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RESEARCH ARTICLE

Hemocompatibility assessment and drug release kinetics investigation from materials based on electrospun nanofibers

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ABSTRACT

Objective(s): Polymeric nanofiber has a huge potential for a various biomedical applications such as wound healing and orthopedic implant. Since most of the future applications of therapeutic nanofiber are interacting with human blood components, it is important to investigate hemocompatibility.

Methods: In this study, nanofibers with antibacterial properties were synthesized by electrospun of polymeric composite of chitosan (CS), poly (ethylene oxide) (PEO) and vancomycin (vanco). The results obtained from scanning electron microscopy (SEM), FTIR spectroscopy, antibacterial and hemolysis tests of nanofiber were evaluated. The kinetic and drug release mechanism of drug loaded electrospun samples were also investigated.

Results: The surface morphology of a composite nanofiber indicated that the nanofiber is flat and smooth. The results of antibacterial tests showed that prepared nanofiber has antibacterial properties. Hemolysis test indicate that this nanofiber has non hemolytic impact on red blood cells (RBCs). Also, it was found that the mechanism of antibiotic release can be described as Fickian diffusion model.

Conclusions: Infections and pathophysiological factors cause delayed healing of wound healing. Therefore, using antibacterial nanofibers for elimination of antibacterial infection from wounds, accelerate wound healing.

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INTRODUCTION

One of the most important usages of nanofibers are in the production of polymeric nanofiber. It is widely used in industrial and medical fields such as for wound dressing, drug and gene delivery, cardiovascular implants, artificial veins, prosthesis, facial mask, filtration, and stitching. Nanofibers and the matrices made of them can deliver drugs directly to the inner tissues and there are a variety applications for them in medicine such as fully solvable bandage and stitch. The nanofiber also decreases infection and bleeding rates [1, 2].

The skin is the largest body limb and a physical barrier against external damages. When damaged, pathogens can penetrate directly into the body and cause infection. To solve this, wound dressings with antibacterial solutions are used [3]. While the wound is exposed to oxygen and cutaneous respiration is facilitated, the small holes of nanofiber matrix prevent penetration of bacteria and pathogens to the wound areas [4].

CS is a natural nontoxic, biocompatible, and biodegradable polymer [5] that is used in drug delivery, cellular delivery system, orthopedics, wound healing, ophthalmology, stitching, hemodialysis, contact lenses, and bone healing [6]. It is also featured with antimicrobial effects against bacteria, fungi and yeasts and coagulation acceleration effects [7, 8].

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Hemocompatibility is a key factor in examining biological effects of nanofibers. Hemolysis means the rapture of erythrocytes (red blood cells) and the release of the contents (e.g. hemoglobin) into the surrounding fluid, which leads to anemia, icterus, and renal failure. Hemolysis tests are widely used to examine hemocompatibility in hematology field. It is an easy and cost effective method to examine cytotoxicity in erythrocyte membrane [9].

CS might induce thrombosis due to its cationic nature. The reaction takes place between amino CS and blood cells and plasma proteins. Positive charge of CS may induce fibrinogen absorption followed by platelet and leucocytes [10]. The present paper is an attempt to survey the antibacterial effects and hemocompatibility of nanofibers of CS/PEO / vanco.

The main purpose of this work has been to study and evaluate the CS/PEO blend nanofibrous mats containing vancomycin for the acceleration of wound healing. On the other hand, developing complex polymeric matrices requires more robust mathematical models to elucidate the solute transport mechanisms. It is believed that the mechanism of vancomycin release from nanofibers is related to Fickian diffusion and some useful information along with interesting results and their interpretations are critically discussed.

MATERIALS AND METHODS

CS with low molecular weight (degree of deacetylation: 75-80%, viscosity: 200–800 CP), gelatin powder and PEO (molecular weight: 900.000) produced by Sigma Aldrich were used. In addition, glacial acid (99.8%; molecular weight: 60.05g/mol) and tween 80 produced by Merck; and Nanoaxma electrospinning device and SIGMA VP-500 FE-SEM device (ZEISS- Germany) were used. *Staphylococcus aureus* (*S. aureus*) (ATCC 29213) and *Escherichia coli* (*E.coli*) (ATCC 8739).

