LETTER

Diameter Control of Electrospun Chitosan-Collagen Fibers

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ABSTRACT: In this article, the effects of fundamental parameters including applied voltage, feed rate of solution, collecting distance of fibers, the ratio of chitosan to collagen in the fibers and polymer solution concentration on the diameter and morphology of electrospun collagen-chitosan complex nanofibers were studied to produce ultrafine polymer fibers. Based on the systematic parametric study, it is possible to control the diameter and morphology of the electrospun polymer fibers. This will also be helpful for electrospinning of various polymers. © 2009 Wiley Periodicals, Inc. J Polym Sci Part B: Polym Phys 47: 1949–1955, 2009
Keywords: biomaterials; biomimetic; chitosan; collagen; diameter; electrospinning; parameter

INTRODUCTION

Electrospining is spinning the fibers in nano-scale by electrostatic force. The detailed mechanism of electrospinning has been investigated comprehensively.[1–3] Because of the unusually high porosity in their nanometer scale architecture and large surface area, these ultrafine polymer nanofibers are of commercial interest, and they are regarded as favorable candidates for many applications such as filtration membranes, tissue engineering scaffolds and protective clothing.[4] As particular interests have been addressed in the tissue engineering, great efforts have been made to study electrospinning of biodegradable polymers.[5,6]

Because the natural extracellular matrix (ECM) is molecular complex made up of proteins and polysaccharides and comprises three-dimensional hierarchical fibrous structures of nanometer scale dimensions,[7] the collagen-chitosan complex nanofibers have been prepared by electrospinning to simulate both the components and the structure of native ECM and to develop novel biocompatible and biomimetic tissue–engineering scaffolds in our lab.[8–10] The growth of cells on the nanofiber scaffolds with large surface area to volume ratio shows that the cells not only interact well with the fibers but also proliferate well on the scaffold, indicating a biological function of the cells within the scaffold.[6] These results strongly support that the collagen-chitosan nanofibrous matrices with the components and structural analogy to the natural ECM have great potential in the application of tissue engineering. Further studies are required to optimize the electrospun nanofibrous scaffold, especially to obtain uniform and nanometer scale fibers.

The electrospun fiber diameter determines properties of the electrospun fiber mats such as mechanical, electrical, hydrophobic and optical properties. It was previously shown that both the strength and the conductivity of the film/mat of fibers produced by electrospinning are sensitive to fiber diameter.[11] Moreover, size of the fibers along with morphology influences the

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hydrophobic behavior of polymers.[12] Filtering applications are also affected by the fiber size.[13,14] Most important of all, fiber size will affect the cell behavior on the fibers. Therefore, it is important to have control over the fiber diameter which is a function of material and process parameters.

The electrospun fiber diameter and its uniformity are dependent on many processing parameters. These parameters can be divided into three groups: solution properties, processing conditions, and ambient conditions. Numerous reports studying the effects of these parameters have been reported and each of the parameters has been found to affect the diameter and morphology of the electrospun fibers.[15,16] Although the parameters of the electrospinning process have been analyzed in many polymers, this information has been inadequate to support the electrospinning of collagen-chitosan nanofibers because there are some controversies on those reports. A parametric effect on electrospinning of collagen-chitosan complex is hence required to investigate systematically to optimize the electrospun nanofibrous scaffold. In this article, the effects of fundamental parameters on the diameter and morphology of electrospun collagen-chitosan complex nanofibers were studied to produce ultrafine polymer fibers, which will also be helpful for electrospinning of various polymers.

EXPERIMENTAL

Materials

Collagen I (mol wt, 0.8–1 $\times$ 10^5 Da) was purchased from Sichuan Ming-rang Bio-Tech Co., Ltd. (China) while Chitosan (85%, deacetylated, M_η, ca. 10^6) was purchased from Ji-nan Hai-de-bei Marine Bioengineering Co., Ltd. (China). Two kinds of solvents, 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) from Fluorochem Ltd. (United Kingdom) and Trifluoroacetic acid (TFA) from Sinopharm Chemical Reagent Co., Ltd. (China) were used to dissolve the collagen, chitosan and their blends.

