Solutions. For the electrospinning, various solutions including neat collagen solution, neat zein solution and various collagen/zein blends were prepared in aqueous 70% (v/v) acetic acid solution, respectively. The total polymer concentration of each solution for the electrospinning was fixed to be 40% (w/v). All polymer solutions for electrospinning were characterized with respect to their apparent viscosity by an advanced rheometric extended system (ARES, TA Co.) fitted with a cylinder measuring system. During the electrospinning, the solution feed was driven using a syringe pump. A 20 kV electrospinning voltage was applied between the needle and the collector (aluminum foil). The positive electrode of a high voltage power supply was connected to a metal capillary by copper wires. The distance between the tip of the needle and the surface of the aluminum foil used as a collector was 15 cm, and the flow rate of the solution was 0.75 mL/h. All electrospinning procedures were performed at room temperature. For the in situ incorporation of berberine drug, a required amount of berberine was first dissolved in the collagen/zein blend and then coelectrospun at the same conditions. To ensure that acetic acid did not remain in the electrospun fibers, we dried the fibers in a vacuum dryer for 48 h at room temperature to remove the solvent.

Characterization of Electrospun Fibers. The fiber morphologies of the electrospun membranes were examined by scanning electron microscopy (SEM, JSM-6330F, Japan). Briefly, each electrospun fiber matrix was sputter-coated with gold and visualized by SEM at an accelerating voltage of 15 kV. To determine the fiber diameters and size distribution, the SEM images were analyzed using the image analyzer software (Image plus 6). The averages and standard deviations of the fiber diameters were calculated from 100 random measurements.

For the resultant electrospun collagen/zein membranes, their surface wettability was characterized on the basis of pure water contact angle measurement. Using a sessile drop method, static water contact angle was measured at room temperature on a contact angle goniometer (KRUSS DSA10-MK) equipped with video capture. A total of 30 μL of deionized water was dropped onto a dried electrospun membrane with a micro syringe in an atmosphere of saturated water vapor. At least 10 contact angles were averaged to get a reliable value.

The mechanical properties of electrospun collagen/zein membranes were tested by a universal testing machine (Hounsfield THE 10K−S) equipped with a 100 N load-cell. The thickness of each membrane for mechanical test is about 0.06 mm. Samples were cut into strips in 60 × 10 mm^2. Each tensile test was operated under a crosshead speed of 5 mm/min at room temperature. The tensile strength and elongation were determined according to the average results of 5 tests.

The in vitro degradation of electrospun collagen/zein membrane was monitored by immersing the fibers in a phosphate buffer (pH 7.4) at 37 °C. The samples were removed from the solutions and weighed at 7, 14, and 21 days after being dried inside an oven for 24 h. The weight retention (WR, %) of the nanofibrous matrix was calculated according to the following equation

$$WR(\%) = W_1/W_0 \times 100 \quad (1)$$

where W_0 and W_1 were the initial weight of the fiber sample and the weight of the fiber sample at different days, respectively.

Cell Culture and Cellular Behavior Studies. Electrospun collagen/zein membranes were cut into the films with the area of approximately 1 cm^2 and sterilized by UV irradiation for 1 h. L929 fibroblast cell were seeding onto the nanofiber film at a density of 1.0×10^5 cells/cm^2 in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (w/v) fetal bovine serum, 100 μg/mL penicillin and 100 μg/mL streptomycin. The dishes were incubated at 37 °C in a humidified atmosphere of 5% CO_2 and the culture medium was changed once every 3 days.

For the cell adhesion assay, the cellular constructs were rinsed with Hank's solution at each time point, and the number of attached cells was determined using a hematocytometer after detaching the cells from the fabrics using 0.1% trypsin. The cell adhesion percentage (CA, %) of the nanofibers was calculated according to the following equation:

$$CA(\%) = (N_1/N_0) \times 100 \quad (2)$$

where N_0 and N_1 were the initial number of cells seeded on the electrospun membranes and the number of cells adhesion on the electrospun membranes at each time point, respectively.

The morphology of the cells on the surfaces of electrospun collagen/zein membranes were observed by SEM (JSM-6330F, Tokyo, Japan) after the culture for various time. The membrane samples were washed 3 times carefully with PBS and the cells were fixed with 2.5% (v/v) glutaraldehyde in 0.1 mol/L PBS (pH, 7.4) for 1 h at 4 °C and were further dehydrated by ethanol.

