RESEARCH ARTICLE

Preparation and characterization of CS/PEO/cefazolin nanofibers with *in vitro* and *in vivo* testing

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ARTICLE INFO

Article History:
Received 25 February 2017
Accepted 5 April 2017
Published 11 May 2017

Keywords:
Chitosan
Polymer composites
Biocompatible polymers
Cefazolin
Nanofibers

ABSTRACT

Objective(s): Electrospinning of chitosan/polyethylene oxide (CS/PEO) nanofibers with the addition of cefazolin to create nanofibers with antimicrobial properties were examined.

Methods: Polymeric nanofibers including CS/PEO and CS/PEO/cefazolin, were produced by electrospinning method. The range of nanofiber was 60-100 nm in diameter and measured with ImageJ software. The morphology of electrospun nanofibers was studied with use of scanning electron microscopy (SEM). Moreover, the chemical structures of the nanofibers were evaluated by FT-IR. The drug release of nanofibers was also investigated by UV-Vis spectrophotometry. The antibacterial activity of scaffolds was tested by two type bacteria including Escherichia coli and Staphylococcus aureus. The healing ability of nanofibers was studied on the rat's wound.

Results: The SEM images indicated that the addition of cefazolin as much as 1wt% brings about the best nanofiber. Also, the morphology of electrospun nanofiber is dependent on the viscosity of the solution and the ratio of CS /PEO/ cefazolin. According to the results of cefazolin releasing from nanofibers, the best results were obtained in the presence of CS /PEO/1wt%cefazolin nanofibers as healing sample. In animal studies, the effect of nanofibers was studied in the burn wound healing of rats and improvement of the wound was observed by nanofibers containing 1%wt cefazolin.

Conclusions: According to these results, it seems that CS /PEO/1wt% cefazolin nanofiber is a good choice as a wound covering agent and hold more moisture in its structure thus the surface of wound remain wet during the healing process that prevent from nanofiber sticking to the wound surface.

How to cite this article:
Sadri M, Arab Sorkhi S, Preparation and characterization of CS/PEO/cefazolin nanofibers with in vitro and in vivo testing. Nanomed Res J, 2017; 2(2):100-110. DOI: 10.22034/nmrj.2017.59850.1061

INTRODUCTION

So far, various methods with different features have been used to cover the wound types. For having wound coatings some features should be considered, such as: biocompatibility, biodegradability, wound healing accelerator and prevention of scarring. Recently, some of the nanoparticles such as silver are receiving considerable attention as an antimicrobial agent. But the implication of nanoparticles on health and environment needs to be assessed completely before their application as antimicrobials. The size of the nanoparticles is small and these can easily access the skin, lungs, and brain and cause adverse effects. Traditional methods have deficiencies and often do not have above characteristics. To achieve this goal, in this study, we prepared the nanofibers of chitosan and PEO polymers with cefazolin additives by electrospinning device. Chitosan is a biocompatible and natural polymer with different properties such

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as: biodegradability, biocompatibility [1,2], lack of toxicity, the coagulability, the antigenicity, the lack of water solubility and gelation properties, antibacterial, anti-fungal and anti-viral effects, has wide application in wound dressing and injuries [3,4], wound healing [5], drug delivery systems [6] and varied applications in tissue engineering [7,8] . Application of chitosan in bone tissue engineering [9, 10], in vitro [11] and living tissue [12] reviewed and the strengthening effect of chitosan was shown in the bone formation.

Nevertheless, electrospinning of alone chitosan is not possible, because it has poor solubility in water and high viscosity in aqueous solutions and can be performed just in the presence of other polymers. PEO has become a very useful synthetic polymer, because it has the diverse molecular weight and substantially solubility in most solvents, especially water [13, 14].The properties of this polymer containing; biocompatibility [15], low toxicity [16], as well as the ability for fibers production in aqueous solution [17-18]. The use of this polymer with natural polymers such as chitosan leads to the formation of uniform fibers with high mechanical ability, without decreasing the wound healing potential of chitosan [2, 19]. On the other hand, it was found that using 50% aqueous acetic acid as solvent, chitosan-based nanofibers with the high amount of chitosan can be fabricated from 2 wt% Chitosan and 4wt% of PEO (Chitosan/PEO with the ratio of 90/10) solutions without any other additives. It was also found that the matrix with a chitosan/PEO ratio of 90/10 retained excellent integrity of the fibrous structure in water [20]. In this study, cefazolin antibiotic was used for increasing the antibacterial properties of nanofibers. Cefazolin (first generation of cephalosporin) is a glycopeptide antibiotic that inhibits the formation of bacterial cell wall and causes cell lysis. Every bacteria need to trans-peptidase and carboxypeptidase enzymes for connected precursors and building their cell walls. Cephalosporin antibiotics prevent of trans-peptidase enzymes activity. Each newer generation of cephalosporins has significantly greater Gram-negative antibacterial properties than the preceding generation, in most cases with decreased activity against Gram-positive organisms. Fourth-generation cephalosporins, however, have true broad-spectrum activity. Resistance to cephalosporin antibiotics can involve either reduced affinity of existing penicillin binding protein (PBP) components or the acquisition of a supplementary β-lactam-insensitive PBP. Cefazolin with 450-500 kDa molecular weight, is water-soluble and relatively stable against changes of pH and temperature and is used for skin infections and soft tissues [21]. As a first-generation cephalosporin antibiotic, cefazolin and other first-generation antibiotics are very active against Gram-positive bacteria and some Gram-negative bacteria. Cefazolin is used in a variety of infections for example: skin and skin structure infections (due to S. aureus penicillin-sensitive and penicillin-resistant, group A beta-hemolytic streptococci, and other strains of streptococci); bone and joint infections (due to S. aureus); urinary tract infections (due to E. coli, P. mirabilis and Klebsiella species).

