Fast and Efficient Electrospinning of Chitosan-Poly(ethylene oxide) Nanofibers as Potential Wound Dressing Agents for Tissue Engineering

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ABSTRACT: In this work, nanofibers of chitosan/poly (ethylene oxide) (PEO) with an average fiber diameter from a few microns down to about 30 nm and a narrow size distribution were fabricated by a fast electrospinning process using a handheld electrospinning device. It was found that the matrix with a formulation of chitosan/PEO ratio of 90/10 (w/w) and 0.3% Triton® X-100 retained excellent integrity of the fibrous structure in 0.5*M* acetic acid solution. The characterization of nanofibrous structure by scanning electron microscope (SEM) imaging showed the homogeneity of nanofibrous structure without considerable bead-like structures. The excellent electrospinnability of the current formulation represents electrospinning of natural biopolymer chitosan as a useful process in various biomedical applications, especially as potential wound dressing agents. Nonwoven mats and polymer solutions with composition chitosan/PEO ratio of 90/10 and 60/40 showed excellent antibacterial activity against *E. coli* and *P. aeruginosa*. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2011

Key words: nanostructures; biomaterials; polymers; SEM

INTRODUCTION

Nanotechnology and its branch nanobiotechnology are highlighted topics for the production of new properties of materials by reduction of dimensions to extremely small sizes.[1] In recent years, considerable research efforts of science and technology have been directed toward the development of safe and efficient nanostructured systems with the use of polymers for diverse applications.[2–4]

Electrospinning is a unique and powerful technology that can produce nonwoven fibrous articles with fiber diameters ranging from tens of nanometers to microns, a size range that is otherwise difficult to access by conventional nonwoven fiber fabrication techniques.[5,6] The increased interest in electrospinning was initiated in the 1990s by the possibility of producing polymeric nanofibers under laboratory conditions.[1] Electrospun nanofibrous scaffolds possess an extremely higher surface area to volume ratios and smaller spaces between individual fibers than larger fibers, tunable porosity, and malleability to conform over a wide variety of sizes and shapes and to offer opportunities for use in different applications. Furthermore, the scaffold composition can be controlled precisely to achieve desired properties and functionalities. Because of these advantages, electrospun nanofibrous scaffolds have been widely investigated in the past several years with materials of different compositions[7–10] for various applications such as filtration,[11,12] optical and chemical sensors,[13–15] electrode materials,[16–18] and biological scaffolds.[19–21]

In comparison with synthetic polymers, natural polymers usually exhibit better biocompatibility and low immunogenicity, when used in biomedical applications. On the other hand, synthetic polymers often offer many advantages over natural polymers. They can be tailored to give a wider range of properties and predictable uniformity. Moreover, some synthetic polymers are cheaper, and represent a more reliable source of raw materials.[22]

Among various natural polymers, polyaminosaccharide chitosan has some unique properties which make it attractive and has been proven to be biologically renewable, biodegradable, nonantigenic, and biocompatible, and used in wound dressing, wound healing,[23] drug delivery systems,[24,25] various tissue engineering applications,[26–30] and the enhancement of bone formation both *in vitro*[31] and *in vivo*.[32]

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Furthermore, the application of chitosan as an antimicrobial agent against fungi, bacteria, and viruses and as an elicitor of plant defense mechanisms has been reviewed.[33]

In spite of the variety of synthetic possibilities, only a rather small number of water-soluble polymers have been electrospun from water or solvent mixtures containing water. The most extensive investigations have been carried out on poly(ethylene oxide) (PEO), because this polymer is readily available in different molecular weights. PEO is versatile for electrospinning, because of its solubility in many solvents especially in water.[34–41] On the other hand, PEO fibers are particularly interesting for devices contacting with living organisms such as biomedical applications, because of their good biocompatibility[42] and low toxicity[43] and also because of its electrospun from aqueous solution and production of fibers with diameters in the range of 500–5000 nm[44] and ordering in the surface layer in PEO fibers observed by atomic force microscopy.[45]

Keeping these facts in mind that chitosan as a natural polymer and PEO as a synthetic polymer are two important and very useful polymers that could serve as precursors in electrospinning of nanofibers for tissue engineering and wound healing and due to the importance of this technology, introduction of new, fast, efficient, and inexpensive techniques for this purpose is of prime importance.