Electrospinning of Collagen and Chitosan Blend Bolution

Collagen and chitosan were dissolved in HFIP/TFA (V/V, 90/10) mixture at various concentrations (6%, 8, and 10%, g/mL), respectively. A series of collagen and chitosan blend solutions (collagen/chitosan = 80/20, 50/50, 20/80, w/w) were prepared by mixing each solution in predetermined ratios. The electrospinning experiments were performed at room temperature. The polymer solution was placed into a 5 mL syringe with a needle having an inner diameter of 0.46 mm. A clamp connected the high voltage power supply (which can supply positive voltage from 0 to 30 kV) to the needle. A piece of aluminum foil was placed directly below the needle and acted as grounded collector. The polymer jets generated from the needle by the high voltage field formed the nanofibers and membrane on the grounded collector. The applied voltage, collecting distance (the distance from the needle tip to the collector) and feed rate of the solution can be changed, respectively. The electrospun fibrous membrane was stored in the vacuum oven at normal room temperature to remove the residual solvents.

Scanning Electron Microscopy Analysis

The morphologies of the electrospun fibers and membrane were observed under a scanning electron microscope (SEM) (Quanta FEG 200, FEI Company, The Netherlands) at an accelerating voltage of 10 or 15 kV. Based on the SEM micrographs, the average diameter and diameter distribution were determined by choosing 100 fibers at random among 1000$\times$ magnification SEM images and analyzing them using image analysis software Adobe Photoshop 7.0.

Statistics and Data Analysis

Fiber diameters were compared by analysis of variance using the SPSS 13.0 software. Differences were considered statistically significant for P values < 0.05.

RESULTS AND DISCUSSION

Effect of Processing Conditions

Parameters for processing condition include the voltage, volume feed rate and collecting distance applied during electrospinning. These parameters effects on the electrospun fiber diameter were summarized below.

Voltage

To study voltage effect, the voltage was increased during electrospinning process from 12 to 28 kV at 4 kV interval while keeping a fixed polymer concentration (8%), feed rate (0.8 mL/h), collecting distance (110 mm) and the ratio of collagen to chitosan (1:1). Figure 1 gave the SEM images of electrospun chitosan-collagen fibers and Figure 2 showed the relationship between the average fiber diameter and voltage. It was observed that the diameter of fiber diameter did not dramatically vary with varied voltage. According to past works, higher voltage was reported to induce not only larger diameter but also smaller diameter. Applied voltage may affect some factors such as mass of polymer fed out from a tip of needle, elongation level of a jet by an electrical force, morphology of a jet (a single or multiple jets), and so forth. A balance among these factors may determine a final diameter of electrospun fibers. Increasing the applied voltage does increase the electrostatic force and