In other investigation, the CS-PEO-1% F. silica-0.5% cefazolin mat was considered as the drug delivery system for biomedical and wound dressing [22].

Therefore, in this study polymer/ cefazolin composite mats were prepared by the electrospinning method for the efficiently controlled delivery of the cefazolin drug.

The combination of the antibacterial properties of chitosan and adding antibacterial properties with an antibiotic drug is a promising strategy for the preparation of nanofibrous materials suitable for wound dressing applications. In this study, a one- step method–electrospinning in common solvent – has been used for the preparation of nanofibers based on chitosan, PEO and cefazolin antibiotic drug. As the spin ability of pure chitosan is challenging, chitosan/PEO blend was used as matrix phase and cefazolin as reinforcing phase in order to improve the wound healing properties of chitosan nanofiber.

The nanofibers with 1%wt cefazolin have suitable antibacterial properties. The new work in this research was the adding the 1%wt cefazolin antibiotic for increasing the antibacterial activity and in vivo test was showing the good performance of nanofibers. We can use this nanofibers as a new gauze pads in skin burn and the other application in medicine.

MATERIALS AND METHODS

Acetic acid, tween 80 and nutrient agar cultivation environment were prepared from Merck.

Cefazolin sodium salt was purchased from Tehran medication. Escherichia coli (E.coli) (ETEC ATCC 35401), Staphylococcus aureus (S.aureus) (ATCC 6538), a medium molecular weight of chitosan and PEO with molecular weight of 900000 g.mol-1 were purchased from Sigma-Aldrich. Electrospinning (model ES100, Fanavaran nano-meghyas Co. of Iran), scanning electron microscope (model MV2300, Camscan SEM Co.,of Czech Republic), FT-IR (model Spectrum GX, Perkin Elmer Co., of USA), UV-Vis spectrophotometer (model U-3010, Hitachi Co., of Japan), incubator (model JTSL 40, Jal Tajhiz Co. of Iran) were used.

Preparation of chitosan/PEO solution (90 to10) with and without antibiotic

The chitosan/PEO mixture was prepared by slowly adding 0.27 g of chitosan powder with medium molecular weight to 0.04 g PEO in the appropriate volume of 50% acetic acid. Then, 0.25mL tween 80 was added to this solution as an emulsifier. The prepared solution was stirred using a magnetic stirrer at 200 rpm for 12 h at room temperature, to yield a homogeneous solution.

In order to prepare chitosan/PEO/cefazolin solution, cefazolin with percentages of 0.5, 1, 2 and 3wt% was added to polymeric solutions of chitosan/PEO and mixed. The antibiotic agent is firstly dissolved in the same solvent and slowly added to the polymer solution while stirring to produce a homogeneous solution prior to electrospinning. This method can accommodate a large amount of the antibiotic to be loaded in the nanofibers by adjusting 1wt% concentration of the drug in the electrospinning solution.

Preparation of nanofibers from polymeric solutions

In this stage, for producing electrospun nanofibers, the prepared solutions were supplied into a 5 mL syringe with a stainless steel capillary needle (inner diameter =0.9 mm). The electrospinning was performed at room temperature and the solutions were injected from the syringe pump with a feed rate of 0.3-0.5 mL/h at 17-20 kV voltage. The electrospun nanofibers were assembled on a collector which was placed at 10 cm from the needle tip and rotate at 400 rpm. In order to obtain nanofiber with the appropriate thickness, electrospinning was performed for 10 h. For the complete evaporation of solvent and water from the nanofibers, they were drying at room temperature.

Biodegradation and swelling of nanofibers

A buffer at pH 5.5 and 37oC temperature was used as a simulation of human skin in order to examine the degradation and swelling of prepared nanofibers.

The degradation and water absorption capacity of scaffolds of the nanofibers were studied by immersing segments of chitosan/PEO/cefazolin nanofibers mats in 500 mL of buffer at 37oC for 24 h. Afterward, the wet nanofibers were placed between two filters to remove the excess amount of buffer solution.