EXPERIMENTAL

Materials and Instruments

All solvents, chemicals and reagents were purchased from international chemical companies. Chitosan (medium M_w, Brookfield viscosity 200.000 cps) and poly(ethylene oxide) (average M_w 900,000) were obtained from Sigma-Aldrich. Triton® X-100 was purchased from Fluka. DMSO, AcOH, and gelatin (for microbiology) were purchased from Merck. Tetracycline was commercial capsule. SEM micrographs of the samples were prepared with a LEO 1455VP scanning electron microscope. The electrospinning system used in this study was briefly a DC voltage of 15–25 kV (high DC power supply, Glassman) applied between the syringe tip and a cylindrical collector covered with aluminum foil. The injector device of electrospinning was Harvard. The antibacterial activities were analyzed in Pasteur Institute of Iran.

Preparation of stock solutions

The chitosan solution (2% w/v) and PEO solution (3% w/v) were first prepared separately by dissolving chitosan or PEO in 0.5*M* acetic acid. Then, the chitosan and PEO solutions of different ratios were mixed to obtain the mixtures with weight ratios of chitosan to PEO ranging from 50/50 to 90/10, and the resultant mixtures were stirred for 12 h. After that, solutions containing 0–1 wt % of Triton® X-100 and 0–15 wt % of DMSO were mixed with chitosan/PEO solutions, and the mixtures were stirred overnight to yield homogeneous solutions before use.

Electrospinning of nanofibers

The prepared stock solutions for electrospinning were fed into a 3-mL disposable syringes fitted with pipette tips of 0.3–1.0 mm in diameters. The solution feeds were driven by the gravity and the feeds speeds were controlled by the tilt angle of the syringe. The electrospinning systems used in this study were two convenient static and portable instruments. A DC voltage of 15–25 kV was applied between the syringe tip and a collector covered with aluminum foil. The typical distance between the syringe tip and the collector was 10–20 cm. During the spinning process, the pendant droplet at the syringe tip was split by a repulsion force set by the charge in the droplet, and formed a jet of a cone-like shape traveling towards the collector, during which time the solvent evaporated and polymer fibers deposited on the collector in form of a nonwoven fibrous mat. All the spinning experiments and drying of as-spun nanofibers were performed at room temperature.

Analysis of nanofibers

Electrospun nanofibers were sputter-coated with P_t, and the morphology of the nanofibers was examined with a SEM at an accelerating voltage of 10 kV. The diameters were presented as the average five standard deviations. To analyze the fibers size distributions, the average diameters of nanofibers were determined by measuring the diameters of the nanofibers at more than 50 different points in each SEM image.

Antibacterial evaluation

Three stock solutions of chitosan (2%)/PEO (3%) with weight ratios of 90/10 (fresh-including 0.3% tetracycline); 90/10 (four months maintained); 60/40 (fresh) all of them including Triton® X-100 (0.3%); DMSO (10%) in aqueous AcOH (0.5*M*) were prepared. Then, their antibacterial activities against gram-negative bacteria *Escherichia coli* (ATCC: 25922) and *Pseudomonas aeruginosa* (ATCC: 27853), and gram-positive bacteria *Staphylococcus aureus* were analyzed in Pasteur Institute of Iran (Tehran).

RESULTS AND DISCUSSION

Combining chitosan with PEO might lead to novel materials suitable for diverse applications in the biomedical field. Therefore, in the present work, the fabrication of chitosan-containing nanofibers by electrospinning from chitosan/PEO blend solutions including Triton® X-100 as an additive and dimethyl sulfoxide (DMSO) as a cosolvent in 0.5*M* acetic acid (AcOH) solution using a handheld electrospinning device[46] is shown. The effect of solution concentration and applied field strength on the diameters and morphology of the fibers has been studied. Also, the effect of adding tetracycline hydrochloride[47]—as an antimicrobial agent and a model drug—on *E. coli* and *P. aeruginosa* and *S. aureus* bacteria has been studied.

Herein, we began our investigations to find an efficient approach and optimized polymers. In this regard, a few natural and synthetic polymers were examined. This study aims to improve solubility of chitosan by modification of the polymeric mixture using PEO, Triton® X-100 and other additives to generate ultrafine fibers by electrospinning of the solutions using two convenient static and portable instruments.

Fiber formation and morphology of nanofibers

It is important to note that the system configuration and operation conditions for fabricating micro and nanofibers differ vastly from one system to another, depending on the material and the choice of solvent. Physical and chemical parameters of polymer solution such as viscosity, electric conductivity and concentration can determinedly affect the formability, processability, and morphology of electrospun fibers. The major challenge in electrospinning and utilizing of chitosan as a material feedstock in biomedical applications is the poor solubility and the high viscosity of its aqueous solution. Usually, electrospinning of various chitosan/PEO from aqueous solutions produces a lot of beads. At low polymer concentrations, the solutions do not contain sufficient material to produce stable solid fibers. With increasing polymer concentration, the number of direct interchain associations of chitosan molecules in the solution increases rapidly and reaches a critical value of forming a 3D network structure-a highly viscous gel, rendering the solution unspinnable. To solve this problem, PEO was introduced in this study to reduce the viscosity of chitosan solution by interacting with chitosan through hydrogen bonding, rendering the solution spinnable at higher polymer concentrations. Because of the importance of solution viscosity on spinnability and morphology of as-spun fibers, solutions of different chitosan/PEO ratios (from 90/10-to-60/40) were investigated and 90/10, 80/20, 60/40 were the better than others. The images of the as-spun products are shown in Figures 1–6.

