

Moxifloxacin-loaded electrospun polymeric composite nanofibers based wound dressing for enhanced antibacterial activity and healing efficacy

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Abstract

Nanofibers were prepared with different ratios of chitosan (CS) and polyethylene oxide (PEO) via the electrospinning technique, and tested for morphological features using scanning electron microscopy (SEM). Drug content, drug loading, release of moxifloxacin (MXF) at pH 4.7 and pH 5.5, and degree of swelling were investigated. Further characterisation was accomplished via X-ray diffraction (XRD), thermogravimetric analysis (TGA) and Fourier-transform infrared spectroscopy (FTIR). Antibacterial activity was studied in comparison with blank nanofibers. Furthermore, wound healing was studied in Sprague-Dawley rats. A formulation consisting of CS/PEO (50/50) exhibited smooth-surfaced and beadless nanofibers having a mean diameter of 138 ± 25 nm. Drug content and drug loading in the optimized formulation, consisting of MXF, CS and PEO (0.5/2.5/2.5, w/w/w), were 99-101% and 9.1%, respectively. MXF release in 48 hours was $79.83 \pm 4.2\%$ and $94.29 \pm 3.9\%$ at pH 7.4 and pH 5.5, respectively. Moreover, degree of swelling was $133 \pm 13\%$. The drug existed in the amorphous state and had no covalent interaction with the polymers. MXF-loaded nanofibers demonstrated greater stability, antibacterial activity and wound healing efficacy than did blank nanofibers. Accordingly, MXF-loaded CS-PEO polymeric composite nanofibers might be a potential wound dressing for effective wound healing.

Keywords: antibacterial, electrospinning, moxifloxacin, polymeric nanofibers, wound dressing, wound healing.

1. Introduction

A wound is a damage of skin structure and function. Wound healing is the progressive repair of injured tissues.^[1] It is an event which consists of following phases: hemostasis, inflammation, migration, proliferation and maturation.^[2] A wound dressing is a natural or synthetic material that (i) remains in close contact with the injured area for an appropriate period of time, (ii) absorbs fluids exuded from damaged skin, (iii) keeps damaged area adequately moist, (iv) allows the air to move across, (v) protects the wound from heat, and (vi) do not cause distress when detached upon need.^[3] For efficient wound healing, absence of microbes at the wound site is necessary; therefore, addition of a suitable antimicrobial agent in a wound dressing is essential.^[4,5] The most notorious culprits exacerbating wound injury include *Staphylococcus aureus*, *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa*.^[6,7] Moxifloxacin (MXF) is a broad-spectrum antibiotic which is very effective against these bacteria when applied directly to the wound.^[8] It possesses anti-inflammatory property as well which facilitates wound healing.^[9]

Fabrication of a wound dressing using nanofibers is a promising way as nanofibers impart most of the properties required for an ideal wound dressing. A wound dressing made from nanofibers provides high surface-to-volume ratio for better drug loading and drug release, high porosity that allows holding of excessive exudates and exchange of gases, greater flexibility which enables adjustment of dressing according to the shape of the wound.^[10] Chitosan (CS) is a promising polymeric biomaterial which exhibits antibacterial properties and facilitates restoration of damaged skin.^[11,12] It is relatively nontoxic, biocompatible and biodegradable in nature. Moreover, it carries anti-inflammatory, antioxidant and hemostatic characteristics which are additional benefits in wound healing.^[13] CS is not spinnable alone owing to its rigid chemical nature and molecular interactions; accordingly, incorporation of another suitable polymer can

improve its spinnability.^[14] Polymers such as genipin, polyvinyl alcohol and polyethylene oxide have been employed in conjunction with CS for this purpose.^[15-18] Polyethylene oxide (PEO) is an approved polymeric matrix for use in food products, cosmetic items and pharmaceuticals. It is added to other polymeric biomaterials for enhancing their spinnability as it is an excellent ion conductive polymeric matrix.^[19]

There are numerous techniques, such as template synthesis, drawing method, phase separation method, thermal-induced phase separation, melt-blowing technique, centrifugal spinning, biocomponent extrusion, self-assembly, electrospinning and so on, which can be exploited for the fabrication of nanofibers. Amongst these methods, electrospinning is the simplest and most sophisticated way to achieve nanofibers. In this technique, drug and polymeric matrices are dissolved in a solvent system to get a clear solution which is then filled in a syringe and pushed at a constant rate towards the steel nozzle with the help of a pump. An electrode is attached with the steel nozzle for applying a high voltage. A specific high voltage results in formation of Taylor cone which splits the ejecting solution into charged fibers which are collected by a conductive collector to which the opposite electrode is attached.^[20] This technique has been used for the formation of several antibiotic-loaded polymeric composite nanofibers.^[21]

The aim of this research work was to fabricate and characterize MXF-loaded CS-PEO composite nanofibers based wound dressing for improved antibacterial activity and healing efficacy. Three CS-PEO based polymeric composites were fabricated via the electrospinning technique, and inspected for shape and surface quality using SEM. Drug content and drug loading, release rate of the drug at pH 4.7 and pH 5.5, and degree of swelling were determined. Further solid-state characterisation was done using XRD, TGA and FTIR spectroscopy. Antimicrobial activity against *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* was also assessed

by determining zone of inhibition and the quantitative measurement of colony-forming units (CFU). Moreover, *in vivo* wound healing was assessed in Sprague-Dawley rats.