The morphology of the nanofibers structures and also their degradation and swelling were studied by scanning electron microscope.

Analysis of surface morphology and measurement of nanofibers diameter

For analyzing the morphology of surface and nanofibers diameter of the samples, scanning electron microscopy was used. For this purpose, the nanofibers had been collected on the aluminum foil on the collector and were cut. Ionic coater E5200 model applying 1kV voltage on prepared samples and they were covered by gold for 5 minutes. Also, "Image J" software was used to determine the average nanofibers diameter.

Drug releasing template from nanofibers by UV-Vis spectrometery

Releasing template of cefazolin from nanofibers scaffold was studied by UV-Vis spectrometery. 5×5 cm segments of chitosan/PEO/cefazolin nanofibers were placed in 5 mL of buffer at 37oCin order to investigate the drug release into the buffer solution. Sampling from this solution was performed at specific times and the UV-Vis spectrum of released active substance was recorded from 200-800 nm wavelengths. The maximum wavelength (λ max) for cefazolin is 273 nm.

FT-IR analysis of prepared nanofibers

FT-IR is one of the commonly used spectroscopic methods for studying polymers. We used this technique for investigation of chemical changes during the nanofibers preparation and confirming the chemical attachment. The FT-IR spectra of the polymeric nanofibers were measured by placing

the samples between two KBr windows spacer with a 0.25 mm thickness. For this purpose, FT-IR spectrum of chitosan/PEO and chitosan/PEO/cefazolin nanofibers were recorded.

In vitro and in vivo testing of nanofibers

For investigating of in vitro antibacterial property of nanofibers, we tested them by using agar plate method. The antibacterial activity of electrospun CS/PEO and CS/PEO/Cefazolin nanofibers, against the Gram positive bacteria S. aureus ATCC 6538 and Gram negative E. coli ATCC 35401 which is commonly found on burn wounds, was assessed by a semi-quantitative way based on diffusion which known as antibiogram on bacterial culture medium, Mueller-Hinton agar, was prepared and used for antibacterial testing. The bacterial species Staphylococcus aureus and Escherichia coli are recommended in most test methods. These two species are potentially pathogenic and therefore require proper physical containment facility in their handling. Many studies have used them as the test microorganism which can be cultured and handled in a standard laboratory with minimal health risk.

Nanofibers were first sterilized by using ethanol 75% treatment. Then these nanofibers mats caught into 5 mm disks and were dropped in the culture on a Mueller-Hinton agar plate. The plates were incubated at 37°C for 24 h, and the inhibition zone of bacteria was measured to determine minimum inhibitory concentration (MIC).

Furthermore, the wound healing activity was evaluated by burn wound model in an adult rat. Healthy male rats weighing 150– 200g were selected for the study. Animals were divided into 4 groups, consisting 2 animals in each group.

The wound healing activity was conducted with the protocol as shown in table 2. The animal was unconscious with chloroform and 1.5 ×1.5 cm segment on the back side of rats was burned by a pipe with a diameter 1.5 cm of boiling water for several minutes and skin wounds has been sterilized with 70% alcohol.

After, the wound was blotted with sterile gauze in control group, the respective nanofibers on the wound of animals in treatment groups (Group- III, IV) and were investigated on days 3, 5, 7, 10 after the injury and wound size was measured (with three repeat tests).

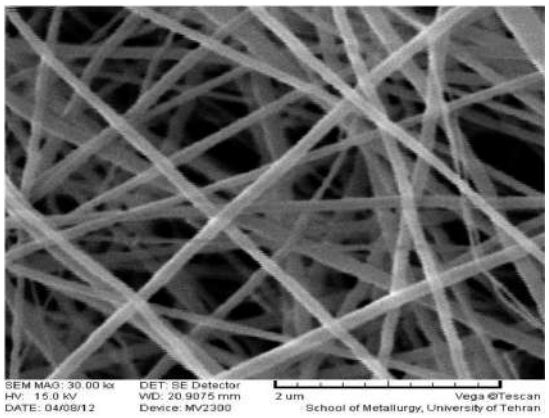

Fig. 1. SEM image of chitosan/PEO nanofibers with ratio of 90 to 10

RESULTS AND DISCUSSION

Electrospinning of chitosan /PEO with a ratio of 90 to 10

SEM images of samples that have been collected on aluminum foil, showed all nanofibers are electrospun regularly and without beads (Fig. 1).

