NATURAL POLYMERS

Influence of Chitosan Molecular Weight and Poly(ethylene oxide): Chitosan Proportion on Fabrication of Chitosan Based Electrospun Nanofibers[1]

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Abstract—Fabrication of electrospun chitosan nanofibers is still a controversial issue in publications. Although regarding the lots of reports, mixtures of chitosan with a hydrophilic synthetic polymer such as polyethylene oxide (PEO) have been electrospun successfully, abundance of partly contradictory protocols in which one variable has been surveyed in each study is unfortunately baffling. In the present study, influence of three considerable parameters including the average molecular weight of chitosan, chitosan solution concentration and the mass ratio of polyethylene oxide to chitosan at the mixtures on electrospinning possibility as well as the quality of as-spun fibers is investigated. Eventually, the necessities for obtaining the best results are introduced followed by further analysis of optimized nanofibers using atomic force microscopy. According to our results, the blend solutions prepared from the low molecular weight (LMW) chitosan and PEO are efficient for reproducible production of bead-free electrospun nanofibers even in low proportion of polyethylene oxide.

INTRODUCTION

Electrospinning is a unique method which makes it possible to fabricate extra cellular matrix (ECM) mimicking nano- and microfibers from natural or artificial polymers. The plenty of controllable variables at this technique including those concerning the polymer solution (type of solvents, polymer concentration, viscosity, surface tension, conductivity and etc.) and those related to spinning process (engaged DC voltage, flow rate of polymer solution, distance between the nozzle and the collector, temperature and etc.), facilitates creation of fibrous mats with desired characteristics including fiber diameters, porosity, orientation and degree of consistency [1, 2]. However, this diversity of the parameters makes it difficult to define the optimum conditions for obtaining the best results.

Chitin as the second abundant naturally occurring polymer after cellulose with a similar carbohydrate-based linear structure and its famous derivative, chitosan, obtained from an incomplete deacetylation

procedure both have the good biocompatibility and versatile biological activities [3, 4]. Nowadays, chitosan is one the most favorite materials for a variety of applications including tissue engineering [5–7], gene and drug delivery [8–11], enzyme immobilization [12, 13], and heavy metals filtration [14–16]. Regarding a large body of scientific reports, among the chitosan various forms, use of its nanofibrous structures have been intensified. Due to high viscosity and high surface tension of chitosan solutions, the feasibility of fabrication of pure chitosan nanofibers via electrospinning technique had been challenging for the years [2, 17, 18]. Albeit the successful electrospinning of pure chitosan solutions has been now reported [19–21], the use of substantially toxic solvents employed in such reports including trifluoroacetic acid (TFA), dichloromethane (DCM) and hexafluoroisopropanol (HFIP) is a remarkable disadvantage. Plenty of reports have shown that preparation of blended solutions with the different ratios of chitosan and some hydrophilic synthetic polymers like polyethylene oxide (PEO) [22–25] and poly vinyl alcohol (PVA) [26, 27] facilitates the electrospinning process and production of