Preparation of CS/PEO nanofiber with 90/10 weight ratio

Deionized water and acid acetic solution (50-50) was poured into a beaker on a heater-stirrer. Then, 0.27g of CS and 0.04g PEO were added to the solution gradually. As emulsifier, 0.25ml tween (80) was also added and the beaker was sealed by parafilm (to prevent evaporation). The beaker remained on the heather-stirrer for 24h at 37°C until the polymer solution was completely dissolved.

Preparation of CS /PEO nanofiber with 0.4% wt of vanco

The same as above while vancomycin was added as the additive.

Electrospinning

The polymer solution was collected in a syringe (5ml) and the syringe was mounted on the device. The voltage was set at 16-20v, a tip-to-target distance of 10 cm and a flow rate of the polymer solution of 0.5 ml/h. Electrospinning of each solution took 3h.

SEM imaging

Morphology of the nanofibers was examined using SEM.

FTIR spectrometry

After the preparation of membrane, a FTIR sample was prepared to examine functional groups and the interactions between nanofibers.

Antibacterial activity

To examine antibacterial activity using the disk diffusion method. A suspension of S. aureus gram positive bacteria and E.coli gram negative equivalent of Mcfarland 0.5 (1.5×108 cfu / ml) was prepared. Then a sterile swab is dipped into the bacterial suspension and was cultured on Muller Hinton Agar plate. The plates were placed into an incubator for 24 h at 37 °C and then the diameter of growth inhibition ring diameter was measured.

Measuring the nanofiber antibacterial activity

To measure the antibacterial effect of the nanofibers produced through electrospinning, they were cut into 1×1cm2 pieces and then a UV-sterilized biologic hood was mounted on the plat surface. The sample plates were placed into an incubator for 24 h at 37 °C. Afterward, the diameter of growth inhibition ring was measured.

Hemolysis test

Blood samples needed for hemolysis test were taken from a healthy individual and the samples were poured into heparin tube. The samples were diluted by 0.9% sodium chloride at 1:1.25 ratio; then 0.2 ml blood sample was poured into falcon tube and the volume was increased using sodium chloride. The samples were incubated for 30 min at 37 °C. Deionized water and blood were taken as positive control and sodium chloride (0.9%) was taken as negative control. Hemolysis percentage

was obtained as follows:

$$HR = (D_t - D_{nc}) / (D_{pc} - D_{nc}) \times 100\%$$

Evaluation of drug release from nanofibers with UV-Vis spectrophotometry

Releasing of vancomycin from composite nanofiber scaffold was studied by UV-Vis spectrophotometry. First, nanofibers of CS/PEO with 0.4 g of vancomycin were prepared. Electrospinning were carried on to an aluminum foil for 5 h. Then nanofiber removed carefully from the aluminum foil using forceps. Afterward, 5×5 cm segments of crosslinked CS/PEO/vanco nanofiber were placed in 5 ml of PBS at 37 °C in order to vancomycin be released 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. For this purpose, Sampling was carried at 2, 4, 12 and 24 h and 2, 4, 7, 10 and 14 days. At each time, 200 µl of each solutions were transferred to the cuvette located in the instrument. According to scientific literature, the vancomycin has absorption at 279-280 nm.

The release kinetics of CS/PEO/vanco can be described using Korsmeyer–Peppas based on the results obtained from all the samples [11].

Where, Mt is the cumulative amount of drug released at time t, M∞ is the initial drug loading, K is a constant characteristic of the drug-polymer system, and n is the diffusion exponent suggesting the nature of release mechanism.

RESULTS AND DISCUSSION

SEM images

CS/PEO solution (90/10 weight ratio) was prepared using nanofiber electrospinning. Electrospinning is a simple and inexpensive method to produce fiber at nanoscales. Based on the literature review, we know that due to high viscosity, CS solution is not suitable for electrospinning. PEO modifies inter molecular and intramolecular interactions of CS and attenuates its viscosity so that the solution becomes suitable for electrospinning [12]. SEM images of CS/PEO nanofiber with and without vancomycin are pictured in Figs 1 and 2. Clearly, uniform tissues without nodes are obtained with the noted ratio. The average diameters of CS/PEO and CS/PEO nanofibers with 0.4% wt of vancomycin are 94.24 and 110.2 nm, respectively.