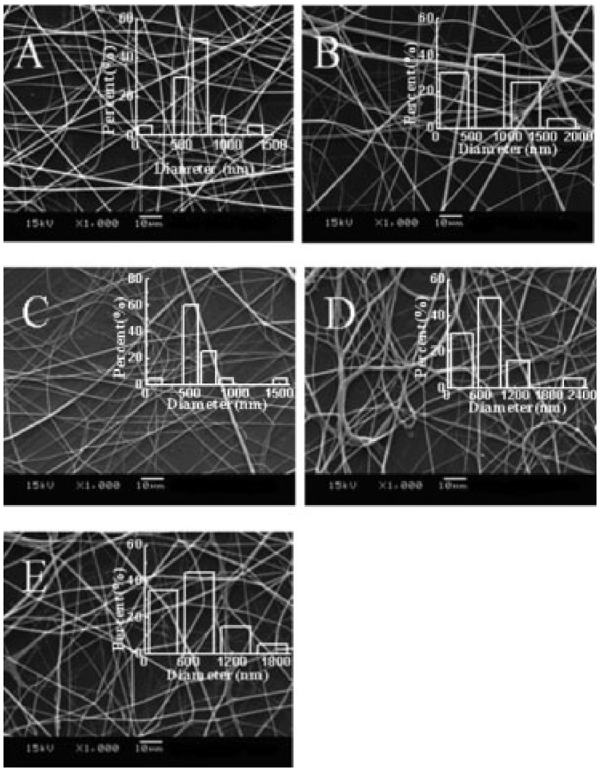

Figure 1. SEM images of electrospun chitosan-collagen fibers at different voltage: (A) 12 kV, (B) 16 kV, (C) 20 kV, (D) 24 kV, (E) 28 kV. The insets show the fiber size distributions. (Collecting distance: 11 cm, Feed rate: 0.8 mL/h, Solution concentration: 8%, The ratio of chitosan to collagen: 1:1.)

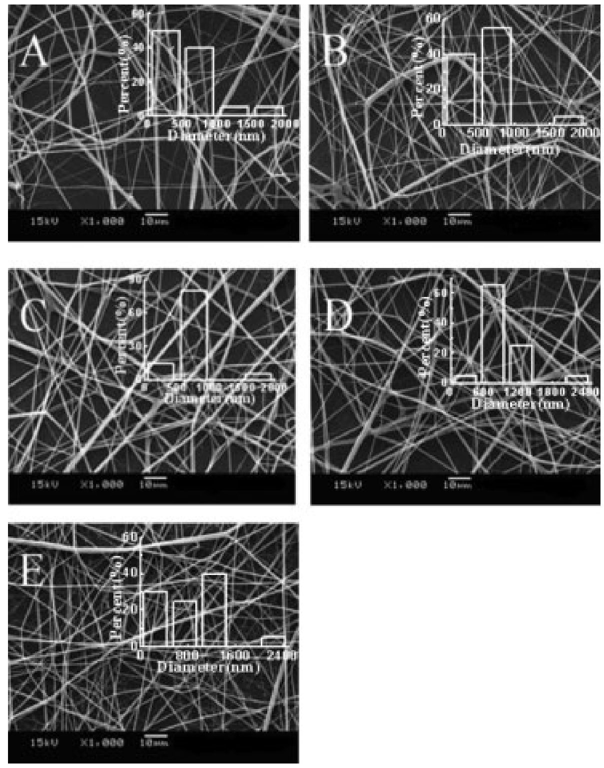

Figure 3. SEM images of chitosan-collagen fibers electrospun at different feed rate: (A) 0.48 mL/h, (B) 0.60 mL/h, (C) 72 mL/h, (D) 0.84 mL/h, (E) 0.96 mL/h. The insets show the fiber size distributions. (Collecting distance: 11 cm, Voltage: 16 kV, Solution concentration: 8%, The ratio of chitosan to collagen: 1:1.)

create smaller diameter fibers, but it also draws more solution out of the spinneret. If the increasing electrostatic force draws much more solution out of the needle, the fiber diameter will increase with increasing voltage, as

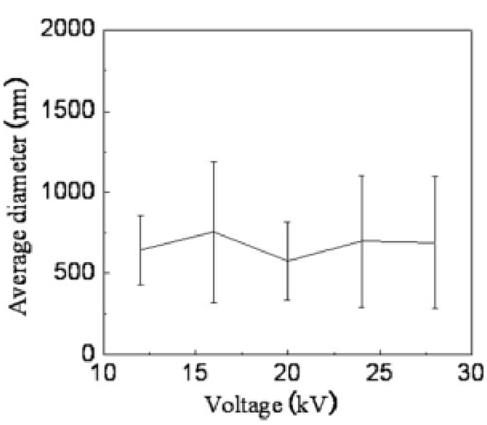

Figure 2. Relationship between the average fiber diameter and voltage. Error bars represent means ± SD for $n = 100$. (Collecting distance: 11 cm, Feed rate: 0.8 mL/h, Solution concentration: 8%, The ratio of chitosan to collagen: 1:1.)