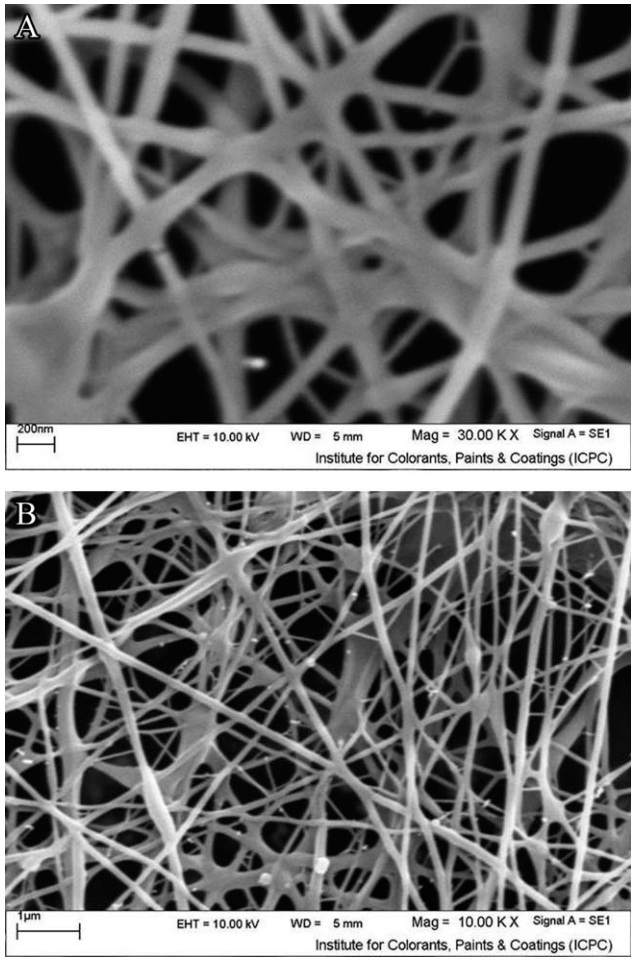

Figure 1 Selected SEM images of nanofibers with chitosan (2%)/PEO (3%) weight ratios of 90/10, containing Triton® X-100 (0.3%) and DMSO (10%) in aqueous AcOH (0.5*M*) with different magnifications (200 nm in A) and (1 μm in B) using stationary instrument.

Comparison of SEM images of the same solutions with two instruments, stationary instrument (Fig. 1) and portable instrument (Fig. 2) shows that portable or handled electrospinning device was suitable for this regard. Therefore, it was chosen as the main used electrospinning device in this study.

A pure chitosan or PEO solutions, could not be directly electrospun to produce stable fibers. It has been shown that the viscosity of the polymer solution decreased monotonically with increasing PEO content. It transformed progressively from a bead-like structure to a fibrous structure as the PEO-to-chitosan ratio increased (from 10/90 to 20/80 to 40/60) (Figs. 2–4).

The high viscosity of chitosan solution is related to the strong hydrogen bonding between polar and free NH_2 and OH groups of chitosan chains. The decrease in viscosity with addition of PEO can be explained by the change in inter and intramolecular

