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RESEARCH ARTICLE

Implants modified with polymeric nanofibers coating containing the antibiotic vancomycin

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ABSTRACT

Objective(s): Implant-related infections are disastrous complications in the clinic. One recent strategy to reduce the rate of infection is using the bioactive coating with an antibiotic. The purpose of these bioactive surfaces is to prevent bacterial adhesion to the implant and, consequently, the development of biofilm. In this study, vancomycin-loaded polymeric coating on implants was prepared using the electrospinning technique.

Methods: We selected polymers, chitosan (CS), and poly ethylene oxide (PEO) to prepare nanofibers. Then for the better attachment of nanofibers on the implant, the first coated the implant with thin film CS-gelatin. The prepared coatings were characterized using Scanning electron microscopy (SEM) and FT-IR spectroscopy. The antibacterial effectiveness of vancomycin-loaded polymeric coating and the bacterial adhesion of Staphylococcus aureus were evaluated in vitro. An elution study was performed with UV-Vis spectroscopy to determine the release behaviour of the vancomycin from the polymeric coating.

Results: The morphology of the vancomycin-loaded polymeric coating implant exhibited nanofibers with diameters 70-130 nm. The vancomycin-loaded polymeric coating titanium significantly reduced the adhesion of the staphylococcus aureus compared with bare implants in vitro. The release of vancomycin showed an initial vancomycin burst effect followed by a slow release. 36%of the drug in first two hours, 70% in first 24 hours and 96% in the first week released.

Conclusions: The vancomycin-loaded polymeric coating, present many advantages and may be considered to prevent and treat implant-associated infections by impeding bacterial adherence to the implant surface or reducing the concentration of bacteria near the implant.

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INTRODUCTION

Bacterial infection after orthopedic surgery is one of the most common complications that is currently difficult to cure with antibiotic treatment. In orthopedic surgery, implant was used to replace a missing joint or bone or to support a damaged bone[1]. This implants used for internal fixation are made from stainless steel and titanium, which are durable and strong. Implant-related infection is a serious complication to treat. Microbial plaque accumulation and biofilm formation on the implant lead to greater bone loss and frequent infections,

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and the most important antibiotics from bacterium staphylococcus aureus is vancomycin which is used to treat related bacterial infection of the implant [2, 3].

Vancomycin is the most effective antibiotic for the treatment of these type of infection which is mainly used against gram-positive cocci as such as enterococci and staphylococci. The bacterial pathogen staphylococcus aureus has the most important contribution in the appearance of this infection. Vancomycin is a type of glycopeptide antibiotic that blocking the construction of a cell wall and causes of cell lysis [4, 5]. Zhang et al.[6] utilized the vancomycin-coated titanium implants in treating the implant-associated infection. Also, Smith et al.[7] investigated CS films containing vancomycin and daptomycin coating on titanium surface and release of the drug.

On the other hand, electrospinning technique that is dependent on electrostatic forces used to production of fibers sizes with diameters in the range of nanometer and micrometer[8]. When the average fiber diameter decreased from several micrometers to several hundred nanometers can be gained amazing properties such as the high surface-area-to-volume ratio of the nanofibers, excellent mechanical flexibility, and high performance. These electrospun nanofibers exhibited excellent antibacterial activity[9, 10].

CS is a suitable substance for use in wound healing and implants because is a non-toxic, biocompatible, biodegradable polymer, antibacterial and antifungal, antithrombogenic[1] and due to the presence of the amino (-NH2) and the hydroxyl (-OH) groups have a good reaction [11, 12]. It is used in the drug release system, an orthopedic implant, wound healing, ophthalmology, hemodialysis, contact lenses and bone repair[13]. Ignatova et al.[14] reported that preparation of CS nanofibers by electrospinning of its aqueous solution was possible in the presence of a second polymer. Also, Hima Bindu et al.[15] applied gelatin and CS for preparation of film. Gelatin has a synergistic effect on some properties of CS. With the increase in gelatin concentration, thickness, folding endurance, water absorption capacity and tensile strength of film were increased. Gelatin has a carboxyl group and have the ability to bind CS to hydrogen bond[16].

