

Preparation of Biopolymeric Nanofiber Containing Silica and Antibiotic

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ABSTRACT

The biocompatible and biodegradable polymer nanofiber with high potential for anti-bacterial coating are used for: multi-functional membranes, tissue engineering, wound dressings, drug delivery, artificial organs, vascular grafts and etc. Electrospinning nanofiber made of scaffolding due to characteristics such as high surface to volume ratio, high porosity and very fine pores are used for a wide range of applications. In this study, polymer composite nanofiber Silica/chitosan/poly (ethylene oxide) /cefepime antibiotic synthesis and antibacterial properties will be discussed. The optimum conditions for preparation of electrospun nanofiber were: voltage; 21 kV, feed rate; 0.5 mL/h, nozzle-collector distance; 10 cm, and chitosan/poly(ethylene oxide) weight ratio 90:10 and the volume ratio of chitosan/silica is 70:30. The antibacterial activity of composite scaffolds were tested by agar plate method by two type bacteria including *Escherichia coli* and *Staphylococcus aureus*. With the addition of the silica to chitosan, the hybrid was more biodegradable and improves the mechanical properties of biopolymer.

INTRODUCTION

Electrospinning is a progressive method which produces fibers ranging from the submicron level to several nanometers in diameter in a high voltage electrostatic field [1]. Electrospinning is an economical method to produce nanofiber. Because of the high specific surface area, tunable pore size, flexibility and the nanofibrous membranes are finding an increasingly wide range of applications. Some particular attention has been devoted to antibacterial nanofiber for applications such as wound dressings. A variety of biocides, e.g., antibiotics, chitosan have been incorporated by various techniques into nanofiber that exhibit strong antibacterial activity in standard assays [2]. Under the influence of the electrostatic field, a pendant droplet of the polymer solution at the capillary tip is deformed into a conical shape (Taylor

cone). If the voltage surpasses a threshold value, electrostatic forces overcome the surface tension and a fine charged jet is ejected. The jet moves towards a ground plate acting as counter electrode. Due to the extensional viscosity of the polymer solution and the presence of entanglements, the jet remains stable and does not transform into spherical droplets as expected for a liquid cylindrical thread. The solvent begins to evaporate immediately after the jet is formed. The result is deposition of a thin polymer fiber on a substrate located above the counter electrode [3]. Electrospinning is a relatively simple method to obtain ultrafine not only polymers, but also inorganic materials can be electrospun to fibers [4]. Silica has widely applications in different fields of catalysts, optical and magnetic devices. These materials also had potential application in the field of nonlinear optical materials due to the nano-confined effects on the interfaces determining large nonlinear optical susceptibilities.

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Since making silica into ordered morphologies has many benefits in the areas of sensors, catalysis, separations and drug delivery, several researches were reported to fabricate shaped mesoporous materials including spheres, rods and fibers [5]. In this research, we hybridized silica with biocompatible and biodegradable chitosan, in order to enhance their mechanical properties and control the profile of drug release. Silica/chitosan hybrids with various silica volume contents (10, 20, 30, and 50%) were synthesized using electrospinning. The silica xerogel system is an attractive material to apply to metallic substrates. In this study, we hybridized silica with a natural polymer chitosan and cefepime for use as a new antibacterial coating for different application in medicine. Chitosan, a kind of polysaccharide, is a deacetylated derivative of chitin and has been widely applied in biomedical applications because of its cell compatibility, biodegradability and nontoxic characteristics. The degradation products of chitosan are metabolized by the action of human enzymes, especially lysozyme, which enables chitosan to be incorporated into glycoproteins, which occurs in connective tissue [6]. These properties of chitosan make it a suitable material for hybridization with silica.

In the presence of a limited amount of acid, chitosan is soluble in water, methanol, ethanol and acetone mixtures. Chitosan has a positively charged polyelectrolyte in pH below 2- 6 for having free amino groups and which contribute to its higher solubility in comparison to chitin. However, this property makes chitosan solutions highly viscous for electrospinning [1]. Chitosan exhibits antimicrobial properties towards bacteria, viruses and fungi, for which many strains have been assayed. The antimicrobial mechanism includes the initial deposition of a chitosan coating on the anionic cell wall, with subsequent alteration of biochemical functions, and damage to internal organelles by internalized chitosan oligomers [7]. Viscosity of a polymer solution is the characteristics of intermolecular interactions between polymer chains. The high viscosity of chitosan solution is due to the strong hydrogen bonding between NH_2 and OH groups of chitosan polymer chains. The decrease in viscosity with addition of PEO can be attributed to the change in inter and intramolecular interactions of chitosan chains. PEO molecules bound onto chitosan backbone disrupt the self-association of chitosan chains by forming new hydrogen bonding. Physically, this modulation in

associative forces by PEO is manifested as an increase in chitosan solubility and a decrease in its solution viscosity [8].