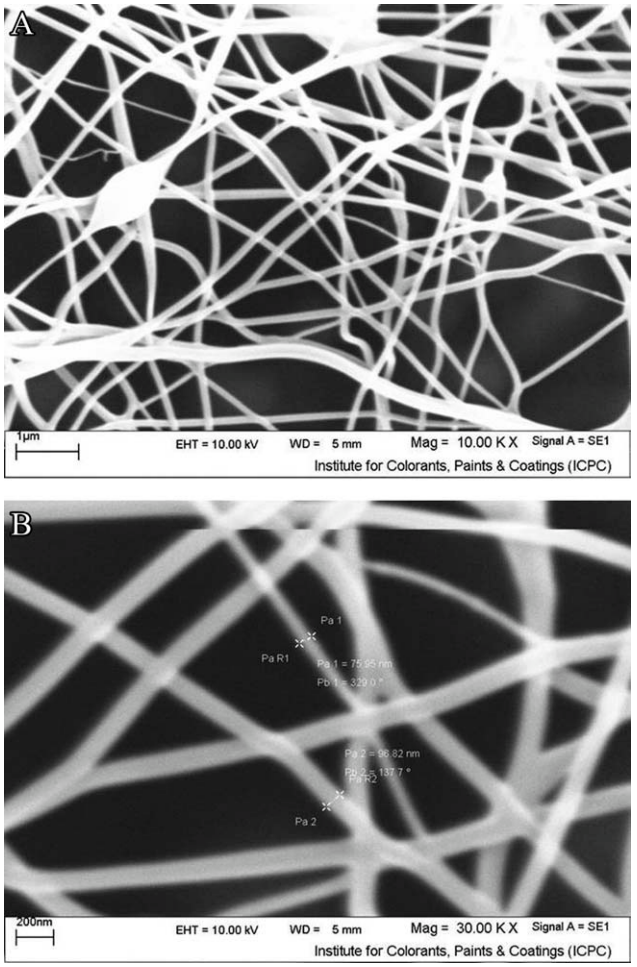

Figure 2 Selected SEM images of nanofibers with chitosan (2%)/PEO (3%) weight ratios of 90/10, containing Triton® X-100 (0.3%) (A) and DMSO (10%) (B) in aqueous AcOH (0.5*M*) using portable instrument.

interactions of chitosan chains. In other words, PEO molecules enter into the chitosan backbone and disrupt the self-association of chitosan chains by forming new hydrogen bonding between its OH groups and water molecules. Therefore, chitosan solubility increases and solution viscosity decreases. As illustrated in Figure 2, the maximum chitosan/PEO ratio for making a spinnable solution is 90/10, above which the spun product exhibited a nonuniform structure or droplets. However, at this chitosan/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. To improve the spinnability of the polymer solution at the chitosan/PEO ratio of 90/10, a small amount of Triton® X-100 (0.3 wt %) as a surfactant was added into the stock solution. Compared to the same solution without the surfactant, the addition of Triton® X-100 substantially improved the electrospun structure [Fig. 2(A)]. Although a fibrous structure was produced as a result of addition of Triton® X-100, the bead-like structure was still seen embedded in the fibers. Further improvement in structural uniformity was