FTIR spectrometry

Fig. 3 illustrates FTIR spectrum of CS/PEO nanofiber and the nanofiber containing vancomycin antibiotic. Clearly, 3300-3500 cm^{-1} region demonstrates stretching peak, which indicates OH carboxyl functional groups. Stretching peak at 2800 cm-1 region is attributed to stretching vibration of C-H group. Peak of carbonyl group (C=O) is visible at 1650-1680 cm^{-1} region.

Antibacterial test

Wound contraction is a key feature of closure of a skin wound, so that the wound area is decreased and the edges of the wound move closer. This is a feature of the skin's healing capability and infection interrupts it. This highlights the necessity of an antibacterial agent for skin wound healing.

Antibacterial characteristic of CS is rooted in its cation groups that enable negative load connection at cellular surface and prevent bacterium and fungus growth. The interaction

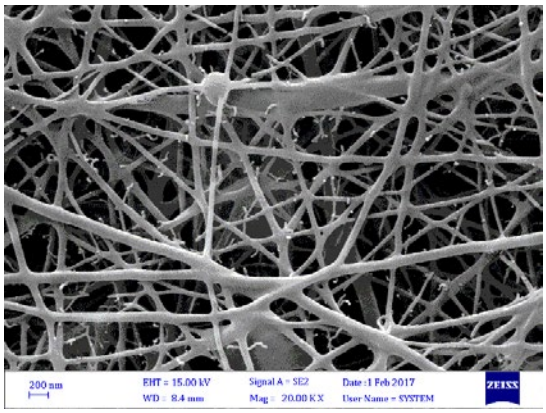

Fig. 1. SEM image of electrospun CS/PEO nanofiber

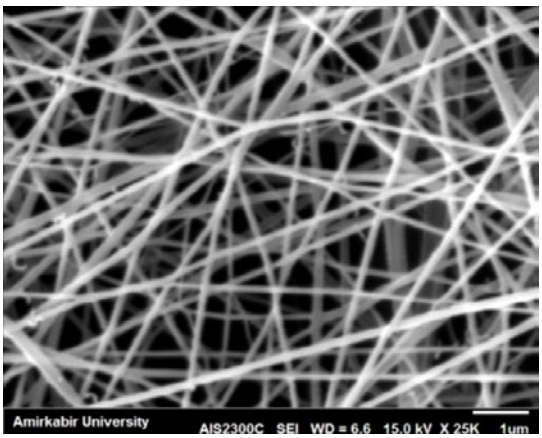

Fig.2. SEM image of electrospun CS/PEO nanofiber with 0.4 g vancomycin

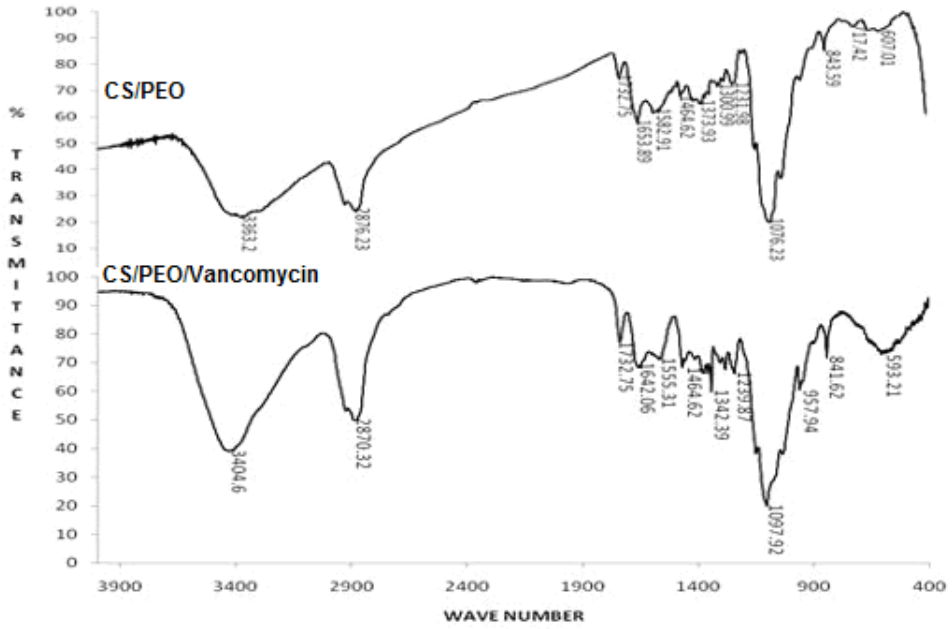

Fig. 3. FT-IR spectrum of CS/PEO nanofiber with and without vancomycin

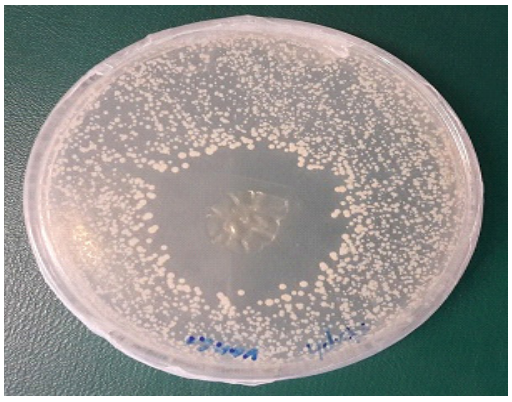

Fig. 4. Antibacterial activity of CS/PEO nanofiber containing vancomycin against S. aureus

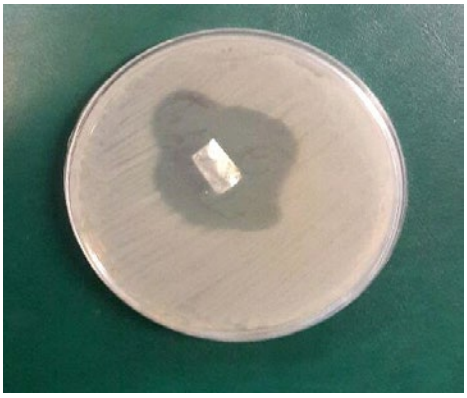