The cell viability was evaluated by the MTT assay as referred by Mossmann.[38] Briefly, the electrospun membranes with the attached cells were incubated in 20 μL of 3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) in DMEM for 4 h at 37 °C and in a 5% CO2 atm. The formazan derivative formed was dissolved in 200 μL of dimethyl sulfoxide for 15 min and the absorbance was measured at 570 nm by a spectrophotometer (Synergy2, USA). At each time point, six samples were used to measure the number of the cells attached on the electrospun membranes.

In vitro Drug Release Assay. For each drug release assay, a piece of electrospun collagen/zein membrane with berberine (about 20 mg) was first placed in a vial filled with a 20 mL phosphate buffer solution (pH 7.4). The release study was carried out at 37 °C and 60 rpm in a thermostatical shaking incubator. In this case, 3.0 mL of sample was taken from the medium after appropriate intervals and then the same volume of fresh release medium was added as replacement. A calibration curve was obtained by high-performance liquid chromatography (HPLC, Agilent 1200, USA) at 263 nm for the determination of berberine drug concentration. In the assessment of drug release behavior, a cumulated amount of the released drug was calculated. The percentages of drug released from the electrospun membrane were plotted against time.

Antibacterial Activity Test. The antibacterial activity of electrospun collagen/zein membranes without and with berberine against *Escherichia coli* and *Staphylococcus aureus* were investigated. The assessment was carried out by a disk agar diffusion method. A cell suspension of 1×10^8 cells/mL was inoculated and spread on an agar plate. All electrospun membranes were cut into circular discs (1 cm in diameter) and placed on the top of the agar plate. The plates were incubated at 37 °C for 12 h. If inhibitory concentrations were reached, there would be no growth of the microbes, which could be seen as a clear zone around the disk specimens. The zone was then recorded as an indication of inhibition against the microbial species.

Wound Healing Assay and Histological Examination. The in vivo wound healing was evaluated using 48 female Sprague−Dawley rats with an average body weight of 400 g. After anesthetization, two full-thickness rectangular wounds of 1 cm × 1 cm were prepared on each of the rat's back, parallel with the vertebral column. The

electrospun collagen/zein membranes were then applied to the wounds of each rat. For a comparison study, a gauze sponge was evaluated. No dressings were replaced during the whole healing process. After various postoperative days, macroscopic photographs of the wounds were taken, and the wound area was measured. For the histological analysis, the covered nanofibrous membranes and cotton gauze were removed from wound areas, and the healed wound skin tissues were dissected when the rat was under anesthesia. The cut tissue was fixed between two glass slides and then immersed in formalin solution. Isometry continuum cut sections were obtained by a microtome in transverse and vertical planes from each wound skin.

RESULTS AND DISCUSSION

Effects of Blending Ratio on Electrospinnability and Membrane Properties. Nanofibrous membranes made of biodegradable and biocompatible polymers are of great interest for wound healing because their structure is similar to that of the native extracellular matrix.[39,40] To obtain electrospun collagen/zein nanofibrous membranes, 70% (v/v) aqueous acetic acid was used as the cosolvent for collagen and zein. We found that such a solvent system could dissolve well two proteins even when their concentrations are higher than 60% (w/v).

Figure 1 shows the SEM images for the electrospun polymeric membranes from neat collagen solution, neat zein solution and collagen/zein blends with different weight fractions (f_{zein}) of zein. In these cases, total polymer concentration was kept to be 60% (w/v) for each electronspun solution. As observed, neat collagen solution did not result in any nano- or microfibers. In contrast, neat zein solution and three collegen/zein blends could be electrospun effectively into the bead-free membranes with fibrous morphology and different fiber diameters. Different from the membrane obtained from neat zein solution, the membranes obtained from three collegen/zein blends were found to have smaller fiber diameters and greater fiber flexibility. With the increase of f_{zein} from 0.33 to 0.67, the average fiber diameter increased from 423 to 910 nm, and the fibers became more rigid. It seems that the addition of zein can increase the electrospinnability of collagen.