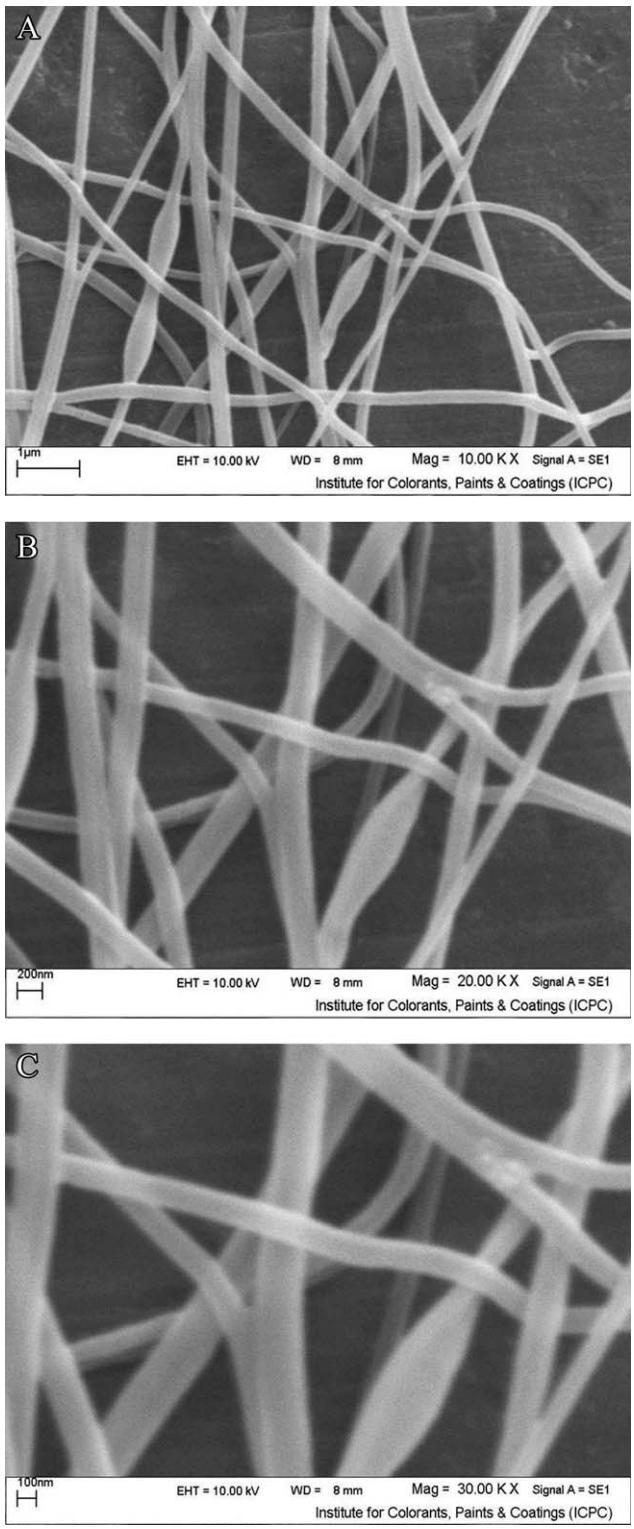

Figure 3 Selected SEM images of nanofibers with chitosan (2%)/PEO (3%) weight ratios of 80/20, containing Triton® X-100 (0.3%) and DMSO (10%) in aqueous AcOH (0.5*M*) with different magnifications (1 µm in A), (200 nm in B) and (100 nm in C) using portable instrument.

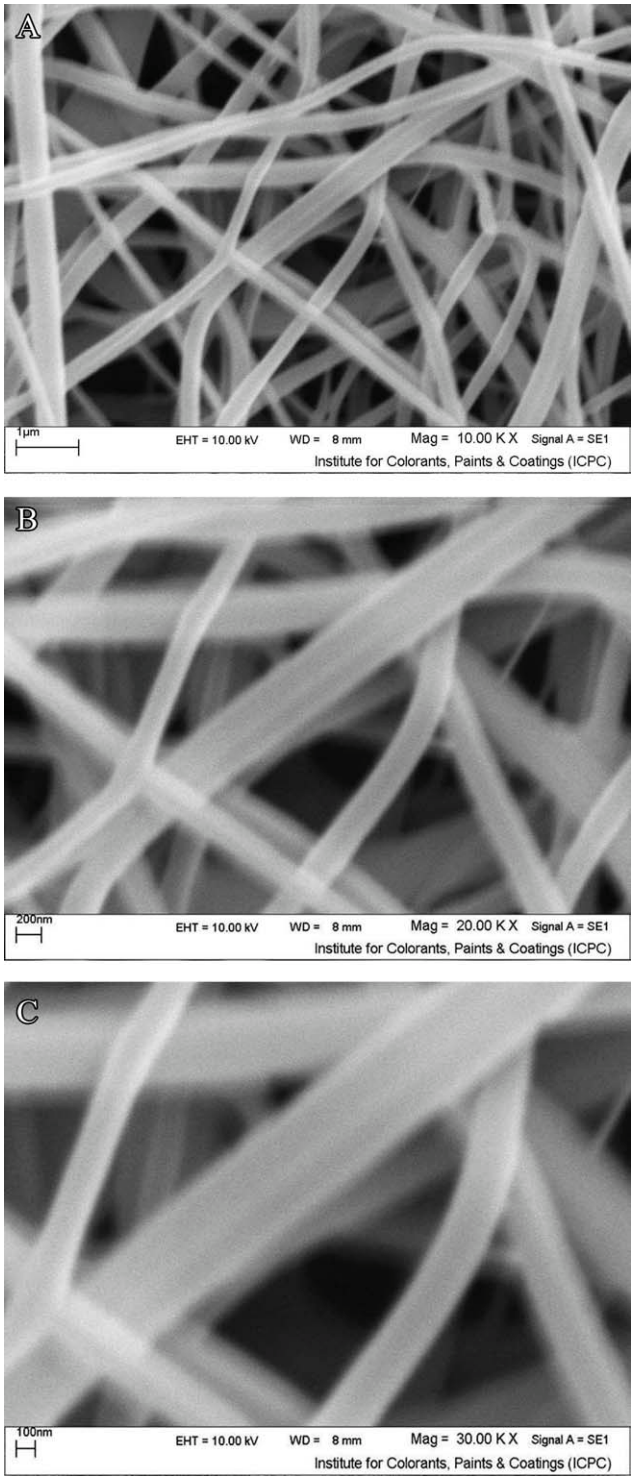

Figure 4 Selected SEM images of nanofibers with chitosan (2%)/PEO (3%) weight ratios of 60/40, containing Triton® X-100 (0.3%) and DMSO (10%) in aqueous AcOH (0.5*M*) with different magnifications (1 μm in A), (200 nm in B) and (100 nm in C) using portable instrument.

achieved by introducing DMSO as a cosolvent in the polymer solution. Figure 2(B) shows the SEM images of nanofibers obtained when DMSO was introduced as a cosolvent. The average fiber diameters, as estimated from the images, were in the range of 30–250 nm. The fibrous structure was substantially improved by the addition of the DMSO, and the fibers were approximately bead-free.