2. Materials and methods

2.1. Materials

Chitosan (95% degree of deacetylation) was acquired from Sigma-Aldrich Co. (St. Louis, MO, USA). Polyethylene oxide (MW 600 kDa) was purchased from Polysciences Inc. (Warrington Township, PA, USA). Moxifloxacin HCl was obtained as a gift sample from Schazoo Zaka Laboratories (Lahore, Punjab, Pakistan). Acetic acid was procured from Daejung Chemical Co. (Siheung, South Korea). All other materials used in this research were of reagent grade.

2.2. Preparation of MXF-loaded CS-PEO polymeric composite nanofibers

CS (5% w/v) and PEO (5% w/v) solutions were prepared separately using double distilled water. For each solution, vortex-mixing (200 rpm) was performed at room temperature overnight until clear solution was obtained. Then, these stock solutions were used in the preparation of mixtures of CS and PEO at the volume ratios of 70/30, 60/40 and 50/50. Each mixture was further vortex-mixed for two hours to obtain maximal homogeneity. In each solution mixture, 0.5 g moxifloxacin was added and vortex-mixing was performed further for one hour in order to achieve uniform blend of the drug and polymeric matrices. Subsequently, the final absolutely clear solution was subjected to electrospinning for nanofibers formation.

Electrospinning was accomplished using LINARI-RT Collector Electrospinning Equipment (Linari NanoTech, Pisa, Italy). A solution of constituents was filled in a glass syringe which was then properly positioned on the equipment. The solution in the syringe was pumped through single-

lumen steel nozzle at a rate of 250 $\mu\text{L/hr}$. The optimized voltage which split the jet to plume beneath the Taylor cone was 10 kV. The distance between the tip of the nozzle and metallic collector was set at 10 cm. The MXF-loaded electrospun nanofibers were carefully collected and kept in a desiccator until constant weight.

2.3. Morphology of MXF-loaded CS-PEO polymeric composite nanofibers

The shape and surface physiognomies of the prepared nanofibers were observed using FEI Quanta 250 SEM (Thermo Fisher Scientific, Waltham, MA, USA). The samples were attached to an aluminum stub using double-side adhesive carbon tape. Then, the sample-loaded stub was placed in the sputter coater for gold coating in order to make the samples conductive for imaging purpose.

2.4. MXF content and MXF loading in CS-PEO polymeric composite nanofibers

Ten milligrams of MXF-loaded nanofibers were completely dissolved in 50 mL of phosphate saline buffer (pH 7.4) by magnetic stirring. The clear solution was filtered and diluted adequately. The concentration of MXF in the diluted sample was quantified at 289 nm using a UV-visible spectrophotometer (UV-1900, BMS, Montreal, Canada). The content of MXF in electrospun polymeric nanofibers was assessed by the following formula: $M_C = M_A/M_T \times 100$. Where, M_C = percentage of MXF content, M_A = actual amount of MXF determined by spectrophotometer, and M_T = theoretical amount of MXF calculated from drug/excipient ratio. Furthermore, the percentage of MXF loading in polymeric nanofibers was calculated by using the following formula: $M_L = M_W/M_N \times 100$. Where, M_L = percentage of MXF loading to nanofibers, M_W = weight of MXF in grams present in M_N of nanofibers, and M_N = weight in grams of MXF-loaded nanofibers.

2.5. Degree of swelling of CS-PEO polymeric composite nanofibers

For determining the degree of swelling, a sample of nanofibers was cut and weighed. It was kept immersed in a phosphate buffer solution (pH 7.4) at 37°C for 24 hours. Then, it was carefully removed and slightly passed on a tissue paper so as to remove excess of liquid.^[22] The swollen sample was weighed again. Degree of swelling (%) was calculated using the following formula: $DS = [(W_2 - W_1) / W_1] \times 100$. Where, DS = degree of swelling, W_1 = initial weight of nanofibers, and W_2 = weight of nanofibers after treatment with liquid.^[22]

2.6. Release of MXF from CS-PEO polymeric composite nanofibers

Release rate of MXF from the optimized CS-PEO polymeric composite nanofibers was determined using the paddle apparatus. Cellulose acetate dialysis membrane (MWCO 10,000 Da) was activated as per protocol. The nanofiber formulation equivalent to 10 mg MXF was properly sealed in activated dialysis membrane pouch and enclosed in the sinker. Then, the sinker was dropped in the vessel containing 500 mL of phosphate buffer (pH 7.4) which was maintained at $37 \pm 1.0^\circ\text{C}$. The speed of paddle was set at 50 rpm. At each specified interval, 5 mL of medium was taken and volume was immediately replaced with fresh phosphate buffer (pH 7.4) to maintain the sink conditions. The sample was filtered using a syringe filter (0.22 μm) and diluted. The diluted sample was assayed at 289 nm using a UV-visible spectrophotometer (UV-1900, BMS, Montreal, Canada) for the quantification of MXF. Likewise, release of MXF in CS-PEO polymeric composite nanofibers was determined at pH 5.5.