In this study, by using electrospinning technique and synthesis of nanofibers of CS/PEO as biocompatible drug carriers and synthesis CS/gelatin thin film as a substrate for CS nanofibers, can be designed antibacterial polymer coatings containing vancomycin on orthopedic titanium implants thus preventing the spread of infections. As well as local release of antibiotics to reduce the dose of the drug consumption as a result the risks of complications can be significantly lowered.

Also, in this work, poly (ethylene oxide) was used to reduce the viscosity of the CS solution. PEO is a synthetic and biocompatible polymer that can be attached with CS by hydrogenic bonding and as a result, increase the solubility of CS, reduce the high viscosity of CS solution and the electrospinning can do easily [17].

MATERIALS AND METHODS

CS with low & medium molecular weight (degree of deacetylation: 75¬-80%, viscosity: 200–800 CP) and PEO with a molecular weight of 900.000 g.mol-1 , glass transition temperature (Tg) of -57°C and melting point of 63-68°C,viscosity:400–800 CP were purchased from Sigma Aldrich (USA). Vancomycin antibiotic were purchased from Jaber Ebne Hayyan Pharmaceutical Company (Iran). Glacial acetic acid purity 96% and molecular weight 60.05g.mol-1, tween 80, PBS (pH 7.2 – 7.4 , 0.01 M), ethanol 70 and 96%, acetone, nitric acid, gelatin, Mueller¬ Hinton Antibiogram medium agar (MHA), were purchased from Merck. Titanium screws (Size 35 × 4 mm) were purchased from Sanat metal Company (Iran). Staphylococcus aureus strain (ATCC 29213).

Preparation of CS/gelatin solution

For preparation solution, CS and gelatin were mixed with deionized water, acetic acid, ethanol, and tween, respectively. First, 50 ml deionized water was mixed with 50 ml acetic acid in a beaker on heater–stirrer at 40°C. Then CS with low molecular weight and gelatin with weight ratio of 1% added to the solution. Tween 80 is used as an emulsifier. The beaker should be tightly sealed with parafilm to prevent evaporation of water and solvent and thus increase the concentration of the solution. Afterward, solutions were stirred for 2h to ensure adequate mixing and centrifuged to remove air bubbles at 2500 rpm for 10 min.

Preparation and sterilization of titanium screws

Titanium screws were sonicated for 10 min in each of the following chemicals in succession: in acetone, ethanol 70% and deionized water, respectively. After sonication, the titanium screws were placed in a 3:7 (v/v) nitric acid–deionized water solution for 30 min at room temperature and the samples were rinsed with deionized water. Then the screws were positioned over a hot plate at 250° C for 10 min to remove moisture and entrapped air from the surface.

Coated the implant with thin film CS/gelatin

We used two methods for coating the screws: -Deposition method, after preparing the CS-gelatin solution and sterilize the screws, the screws placed in a petri dish and the solution was poured over the screwed ensuring complete coverage of the screws. Then transfer the product into a petri dish in an oven at 37 °C for 24 h. The solution was then allowed to evaporate after which time, a transparent coating was seen on the surface of the titanium screws. The screws were placed into the ethanol 90% for 10 min to stabilize the film.

-Casting method, after preparing the CS-gelatin solution and sterilize the screws, the screws placed in a petri dish and the polymer solution was poured over the screwed ensuring complete coverage of the Ti screws and left for 10 min. The screw was removed using forceps then placed in an oven at 37 °C for 12 h. The solution was then allowed to evaporate after which time, a clear coating was seen on the surface of the Titanium screws. The Ti screws were then immersed into the ethanol 90% for 10 min to stabilize the film.

Scanning electron microscope (SEM)

Investigation and comparison coated screws by deposition and casting methods, and selection better method was recorded by scanning electron microscope.