Electrospinning of chitosan/PEO/cefazolin solution

SEM images showed the morphology of electrospun nanofibers of chitosan/PEO containing the different concentration of cefazolin (Fig.2). In 0.5 and 1 wt% concentration of cefazolin, the uniform nanofibers were formed on the surface of the collector. The addition of 2 and 3wt% of the drug caused formation a significant bead between the nanofibers, because there is no perfect dissolution of antibiotic in solutions and distribution of antibiotic could not produce homogenous solutions throughout the mats that contained 2 and 3wt% antibiotic. The SEM image of these nanofibers showed white beads and nanofibers diameter and the number of beads in the nanofibers structure increased in 3wt% and more of drug. We have chosen 1wt% of antibiotic for using in next tests.

The inclusion of antibiotics in the polymer solution can have some effect on the electrospinnability of the polymer and the morphology of the nanofibers, due to the changes in viscosity, surface tension, and conductivity of the solution. The determination of nanofibers diameters was achieved with "Image J" software regardless of the addition of higher amounts of antibiotics. By using this software, the diameter of at least 50 nanofibers are determined and an average diameter is calculated. The average

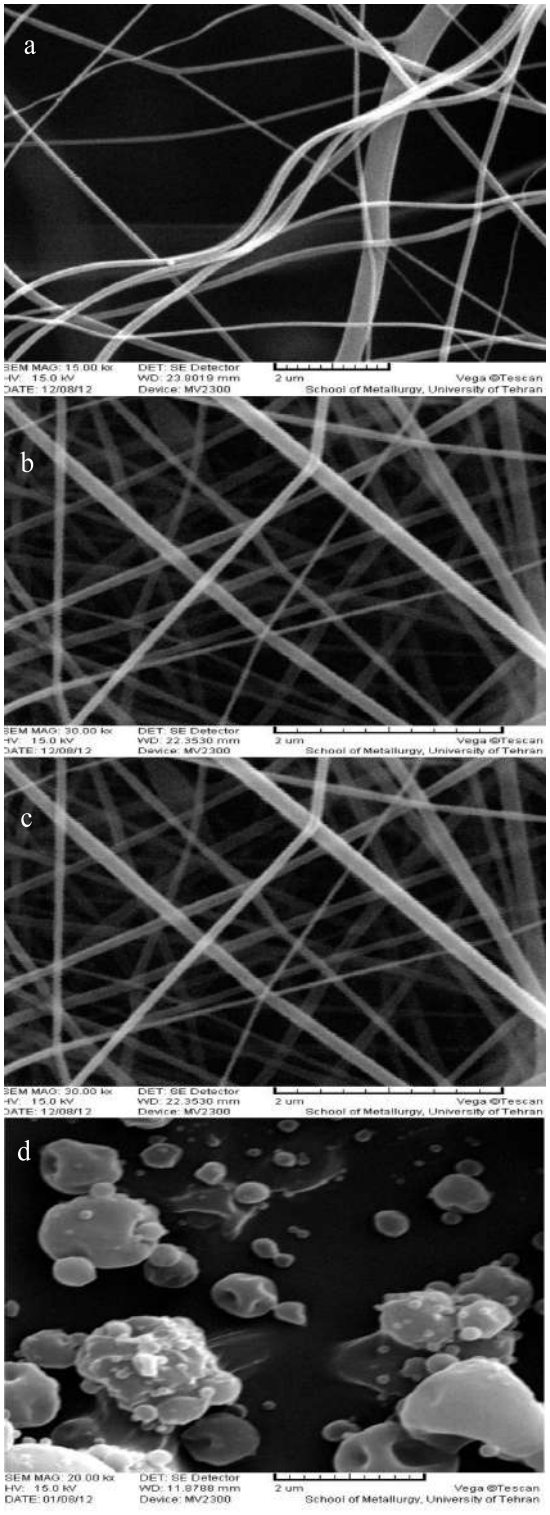

Fig. 2. SEM images of electrospun chitosan/PEO nanofibers containing; a)0.5 b)1 c)2 and d) 3wt% of cefazolin

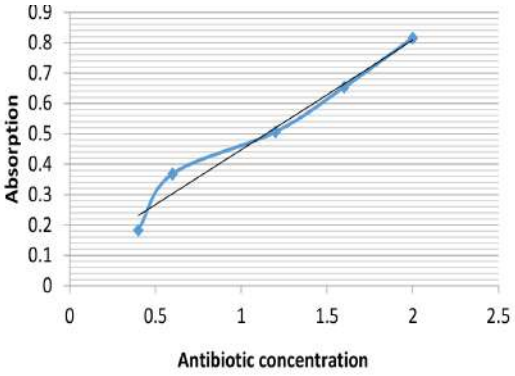

Fig. 3. Calibration curve of cefazolin antibiotic

diameter of the nanofibers containing 1wt% of the drug is 92.5 nm. This diameter is a suitable diameter for nanofibers and is lower than the other research of electrospinning of CS /PEO/1wt% antibiotic nanofibers that is a good result [22]. The morphology of nanofibers with 0.5 wt% of cefazolin was suitable and without bead but in 2 and 3 wt% of cefazolin the nanofibers cannot appear very suitable. The low diameter of nanofiber has a good advantage such as more surface area to volume ratio, the better interaction of nanofibers with cells, more control of the humidity wound and more ability for loading the drugs in pores and finally increasing the antibacterial properties.