reported by Demir et al.[15] Baumgarten[17] thought that the diameter of the jet initially decreases as the field strength increases and then begins to increase as the field strength further increases. This, in fact, means an increase in flow rate as the applied voltage increases beyond a certain level. The phenomenon is related to the fact that increasing the field increases the electrostatic stresses, which in turn, draws more material out of the syringe.[18] Although voltage effects show different tendencies, the voltage did not show a significant role in controlling the fiber morphology. It was already reported that a diameter of electrospun fibers was not significantly affected by the applied voltage.[6,15,16]

Feed Rate

The solution feed rate is another factor to influence electrospun fiber diameters. Figure 3 showed SEM images of fibers electrospun from collagen-chitosan solutions at different feed rate while keeping a fixed voltage (16 kV), collecting distance (110 mm), the ratio of collagen to chitosan (1:1) and polymer solution concentration (8%) and the relationship between the average fiber diameter and feed rate was shown in Figure 4. When the flow rate is

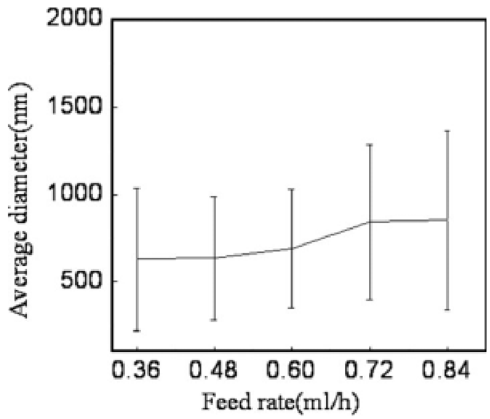

Figure 4. Relationship between the average fiber diameter and feed rate. Error bars represent means ± SD for $n = 100$. (Collecting distance: 11 cm, Voltage: 16 kV, Solution concentration: 8%, The ratio of chitosan to collagen: 1:1.)

increased, there is a corresponding increase in the fiber diameter simply because a greater volume of solution is drawn away from the spinneret.

Collecting Distance

The voltage (16 kV), feed rate (0.6 mL/h), the ratio of collagen to chitosan (1:1) and polymer solution concentration (8%) were kept constant and the tip-to-collector distance was altered from 80 mm to 160 mm at 20 mm interval to study the effect of collecting distance on electrospinning of collagen and chitosan blend. From Figures 5 and 6, the fiber can be obtained within the collecting distance range and the diameters of fiber do not vary significantly with the difference of collecting distance. Collecting distance may affect some factors such as the evaporation of solvent, electric field strength, and so forth. A smaller collecting distance leads to greater jet stretching and elongation by increasing the electric field strength, which results in smaller fiber diameters. When adjusting collecting distance, it is very important to ensure the polymer solution jet has enough flight time for the solvents to evaporate. When the collecting distance is too small, the jet may not have enough time to dry, leading to a nonuniform fiber samples. On the other hand, when the distance is too long, the jet has enough time to dry, but the reduced electric field strength causes the nonuniform fiber morphology, which is similar to the case of high feed rate.[19] In addition, increasing the distance from the needle to the collector does not necessarily enhance the evaporation but does reduce the field strength. The fiber formation is related to both the ambient humidity and the distance from the needle to the collector. If the ambient humidity is low, the increased distance will reduce the fiber diameters as more solvent evaporates over the longer distance. When the ambient humidity is high, the increased distance does not increase the evaporation but only reduces the field strength.[20] A balance among these factors may determine a final diameter of electrospun fibers.