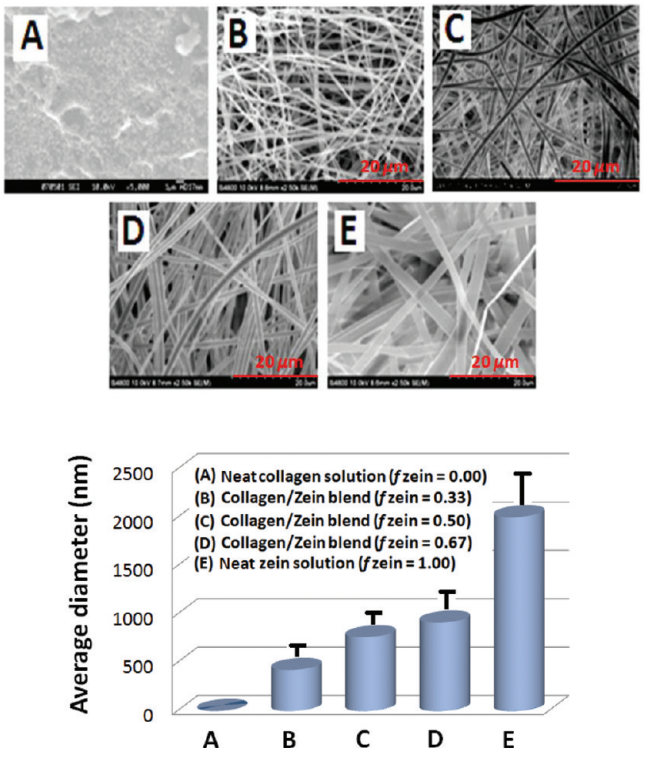


Figure 1. Morphology and average fiber diameters of electrospun membranes from neat collagen solution, neat zein solution and collagen/zein blends with different weight fractions (f_{zein}) of zein. Total polymer concentration, 60% (w/v); solvent, aqueous 70% acetic acid.

The bad electrospinnability of neat collagen solution may be due to inadequate chain entanglements in aqueous acetic acid solution.[41,42] To confirm this, we measured the apparent viscosity of each electrospun solution at a fixed shear rate of 1.0 1/s. As a result, the apparent viscosity was found to be 35.2 mPa s for neat collagen solution, 50.44 mPa.s for the blend with the f_{zein} of 0.33, 99.76 mPa s for the blend with the f_{zein} of 0.50, 233.26 mPa s for the blend with the f_{zein} of 0.67 and 681.17 mPa s for neat zein solution, respectively. Among them, neat collagen solution has the lowest viscosity and neat zein solution has the highest viscosity. In addition, an increase of f_{zein} results in a remarkable increase of blend viscosity, which would be favorable for electrospinning.[43]

For the resultant collagen/zein nanofibers, their fiber properties were investigated with respect to the effects of blending ratio in term of f_{zein}. Figure 2 gives the tensile strength and elongation at break of three electrospun nanofibers. With the increase in f_{zein} from 0.33 to 0.67, the tensile strength increased from 0.2 to 6.3 MPa, whereas the elongation at break decreased from 39.5 to 12.8%. As observed from the SEM images in Figure 1, randomly oriented nanofibers became more bulky and stiff when the amount of zein increased, which could be attributed to enhanced chain entanglements in electrospun collagen/zein blend with higher visicosity. Such a structure change would induce simultaneously the increase of tensile strength and the decrease of elongation at break. A reduced elongation at break implies that the electrospun nanofibers have a more brittle structure. From the viewpoint of wound dressing application, electrospun collagen/zein membranes should have reasonable tensile strength and elongation at break. A higher tensile strength and a greater elongation at break will be favorable for practical handling during the wound healing process.

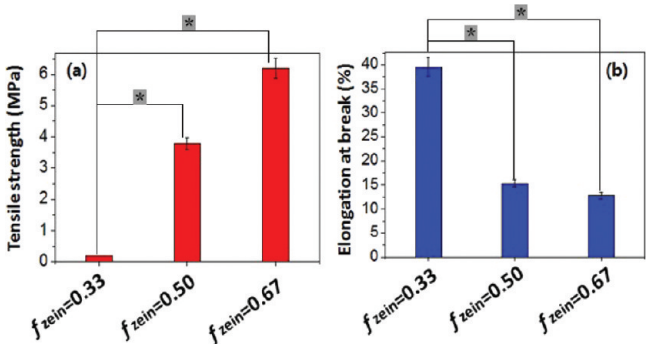
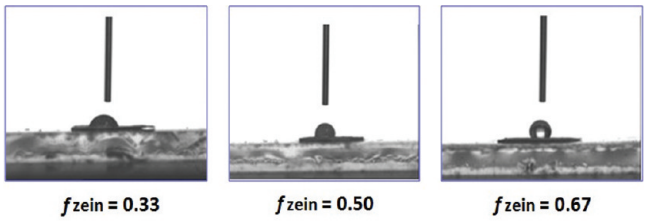


Figure 2. (a) Tensile strength and (b) elongation at break of electrospun nanofibers from collagen/zein blends with different weight fractions (f_{zein}) of zein. Data represent mean ± SD (n = 3, Student's t-test, *p < 0.05).