Antibiotic releasing

In order to investigate the releasing of nanofibers containing antibiotic, nanofibers were immersed in phosphate buffer with pH 5.5 and 37°C. In the period of time, the releasing of the drug was measured. First, it is necessary to draw the calibration curve and then, the calibration curve of cefazolin was determined by taking the absorbance of cefazolin concentrations between 0 and 2 mg/mL as parameters as well as the calibration curve was fitted to the Lambert–Beer law. A linear relationship between the cefazolin concentration (y) and the optical absorbance (x) was obtained (y =0.925x - 0.187 and R2= 0.855). All experiments were repeated three or more times and the experimental data are expressed as means ± standard error deviation. Fig.3 shows the relationship between absorption and antibiotic concentration.

To evaluate the amount of drug loaded by nanofibers, a UV-Vis spectrometer was used for

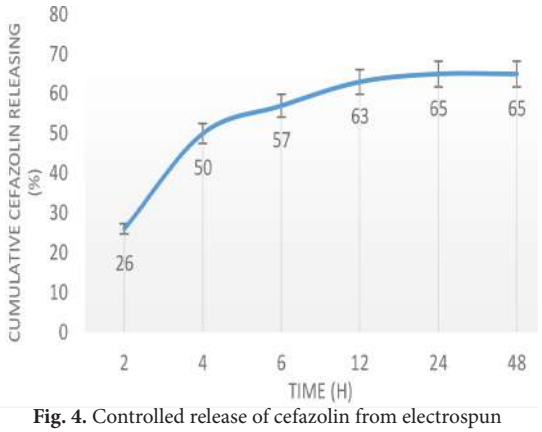

Fig. 4. Controlled release of cefazolin from electrospun nanofibers containing 1 wt% drug on time

analysis.

Fig.4 shows the release profile of cefazolin in time. Cefazolin wasn't released rapidly, its delivery happened moderately during the two days. In the early times, most of the drug releasing from nanofibers containing 1wt% antibiotic, was approximately 26% and increased in the next times. This release continued until around two days. Probably the connection between drug and chitosan is hydrogen bonds and maximum releasing of nanofibers are about 65% and then remain constant for the next time.

This releasing template can be attributed to the porous structure of prepared nanofibers that cause to penetration of buffer to the nanofibers pores thus the releasing process occurs slowly. Moderate delivery of active substance during some days results in better healing and curing of the wound.

The release of the agent in an aqueous environment was found to follow a biphasic profile: an initial burst release followed by a much slower process thereafter. The high burst release can be ascribed to two reasons. First, the very small diameter and the high surface area in the nanofibers provide short diffusion pathway and are conducive to mass transfer of the drug. Second, during electrospinning, the majority of cationic drugs is likely to be localized on the surface of the nanofibers due to their ionic strength. In such a spatial arrangement, the drug can easily be dissolved and released into a solution.

FT-IR spectroscopy of nanofibers

FT-IR spectroscopy of electrospun nanofibers in a range from 400 to 4000cm^{-1} at room temperature were shown in Fig. 5. Fig. 5a shows the band between 3600-3200cm^{-1} assigned to the stretching mode of the O-H and N-H bonds of chitosan and O-H bond of PEO. N-H bending vibration of the NH_2 groups appears at about 1569cm^{-1}. Moreover, the bands located between 2960-2874 cm^{-1}are ascribed to the symmetrical stretching mode of the C-H bond and band in the 1243 cm^{-1} is assigned to the symmetrical bending vibration of the methylene groups. The band at 1736 cm^{-1} correspond to C=O stretching vibration of the carbonyl group of chitosan. Also, the band of 1342 cm^{-1} is assigned –C–O stretching of the primary alcoholic group in chitosan. As shown in Fig. 5b, the broad band at around 3359 cm^{-1} is assigned to the stretching mode of the O-H and N-H bonds in the chitosan and cefazolin and O-H bond in the PEO backbone. Moreover, the bands located in 1630 cm^{-1} ascribed to the stretching mode of the carboxyl group in the cefazolin.

Also, as shown in Fig. 5b and 6, the absorptions bands of thiodiazole part of cefazolin are stretching vibrations of C=N (1399 cm^{-1}), C–S (561 cm^{-1}) and in-plane vibrational mode involving NCS moiety (1034cm^{-1}).The other band at 920 cm^{-1} may be related to the stretching vibration of C-C group. The band at 839 cm^{-1} correspond to N-H stretching and bending vibration of the tetrazole ring.