2.7. XRD analysis

Assessment of the crystalline property of the samples was accomplished using an X-ray diffractometer (PANalytical X`Pert PRO, Malvern Panalytical; Doncaster, UK). The equipment was having a Cu $K\alpha_1$ monochromatic radiation source ($\lambda = 0.15406$ nm). The analysis was performed using a voltage of 40 kV and 40 mA current. The patterns were recorded in the range of 10-70° at a scanning speed of 1°/minute, with 2 θ scanning mode and a step size of 0.02°/second.

2.8. Thermal analysis

The changes in weight of a sample occurring with gradual elevation in temperature were investigated using a thermogravimetric analyzer (TGA Q50, TA Instruments; New Castle, Delaware, USA). A sample (5-15 mg) placed on the hangable platinum plate was lowered into the furnace by the moving arm. The sample enclosed in the furnace was gradually heated in the range of 30-600°C at the rate of 10°C/min using nitrogen flow of 25 cm³/min. The change in weight (%) of the sample was noted.

2.9. FTIR spectroscopic analysis

FTIR spectrum of each sample was achieved by scanning the individual sample in the range of 4000-600 cm⁻¹ with 2 cm⁻¹ resolution. An FTIR spectrophotometer (Spectrum-Two, Perkin Elmer, San Diego, CA, USA), using a ZnSe based ATR module and LiTaO₃ type detector, was employed for this purpose.

2.10. Culture Collection

The clinical isolates were acquired from Allied Hospital Faisalabad (Faisalabad, Punjab, Pakistan). The microbial cultures were collected from the selected patients suffering from chronic

skin wound infections. The samples were collected using sterile cotton swabs previously dipped in sterile saline. The microbial samples were transferred aseptically to nutrient broth. All the microbes were identified by biochemical tests using analytical profile index kits. The selected organisms were preserved in Mueller-Hinton agar media which were placed in a refrigerator and refreshed weekly. All the procedures were done in accordance with the ethical guidelines outlined in the Declaration of Helsinki. The protocol (Ref. No. GCUF/ERC/4145) was also approved by the Institutional Clinical Ethics Committee of Government College University Faisalabad.

2.11. Evaluation of antibacterial activity

For investigating activity of nanofibers against *Staphylococcus aureus* (ATCC 29213), *E. coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 9027), disc diffusion method was employed.^[23] Mueller-Hinton agar plates were prepared and culture was streaked on the plates. Electrospun nanofibers, cut into 8 mm diameter swatches, were positioned on these agar plates. Subsequently, incubation at 37°C for 24-48 hours was performed. The zone of inhibition corresponding to each microbe type was measured.

The quantitative measurement of colony-forming units (CFU) was also done. Mueller-Hinton broth was taken in tubes and 10⁴ CFU/mL of freshly grown bacterial cultures were added. A nanofiber swatch, possessing a dimension of 3.5 cm², was placed in each tube containing broth and bacterial culture. Samples were taken at predetermined time intervals of 0 h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 18 h and 24 h. Each sample was spread aseptically on a separate Mueller-Hinton agar plate. All the plates were incubated at 37°C for 24 hours. The negative control was consisting of bacterial suspension in Mueller-Hinton broth while positive control was consisting of blank nanofibers-treated sample. The reduction (%) in growth of the test organisms was calculated using

the following formula: $R = [(X_1 - X_2) \times 100]/X_1$. Where, R = percentage reduction in the selected bacterial type, X_1 = number of bacteria recovered from control sample, and X_2 = number of bacteria recovered from the drug-loaded nanofibers-treated sample.

2.12. In vivo wound healing study

Experimental organisms – Twelve male Sprague-Dawley rats, each having 6-8 weeks of age and weighing 250-270 g, were divided into three equal groups. All the rats were acclimated prior to experimentation. They were free to enjoy laboratory standard food and water in cages. One group was for acting as a negative control (untreated), the other group was for testing effect of positive control (blank CS-PEO polymeric composite nanoweb), and the third group was for investigating the influence of MXF-loaded CS-PEO polymeric composite nanoweb. The hair from the dorsal side of each anesthetized animal were removed by trimming and shaving. All the animal handling, care and surgical procedures performed in the whole study were conducted in accordance with the ethical protocol for exploitation of animals in research which was approved by the Institutional Animal Care and Use Committee (IACUC) of Government College University Faisalabad (Ref. No. GCUF/ERC/4146).

Formation of an infection wound model – To each anesthetized rat, a full thickness wound (1 cm^2) was inflicted at shaved skin on the dorsal side. Then, each wound was infected intentionally by introducing 30 μL of *Staphylococcus aureus* (KCCM 40050) solution ($3.2 \times 10^8/\text{mL}$) into the wound. Test samples were applied to wounds, and all wounds were protected using an elastic adhesive bandage (Soft cloth tape). Each rat was stayed in a single cage. The pictures of wounds were taken at 0, 1, 5 and 10 days using a digital camera.