Mesoporous silica nanoparticles are viewed as a promising and flexible platform for numerous biomedical applications. These types of nanoparticles can feature a well-defined and tunable porosity at the nanometer scale, high loading capacity, and multiple functionality for targeting and entering different types of cells [9]. The most recent research progress on silica-based controlled drug delivery systems, including: (i) pure mesoporous silica sustained-release systems, (ii) magnetism and/or luminescence functionalized mesoporous silica systems which integrate targeting and tracking abilities of drug molecules and (iii) stimuli responsive controlled release systems which are able to respond to environmental changes, such as pH, redox potential, temperature, photo irradiation and biomolecules [10]. Cefepime is an antibacterial agent belonging to the cephalosporin class of antibacterial with *in vitro* antibacterial activity against facultative Gram-positive and Gram-negative bacteria [11]. It is highly soluble in water. Cefepime is a bactericidal agent that acts by inhibition of bacterial cell wall synthesis. Cefepime has a low affinity for chromosomally encoded beta- lactamases. Cefepime is highly resistant to hydrolysis by most beta-lactamases and exhibits rapid penetration into Gram-negative bacterial cells [12].

MATERIALS AND METHODS

Chitosan with medium molecular weight and degree of deacetylation 75-80%, PEO with a molecular weight of 900.000, dimethyl sulfoxide (DMSO) and tetra ethyl ortho silicate (TEOS) were purchased from Sigma-Aldrich, glacial acetic acid purity 99.8% and molecular weight 60.05 g/mol, twice distilled and deionized water, ethanol and tween 80 buffer were prepared from Merck, Mueller- Hinton Antibioqram medium agar (MHA), *Escherichia coli* bacteria, *Staphylococcus aureus*, hydrochloric acid (HCl 37%), cefepime hydrochloride antibiotic.

Preparation solution

For preparation the silica solution that gave fibers with the least beads and smallest fiber diameter, TEOS was mixed with water, ethanol and HCl, respectively with a molar ratio of 1:3:8:0.04. First, TEOS was mixed with ethanol in a beaker. Then, HCl/water solution was added drop by drop to the TEOS/ethanol solution under

vigorous stirring. The solution was heated at 60°C for 1h and then cooled down to room temperature [13].

A 4 wt. % PEO solution was prepared separately in 0.5 M acetic acid and stirred for a period of 24 h. Then, 3 wt. % of chitosan (Cs) solution and 4 wt. % PEO solution in acetic acid were blended with Cs/PEO weight ratio of 90:10 with 0.5 acetic acid concentration. This ratio was kept stable for the mixed Cs/PEO/TEOS solutions. We used tween 80 buffer is used as an emulsifier. The maximum Cs/PEO ratio for making a spin able solution is 90/10(w/w), above which the spun product exhibited a no uniform structure or droplets. However, at this Cs/PEO ratio, the electrospinning did not produce the desired fibrous structure; instead, a structure of short fibers embedded with a considerable amount of beads was seen [8]. To improve the spin ability of the polymer solution at the Cs/PEO ratio of 90/10(w/w), a small amount of DMSO was introduced into the stock solution as a cosolvent.

Afterwards, the Cs/PEO solution was added with volume ratio 10, 20, 30 and 50% of TEOS. The resulting solutions were stirred for 3h after the addition of Cs to ensure adequate mixing. Then, different amounts of 0.3-2% of cefepime added to the Cs/TEOS polymeric solution and for 3h stirred.

Electrospinning

A variable high voltage power supply was used to provide voltage to the electrospinning solution. To produce electrospun nanofiber, the solution was loaded in a 5mL syringe with a stainless steel capillary metal hub needle. The positive electrode of the power supply was attached to the needle tip, while the grounded electrode was connected to a metallic collector wrapped with aluminum foil. All nanofiber were spun at 21kV, keeping a constant tip-to-collector distance of 10 cm and the flow rate was kept at 0.5 mL/h.