Biodegradation and swelling of nanofibers

The order of degradation of nanofibers is the loss of their structure into a polymeric mass without a

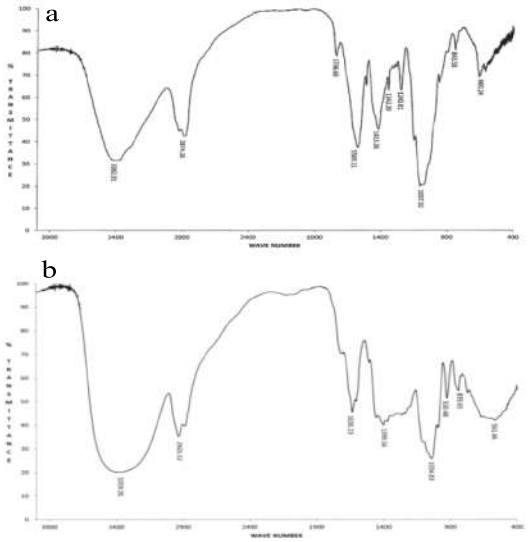

Fig. 5. FT-IR spectrum of;a) chitosan/PEO nanofibers b) chitosan/PEO/cefazolin nanofibers

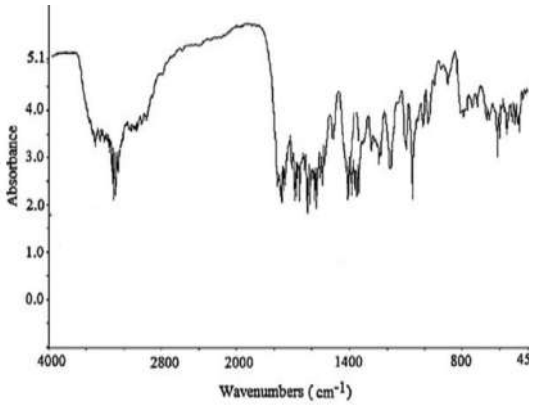

Fig. 6. FT-IR spectrum of cefazolin

specific form. Fig.7 shows SEM images of CS/PEO containing the different concentration of cefazolin nanofibers after immersion in the buffer. Neither chitosan, nor PEO can degrade in the buffer for 24 h. Electrospun nanofibers of chitosan/PEO/ 3 wt% cefazolin have been completely resolved in the buffer. This result is probably due to insertion of cefazolin into nanofibers. Cefazolin is water-soluble and could possibility increase the solubility of nanofibers within the buffer and water. So that by increasing the percentage of cefazolin disturbing of the structure can be seen more.

Also, CS and PEO are both hydrophilic polymers. The great tendency to interact with water is probably due to the increased H-bond interactions between the functional groups of the CS, PEO and cefazolin with the water molecules.

It is notable that since CS and PEO are both hydrophilic polymers which are soluble in an acidic aqueous environment, in order to apply the CS-PEO nanofibrous mats for biomedical applications, an appropriate mat with desirable wettability must be prepared.

Finally, swelling and degradation test showed that the most of degradation and swelling related to CS/ PEO containing 3wt% cefazolin nanofibers, and least of degradation and swelling related to CS/ PEO containing 0.5wt% cefazolin nanofibers.

The water uptake or swelling percent of samples was calculated by using following formula, where W0 is initial weight of the dry sample and Ws is a swollen weight of the sample at equilibrium.

$$\text{Swelling percent} = (Ws - W0) / W0 \times 100$$

The plot of swelling percents is presented in Fig. 8.