Measurement of wound size - The percent reduction in wound size was calculated using the

following formula: $[(S_1 - S_2)/S_1] \times 100$. Where, S_1 = initial wound size, and S_2 = wound size at a specific time “t”.^[5]

3. Results and discussion

In the preparation of MXF-loaded polymeric composite nanofibers, PEO was used in combination with CS as CS is not spinnable alone because of its rigid chemical nature.^[14] PEO mitigates its rigidity and enhances spinnability.^[17] Moreover, viscosity and conductivity of the solution to be electrospun are the important properties which greatly influence the spinnability and contribute in the morphological characteristics of nanofibers. SEM images of the samples are shown in Fig. 1. Moxifloxacin plain drug was consisting of elongated crystals having irregular shapes (Fig. 1A). Electrospinning of the solution mixture consisting of 5% (w/v) CS and 5% (w/v) PEO at the volume ratio of 70/30 resulted in somewhat rough-surfaced fibers bearing enlarged beads (Fig. 1B). As the quantity of PEO increased in 60/40 (v/v) mixture, spinnability was improved remarkably and beads appeared shrunken (Fig. 1C). Further increase in quantity of PEO in 50/50 (v/v) mixture, revealed sufficiently beadless and smooth-surfaced nanofibers (Fig. 1D); therefore, this ratio was adopted for further explorations. The viscosity and conductivity of this solution mixture for the preparation of MXF-loaded CS-PEO polymeric composite nanofibers were 2242 cP and 2379 μ S, respectively. Furthermore, the mean diameter of the fibers, calculated using SEM and ImageJ software concomitantly,^[24] was 138 ± 25 nm.

The drug content in the optimized MXF-loaded CS-PEO polymeric composite nanofibers was within 99-101% range. This high drug content might be ascribed to absolutely clear solution of MXF, CS and PEO prepared for electrospinning. The clearness suggested that the drug, CS and PEO were completely dissolved and homogeneously intermingled in the solution.^[25] The drug

loading calculated was 9.1%.

Water absorption capacity is important for release of the drug at the wound site. Also, it is responsible for absorption of excessive exudate oozing out from the wound. Both the factors enhance effectiveness of wound management. Water absorption capacity can be assessed by determining degree of swelling. The degree of swelling found in the optimized MXF-loaded CS-PEO polymeric composite nanofibers was $133 \pm 13\%$ while observed in the corresponding blank CS-PEO polymeric composite nanofibers was $120 \pm 17\%$. The rate of drug release from this formulation is shown in Fig. 2. The drug release was superior at pH 5.5 than that observed at pH 7.4. Over the period of initial 8 hours, the release profiles obtained at the both pH were not significantly different ($P > 0.05$) from one another; however, they became significantly different ($P < 0.05$) after 8 hours. In 48 hours, the drug release at pH 7.4 and pH 5.5 was $79.83 \pm 4.2\%$ and $94.29 \pm 3.9\%$, respectively. The relatively faster release at pH 5.5 might be attributed to greater affinity of CS for lower pH which enhances its solubility.^[26] The greater release of the drug at pH 5.5 was advantageous as the pH of skin is 5.0-6.0.^[27]

XRD patterns are shown in Fig. 3. MXF plain drug powder furnished typical crystalline peaks at 14.43° , 15.55° , 16.86° , 17.37° , 17.87° , 18.49° , 19.54° , 20.31° , 23.56° , 24.11° , 24.49° , 26.73° , 27.44° , 29.11° , 31.49° , 35.17° , 36.67° and 38.74° (Fig. 3A). CS was amorphous in nature as it did not show any sharp spike (Fig. 3B). PEO also generated sharp spikes at 19.12° , 23.25° , 26.17° and 26.87° (Fig. 3C) which suggested that it was a crystalline polymer.^[28] The distinguishing peaks corresponding to the drug and PEO were also observed in the pattern produced by the physical mixture (Fig. 3D). This suggested that the crystalline components maintained their crystalline integrity in the physical mixture. The physical mixture was prepared by simply mixing MXF, CS and PEO (0.5/2.5/2.5, w/w/w) using a pestle and mortar. Blank CS-

PEO polymeric composite nanofibers, prepared using CS and PEO (2.5/2.5, w/w), showed two sharp peaks pertaining to PEO suggesting that PEO was present in the crystalline or semi-crystalline state in the formulation (Fig. 3E). The drug existed in the amorphous state in MXF-loaded CS-PEO polymeric composite nanofibers as no peak associated with the drug was found in the pattern; however, emergence of two peaks related to PEO (Fig. 3F) suggested that it was present in the crystalline or semi-crystalline state.^[29]

TGA thermograms obtained in the range of 30-600°C are shown in Fig. 4. There is only 10% weight loss in initial 260°C suggesting evaporation of moisture and volatile components. About 30% rapid degradation of MXF plain drug powder occurred in the range of 280-400°C (Fig. 4A). CS was rapidly degraded in the range of 270-400°C resulting in 40% weight loss (Fig. 4B). In the case of PEO, approximately 80% sudden loss in weight was observed in the range of 350-420°C (Fig. 4C). In the range of 250-430°C, about 50%, 60% and 45% weight loss were observed in physical mixture (Fig. 4D), blank CS-PEO polymeric composite nanofibers (Fig. 4E) and MXF-loaded CS-PEO polymeric composite nanofibers (Fig. 4F), respectively. This weight loss might be attributed to breakage of inter and intra chain linkages owing to thermal degradation.^[30] Furthermore, the segments of thermograms in the range of 400-600°C suggested that incorporation of MXF resulted in thermally more stable nanofibers.