The hydrophilic/hydrophobic characteristics of the nanofibrous membranes can influence the initial adhesion of the cells and their proliferation to a higher extent.[44] For this reason, water contact angles of electrospun collagen/zein nanofibers were determined, as shown in Figure 3. With the increase of f_{zein} from 0.33 to 0.67, the water contact angle was found to decrease from 120.9 ± 5.0 to 75.7 ± 10.7. This result demonstrates that



Electrospun nanofiber	Contact angle (°)
fzein = 0.33	120.9 ± 5.0
fzein = 0.50	106.7 ± 6.2
fzein = 0.67	75.7 ± 10.7

Figure 3. Water contact angles of electrospun nanofibers from collagen/zein blends with different weight fractions (f_{zein})of zein.

an increase of zein amount would result in a decrease of surface wettability for the electrospun nanofibers. It is well-known[45] that zein has a more hydrophobic character than other proteins as a consequence of the presence of the apolar amino acids of proline and glutamine, which are the main constituents of zein. A more hydrophilic nanofiber surface will be helpful for the cell attachment but may result in poor fiber stability. Therefore, an optimized amount of zein should be taken in order to keep good surface wettability and fiber stability.

To understand the cell attachment and proliferation on the electrospun collagen/zein nanofibers, mice skin fibroblast (L929) cells were cultured for various time and evaluated in terms of cell adhesion percentage and cell viability, as shown in Figure 4. Among three electrospun nanofibers investigated, the

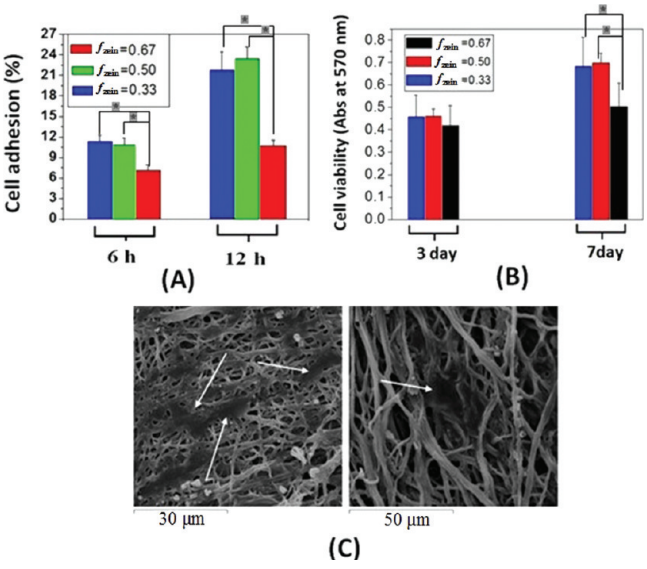


Figure 4. (a) Cell adhesion and (b) cell proliferation on the electrospun nanofibers from collagen/zein blends with different weight fractions (f_{zein}) of zein and (C) typical SEM images for the cell interactions with the nanofibers (f_{zein} =0.50). Data represent mean ± SD (n = 3, Student's t-test, *p < 0.05).

membrane containing the highest zein content (f_{zein} = 0.67) provided the lowest cell adhesion percentage and cell viability, regardless of culture time (Figure 4A,B). These results could be attributed to the lower surface wettability of this membrane, as shown in Figure 3. In contrast, other two membranes containing lower zein contents (f_{zein} = 0.33, 50) have higher cell adhesion percentage and cell viability. In particular, the membrane with the f_{zein} of 0.50 was observed to have appropriate or ever higher cell adhesion percentage and cell viability when compared to the membrane with the f_{zein} of 0.33. It has been reported that increasing fiber stiffness promotes cell attachment and growth.[46] Compared to the membrane with the f_{zein} of 0.33, the membrane with the f_{zein} of 0.50 has stiffer fiber structure in spite of lower surface wettability. In addition, the membrane with the f_{zein} of 0.50 had improved water stability and retained their high surface to volume ratio and the large volume of interconnected pores, which would also facilitate the cell attachment.[47] SEM examination at two magnifications of fibroblast cultures (Figure 4C) confirmed good attachment and spreading of L929 cells on the electrospun nanofibrous membrane with the f_{zein} of 0.50.