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bead-free nanofibers with varying degrees of success. Apparently, the key cause of this achievement is the hydrogen bonds forming between chitosan strands and PEO or PVA chains which decrease the viscosity and surface tension of the mixtures compared to the pure chitosan solutions. For the same reason, the increment of PEO (or PVA) : chitosan ratio may lead to the better results however the increased water solubility of the as-electrospun fibers would make them inappropriate for the cell culture and tissue engineering applications [22]. Cross linking of the fibers via thermal treatment [28] or reagent based methods [29, 30] have been proposed to diminish this solubility, nevertheless these approaches are not sometimes completely advantageous. For example, arose from lack of chemically functional groups, polyethylene oxide (PEO) cannot participate in conventional cross linking procedures except those recruiting gamma or UV radiation in the presence of a photo-initiator [31, 32]. Therefore, cross interaction between functional groups of chitosan via thermal treatment or by using gluteraldehydeorgenipin [33] cannot inhibit swelling of PEO components in water. This phenomenon induces loss of fibrous structure in the samples having high mass ratio of PEO in an aqueous environment [22]. Therefore, fabrication of chitosan as-electrospun nanofibers with the minimum amounts of PEO is noteworthy. Whereas some groups have claimed the production of uniform nanofibers from the blend solutions with the PEO : chitosan ratio of 1 : 9 as the least proportion of PEO yielding a spinnable solution [22, 34], Pakravan et al. refuted the fabrication of bead-free nanofibers with that little content of PEO except with doing electrospinning at high temperatures [23]. Furthermore, addition of some co-solvents or/and surfactants has been shown to be useful in quality improvement of as-spun chitosan-PEO fibers having the low amounts of polyethylene oxide [18, 22, 35, 36]. On the other hand, since the chitosan with the various molecular weights are presented by the manufacturers, choosing the appropriate ones to become successfully electrospun is indispensable. However Klossner et al. reported the impossibility of manufacturing electrospun nanofibers from the pure chitosan solutions with the different molecular weights, they didn't compare their spinnability in a blend with PEO [37]. In another work, Zhang et al. characterized the nanofibers obtained from the blend solutions of PEO and different chitosan samples. Nevertheless, all chitosan types except one had low molecular weights. Moreover the mass ratio of PEO to chitosan was very high which made the nanofibrous products useless [38]. In one of the most comprehensive work, Desai et al. studied the effect of chitosan and PEO molecular weights as well as PEO : chitosan ratios on morphology of electrospun nanofibers [39]. For this purpose, they employed chitosan samples with two molecular weights. Although they reported making solutions from the chitosan with the molecular weight of

1400 kDa as the high molecular type, we absolutely disclaim the possibility of producing homogenous solutions from such a high molecular weight chitosan with the reported concentrations. Furthermore, since the PEO and chitosan samples were dissolved in water and acetic acid respectively, blending of polymer solutions had resulted in change of the acetic acid concentration. This had a distinctive impact on the electrospinning process as reported by Geng et al. [40] which was overlooked when evaluating influence of PEO : chitosan ratios [39]. In some works, merely one type of chitosan was used while other variables such as PEO : chitosan ratio [22, 25], PEO molecular weight [24] or influence of salts [41], surfactants [36] and heat [41] on electrospinning of chitosan-PEO solution were evaluated.

In the present study, electrospinning of chitosan-PEO blend solutions with different proportions was investigated. The main aim of our research was defining optimum conditions for reproducible production of bead-free chitosan nanofibers with the least PEO : chitosan mass ratio. In order to minimize the tunable parameters, some of them whom were studied formerly, were disregarded herein by fixing them at the relatively appropriate amounts reported previously. For the first time, we evaluated *exclusively and simultaneously* three main variables including the average molecular weight of chitosan (by using high, medium and low molecular types), chitosan concentration and the ratio of polyethylene oxide (PEO) to chitosan in the mixed solutions. Scanning electron microscopy was recruited firstly to evaluate each as electrospun case in terms of bead formation, fibers thickness and homogeneity. Afterwards, the best qualified samples were further analyzed by atomic force microscopy. Furthermore, electrical conductivity as well as rheological properties of all the used polymer solutions were measured.

EXPERIMENTAL

Materials

High, medium and low molecular weight chitosan (75−85% deacetylated with the molecular weight ranges of $(3.10-3.75) \times 10^5$, $(1.90-3.1) \times 10^5$ and $(0.5-1.9) \times 10^5$ Da respectively), polyethylene oxide (PEO) with the average molecular weight of 9×10^5 Da, glacial acetic acid, dimethyl formamide (DMF) and Triton X-100 were altogether obtained from Sigma-Aldrich Company.

Preparation of Polymer Solutions

Chitosan solutions with different concentrations (from 1.5 up to 2.5 wt%) were prepared by dissolving calculated amount of high, medium and low molecular weight chitosan powders in 50% (v/v) aqueous acetic acid, and stirring the sealed mixtures at room tem-

perature for 48 h followed by centrifugation (4800 rpm for 20 min). Afterwards, the supernatants were filtered by the sintered glass funnel (with P1 degree of porosity, Gupta scientific industries) to yield the clear solutions without any unsolved microparticles. At the same time, polyethylene oxide (PEO) powder was dissolved separately in 50% (v/v) aqueous acetic acid and stirred at room temperature for 24 h to produce a 3 wt % homogenous solution. The chitosan and PEO solutions were then blended with different proportions and stirred gently for 12 h. In one case, Triton-x-100 and DMF were added to the polymer solution in the final concentrations of 0.4 and 10 wt% respectively, followed by stirring overnight as described before [22]. The blend polymer solutions were used immediately at the next procedures to avoid aging phenomenon affecting chitosan structure [42, 43] as well as a documented event arose from time-dependent phase separation of two mixed polymers [44].