The surface morphology and fiber Diameter:

Scanning electron microscope (SEM) was employed to study the surface of nanofiber. Image J software was used to determine the average fiber diameter and standard deviation by measuring the diameter of at least 50 nanofiber.

Microbiological Test

The effect of the incorporation of cefepime in nanofiber and anti-bacterial properties in coatings was

investigated by conducting agar disc diffusion tests on Cs/%30TEOS and Cs/%30TEOS/%1Ant. nanofiber by culturing of the bacteria *E. coli* (ETEC ATCC 35401) and *S. aureus* (ATCC 6538). Nanofiber were first sterilized using ethanol 75% treatment. Each bacteria separately suspensions of 0.5 McFarland number of bacteria is prepared and containing $1-2 \times 10^8$ CFU/mL of each series were removed and placed on the surface of Mueller- Hinton agar plates seeded with *S. aureus* and *E.coli* and were inoculated at 37°C. After 24h of incubation, the zones of inhibition (diameter of the inhibition circle around paper disks) were measured.

FTIR spectroscopy

Infrared (IR) spectra of the composite nanofiber were recorded with 8400S FT IR spectroscopy in transmittance mode at room temperature. Samples was scanned from 4000 to 400 cm^{-1} .

RESULTS AND DISCUSSION

Fig. 1 shows that all nanofiber are electrospun regularly and without knots. But the diameter of the nanofiber in solution 50:50 Cs/TEOS is more than others and the ratio 50:50 solution have low viscosity and it is more difficult electrospinning. For this reasons, we selected ratio 70:30 Cs/TEOS for perform next steps, means adding drug to it and microbial testing.

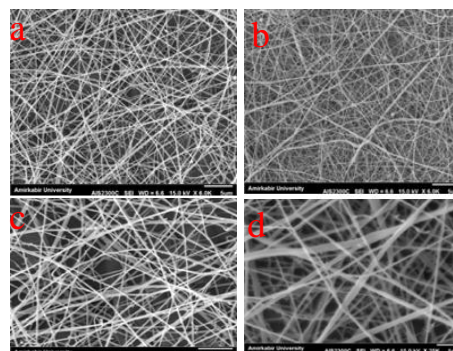


Fig. 1. SEM images of the electrospun nanofiber of Cs/PEO solutions with a) % 10 TEOS, b) %20 TEOS, c) % 30 TEOS and d) % 50 TEOS

Fig. 2 shows the SEM images of electrospinning nanofiber with different percentages of antibiotic. Nanofiber in 1wt.-% and lower antibiotic concentrations solutions are uniform and without bead. In 2wt. % antibiotic concentration many beads on structure of nanofiber is created. Because particles of antibiotic not completely dissolved in the solution of

Cs/TEOS. As a result, we selected Cs/PEO/%30 TEOS /%1 Ant. nanofiber as an appropriate fibers. Using software Image J, the diameter of 50 number of nanofiber measured and their average diameter was calculated. Uniform nanofiber with smooth surface were obtained within a composition of Cs/PEO/%30TEOS/%1Ant. (see Fig. 2c). Spinning conditions as voltage, constant tip-to-collector distance, flow rate, temperature and relative humidity were kept fixed so that only the polymer, solvent, TEOS and drug concentrations could influence on the fiber morphology. The average diameters of the as-spun nanofiber are 112nm.

Microbial tests showed that chitosan/TEOS/Ant. nanofiber effective on Gram- positive and negative bacteria. Scaffolding nanofiber with and without antibiotics were effective on Gram- positive and negative bacteria and have an inhibitory effect on them. The mechanism of inhibitory effect of antibiotics by way of effect on bacterial growth on walls bacteria. In higher pH of 6.5 chitosan solubility decreases and is losing poly cationic properties. As a result of the reaction between positive and negative charges of the cell surface of Gram- negative bacteria and surface charge of chitosan is causing damage to the growth of the bacterial cell wall. The zone diameter formed around of nanofiber as an index of antimicrobial activity is considered.