Fig.5. Antibacterial activity of CS/PEO nanofiber containing vancomycin against E. coli

modifies penetrability of cellular membrane of microorganisms so that it leads to cell death after when its content is discharged [7]. Vancomycin was used to improve the antibacterial effect. It is an antibiotic that inhibits the cellular wall of bacterium and it is widely used to treat nosocomial infection and prevent biofilm formation.

The results for CS/PEO nanofiber scaffold containing vancomycin are pictured in Figs. 4-5. Clearly, the scaffold has antibacterial effects against gram positive S. aureus and gram negative E.coli. The diameter of the zone is 19 mm for S. aureus and 20 mm for E.coli.

Hemolysis test

Hemocompatibility and toxicity of the nanofibers were examined using hemolysis test. The results showed that hemolysis of the nanofiber was less than 2% and according to the protocol, the nanofiber is free of hemolysis effect for red blood cells. It also showed good hemocompatibility (Fig.6 and Table 1).

Evaluation of Drug Release of CS/PEO/vanco Nanofibers

Fig. 7 shows the release profile of vancomycin into buffer at 37°C and in the wavelength range

Table 1. Hemolysis ratio of the samples.

Samples	Optical density at 540 nm	Hemolysis rate (%)
Deionized water	0.894	Positive control
Sodium chloride 0.9 %	0.062	Negative control
Nanofiber with vancomycin	0.076	1.6