Conductivity of Solutions

Electrical conductivity of the polymer solutions was determined using a conductometer (712 conductometer, Metrohm, Switzerland) with three independent measurements at the equal temperatures (25 ± 0.5°C). The results were averaged and depicted as Mean ± SD in a column graph using Graph Pad Prism 5 software.

Viscosity of Solutions

To evaluate rheological properties of the polymer solutions, a Brooke viscometer (DV-III ULTRA) was employed at the equal temperatures for each examination (30.12 ± 0.36°C). Eventually, the dynamic (shear) viscosity versus shear rate in the range between 6.8 and 13.6 S^{-1} was illustrated using Graph Pad Prism 5 software.

Electrospinning

The polymer solutions were poured into a 10 mL disposable syringe and continually driven by a programmable pump at the typical flow rates of 0.3−0.5 mL/h. The electrospinning system utilized in this study engaged DC voltages 15−20 kV between the injection nozzle and a cylindrical rotating collector covered by an aluminum foil. The distance maintained between the nozzle and the collector was around 10−15 cm and the rotating rate of the collector was adjusted at 300 rpm. The electrospinning experiments were performed at room temperature and relative humidity of 15−25%. Finally, the as-spun fibers were

air dried at room temperature and maintained in a dry place before the next examinations.

Morphology Examination by Scanning Electron Microscopy

The dried electrospun nanofibers were sputter-coated by an ultrathin layer of Au, and the morphology of the nanofibers was characterized by SEM (KYKY-EM3200) at an accelerating voltage of 26 kV. The nanofibers were visually evaluated for degree of consistency and existence of likely defects including beads, droplets and other heterogeneous structures. Subsequently the average diameter of nanofibers was estimated with measuring the diameters of at least 50 different fibers for each case. Fibers size distributions were analyzed and depicted by Easy Fit 5.6 software.

Surface Analysis by Atomic Force Microscopy

In the cases of bead-free nanofibers proven by SEM images, the samples were further analyzed by an Atomic Force Microscopy (AFM- Ara Pajuhesh) at the tapping (semi-contact) mode in the scan sizes of 5 and 15 μm^2. The analysis was done by a silicon cantilever with a spring constant of 1−5 N/m and an estimated tip radius of 5−10 nm. Topo (height) images were acquired followed by estimating average diameter of nanofibers and their roughness measurement using Imager software.

RESULTS AND DISCUSSION

Preparation of Polymer Solutions

Chitosan has a poor solubility in pure water as well as in common organic solvents even those having high dielectric constant such as DMSO and DMF. This behavior mainly arises from amplitude of some chemical groups within chitosan structure including primary amines, hydroxyls and carbonyls which participate in formation of intermolecular hydrogen bonding. It has been shown that low amounts of pH increase water solubility of chitosan due to protonation of its primary amines [45]. Whereas positive ammonium groups are unable to form hydrogen bonds with each other, electrostatic repulsive forces between them, moreover their hydrogen bonds formation with water molecules enhance chitosan solubility in aqueous acid solutions. Among the acidic solvents, diluted acetic acid is the most favorite one for making chitosan solutions especially when electrospinning of chitosan-PEO blend is aimed. It has been documented by Geng et al. that increment in acetic acid percentage in aqueous solutions have a contradictory influence on chitosan spinnability by increase in viscosity of chitosan solutions parallel to decrease in their surface tension [40]. In the present study a mean percentage of acetic acid (50%) was used to make chitosan solutions as formerly described [23]. Interestingly, even after 48−72 h
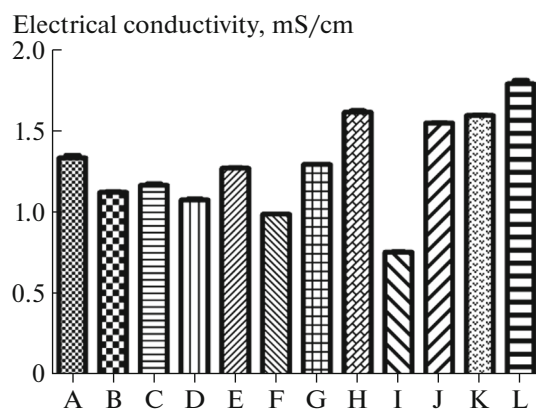
Electrical conductivity, mS/cm