Cs/PEO/TEOS/Ant. nanofiber having the most antibacterial activity means 25 and 40mm inhibition zone against *S. aureus* and *E. coli*, respectively. But nanofiber without antibiotic show 12 and 5mm inhibition zone against *S. aureus* and *E. coli*, respectively

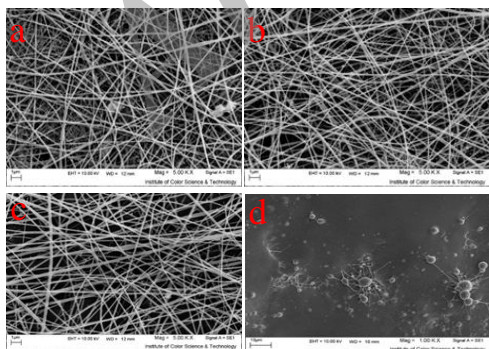


Fig. 2. SEM images of the electrospun nanofiber: Cs/PEO/%30 TEOS/Ant. solution with different amounts of the antibiotic a) 0.3 wt. %, b) 0.5wt. %, c) 1wt. % and d) 2wt. %

The chemical structures of nanofiber were evaluated by Fourier transform infrared spectroscopy. FT IR spectrum of Cs/PEO/TEOS nanofiber is shown in the Fig. 3. The broad band in 3355 cm^{-1} is assigned to the stretching mode of the O-H and N-H bonds in the chitosan and O-H bond in the PEO backbone. The characteristic bands of silica are observed: those at 1068 , 950 cm^{-1} and 792 cm^{-1} are attributed to Si-O-Si and Si-OH stretching vibrations, respectively, which is consistent with the reported results. The medium band at 2917 cm^{-1} is attributed to the C-H stretch and C-O-C accordingly of the PEO. Covalent bonds between silanes and chitosan proceed via the hydroxyl groups of chitosan, due to the formation of strong Si-O bonds rather than weaker Si-N bonds [14, 15].

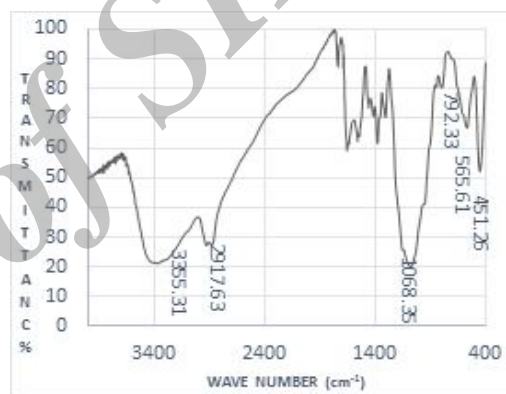


Fig. 3. FT IR spectrum of Cs/PEO/TEOS nanofiber

FT IR spectra of as-spun nanofiber indicate which bonds are formed or lost during electrospinning processes and elucidate whether Cs/PEO/TEOS/Ant. nanofiber are a physical mixture or covalently bound network. Fig. 4 showed in the wide peak at 3333.63 cm^{-1} is assigned to the stretching of the Cs hydroxyl groups. The 1622 cm^{-1} peak shown stretching of COOH of carboxylic acid and B-lactam group of cefepime. Silica shows characteristic peaks at 1074 , 559 , and 443 cm^{-1} corresponding to the asymmetrical stretching, symmetric stretching, and bending vibrations in $\text{Si}''\text{O}''\text{Si}$ bonds, respectively. The medium band at 2921.58 cm^{-1} showed to the C-H stretch and C-O-C accordingly of the PEO [16- 18].

CONCLUSION

We have prepared refining silica nanofiber by electrospinning method. Cs/PEO/%30 TEOS/%1 Ant.

is as an appropriate nanofiber, this as-spun nanofiber presented also smooth surfaces and possessed a smaller average diameter of 112nm chitosan acted as a natural polymer and PEO is as a synthetic polymer that decreases the viscosity of chitosan. This composite nanofiber used for applications such as: tissue repair or remodeling, coating for implants and etc. Cs/PEO/TEOS/cefepime antibiotic nanofiber effective on Gram-positive and negative bacteria and nanofiber without cefepime have less antibacterial activity against *S. aureus* and *E. coli*. The hybrid of Cs/TEOS was more biodegradable and improves the mechanical properties of the controlled and release of antibiotic.

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CONFLICT OF INTEREST

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

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