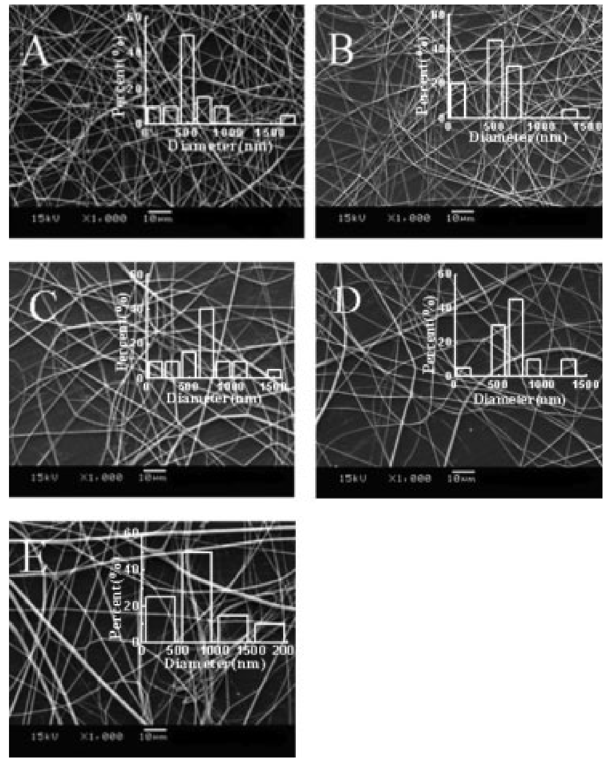

Figure 5. SEM images of chitosan-collagen fibers electrospun at different collecting distance: (A) 80 mm, (B) 100 mm, (C) 120 mm, (D) 140 mm, (E) 160 mm. The insets show the fiber size distributions. (Feed rate: 0.8 mL/h, Voltage: 16 kV, Solution concentration: 8%, The ratio of chitosan to collagen: 1:1.)

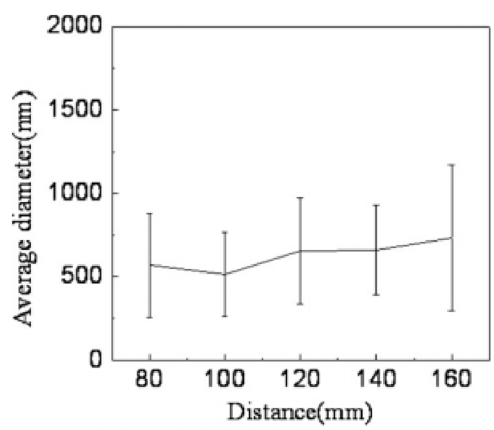

Figure 6. Relationship between the average fiber diameter and spinning distances.. Error bars represent means ± SD for $n = 100$. (Feed rate: 0.8 mL/h, Voltage: 16 kV, Solution concentration: 8%, The ratio of chitosan to collagen: 1:1.)