FTIR spectra are shown in Fig. 5. The spectrum of MXF (Fig. 5A) exhibited stretching of C=O at 1705 cm⁻¹ and stretching of C-N at 1319 cm⁻¹. The aromatic C=C asymmetric stretching was demonstrated at 1620 cm⁻¹, 1517 cm⁻¹ and 1453 cm⁻¹. The N-H stretching of primary amine was depicted at 3469 cm⁻¹ while O-H stretching appeared at 3525 cm⁻¹.^[31] In the spectrum of CS (Fig. 5B), peak appearing at 3373 cm⁻¹ was corresponding to N-H and O-H stretching frequencies. The O-H bond stretching pertaining to glucopyranose rings was represented by the peak at 3593

cm^{-1} . The peaks at 3004 cm^{-1} and 2874 cm^{-1} appeared due to C-H stretching. The amide II band related peaks at 1655 cm^{-1} and 1592 cm^{-1} emerged owing to C-O stretching and N-H stretching, respectively. The peak arising at 1405 cm^{-1} was generated due to asymmetric C-H bending of CH_2 group. The C-O stretch of glucosamine also generated a peak at 1078 cm^{-1} due to skeletal vibrations.^[32] In the spectrum of PEO (Fig. 5C), the peak at 2877 cm^{-1} showed C-H stretching of alkane, C=C stretching was shown by the peak at 1617 cm^{-1} , C-H bending was represented by the peak at 1458 cm^{-1} and O-H bending of alcohol was observed at 1341 cm^{-1} . The peaks at 1280 cm^{-1} , 1241 cm^{-1} , 1145 cm^{-1} , 1103 cm^{-1} and 1060 cm^{-1} appeared owing to C-O stretching of $-\text{CH}_2\text{-O}-\text{H}$. The C=C bending was represented by the peaks at 958 cm^{-1} and 845 cm^{-1} . The peaks of MXF which appeared at 1705 cm^{-1} , 1620 cm^{-1} , 1517 cm^{-1} , 1319 cm^{-1} , 799 cm^{-1} and 724 cm^{-1} were declared as chief distinguishing peaks of MXF because they were differentiable in the spectrum of physical mixture (Fig. 5D). These peaks were absent in the spectrum of blank CS-PEO polymeric composite nanofibers (Fig. 5E) as no drug was present in these nanofibers. On the other hand, these peaks were witnessed in the spectrum of MXF-loaded CS-PEO polymeric composite nanofibers (Fig. 5F). Moreover, spectra of the physical mixture and MXF-loaded CS-PEO polymeric composite nanofibers were overlapping to each other. The comparison of spectra suggested that no strong interaction existed among the constituents of MXF-loaded CS-PEO polymeric composite nanofibers.

CS possesses some antibacterial properties; thus, it can speed up healing of damaged skin.^[11,12] Also, it produces anti-inflammatory, antioxidant and hemostatic effects which are extra advantages in wound healing.^[13] Therefore, antibacterial activity was also determined using blank CS-PEO polymeric composite nanofibers as well. Blank CS-PEO polymeric composite nanofibers produced $16.16 \pm 2.02 \text{ mm}$, $19.33 \pm 3.75 \text{ mm}$ and $19.50 \pm 1.32 \text{ mm}$ zone of inhibition

measurements ($n = 3$) against *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa*, respectively. MXF-loaded CS-PEO polymeric composite nanofibers produced 32.33 ± 1.15 mm, 35.67 ± 1.53 mm and 36.83 ± 2.56 mm zone of inhibition measurements ($n = 3$) against *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa*, respectively. This suggested that MXF-loaded CS-PEO polymeric composite nanofibers were more effective against these microbes than blank CS-PEO polymeric composite nanofibers. Furthermore, percent reduction in CFU after 24 hours of sample exposure is shown in Fig. 6. The control sample (Fig. 6A) was kept untreated. The comparison of blank CS-PEO polymeric composite nanofibers (Fig. 6B) with the control sample confirmed that CS possessed some antibacterial properties. As compared with blank CS-PEO polymeric composite nanofibers, MXF-loaded CS-PEO polymeric composite nanofibers (Fig. 6C) inhibited the growth of *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* about 8-fold, 18-fold and 30-fold, respectively. Thus, MXF-loaded CS-PEO polymeric composite nanofibers exerted more antibacterial effect than did blank nanofibers.