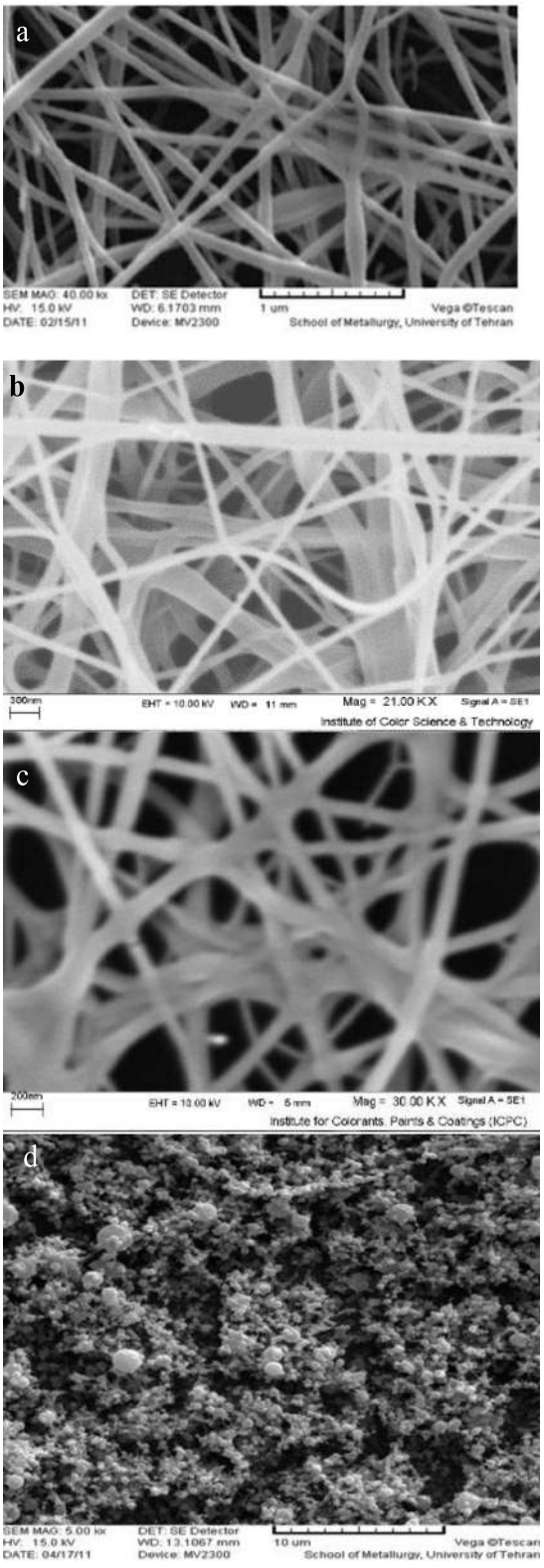

Fig. 7. SEM image of chitosan/PEO with a)0.5, b)1, c)2 and d)3 wt% cefazolin nanofibers after immersion in buffer

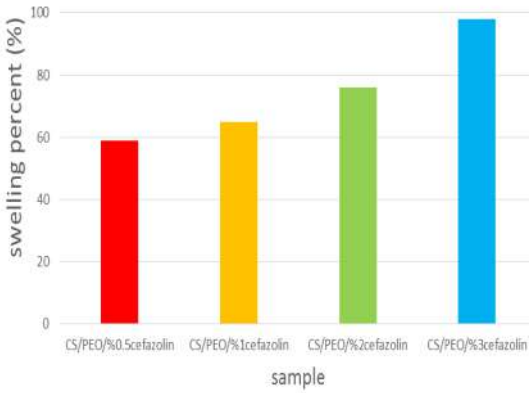

Fig. 8. The swelling diagram of nanofibers in buffer with pH 5.5 and 37oC temperature

In vitro and in vivo testing of nanofibers

In the microbial test, the zone diameter formed around of nanofibers is considered as an index of antibacterial activity. Quantitative methods are used to determine antimicrobial minimum inhibitory concentrations (MICs). These MICs provide estimates of the susceptibility of bacteria to antimicrobial compounds. Quantitative methods that require measurement of zone diameters provide reproducible estimates of the susceptibility of bacteria to antimicrobial compounds. One such standardized procedure requires the use of standardized inoculum concentrations. This procedure uses paper disks impregnated with 30-mg cefazolin to test the susceptibility of microorganisms to cefazolin.

The size of the zone is indicative of the level of antibacterial activity in the nanofibers and is affected by the potency of the antibacterial agent, the amount that has been leached into the agar and the rate of release. However, the zone of inhibition should not be expected if the antibacterial agent in the sample cannot diffuse into the agar, such as the inherently antibacterial chitosan nano membranes, or textiles on which the antibacterial agents have been durably attached. This zone of inhibition method has been used to examine the antibacterial activity of electrospun nanofibers loaded with an antibiotic.

The antibacterial study of chitosan/PEO/1wt%cefazolin nanofibers (Fig.9) shows that this scaffold has the antibacterial effect and inhibition zone was created in the presence of both of Gram-negative and Gram-positive bacteria. This means that cefazolin destroyed both type bacteria membranes but cefazolin inhibitory effect on the growth of Gram-positive bacteria is more than that of Gram-negative bacteria.

Table 1 shows the antibacterial activity of cefazolin and chitosan/PEO nanofibers with and without cefazolin.

The wound contraction is an important feature in healing which helps to close the wound by decreasing the gap between its dermal edges and reducing the wound surface area. It mainly depends on the repairing ability of tissue which may be reduced due to infections. It was measured to find the extent of reduction in wound area at different periods of treatment by graphical method. Wound area was calculated on 3rd, 5th, 7th, and 10th post wounding day by counting a number of squares of retraced wound area on graph paper.