Figure 5 shows the in vitro degradation profiles of electrospun nanofibers from collagen/zein blends with different blending

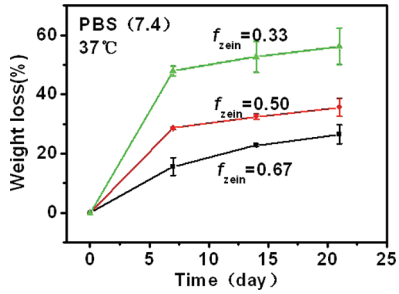


Figure 5. In vitro degradation profiles of electrospun nanofibers from collagen/zein blends with different weight fractions (f_{zein}) of zein in a phosphate buffer solution (pH7.4) at 37 °C.

ratios in a phosphate buffer solution (pH 7.4) at 37 °C. As seen, the electrospun membranes were degraded with the increase of time. Depending on the blending ratio, the electrospun membrane has various degradation rates. For example, the in vitro degradation in 14 days reached approximately 23% for the membrane with the f_{zein} of 0.67, 33% for the membrane with the f_{zein} of 0.50 and 47% for the membrane with the f_{zein} of 0.33, respectively. The extent of the degradation decreased when the content of zein in the nanofibers increased, which could be due to stronger mechanical property and weaker surface wettability of the electrospun membrane in the case of higher zein content. Therefore, we may modulate the degradation rate of electrospun collagen/zein nanofibers by the change of the collagen/zein blending ratio during the electrospinning process in order to meet the applicable requirements for wound dressings.

In situ Incorporation of Antibacterial Drug into Electrospun Membranes. Nanofibrous membranes are highly soft materials with high surface-to-volume ratios, and therefore can serve as excellent carriers for therapeutic agents that are antibacterial or accelerate wound healing.[48] In this context, an electrospun polycaprolactone nanofibre membrane containing a model antibiotic (biteral) has been used as a barrier to prevent the postsurgery abdominal adhesions for wound healing.[49] Another study was done by embedding silver nanoparticles into the electrospun polyvinyl alcohol membranes for in vivo wound healing and antibacterial performances.[50] In this work, we investigated the feasibility of incorporating berberine drug into electrospun collagen/zein membrane during the electrospinning.

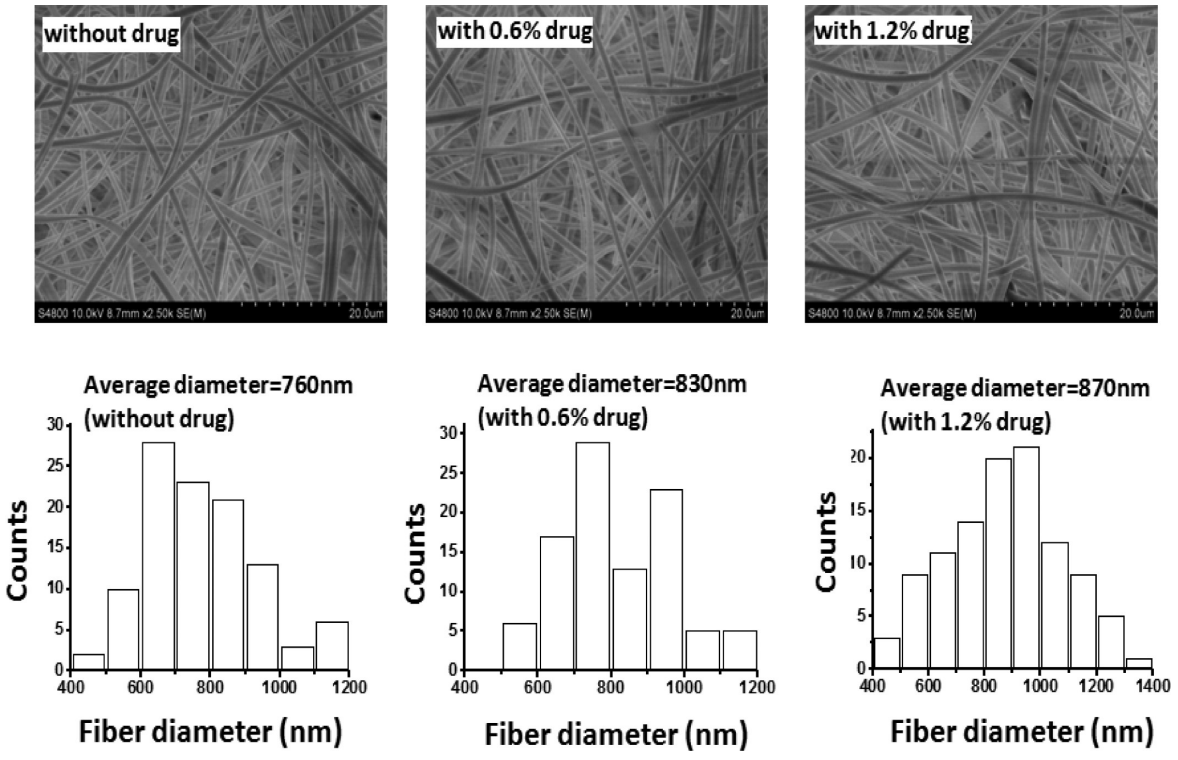


Figure 6. Effects of drug incorporation on the morphology and fiber diameter of electrospun nanofibrous membrane from collagen/zein blend with the f_{zein} of 0.50.