Fig. 1. Electrical conductivity of polymer solutions. (A) PEO (3%) + MMW-Chitosan (2%) with the mass ratio of 1 : 9. (B) PEO (3%) + MMW-Chitosan (2%) with the mass ratio of 1 : 9 + Triton X-100 (0.4%) + DMF (10%). (C) PEO (3%) + MMW-Chitosan (2%) with the mass ratio of 1 : 3. (D) PEO (3%) + MMW-Chitosan (2%) with the mass ratio of 1 : 2. (E) MMW-Chitosan (1.5%). (F) PEO (3%) + MMW-Chitosan (1.5%) with the mass ratio of 1 : 2. (G) PEO (3%) + LMW-Chitosan (2%) with the mass ratio of 1 : 3. (H) PEO (3%) + LMW-Chitosan (2.5%) with the mass ratio of 1 : 9. (I) PEO (3%). (J) MMW-Chitosan (2%). (K) LMW-Chitosan (2%). (L) LMW-Chitosan (2.5%).

Shear viscosity, Pa s

Fig. 2. Shear (dynamic) viscosity versus shear rate of the polymer solutions. (*1*) PEO (3%) + MMW-Chitosan (2%) with the mass ratio of 1 : 9. (*2*) PEO (3%) + MMW-Chitosan (2%) with the mass ratio of 1 : 9 + Triton X-100 (0.4%) + DMF (10%). (*3*) PEO (3%) + MMW-Chitosan (2%) with the mass ratio of 1 : 3. (*4*) PEO (3%) + MMW-Chitosan (2%) with the mass ratio of 1 : 2. (*5*) MMW-Chitosan (1.5%). (*6*) PEO (3%) + MMW-Chitosan (1.5%) with the mass ratio of 1 : 2. (*7*) PEO (3%) + LMW-Chitosan (2.5%) with the mass ratio of 1 : 9. (*8*) PEO (3%) + LMW-Chitosan (2%) with the mass ratio of 1 : 3.

of magnetic stirring of solutions, some unsolved floating microparticles were observed. To the best of our knowledge, methods for elimination of these particles were not previously discussed whereas any unsolved bit may disturb the continuous flow of the polymer jet ejected from the needle of nozzle at the electrospinning process. In order to remove afore-mentioned particles, centrifugation at 4800 rpm followed by filtration using the sintered glass funnel was performed. Notably, high viscosity of chitosan solutions inhibits its pass through the filters which have narrow pore sizes. Sintered glass funnels with the porosity grade of G0 or G1 having the pore size of 160−250 and 100−160 μm respectively were found to be suitable for filtration of chitosan solutions regarding the polymer concentrations and apparent viscosities. Albeit, polyethylene oxide has a high solubility in pure water, it was also dissolved in 50% aqueous acetic acid to maintain the acid concentration unchanged at the blend solutions made from different proportions of PEO and chitosan primary solutions. The stoichiometric calculations were precisely done for making the blend solutions with different mass ratios rather than volumetric ratios.