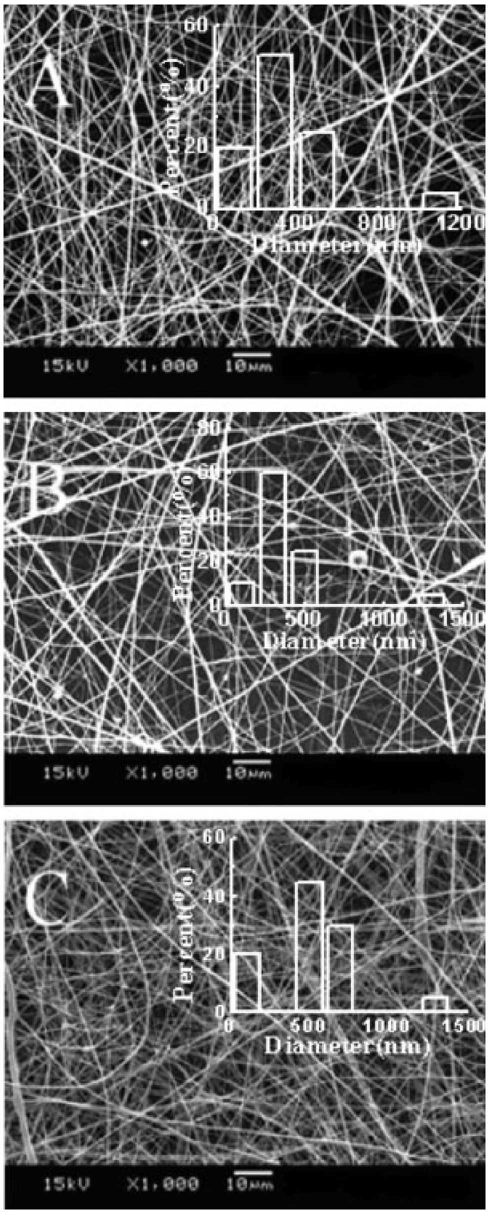

Figure 7. SEM images of chitosan-collagen fibers electrospun with different solution concentration: (A) 6%, (B) 8%, (C) 10%. The insets show the fiber size distributions. (Feed rate: 0.8 mL/h, Voltage: 16 kV, Collecting distance: 110 mm, The ratio of chitosan to collagen: 1:1.)

Effect of Polymer Solution Concentration

The voltage (16 kV), feed rate (0.6 mL/h), the ratio of collagen to chitosan (1:1) and collecting distance (110 mm) (8%) were kept constant and polymer solution concentration was altered from 6 to 10% at 2% interval to study the effect of solution concentration on electrospinning.

From Figures 7 and 8, the average diameter of fibers increases significantly with the increase of polymer solution concentration. The same result has been given in our previous research.[10] No or beaded fibers were formed at too low polymer solution concentration (less than 4%) for any electric field and spinning distances. Surface tension effects could be dominant with decreased polymer concentration/solution viscosity and beaded fibers were consequently produced. Hence, despite the capability to shrink the size of the fibers by decreasing the polymer concentration, this success of obtaining finer fibers was compromised by the change of the fiber uniformity.[16]

Effect of the Ratio Chitosan to Collagen

Figure 9 gave the SEM images of fibers obtained at chitosan content of 20, 50, and 80% by keeping the voltage (16 kV), feed rate (0.6 mL/h), collecting distance (110 mm) and polymer solution concentration (8%) constant and Figure 10 showed the relationship between the average fiber diameter and chitosan content. The average diameter of fibers decreases significantly with the increase of chitosan content. The reason may be that the organic salt formed between TFA acid and the amino groups on chitosan increase the charge density and electrical conductivity of the electrospun polymer solution, which results in higher draw ratio in electrospinning process.[10]

CONCLUSION

Parameter effects on the diameter and morphology of electrospun chitosan-collagen fibers were studied. The results showed polymer solution concentration and the ratio of chitosan to collagen played a significant role in

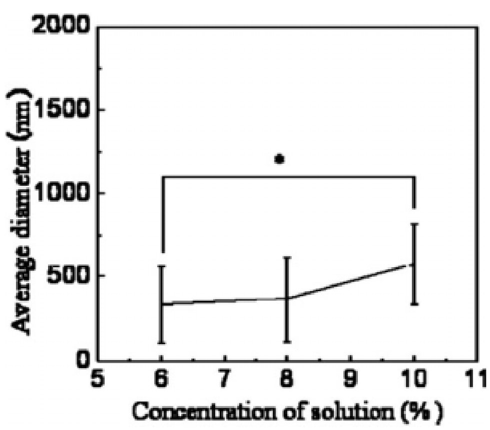

Figure 8. Relationship between the average fiber diameter and solution concentration. Error bars represent means ± SD for $n = 100$. $^{*}P < 0.05$. (Feed rate: 0.8 mL/h, Voltage: 16 kV, Collecting distance: 110 mm, The ratio of chitosan to collagen: 1:1.)

controlling the diameter and morphology of the electrospun collagen-chitosan nanofibers while voltage, feed rate and collecting distance were less effective compared to those parameters. Therefore, it is summarized that electrospun fibers with smaller diameter can be produced with lower polymer concentration, higher electrical conductivity of solution and lower solution feed rate. Voltage and collecting distance relate to many effective factors. A balance among these factors may also determine a final diameter of electrospun fibers. Based on the systematic parametric study, it is possible to control the diameter and morphology of the electrospun polymer fibers.