It is known[51−53] that berberine is an isoquinoline alkaloid with extensive antimicrobial effects on *Escherichia coli*, *Staphylococcus aureus*, Bifidobacterium adolescentis, *Candida albicans* and *Shigella dysenteriae*. For this purpose, two concentrations (0.6 and 1.2 wt %, relative to the polymers) of berberine were dissolved in collagen/zein blend with the f_{zein} of 0.50 and then coelectrospun under the same conditions.

Figure 6 shows the effects of berberine incorporation on the fiber diameter and morphology of electrospun collagen/zein nanofibrous membrane. The electrospun nanofibers possess the common features of being round-shaped with smooth surface and no drug crystals were detected on the polymer surface, which suggested that the incorporated drug was dispersed homogeneously in the electrospun membrane. Compared to the electrospun membrane without the drug, two electrospun membranes with different berberine contents showed slightly higher fiber diameters. However, the incorporation of the drug in the collagen/zein blend did not affect the morphology of the resulting nanofibers. Further investigation was dealt with the effects of berberine incorporation on the cell viability of electrospun collagen/zein nanofibrous membrane, as shown in Figure 7. It was found that there was no obvious difference in the cell viability before and after the drug loading.

For two drug-loaded electrospun collagen/zein nanofibrous membranes, the cumulative release percentages for incorporated berberine were in vitro examined for a period of 4 days in a phosphate buffer solution (pH 7.4) at 37 °C, and the relationships between the cumulative percentage and releasing time were plotted in Figure 8. As seen, these membranes showed similar drug release behavior. An initial fast release for the incorporated berberine was followed by a sustained and slow release. The diffusion of the drug nearby the surface layer of electrospun collagen/zein nanofibers could contribute to the initial fast release. In contrast, the nanofibrous membrane with

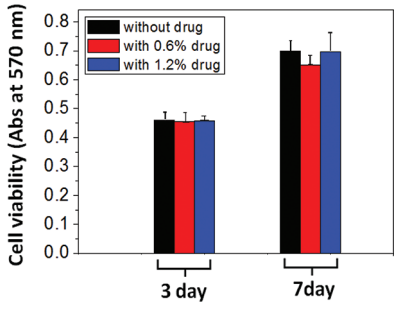


Figure 7. Effects of drug incorporation on the cell viability of electrospun nanofibrous membrane from collagen/zein blend with the f_{zein} of 0.50.

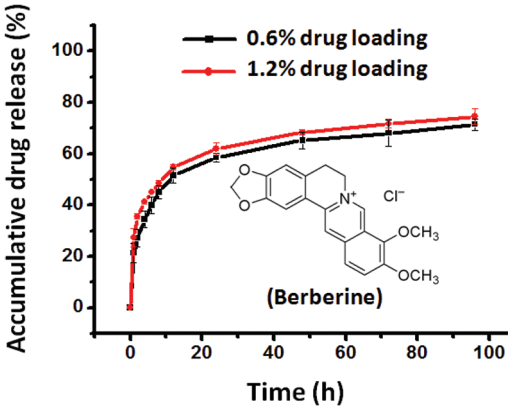


Figure 8. In vitro release profiles of berberine drug from electrospun nanofibrous membrane from collagen/zein blend with the f_{zein} of 0.50 in a phosphate buffer solution (pH 7.4) at 37 °C.

a lower loading amount of berberine was observed to have a slightly slower release rate. To understand the release

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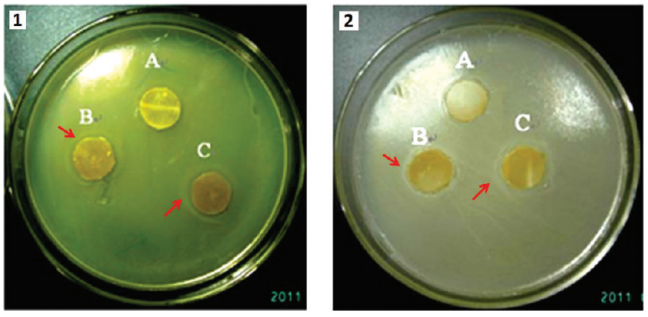