Conductivity of Solutions

According to the Fig. 1 the highest and lowest electrical conductivities were observed in the solutions containing 2.5 wt% low molecular weight chitosan (column L) and 3 wt% PEO (column I) respectively.
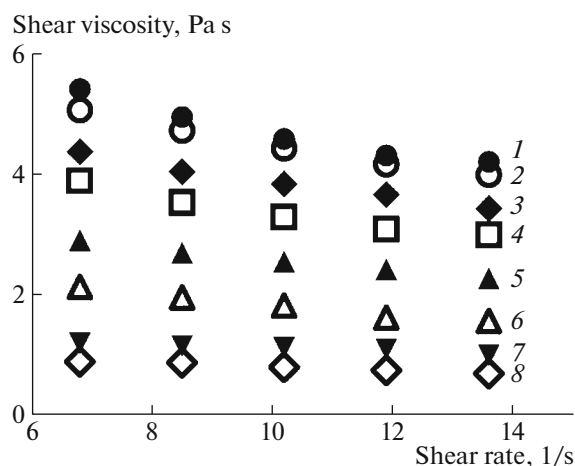
Comparison between columns A, C and D revealed that increase in PEO content in the blended solutions caused decrease in conductivity as expected because of the neutral structure of polyethylene oxide. This event can enhance stability of spinning jet as reported previously [23]. Conversely, comparison between columns E and J as well as between columns K and L showed clearly that increase in chitosan percentage in solution elevated the conductivity due to its polycationic nature as reported before [36]. Interestingly, the low molecular weight (LMW) chitosan solution had more electrical conductivity compared to the solution containing medium molecular weight (MMW) chitosan with the same concentration and degree of deacetylation (compare either the columns J and K or the columns C and G). Actually, in comparison between LMW and MMW chitosan samples with the same weights, the LMW one contains more number of cationic discrete strands with more mobility arose from weaker intermolecular attractions. Considering the columns A and B, addition of DMF resulted decline in solution conductivity which reflects its low conductivity as reported previously [46]. Generally in the present study, the electrical conductivity of examined polymer solutions was not a determinant factor in their spinnability and quality of spun nanofibers.

Viscosity of Solutions

Undoubtedly solution viscosity has an indispensable impact on spinnability and outcome of the elec-
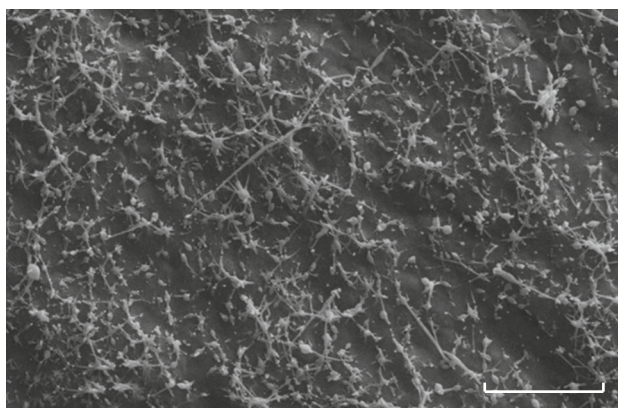
Fig. 3. SEM micrograph of the sample obtained from electrospinning of 1.5 wt % MMW-chitosan solution without PEO. Scale bar represents 10 μm.

trospinning process. It has been shown that increase in viscosity of chitosan solution limits its spinnability [22, 47, 48]. Figure 2 compares the dynamic viscosity of polymer solutions as a function of shear rate. Obviously the blend solutions produced from the low molecular weight chitosan exhibited lower shear viscosity at the shear rates between 6.8 and 13.6 S^{-1} than those composed from medium molecular weight one even in lower chitosan percentage (i.e. 1.5%). This finding was logical according to the fact mentioned above in which intermolecular interactions are weaker within the chitosan sample with the lower molecular weight. Addition of Triton-X100 as a surfactant and DMF as an aprotic co-solvent decreased slightly the viscosity of MMW-chitosan /PEO solution. Furthermore, increase in PEO proportion caused diminution in solution viscosity as reported by Bhattarai et al. [22]. It was interesting that this decline in viscosity was more drastic in those solutions containing MMW-chitosan than ones made by LMW-chitosan because of the low viscosity of LMW-chitosan solution which approximates that of PEO solution. It is worth noting

that all primary solutions of PEO and chitosan were prepared in 50% aqueous acetic acid, hence all the blend solutions were similar in acid concentration moreover the viscosities arose from it.