Preparation of CS/PEO nanofibers with weight ratio 90/10 for electrospinning

For preparation the solution, the 50ml water was poured into beaker then 50ml acetic acid added to it. The solution mixing placed on heater–stirrer. Then 0.27g of CS powder with medium molecular weight and 0.04 g PEO was added slowly to mixture. Afterward, Tween 80 was added to this solution as an emulsifier. The beaker was then sealed with parafilm. Then this solution was kept under vigorous stirring at 37 °C for 3h to ensure complete dissolution of the solution.

Preparation of CS/PEO nanofibers with weight ratio 90/10 containing vancomycin for electrospinning

The solution was prepared as above. Then vancomycin was added to the solution and this mixed solution was stirred for 3h. Five solutions were tested. One of the solution was prepared without vancomycin and the other solutions containing 0.2, 0.4, 0.6, 0.8 g of vancomycin. The solutions were electrospun at the same conditions, and the results were compared.

Electrospinning

The electrospinning parameters were used for the experiments: a high voltage of 20 kV, a syringe of 5ml, a tip-to-target distance of 10 cm and a flow rate of the polymer solution of 0.5 ml/h.

To produce electrospinning nanofiber, the solution was loaded in a 5ml syringe. Then were placed on the embedded pump on the system. The distance between the spinneret and collector was 15 cm and the flow rate was kept at 0.5 ml/h. The solution was electrospun at 20kV for 10 minas well the formation of nanofibers were examined.

Fourier transform infrared (FT-IR) spectra

To investigate the functional groups and interactions bonding between CS-PEO with vancomycin, CS-PEO without vancomycin and vancomycin using a FT-IR Spectrometer.

The surface morphology and fiber diameter

Scanning electron microscope (SEM) was employed to study the surface of nanofiber and was used measuring the diameter of the nanofiber. In this study, the electrospun fibers were collected on the aluminum foil. After the investigation, we adopted the best solution for electrospinning.

Antibacterial susceptibility testing

To antibacterial susceptibility testing, electrospinning was performed on titanium screw for 5h. In this investigation we used Mueller Hinton Agar is used in antibacterial susceptibility testing by the disk diffusion method.

Preparation of culture media

The method for the preparation of culture media is given below: Mueller- Hinton agar was prepared according to the manufacturer's instructions then the pH was adjusted to 7.2 and 7.6 after hydrochloric acid was added and sterilized by autoclaving. It was then inoculated on sterilized plates with a depth of 4mm.

Preparation of McFarland standard

A 0.5 McFarland standard was prepared by mixing 0.05 ml of 1.175% barium chloride di hydrate (BaCl2. 2H2O), with 9.95 mL of 1% sulfuric acid (H2SO4). The solution was kept in the dark for six months. 0.5 McFarland standard contains approximately 1 to 2 x 108 CFU/mL for S. aureus strain ATCC 29213.

Preparation of the bacterial suspension

For the preparation of microbial suspension, selected pure colony of bacteria from the solid culture medium and suspended into a tube containing serum. McFarland Standards are used to standardize the approximate number of bacteria in a liquid suspension by comparing the turbidity of the test suspension with that of the McFarland Standard. If the bacterial suspension is too turbid, it can be diluted with more diluent. If the suspension is not turbid enough, more bacteria can be added. The absorbance of the suspension at 625 nm is 0.08 – 0.1. At this absorbance, the actual numbers of bacteria present in suspension to 0.5 McFarland is 1-2×108 cfu/ml.

Antibacterial susceptibility testing (Antibiogram)

Bacterial suspensions (0.5 McFarland standard) prepared for S. aureus. A sterile swab is dipped into the bacterial suspension, rotated. Excess fluid was removed from the swab by pressing the swab against the wall of the tube. Use the swab to inoculate the entire surface of the Mueller Hinton agar plate three times rotating the plate 60 degrees between each inoculation to ensure an even distribution of the inoculum. Finally, swab around the entire edge of the agar surface. Then the screw coatings with and without antibiotics sterilized with 70% ethanol for 1h. After sterilizing, dried the disks under the laminar flow hood and were placed on the plates. For negative controls, standard discs (screw Uncoated) were used. All the plates were incubated at 35-37°C for 18-24 h. Then measured inhibition zone diameters with a ruler and the results were compared with each other.