Figure 9. In vitro testing of electrospun collagen/zein nanofibrous membranes with no drug (A), 0.6% berberine (B) and 1.2% berberine (C) for antimicrobial activity against (1) *Escherichia coli* and (2) *Staphylococcus aureus* after 12 h contact intervals.

mechanism for the incorporated berberine, we fitted the release curves using the following semiempirical equation[54,55]

$$M_t/M_\infty = kt^n \quad (3)$$

where k is the kinetic constant and n is an exponent characterizing the diffusional mechanism, M_t and M_∞ are the cumulative amount of the drug released at t and equilibrium, respectively. Only in two cases of n = 0.5 (pure diffusion controlled drug release) and n = 1 (swelling-controlled drug release or case II transport), eq 3 becomes physically realistic. Other n values indicate anomalous transport kinetics. In this study, the n value was obtained to be 0.27 with the determination coefficient of 0.98 for the electrospun membrane loading with 0.6% drug, and 0.24 with the determination coefficient of 0.98 for the electrospun membrane with 1.2% drug, respectively. It seems that the diffusion behavior of incorporated berberine drug from electrospun collagen/zein nanofibrous membrane belongs to anomalous transport kinetics.

To be an efficient wound dressing, electrospun collagen/zein nanofibers loaded with berberine should be able to inhibit the growth of the bacterial strains responsible for severe wound infection thereby aiding wound healing. The predominant pathogenic bacteria responsible for severe burn wound infections are *Escherichia coli* and *Staphylococcus aureus*.[32] Hence antibacterial activities of electrospun collagen/zein nanofibers without and with berberine drug were assessed respectively against these typical pathogenic bacteria, as shown in Figure 9. Different from the electrospun nanofibers without berberine, two electrospun nanofibers loaded with berberine drug could show obvious zones of the inhibition for two bacterial strains after 12 h contact intervals. Moreover, the inhibition zone was more remarkable for the electrospun nanofibers loaded with a higher content of berberine drug. These results indicate that electrospun collagen/zein nanofibers loaded with berberine possess efficient antibacterial property and can be used in the treatment of wound healing or dermal bacterial infections.

Promotion of Wound Healing by Eelectrospun Nanofibrous Membranes. Figure 10 shows the photographic images of skin wounds after treated with electrospun collagen/zein nanofibrous membranes (f_{zein} = 0.50) without and with antimicrobial berberine drug as well as the corresponding wound area retentions at different time points after treatment. For a comparison study, conventional cotton gauze was investigated also. As seen, the electrospun nanofibrous membranes exhibited faster and better wound healing than normal cotton gauze. After the treatment for 7 days, the wound area retention was observed to be about 37% in the case of cotton gauze and 12% in the case