The wound closure was measured as wound contraction percentage (WC %) by using following formula:

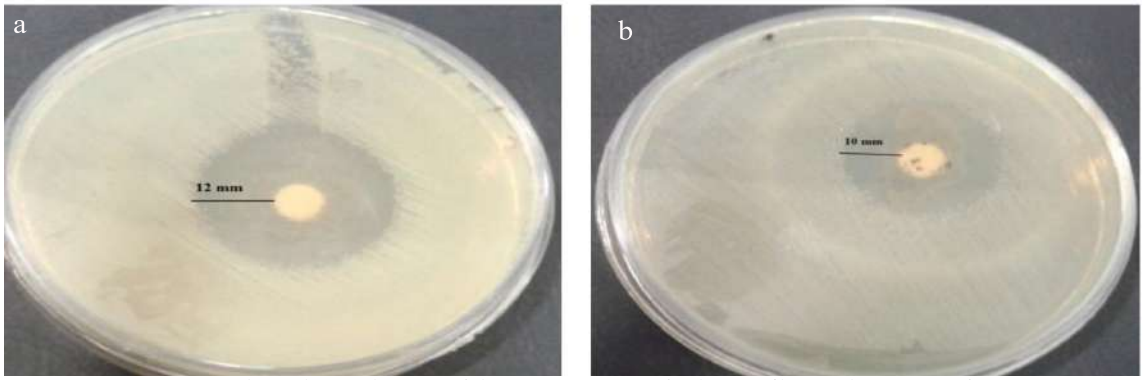

Fig. 9. The antibacterial activity of chitosan/PEO/1wt%cefazolin nanofibers in the presence of; a) S.aureus and b) E.coli bacteria

Table 1. MIC of antibiogram tests for E. coli and S. aureus

Sample	Zone diameter (mm)	
	S. aureus ATCC 6538	E.coli ATCC 35401
CS/PEO nanofiber	Decrease growing up	0
CS/PEO/ 1wt%cefazolin nanofiber	12	10
Cefazolin	14	11

Percent Wound Contraction=((Initial wound size –Specific day wound size)/Initial wound size)x100

The assessment criteria for this method were to calculate percentage wound contraction by measuring internal diameter of the wound in mm. The percentage of wound contraction in untreated and treated groups was measured on 3rd, 5th, 7th, and 10th post wound day and the results are shown in table 2.

65% of wound contraction was observed in the untreated group (group II). Wound contraction in wounds treated with CS/PEO nanofibers was 87% (group III) and CS/PEO/1wt% cefazolin nanofibers was 99% (group IV) which were more than in untreated wound as given in table 2.

According to table 2 and the results of cefazolin releasing from nanofibers, the best results are obtained in the presence of chitosan/ PEO/1wt%cefazolin nanofibers as healing sample. The high swelling property of these nanofibers causes them to hold more moisture in this structure thus the surface of wound remain wet during the healing process that prevent from nanofiber sticking to the wound surface.

CONCLUSIONS

Electrospinning is a simple and inexpensive technique for producing polymer nanofibers. In this work, polymeric nanofibers of chitosan /PEO with the weight ratio of 90 to 10 containing cefazolin, were studied by the electrospinning process. In this study, degradability tests showed that the most of the degradation related to chitosan/PEO containing 3wt% cefazolin nanofibers, and least degradation related to chitosan/PEO containing 0.5wt% cefazolin nanofibers. Also in evaluation water absorption tests, chitosan/PEO containing 0.5wt% cefazolin nanofibers have the lowest water absorption and maximum water absorption was related to chitosan/PEO containing 3 wt % cefazolin. According to these results, it seems that nanofiber of chitosan/PEO containing 1% cefazolin is a good choice as a wound covering and hold more moisture in its structure thus the surface of wound remain wet during the healing process that prevents from nanofibers sticking to the wound surface. Moreover, the oxygen can travel more

Table 2. The results of in vivo testing of nanofibers

S.No.	Group type	Purpose	Healing percentage			
			Day 3	Day 5	Day7	Day 10
1	I	Normal control	-	-	-	-
2	II	Wound control	9	21	53	65
3	III	Treatment with CS/PEO nanofibers	28	48	84	87
4	IV	Treatment with CS/PEO/1wt%cefazolin nanofibers	45	72	93	99

easily between the wound and dressing. These benefits result in more rapid healing and recovery of the wound. FT-IR spectroscopy experiments showed that the cefazolin was present in composite nanofibers. The study of drug release from this nanofiber showed that the liberation level is relatively high during the early hours and over time, high amounts of the drug diffuse from the inside of nanofiber into the aqueous environment. Cefazolin delivery happened moderately during the two days. In the early times, most of the drug releasing from nanofibers was approximately 26% and increased in the next times. The Bacterial test has shown that chitosan/PEO nanofiber has a bacteriostatic effect against Gram-positive bacteria. Scaffolding nanofibers containing cefazolin are effective on both Gram-positive and Gram-negative bacteria. Animal tests results showed that both nanofibers of chitosan/PEO and nanofibers of chitosan/PEO containing cefazolin could lead to wound healing, but the healing percentage of nanofiber containing cefazolin was higher than chitosan/PEO nanofiber. All of these benefits results in rapid and better healing and recovery of the wound.

ACKNOWLEDGEMENT

The authors are grateful to the University of Malek Ashtar for financial support.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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