The representative images of wounds are shown in Fig. 7. It is obvious that the size of wound is the largest in the case of untreated rats (Fig. 7A) on each specific day as compared to wounds of rats treated with blank CS-PEO polymeric composite nanofibers (Fig. 7B) and MXF-loaded CS-PEO polymeric composite nanofibers (Fig. 7C). Similarly, size of wound in rats treated with MXF-loaded CS-PEO polymeric composite nanofibers is smaller than that of rats treated with blank CS-PEO polymeric composite nanofibers. The results of wound healing shown in Fig. 8 are in line with the above observations. In 5 days, wound healing was $18 \pm 7.9\%$ in untreated rats (Fig. 8A). It was $40 \pm 4.1\%$ in rats treated with blank CS-PEO polymeric composite nanofibers (Fig. 8B) while $53 \pm 5.9\%$ in the rats treated with MXF-loaded CS-PEO polymeric composite nanofibers (Fig. 8C).

4. Conclusion

MXF-loaded CS-PEO polymeric composite nanofibers, consisting of MXF, CS and PEO (0.5/2.5/2.5, w/w/w), furnished smooth surfaced and beadles nanofibers of 138 ± 25 nm thickness. MXF content and MXF loading in the electrospun nanofibers were 99-101% and 9.1%, respectively. The drug was present in the amorphous state in the nanofibers and had no covalent interaction with the polymeric excipients. About $94.29 \pm 3.9\%$ and $79.83 \pm 4.2\%$ MXF was released from the nanofibers in 48 hours at pH 5.5 and pH 7.4, respectively. The degree of swelling demonstrated by the nanofibers was $133 \pm 13\%$. As compared with blank nanofibers, MXF-loaded nanofibers showed better stability, antimicrobial activity against *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa*, and wound healing efficacy. Therefore, MXF-loaded CS-PEO polymeric composite might be a promising wound dressing for effective restoration of damaged skin.

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Conflict of interest

The authors report no conflicts of interest.

Reference

- [1] Boateng, J.; Catanzano, O., Advanced therapeutic dressings for effective wound healing—a review. *J. Pharm. Sci.* **2015**, *104* (11), 3653-3680. doi: 10.1002/jps.24610
- [2] Velnar, T.; Bailey, T.; Smrkolj, V., The wound healing process: an overview of the cellular and molecular mechanisms. *J. Int. Med. Res.* **2009**, *37* (5), 1528-1542. doi: 0.1177/147323000903700531
- [3] Padol, A. R.; Jayakumar, K.; Shridhar, N.; Swamy, H. N.; Swamy, M. N.; Mohan, K., Safety evaluation of silk protein film (a novel wound healing agent) in terms of acute dermal toxicity, acute dermal irritation and skin sensitization. *Toxicol. Int.* **2011**, *18* (1), 17-21. doi: 10.4103/0971-6580.75847
- [4] Jin, S. G.; Kim, K. S.; Yousaf, A. M.; Kim, D. W.; Jang, S. W.; Son, M.-W.; Kim, Y. H.; Yong, C. S.; Kim, J. O.; Choi, H.-G., Mechanical properties and in vivo healing evaluation of a novel *Centella asiatica*-loaded hydrocolloid wound dressing. *Int. J. Pharm.* **2015**, *490* (1-2), 240-247. doi: 10.1016/j.ijpharm.2015.05.058
- [5] Jin, S. G.; Yousaf, A. M.; Jang, S. W.; Son, M. W.; Kim, K. S.; Kim, D. W.; Li, D. X.; Kim, J. O.; Yong, C. S.; Choi, H. G., In Vivo Wound-Healing Effects of Novel Benzalkonium Chloride-Loaded Hydrocolloid Wound Dressing. *Drug Dev. Res.* **2015**, *76* (3), 157-165. doi: 10.1002/ddr.21253
- [6] Serra, R.; Grande, R.; Butrico, L.; Rossi, A.; Settimio, U. F.; Caroleo, B.; Amato, B.; Gallelli, L.; de Franciscis, S., Chronic wound infections: the role of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Expert Rev. Anti. Infect. Ther.* **2015**, *13* (5), 605-613. doi: 10.1586/14787210.2015.1023291

- [7] Wilkinson, J. M.; Cavanagh, H. M., Antibacterial activity of 13 honeys against *Escherichia coli* and *Pseudomonas aeruginosa*. *J. Med. Food* **2005**, *8* (1), 100-103. doi: 10.1089/jmf.2005.8.100
- [8] Jacobsen, F.; Fisahn, C.; Sorkin, M.; Thiele, I.; Hirsch, T.; Stricker, I.; Klaassen, T.; Roemer, A.; Fugmann, B.; Steinstraesser, L., Efficacy of topically delivered moxifloxacin against wound infection by *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **2011**, *55* (5), 2325-2334. doi: 10.1128/AAC.01071-10
- [9] Donnarumma, G.; Paoletti, I.; Buommino, E.; Iovene, M. R.; Tudisco, L.; Cozza, V.; Tufano, M. A., Anti-inflammatory effects of moxifloxacin and human β -defensin 2 association in human lung epithelial cell line (A549) stimulated with lipopolysaccharide. *Peptides* **2007**, *28* (12), 2286-2292. doi: 10.1016/j.peptides.2007.09.009
- [10] Jin, S. G.; Yousaf, A. M.; Kim, K. S.; Kim, D. W.; Kim, D. S.; Kim, J. K.; Yong, C. S.; Youn, Y. S.; Kim, J. O.; Choi, H.-G., Influence of hydrophilic polymers on functional properties and wound healing efficacy of hydrocolloid based wound dressings. *Int. J. Pharm.* **2016**, *501* (1-2), 160-166. doi: 10.1016/j.ijpharm.2016.01.044
- [11] Sudarshan, N.; Hoover, D.; Knorr, D., Antibacterial action of chitosan. *Food Biotechnol.* **1992**, *6* (3), 257-272. doi: 10.1080/08905439209549838
- [12] Howling, G. I.; Dettmar, P. W.; Goddard, P. A.; Hampson, F. C.; Dornish, M.; Wood, E. J., The effect of chitin and chitosan on the proliferation of human skin fibroblasts and keratinocytes in vitro. *Biomater.* **2001**, *22* (22), 2959-2966. doi: 10.1016/S0142-9612(01)00042-4
- [13] Kim, S., Competitive biological activities of chitosan and its derivatives: Antimicrobial,