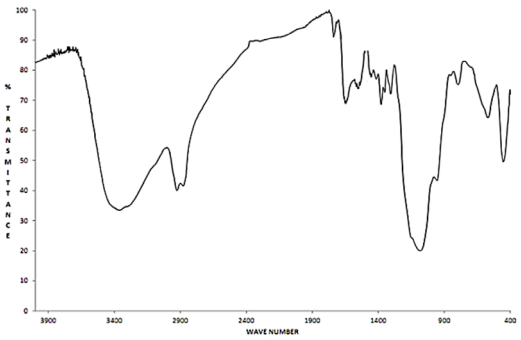

Fig. 1. FTIR spectrum of CS/gelatin film

Evaluation of drug release from nanofibers with UV-visible

First, nanofiber CS/PEO with 0.4 g of vancomycin were prepared (electrospinning were carried onto an aluminum foil for 5 h then nanofiber removed carefully from the aluminum foil using forceps) and then sterilized (placed in 70% ethanol for 1h). Moreover, the prepared sample was cut with the dimension of 5×5. Then was placed into beaker containing 5ml PBS and heated in an oven at 37° C. The lid of beaker had been covered with parafilm to prevent the evaporation of PBS. Sampling was carried at 2, 4, 12 and 24 h and 2, 4, 7, 10 and 14 days. At each time, 200µl of each solutions were transferred to the cuvette located in the instrument. The samples were scan in the wavelength range of 200–800 nm to observe the peak when the drug releases. According to scientific literature, the vancomycin has absorption at 279-280nm. After sampling and taking spectra, the results were evaluated.

RESULTS AND DISCUSSION

Evaluation and interpretation of FTIR spectra of the thin film

In this study, the screws coated with CS/gelatin thin film. To investigate the functional groups and interactions bonding between CS/gelatin polymer using FT-IR spectroscopy. The result is shown in Fig.1. As shown in Fig.1, the CS peaks located at 1655 cm^{-1} represented the stretching vibrations of the C=O bonds. As well as the stretching vibrations

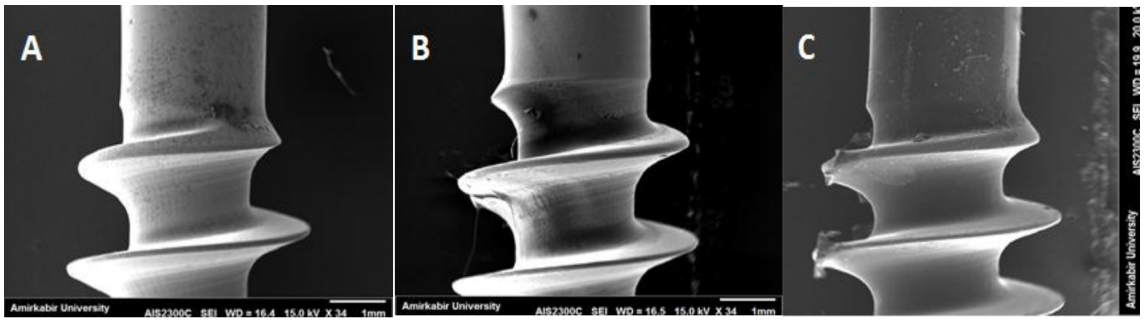

Fig. 2. SEM images of the various screw surfaces (A) uncoated titanium screw, (B) coated screw by deposition methods, (C) coated screw by casting method

of hydroxyl groups in the hybrid CS/gelatin polymer are to be observed at 3300-3500 cm^{-1}. Spectra taken from CS and gelatin were similar to the scientific literature.