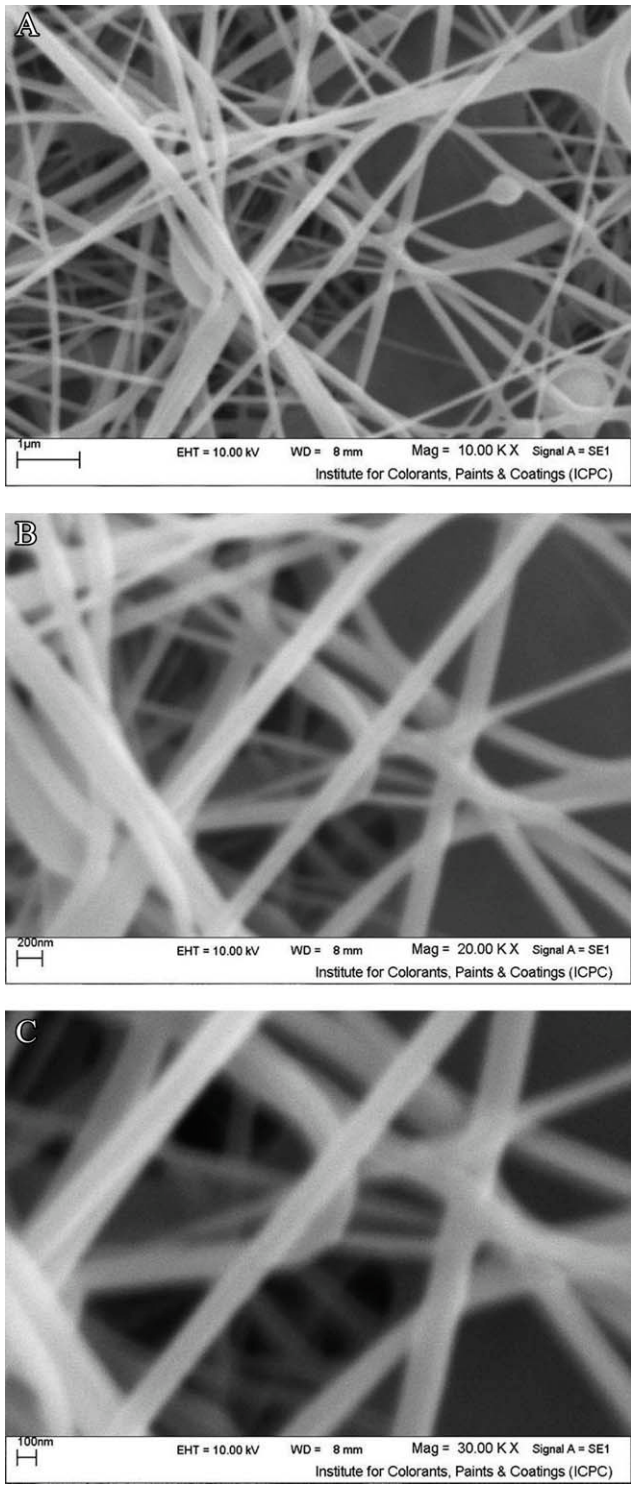

Figure 5 Selected SEM images of nanofibers with chitosan (2%)/PEO (3%) weight ratios of 90/10 (After maintaining solution for four months) are containing Triton® X-100 (0.3%) and DMSO (10%) in aqueous AcOH (0.5*M*) with different magnifications (1 μm in A), (200 nm in B) and (100 nm in C) using portable instrument.