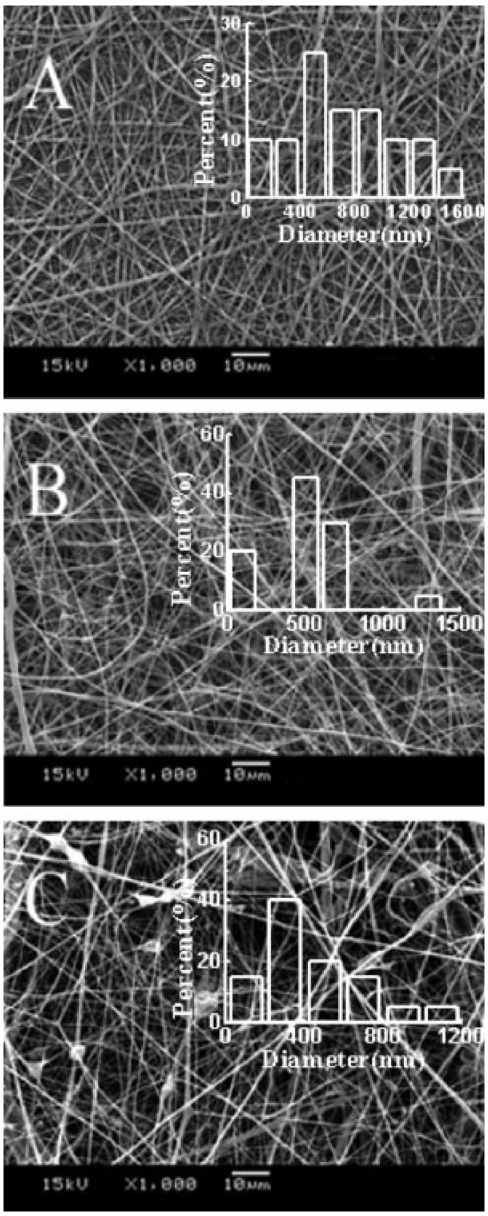

Figure 9. SEM images of chitosan-collagen fibers electrospun with different chitosan content: (A) 20, (B) 50, (C) 80%. The insets show the fiber size distributions. (Feed rate: 0.8 mL/h, Voltage: 16 kV, Collecting distance: 110 mm, Solution concentration: 8%.)

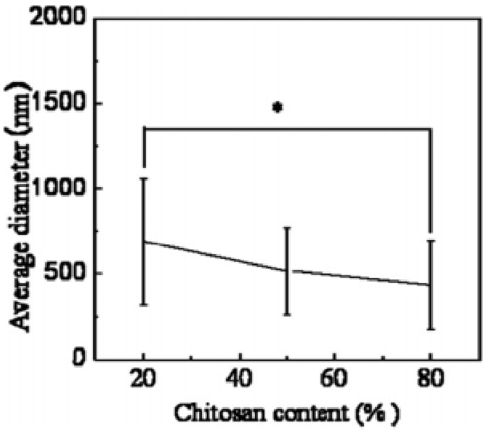

Figure 10. Relationship between the average fiber diameter and chitosan content. Error bars represent means ± SD for $n = 100$. *$P < 0.05$. (Feed rate: 0.8 mL/h, Voltage: 16 kV, Collecting distance: 110 mm, solution concentration: 8%.)

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