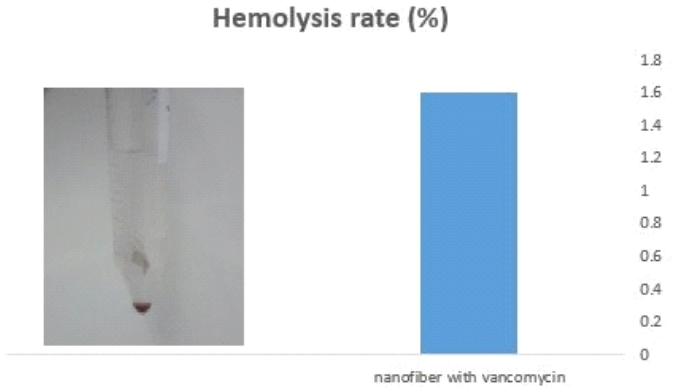

Fig. 6. Curve of hemolysis rate of thin film

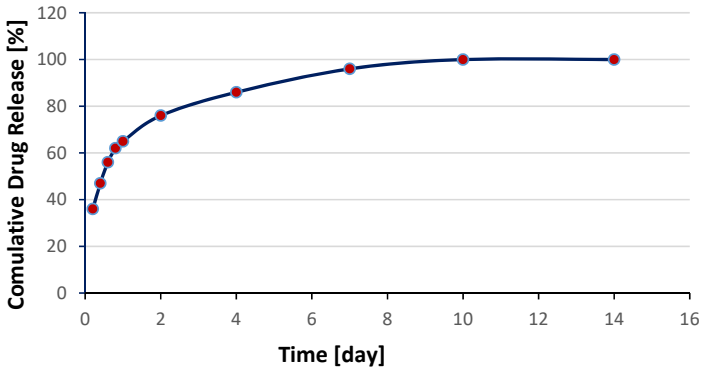

Fig.7. Release profile of vancomycin from the nanofiber scaffold with time

of 279–280 nm after 14 days.

As shown in Fig.7, the drug first has a rapid release, that called a burst effect, and then release rate decreased. The quick release is due to the porosity of CS and release of the superficial drug. As a result, 36% of the drug in the first two hours, 66% in the first 24 hours, and 96% in the first week released. No release was observed after two weeks.

The initial burst release was observed in sample. The released vancomycin reached a plateau after 7 days. Overall, the trend of the release of the drug from the nanofibers scaffold consists of four stages. The sudden release of the vancomycin occurs at the first stage. The second stage in the release of the drug from the polymeric nanofibers bed, is where the slow trend of release starts and the drug release takes place according to diffusion. In this time the drug passes through pores of the polymeric nanofibers bed and enters the buffer solution. After this stage comes the stage of drugs release that have been trapped in the polymeric nanofiber bed. The drugs deeply trapped inside the bed are released due to polymer destruction and breakdown of bonds and preparation of oligomers, which then enter the buffer solution [13]. One can not specify a specific boundary between the steps mentioned.

In the other words, after the onset of the release experiment, the polymer starts to degrade and reaches its maximum following the second stage. The diffusion continues and eventually in the final stage of drug release, the concentration of the vancomycin in the polymeric nanofibers declines. Even with the degradation of polymeric nanofiber,

the vancomycin release rate is slow and the slope of diagram is small, though the drug is still remained in the polymeric nanofiber.

Release kinetics from polymeric nanofibers

Generally, erosion, diffusion, and degradation are the most important mechanisms for drug release from polymeric nanofiber. Major studies were done on vancomycin release rate mechanisms and the release data were analyzed by Korsmeyer–Peppas model. Based on this relationship, the regression coefficients were calculated and the results showed that n=0.38. As a result, the release of drug from the polymer takes place through Fickian diffusion mechanism, which is associated with diffusion distance, the degree of swelling and concentration gradient.

CONCLUSION

CS/PEO nanofiber containing vancomycin was prepared using electrospinning method. The results of SEM images, FTIR analysis, antibacterial tests, and hemolysis tests were examined and compared. SEM images showed uniform fibers without any nodes. Antibacterial tests supported antibacterial effects of the nanofiber against S. aureus and E-coli. Moreover, hemolysis test indicated none-hemolytic properties of the nanofiber. Also, kinetic studies of this drug release system indicated that it has greater resemblance with the Korsmeyer - Peppas model, and according to this model, the kinetic degree of the drug release is estimated to be around 0.38. The results of vancomycin releasing from nanofiber scaffold shown that vancomycin wasn't released suddenly and it delivery was happened moderately during the 14 days.Therefore, it can be used as wound dressing pad to prevent biofilm formation.

CONFLICT OF INTEREST

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

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