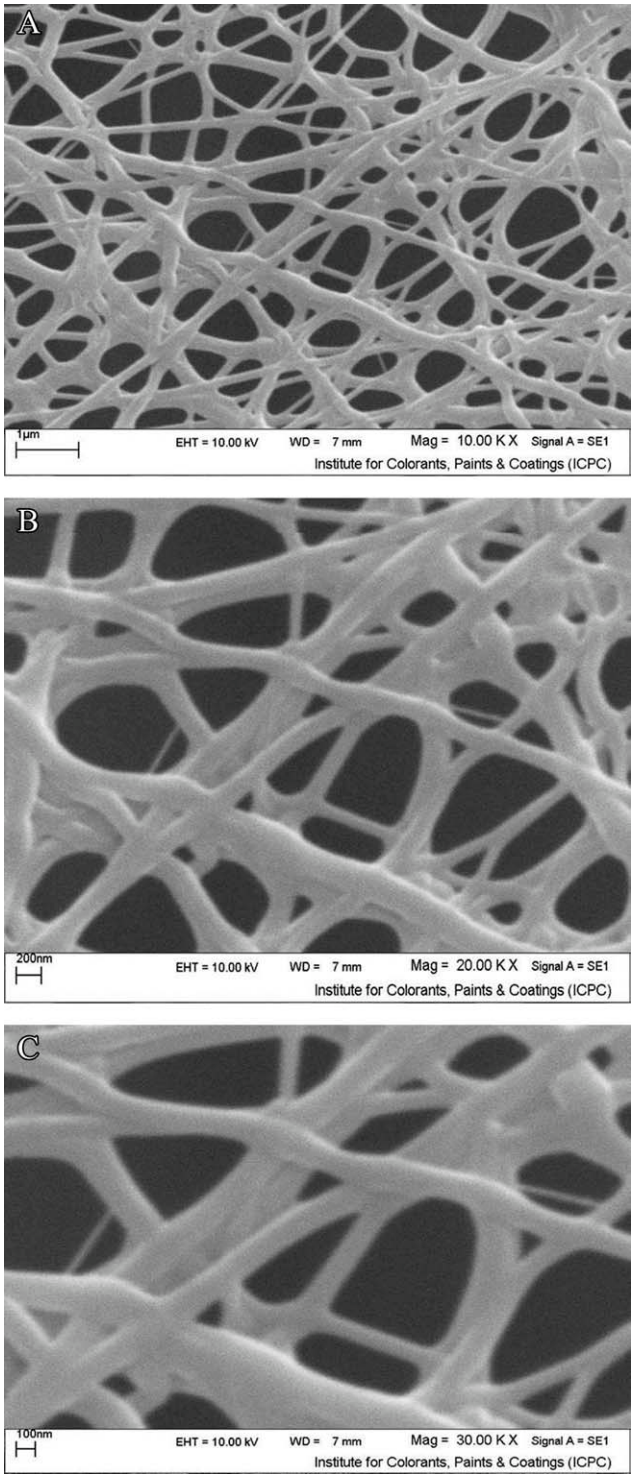

Figure 6 Selected SEM images of nanofibers with chitosan (2%)/PEO (3%) weight ratios of 90/10, containing Triton® X-100 (0.3%) and DMSO (10%) and tetracycline (0.3%) in aqueous AcOH (0.5*M*) with different magnifications (1 μm in A), (200 nm in B) and (100 nm in C) using portable instrument.

The ability to obtain high degree of electrospun fibers alignment has interesting implications in tissue engineering. In this work, it was achieved by twisting an aluminum foil as an efficient stationary collector. In this study, the applied and optimized distances between the tip of the syringe and the collector were 10–20 cm. All other experimental conditions were the same as those under which the fibers shown in Figure 2 were produced. After 10 min of fiber deposition, a filament structure with aligned nanofibers aggregates was formed on the surface of the collector. The degree of the fiber alignment increased initially with increasing voltage, and the highest attainable degree of alignment was achieved at 20 kV above which no apparent improvement in fabrication was observed.

PEO has a high solubility in water, and electrospun pure PEO fibrous membranes dissolve easily in water at room temperature. Thus, it is of practical interest to study the effect of the amount of PEO in chitosan/PEO nanofibers on the integrity of the nanofibrous structure in water. As shown in Figures 3 and 4, nanofibers containing 20 and 40 wt % PEO in solutions as same as nanofibers with 10 wt % PEO—after four months maintaining its solutions) (Fig. 5), no significant change was observed in fibrous morphology. This implies that the stability of polymeric solutions was long enough for their maintaining or storage in unspecialized conditions. This advantage of the current formulation is very important, especially for emergency or critical conditions.

TABLE I
Antibacterial Activity of the Multicomponent Polymers of Chitosan (2%)/PEO (3%) Including Triton® X-100 (0.3%); DMSO (10%) in Aqueous AcOH (0.5 M) Under Various Conditions

				Number of recovered bacteria	
Entry	Bacteria	Concentration of bacteria (CFU/mL)	Group	Starting time (0 h)	Final time (3 h)
1[a]	E. coli	1.6×10^8	Blank	1.6×10^8	3.2×10^8
			Sample	1.6×10^8	0
	P. aeruginosa	1.6×10^8	Blank	1.6×10^8	3.2×10^8
			Sample	1.6×10^8	0
	S. aureus	1.6×10^8	Blank	1.6×10^8	3.2×10^8
			Sample	1.6×10^8	3.2×10^8
2[b]	E. coli	1.6×10^8	Blank	1.6×10^8	3.2×10^8
			Sample	1.6×10^8	0
	P. aeruginosa	1.6×10^8	Blank	1.6×10^8	3.2×10^8
			Sample	1.6×10^8	0
	S. aureus	1.6×10^8	Blank	1.6×10^8	3.2×10^8
			Sample	1.6×10^8	3.2×10^8
3[c]	E. coli	1.6×10^8	Blank	1.6×10^8	3.2×10^8
			Sample	1.6×10^8	0
	P. aeruginosa	1.6×10^8	Blank	1.6×10^8	3.2×10^8
			Sample	1.6×10^8	0
	S. aureus	1.6×10^8	Blank	1.6×10^8	3.2×10^8
			Sample	1.6×10^8	3.2×10^8