Electrospinning and Analysis of Products by SEM

It was previously well evidenced that increase in chitosan concentration leads to diminished beads in chitosan as electrospun nanofibers as well as decrease in fibers thickness [37]. On the other hand, higher concentrations cause elevated viscosities in chitosan solutions making them inapplicable for electrospinning [47]. To resolve this paradox, some approaches such as alteration in acetic acid concentration [40], mixing of chitosan solution with other polymer solutions [17, 24, 44], alkali treatment [20], and increase in temperature [23] or voltage [49] during electrospinning process have been proposed to decline viscosity of chitosan solutions or overcome its negative effect. In the present study, preparation of chitosan solutions with the concentration ≥1.5 wt % was impossible when the high molecular weight (HMW) chitosan were engaged. In accordance with previous studies [47] this low concentration of polymer was not sufficient for formation of the Taylor cone and a continuous spinning jet. In the next step, chitosan with the medium molecular weight (MMW) was used with or without polyethylene oxide (PEO). Experimentally, making homogenous solutions from MMW-chitosan with the concentration above 2 wt % was not feasible even after 72 hours of stirring at room temperature as reported by Homayoni et al [48]. Predictably, electrospinning of pure chitosan solutions was not prosperous so that 2 wt % chitosan solution failed in formation of polymer jet. However making the spinning jet from 1.5 wt % chitosan was apparently tenable, the SEM image of the product indicated failure in fabrication of spun fibers (Fig. 3). In the present study, polyethylene oxide (PEO) was recruited to make the chitosan spinnable. The blend solution of PEO (3 wt %) and MMW-chitosan (2 wt %) when the chitosan mass was
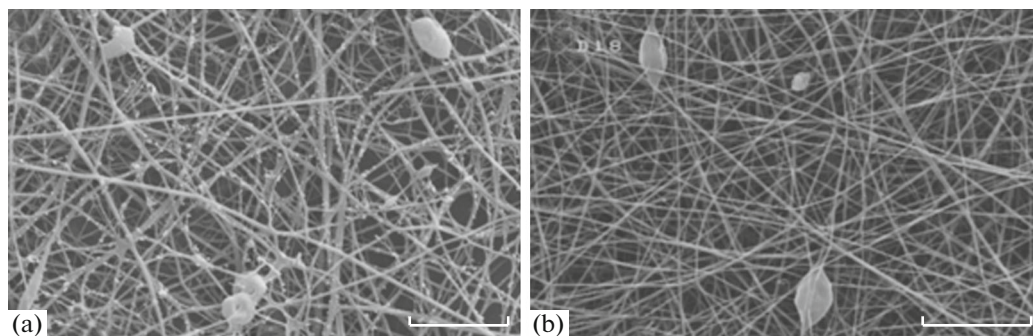

Fig. 4. SEM micrograph of the samples obtained from electrospinning of the blend solutions composed of 1 : 9 mass ratio of PEO to MMW-chitosan. The primary solutions of PEO and chitosan were 3 and 2 wt %, respectively. (a) without and (b) with DMF/ Triton x-100. Scale bars represent 5 micrometer.
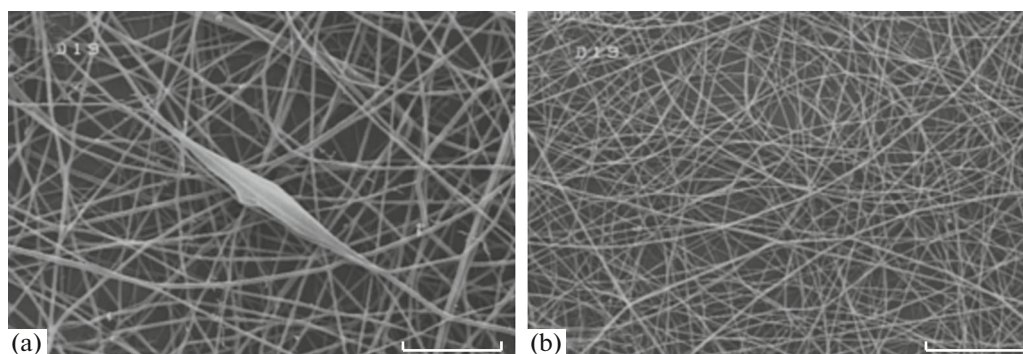
Fig. 5. SEM micrograph of the samples obtained from electrospinning of the blend solution composed of (a) 1 : 3 and (b) 1 : 2 mass ratio of PEO to MMW-chitosan. The primary solutions of PEO and chitosan were 3 and 2 wt % respectively. Scale bars at (a) and (b) represent 5 and 10 micrometers, respectively.