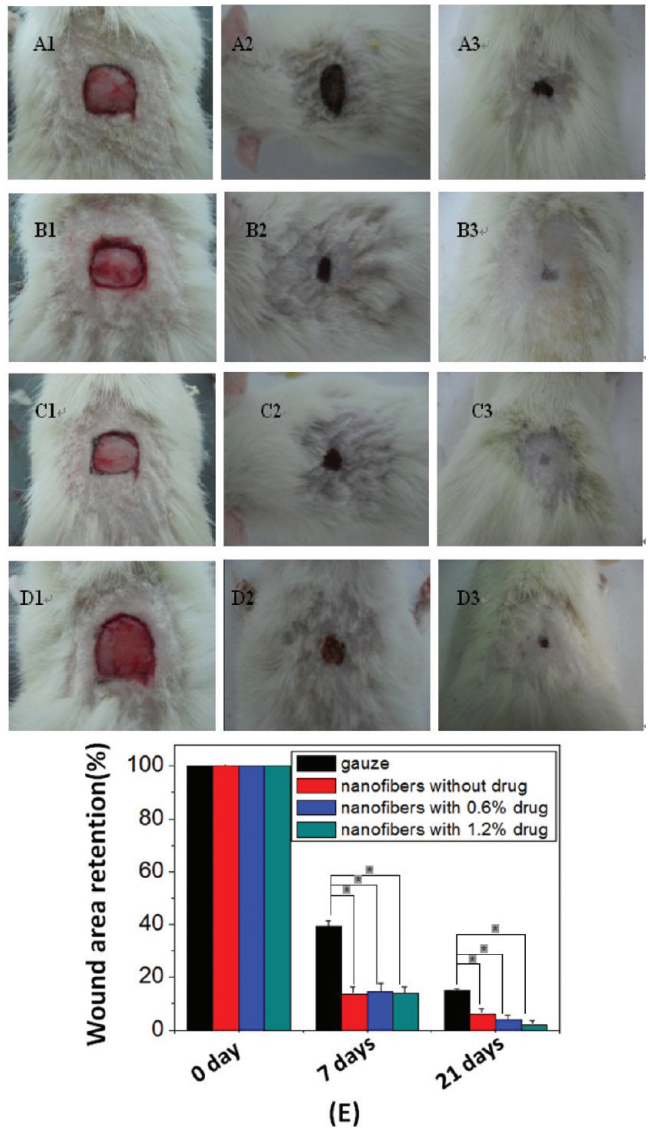

Figure 10. (A) Photographic images of the skin wounds after treated with cotton gauze, electrospun collagen/zein nanofibrous membranes (f_{zein} = 0.50) with (B) no drug, (C) 0.6% berberine, and (D) 1.2% berberine for (1) 0, (2) 7,and (3) 21 days as well as the corresponding wound area retentions. Data represent mean ± SD (n = 3, Student's t-test, $^*p < 0.05$).

of the electrospun nanofibrous membrane (f_{zein} = 0.50, without drug), respectively. After the treatment for 21 days, the wound area retention was observed to be about 13% in the case of cotton gauze and 5% in the case of the electrospun nanofibrous membrane (f_{zein} = 0.50, without drug), respectively. Moreover, the wounds covered with the electrospun nanofibrous membranes showed better fluid retaining when compared to the wound covered with cotton gauze. It was also found that the soft and flexible nanofibrous membranes were easier to be handled than the gauze.

Figure 11 shows the histological images of skin wounds treated for 21 days by cotton gauze and the electrospun collagen/zein nanofibrous membranes (f_{zein} = 0.50). For the skin wounds treated with three electrospun nanofibrous membranes, the maturing of wound healing with a sign of thick collagen fiber bundles and follicular regeneration was clearly observed. In these cases, the epidermal layers had almost regenerated in most

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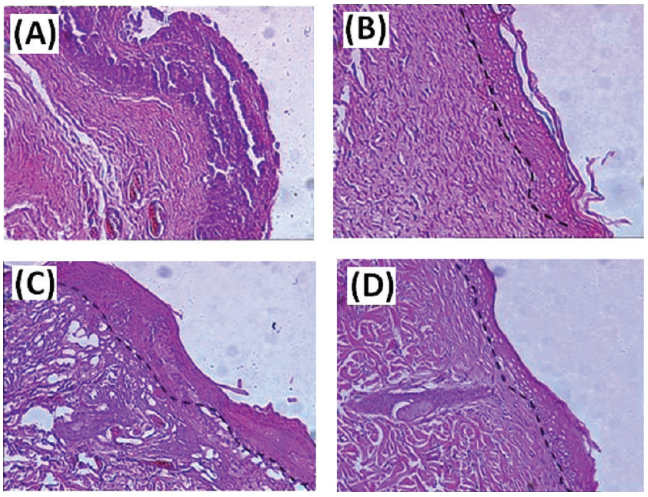

Figure 11. (A) Histological images of the skin wounds treated for 21 days by cotton gauze, the electrospun collagen/zein nanofibrous membranes (f_{zein} = 0.50)(B) without drug as well as loaded with (C) 0.6% berberine and (D) 1.2% berberine.

animals. The wound area was observed to be filled with dense connective tissues and was surrounded by a new dermal layer. In contrast, the bundles of collagenous fibers were observed to be loose and wavy for the dermis tissue after treated with cotton gauze. These results confirm further the effectiveness of wound healing by electrospun collagen/zein nanofibrous membrane.

CONCLUSIONS

The coelectrospinning of collagen and zein in aqueous acetic acid solution could result in biocompatible nanofibrous membranes. Depending on the blending ratio, the electrospun membrane has adjustable fiber diameter, surface wettability, mechanical and in vitro degradable properties as well as cell adhesive ability. The combination of zein is favorable for the improvement of the electrospinnability and fiber tensile strength, while the addition of collagen is useful for the enhancement of surface wettability, in vitro degradability and cell adhesive ability. The in situ incorporation of berberine drug into the electrspun membrane during the electrospinning showed little effects on the fiber morphology and cell viability, and could provide with antibacterial activity. When used as a dressing covering full-thickness skin wounds in mice, such a nanofibrous membrane was observed to induce fast tissue regeneration. This study confirmed the feasibility of electrospun collagen/zein nanofibrous membrane for wound healing.

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Notes

The authors declare no competing financial interest.

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