[a] Chitosan/PEO, 90/10 (4 months maintained).
[b] Chitosan/PEO, 60/40 (fresh).
[c] Chitosan/PEO, 90/10 (fresh-including 0.3% tetracycline).

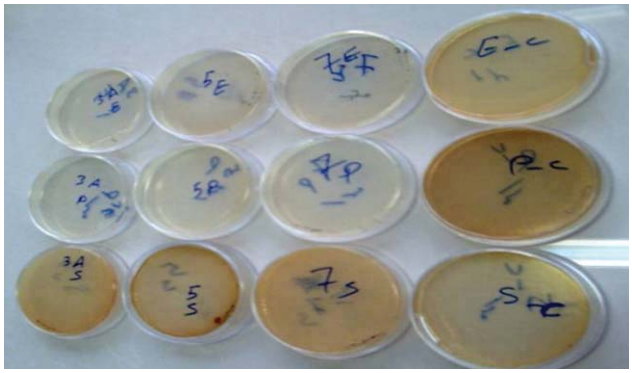

Figure 7 Blank and three samples before (colorful-active bacteria) and after (colorless-inactive bacteria); **1** (second left column); **2** (first left column); **3** (second right column); Blank (first right column); *E. coli* (top row); *P. aeruginosa* (middle row), and *S. aureus* (bottom row). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

One of the most important factors that has been examined in this study was the addition of an external known antibacterial agent, tetracycline. It was added to the optimized polymeric solution (chitosan (2%)/PEO (3%) weight ratios of 90/10; Triton® X-100 (0.3%); DMSO (10%) in aqueous AcOH (0.5*M*)) with different weight ratio and their fiber formation possibility was screened. Finally, the best fibrous structure was obtained from 0.3 wt % of tetracycline. Figure 6 illustrates the nanofibers of 90/10 ratio of chitosan/PEO including optimized amount of tetracycline (0.3 w%) which produced homogeneous and appropriate nanofibers. Below and/or above this ratio we did not obtain suitable nanofibers.

Analysis of antibacterial activity of the polymers

As can be seen from the results summarized in Table I, all three nonwoven mats and polymer solutions with composition chitosan/PEO ratio of 90/10 (fresh), 90/10 (four moths maintained) and 60/40, in the presence or absence of tetracycline, had excellent antibacterial activities against gram-negative bacteria *E. coli* and *P. aeruginosa* but they did not show good response against gram-positive bacteria Staphylococcus aureus after incubating for 72 h at 37°C. Figure 7 shows changes of bacteria population before (colorful fluorescence-active) and after (colorless-inactive) containers in blank and three samples prepared for this purpose.

CONCLUSIONS

In summary, we have developed a simple and efficient method for the electrospinning of multicomponent polymer system of chitosan/PEO nanofibers with an average diameter controllable from a few microns down to a few nanometers. SEM characterization revealed that the spinnability of chitosan solution was substantially improved when the solution viscosity was reduced. Introducing Triton® X-100 as a surfactant and DMSO as a cosolvent into chitosan solution allowed the solution to be spinnable even at high chitosan/PEO ratios (90/10), and substantially improved the spinnability of the solution and the fibrous structure of as-spun nanofibers. The characterization of the optimized nanofibrous structures by SEM imaging showed the homogeneity of them without considerable bead-like structures. The efficient electrospinnability of the current formulation may represent a usefulness process in various

Figure 8 Photographs of the equipments: (A): portable instrument and (B): stationary instrument. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

biomedical applications, especially for constructing of biomimetic and bioactive nanofibrous artificial extracellular matrix for engineering various tissues and a fast method of wound dressing for skin regeneration. Figure 8 shows two photographs of the portable (A) and stationary (B) instruments. Antibacterial activity investigation of nonwoven mats and polymer solutions with composition chitosan/PEO ratio of 90/10 and 60/40 in the presence or absence of antibiotic tetracycline showed efficient antibacterial activity against gram-negative bacteria *E. coli* and *P. aeruginosa*.

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