- antioxidant, anticancer, and anti-inflammatory activities. *Int. J. Polym. Sci.* **2018**, 2018. 708172. doi: 10.1155/2018/1708172
- [14] Sun, K.; Li, Z., Preparations, properties and applications of chitosan based nanofibers fabricated by electrospinning. *Express Polym. Lett.* **2011**, 5 (4). doi: 10.3144/expresspolymlett.2011.34
- [15] Mirzaei, E.; Faridi-Majidi, R.; Shokrgozar, M. A.; Asghari Paskiabi, F., Genipin cross-linked electrospun chitosan-based nanofibrous mat as tissue engineering scaffold. *Nanomed. J.* **2014**, 1 (3), 137-146. doi: 10.7508/NMJ.2014.03.003
- [16] Gonçalves, R. P.; Ferreira, W. H.; Gouvêa, R. F.; Andrade, C. T., Effect of chitosan on the properties of electrospun fibers from mixed poly (vinyl alcohol)/chitosan solutions. *Mater. Res.* **2017**, 20 (4), 984-993. Doi: 10.1590/1980-5373-mr-2016-0618
- [17] Pakravan, M.; Heuzey, M.-C.; Aiji, A., A fundamental study of chitosan/PEO electrospinning. *Polym.* **2011**, 52 (21), 4813-4824. doi: 10.1016/j.polymer.2011.08.034
- [18] Farzinfar, E.; Paydayesh, A., Investigation of polyvinyl alcohol nanocomposite hydrogels containing chitosan nanoparticles as wound dressing. *Int. J. Polym. Mater. Polym. Biomater.* **2019**, 68 (11), 628-638. doi: 10.1080/00914037.2018.1482463
- [19] Zhou, X.; Zhang, T.; Guo, D.; Gu, N., A facile preparation of poly (ethylene oxide)-modified medical polyurethane to improve hemocompatibility. *Colloids Surf. A Physicochem. Eng. Asp.* **2014**, 441, 34-42. doi: 10.1016/j.colsurfa.2013.08.070
- [20] Yousaf, A. M.; Mustapha, O.; Kim, D. W.; Kim, D. S.; Kim, K. S.; Jin, S. G.; Yong, C. S.; Youn, Y. S.; Oh, Y.-K.; Kim, J. O., Novel electrospayed nanospherules for enhanced aqueous solubility and oral bioavailability of poorly water-soluble fenofibrate. *Int. J. Nanomedicine* **2016**, 11, 213-221. doi: 10.2147/IJN.S97496

- [21] Qi, R.; Guo, R.; Zheng, F.; Liu, H.; Yu, J.; Shi, X., Controlled release and antibacterial activity of antibiotic-loaded electrospun halloysite/poly (lactic-co-glycolic acid) composite nanofibers. *Colloids Surf. B Biointerfaces* **2013**, *110*, 148-155. doi: 10.1016/j.colsurfb.2013.04.036
- [22] Jannesari, M.; Varshosaz, J.; Morshed, M.; Zamani, M., Composite poly (vinyl alcohol)/poly (vinyl acetate) electrospun nanofibrous mats as a novel wound dressing matrix for controlled release of drugs. *Int. J. Nanomedicine* **2011**, *6*, 993-1003. doi: 10.2147/IJN.S17595
- [23] Shalumon, K.; Anulekha, K.; Nair, S. V.; Nair, S.; Chennazhi, K.; Jayakumar, R., Sodium alginate/poly (vinyl alcohol)/nano ZnO composite nanofibers for antibacterial wound dressings. *Int. J. Biol. Macromol.* **2011**, *49* (3), 247-254. doi: 10.1016/j.ijbiomac.2011.04.005
- [24] Jain, T. K.; Richey, J.; Strand, M.; Leslie-Pelecky, D. L.; Flask, C. A.; Labhasetwar, V., Magnetic nanoparticles with dual functional properties: drug delivery and magnetic resonance imaging. *Biomater.* **2008**, *29* (29), 4012-4021. doi: 10.1016%2Fj.biomaterials.2008.07.004
- [25] Sun, R.; Shen, C.; Shafique, S.; Mustapha, O.; Hussain, T.; Khan, I. U.; Mehmood, Y.; Anwer, K.; Shahzad, Y.; Yousaf, A. M., Electrospayed Polymeric Nanospheres for Enhanced Solubility, Dissolution Rate, Oral Bioavailability and Antihyperlipidemic Activity of Bezafibrate. *Int. J. Nanomedicine* **2020**, *15*, 705-715. doi: 10.2147/IJN.S235146
- [26] Qin, C.; Li, H.; Xiao, Q.; Liu, Y.; Zhu, J.; Du, Y., Water-solubility of chitosan and its antimicrobial activity. *Carbohydr. polym.* **2006**, *63* (3), 367-374. doi:

10.1016/j.carbpol.2005.09.023

- [27] Lambers, H.; Piessens, S.; Bloem, A.; Pronk, H.; Finkel, P., Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *Int. J. Cosmet. Sci.* **2006**, *28* (5), 359-370. doi: 10.1111/j.1467-2494.2006.00344.x
- [28] Wang, H.; Keum, J. K.; Hiltner, A.; Baer, E.; Freeman, B.; Rozanski, A.; Galeski, A., Confined crystallization of polyethylene oxide in nanolayer assemblies. *Sci.* **2009**, *323* (5915), 757-760. doi: 10.1126/science.1164601
- [29] Yousaf, A. M.; Ramzan, M.; Shahzad, Y.; Mahmood, T.; Jamshaid, M., Fabrication and in vitro characterization of fenofibric acid-loaded hyaluronic acid–polyethylene glycol polymeric composites with enhanced drug solubility and dissolution rate. *Int. J. Polym. Mater. Polym. Biomater.* **2019**, *68* (9), 510-515. doi: 10.1080/00914037.2018.1466137
- [30] Fu, R.; Li, C.; Yu, C.; Xie, H.; Shi, S.; Li, Z.; Wang, Q.; Lu, L., A novel electrospun membrane based on moxifloxacin hydrochloride/poly (vinyl alcohol)/sodium alginate for antibacterial wound dressings in practical application. *Drug Deliv.* **2016**, *23* (3), 818-829. doi: 10.3109/10717544.2014.918676
- [31] Dandagi, P. M.; Belekar, A. M.; Mastiholimath, V. S.; Gadad, A. P.; Sontake, V. W.; Salian, P. S., An improvement of the efficacy of moxifloxacin HCl for the treatment of bacterial keratitis by the formulation of ocular mucoadhesive microspheres. *Sci. Pharm.* **2013**, *81* (1), 259-280. doi: 10.3797/scipharm.1204-08
- [32] Semwal, A.; Ramandeep, S., Preparation, characterization and biological evaluation of chitosan-moxifloxacin prodrugs for pharmaceuticals. *Marmara Pharm. J.* **2014**, *18*, 36-42. doi: 10.12991/201414124

Figure Legends

Fig. 1. SEM images: (A) moxifloxacin ($\times 1000$), (B) MXF-loaded CS-PEO (70/30, v/v) polymeric composite nanofibers ($\times 10,000$), (C) MXF-loaded CS-PEO (60/40, v/v) polymeric composite nanofibers ($\times 10,000$) and (D) MXF-loaded CS-PEO (50/50, v/v) polymeric composite nanofibers ($\times 10,000$).

Fig. 2. Release rate of MXF-loaded CS-PEO polymeric composite nanofibers at pH 7.4 and pH 5.5. Each value shows the Mean \pm SD ($n = 6$).

Fig. 3. XRD patterns: (A) moxifloxacin, (B) chitosan, (C) polyethylene oxide, (D) physical mixture, (E) blank electrospun CS-PEO polymeric composite nanofibers and (F) MXF-loaded CS-PEO polymeric composite nanofibers.

Fig. 4. TGA thermograms: (A) moxifloxacin, (B) chitosan, (C) polyethylene oxide, (D) physical mixture, (E) blank electrospun CS-PEO polymeric composite nanofibers and (F) MXF-loaded CS-PEO polymeric composite nanofibers.

Fig. 5. FTIR spectra: (A) moxifloxacin, (B) chitosan, (C) polyethylene oxide, (D) physical mixture, (E) blank electrospun CS-PEO polymeric composite nanofibers and (F) MXF-loaded CS-PEO polymeric composite nanofibers.

Fig. 6. Antibacterial activity (% reduction in CFU after 24 hours) against *S. aureus*, *E. coli* and *P. aeruginosa* exerted by (A) negative control (untreated), (B) positive control (blank CS-PEO polymeric composite nanoweb) and (C) MXF-loaded CS-PEO polymeric composite nanoweb.

Fig. 7. Representative images of infection wound: (A) negative control (untreated), (B) positive control (blank CS-PEO polymeric composite nanoweb) and (C) MXF-loaded CS-PEO

polymeric composite nanoweb.

Fig. 8. Wound healing effect: (A) negative control (untreated), (B) positive control (blank CS-PEO polymeric composite nanoweb) and (C) MXF-loaded CS-PEO polymeric composite nanoweb. Each value shows the mean \pm S.D. (n=4). * P <0.05 compared with negative control. # P <0.05 compared with positive control.