Evaluation and interpretation of SEM images of CS-gelatin membrane

Investigation and comparison coated screw by deposition and casting methods, and selection better method was done using SEM.

As you see in the Fig. 2.B, by using the deposition methods it is hard to form the membrane on the eventual part of the screw which is spiral; but we observe better coating. In the Fig.2. C, It coated the flat part better than the spiral.

Evaluation and interpretation of FTIR spectra of nanofibers

To investigate the functional groups and interactions between CS-PEO with vancomycin, CS-PEO without vancomycin and vancomycin, FTIR spectroscopy was performed. In Fig. 3a, in the CS-PEO polymer the broad band at 3355cm^{-1} is assigned to the stretching mode of the O–H and N–H bonds in the CS and the O–H bond in the PEO backbone. The medium bands at 2862 cm^{-1} and 2917 cm^{-1} are attributed to the C–H stretch and C–O–C of the PEO, respectively.

The FT-IR spectrum of CS-PEO containing vancomycin electrospun nanofibers is demonstrated in Fig. 3b. The broad band between 3500–3200 cm^{-1} is assigned to the stretching mode of the O–H and N–H bonds in the CS and vancomycin and also the O–H bond in the PEO backbone. The bands located between 2900–2960 cm^{-1} are ascribed to the symmetrical stretching mode of the C–H bond and C–O–C of the PEO and vancomycin. These results imply that hydrogen bonding occurs between CS, PEO and drug molecules.

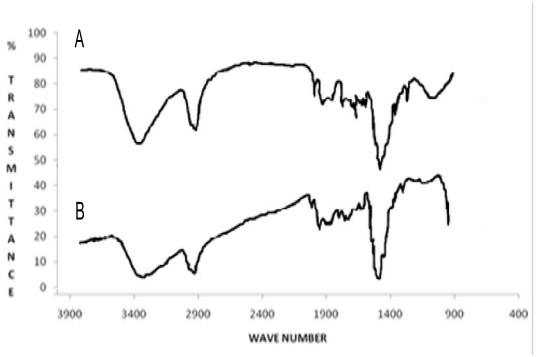

Fig. 3. FTIR spectrum of : (a) CS/PEO without vancomycin (b)CS/PEO with vancomycin

Evaluation and interpretation of SEM images of nanofibers

Scanning electron microscope (SEM) was employed to study the surface of nanofiber and was used measuring the diameter of nanofiber. In this study, the electrospun of fibers were collected on the aluminum foil.

As shown in Fig.4. (D, E), with increasing the vancomycin concentration, the drug not dissolved entirely in solutions and the white beads (vancomycin) were observed. The white beads in Fig.4. E is more than Fig.4. D. thus we pass up the addition of 0.6 and 0.8 g of vancomycin.

In Fig.4 (B,C), the white beads not were observed. Quality and diameter of the nanofibers are suitable for both concentration. Thus 0.4g vancomycin was used for the preparation of nanofibers. At this concentration, good fibers are obtained as well as we have high levels antibiotics. The Image J

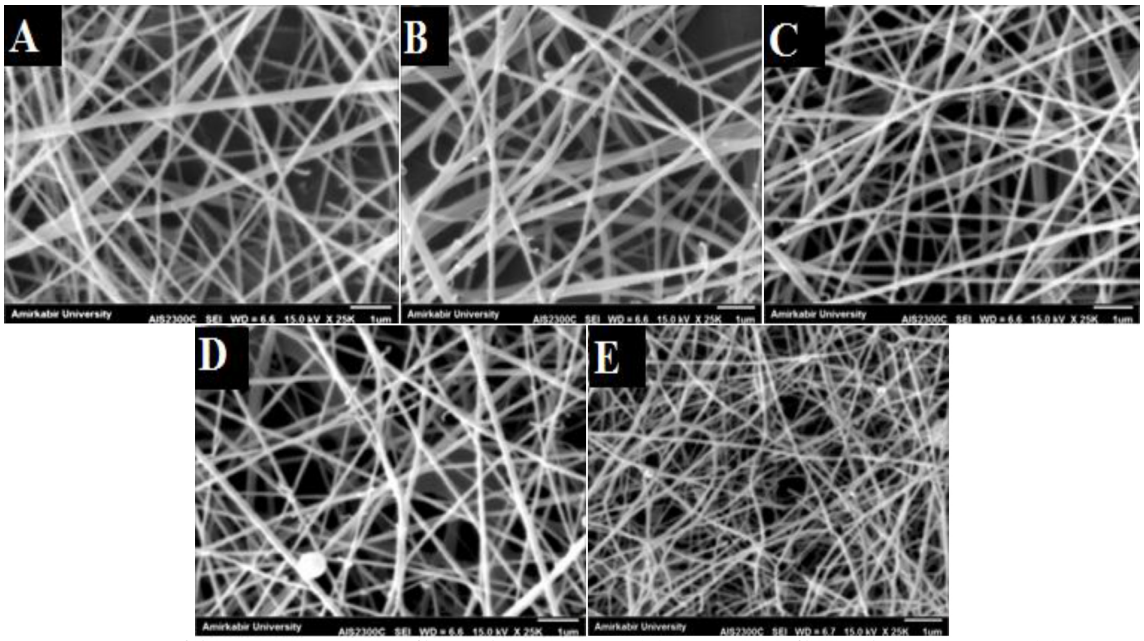

Fig. 4. SEM images of electrospun CS/PEO nanofibers without vancomycin(A), CS/PEO nanofibers with 0.2 g vancomycin(B), CS/PEO nanofibers with 0.4 g vancomycin(C), CS/PEO nanofibers with 0.6 g vancomycin(D) , CS/PEO nanofibers with 0.8 g vancomycin(E).

software was applied to calculate the average size of nanofibers in Fig.4C. The average size of nanofibers was about 92.5nm.

Analysis of the data antibacterial susceptibility testing

After selecting the appropriate nanofiber and film, first coated the screw with a polymeric film using a deposition technique. Then electrospinning of CS /PEO solution with 4.0g vancomycin were performed on titanium screws for 5h. Antibacterial susceptibility testing was conducted using the antibiogram method. Images are shown in below (Fig.5).

As shown in Fig. 5.A, uncoated titanium screw showed no signs of antibacterial properties (negative control). In Fig. 5.B, there is a small zone of growth inhibition around of the nanofiber coated screws. The zone of inhibition might be related to antibacterial activity of CS. CS has an antibacterial activity that is a complicating process that differs between Gram-positive and Gram-negative bacteria due to different cell surface characteristics. In several studies, stronger antibacterial activity was apparent against Gram-negative bacteria than Gram-positive bacteria, while in another study Gram positive bacteria were more susceptible. Still many workers demonstrated there were no significant differences observed between the antibacterial activities against the bacterium. Despite the distinction between Gram-negative and Gram-positive bacterial cell walls, antibacterial modes both begin with interactions at the cell surface and compromise the cell wall or outer membrane first.

The diameter of the zone is measured with a ruler. As shown in Fig. 5.B, the size of the zone is 2 mm. The image shown in Fig.5. C, vancomycin-coated titanium screw had large zones of inhibition compared with Fig.5 B. diameter of the zone of inhibition of vancomycin-loaded coating is 10mm. This zone related to the antibacterial activity of CS and presence of the antibiotic. Thus, the result indicating successful of coated in inhibiting bacterial growth on the surface of the implant.

Evaluation and interpretation of data of drug release rate

Table.1 shows the results of UV-Vis spectrum of a sample in the wavelength range of 279–280 nm after 14 days. According to scientific literature, the vancomycin has absorption at wavelengths of 279 -280 nm. As shown in Table.1, the drug first has a rapid release, that called a burst effect, and then

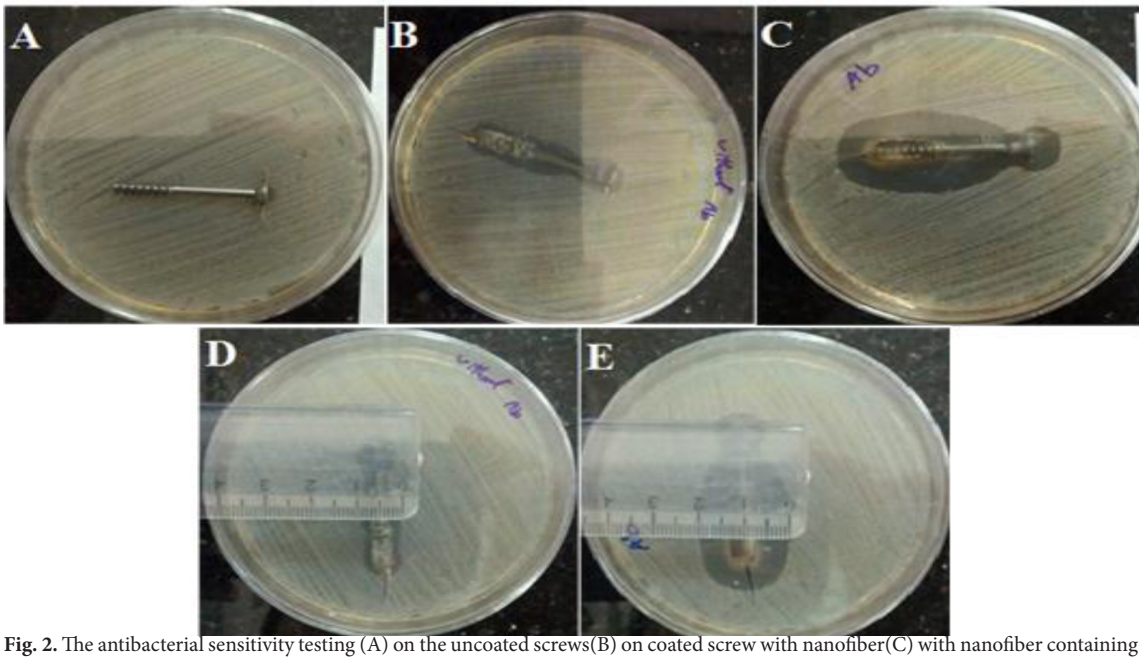

Fig. 2. The antibacterial sensitivity testing (A) on the uncoated screws(B) on coated screw with nanofiber(C) with nanofiber containing vancomycin (D,E) measuring zone of inhibition bacteria

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, 70% in the first 24 hours, and 96% in the first week released. No release was observed after two weeks.

CONCLUSIONS

In this study, for placing nanofiber on the screw, we synthesis thin film on the screw with two methods. After an investigation, we selected deposition method as a better method and coated the screw completely. Then prepared the CS-PEO nanofibers with different concentrations of vancomycin and chose the appropriate concentrations by using the SEM. Polymer nanofibers containing 0.4 g of vancomycin showed better morphology compared to other polymer nanofibers containing 0.6 and 0.8 g of vancomycin. The nanofibers were placed on the thin film very well. The inhibition zone of bacterial growth was detected around the coated screw. At the end, drug release was measured by

Table 1. The results of UV-vis Spectrum of sample in the wavelength range 279–280 nm

Column1	abs+blank	blank	abs-blank	%abs	time
1	0.623	0.236	0.387	36%	2h
2	0.748	0.236	0.512	47%	4h
3	0.862	0.236	0.626	58%	6h
4	0.925	0.236	0.689	64%	12h
5	0.988	0.236	0.752	69%	24h
6	1.005	0.236	0.769	76%	2d
7	1.147	0.236	0.911	84%	4d
8	1.278	0.236	1.042	96.00%	7d
9	1.32	0.236	1.084	100%	10d
10	1.32	0.236	1.084	100%	14d

means of UV-Vis spectroscopy during 14 days. Investigation on drug release rate, after a high start peak that called "burst effect" the release rate decreased. As a result, 36% of the drug in the first two hours, 70% in the first 24 hours, and 96% in the first week released. According to this study, polymeric coating by the described method can be applied for medical implants.

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CONFLICTS OF INTEREST

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

REFERENCES

1. Tobin EJ. Recent coating developments for combination devices in orthopedic and dental applications: A literature review. Advanced Drug Delivery Reviews, 2017;112:88-100.
2. Darouiche RO. Antimicrobial approaches for preventing infections associated with surgical implants. Clinical infectious diseases,2003;36 (10):1284-1289.
3. Song Z, Borgwardt L, Høiby N, Wu H, Sørensen TS, Borgwardt A. Prosthesis infections after orthopedic joint replacement: the possible role of bacterial biofilms. Orthopedic reviews, 2013;5 (2):65-71.
4. Rocha JLL, Kondo W, Baptista MIDK, Cunha CAd, Martins LTF. Uncommon vancomycin: induced side effects. Brazilian Journal of Infectious Diseases,2002;6 (4):196-200.
5. Micek ST. Alternatives to vancomycin for the treatment of methicillin-resistant Staphylococcus aureus infections. Clinical infectious diseases,2007;45 (3): 184-190.
6. Zhang L, Yan J, Yin Z, Tang C, Guo Y, Li D, Wei B, Xu Y, Gu Q, Wang L. Electrospun vancomycin-loaded coating on titanium implants for the prevention of implant-associated infections. International journal of nanomedicine, 2014;9: 3027-3036.
7. Smith JK, Bumgardner JD, Courtney HS, Smeltzer MS, Haggard WO. Antibiotic-loaded CS film for infection prevention: A preliminary in vitro characterization. Journal of Biomedical Materials Research Part B: Applied Biomaterials,2010;94 (1):203-211.
8. Garg K, Bowlin GL. Electrospinning jets and nanofibrous structures. Biomicrofluidics, 2011;5 (1): 13403-13419.
9. Martins A, Reis R, Neves N. Electrospinning: processing technique for tissue engineering scaffolding. International Materials Reviews, 2008;53 (5):257-274.
10. Li X, Cui R, Liu W, Sun L, Yu B, Fan Y, Feng Q, Cui F, Watari F. The use of nanoscaled fibers or tubes to improve biocompatibility and bioactivity of biomedical materials. Journal of Nanomaterials, 2013;2013: 1-16.
11. Dutta PK, Dutta J, Tripathi V. Chitin and CS: Chemistry, properties and applications, 2004.
12. Queen H. Electrospinning CS-based nanofibers for biomedical applications, 2006.
13. Lee DW, Lim H, Chong HN, Shim WS. Advances in CS material and its hybrid derivatives: a review. The Open Biomaterials Journal,2009;1 (1):10-20.
14. Ignatova M, Manolova N, Rashkov I. Novel antibacterial fibers of quaternized CS and poly (vinyl pyrrolidone) prepared by electrospinning. European polymer journal, 2007;43 (4):1112-1122.
15. Tvl HB, Vidyavathi M, Kavitha K, Sastry T, RV SK. Preparation and evaluation of ciprofloxacin loaded CS-gelatin composite films for wound healing activity. International Journal of Drug Delivery,2010;2 (2):173-182.
16. Cheng M, Deng J, Yang F, Gong Y, Zhao N, Zhang X. Study on physical properties and nerve cell affinity of composite films from CS and gelatin solutions. Biomaterials, 2003;24 (17):2871-2880.
17. Bhattarai N, Edmondson D, Veiseh O, Matsen FA, Zhang M. Electrospun CS-based nanofibers and their cellular compatibility. Biomaterials, 2005;26 (31):6176-6184.