nine fold (PEO : chitosan ratio of 1 : 9) was able to fabricate nanofibers via electrospinning however the bead formation as observed by Pakravan et al. [23] was noticeable (Fig. 4a). Moreover, resembling what reported by Bhattarai et al. [22] co-addition of DMF as a co-solvent and Triton X-100 as a surfactant to the blend solution which decreases its surface tension, improved nanofibers quality, however was not effective enough for eliminating the beads from the texture (Fig. 4b). Increase of PEO proportion in the blend solutions resulted decrease in bead existence as well as increase in fibers diameter as already described [37]. When the PEO : chitosan mass ratio was about 1 : 3, merely few beads in spun fibers was detected (Fig. 5a) so that enhanced proportion of PEO to chitosan as 1 : 2, leaded to disappearance of the beads within the nanofibrous network (Fig. 5b). Decrement of chitosan concentration to 1.5 wt % in primary solution eventuated expanded bead formation rather than fiber fabrication in as electrospun products (Fig. 6). This finding
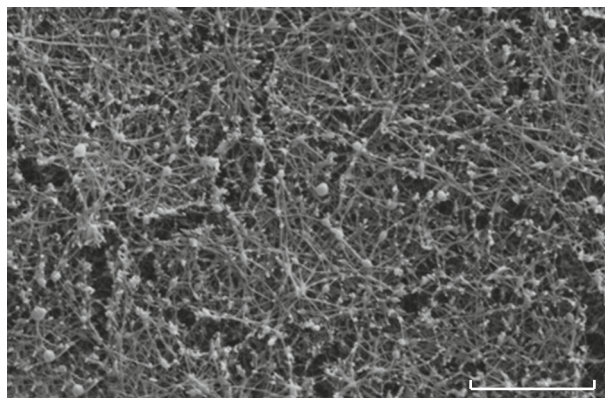
resulted from diminished total polymer concentration at the blend solution was formerly reported as mentioned above [37]. In the present research fabrication of nanofibers from medium molecular weight (MMW) chitosan faced some major problems. Firstly as depicted herein, it was impossible to lower PEO ratio in the blend solution when bead-free products were desired. Secondly, due to high viscosity of MMW-chitosan solution, its filtration for removing undissolved microparticles by the sintered glass funnel and even by employment of high vacuum pressure was absolutely difficult. Furthermore using that high vacuum pressure during long time filtration process might lead to the mild evaporation of the solvent resulting irreproducibility in the next steps because of the change in concentration of solutions.

Chitosan with the low molecular weight (LMW) was also studied to probe its efficiency for formation of spun nanofibers. This type of chitosan contains shorter polymer strands compared to MMW ones which leads to the weaker inter-molecular bonding. As a result of this fact, solubility of LMW-chitosan is more than MMW and HMW chitosan sorts [47]. Arising from the same reason viscosities of LMW-chitosan solutions were low enough for making them prone to get filtered simply by the sintered glass funnel using gentle vacuum pressure. Nevertheless, resembling those resulted using MMW-chitosan, fabrication of electrospun nanofibers from pure LMW-chitosan solutions was impossible. Briefly, LMW-Chitosan solution with the concentration of 2.5 wt % was incapable to make polymer jet during electrospinning procedure. Whereas by lowering the chitosan concentration toward 2 wt %, spinning jet formation was almost observed, the SEM photographs demonstrated failure in fiber formation as noticed in the case of using MMW-chitosan without PEO. Eventually, the mixtures of LMW-chitosan and polyethylene oxide solutions were examined. Firstly, primary solution of chitosan with 2 wt % concentration was blended with PEO solution with the concentration of 3% as was uti-
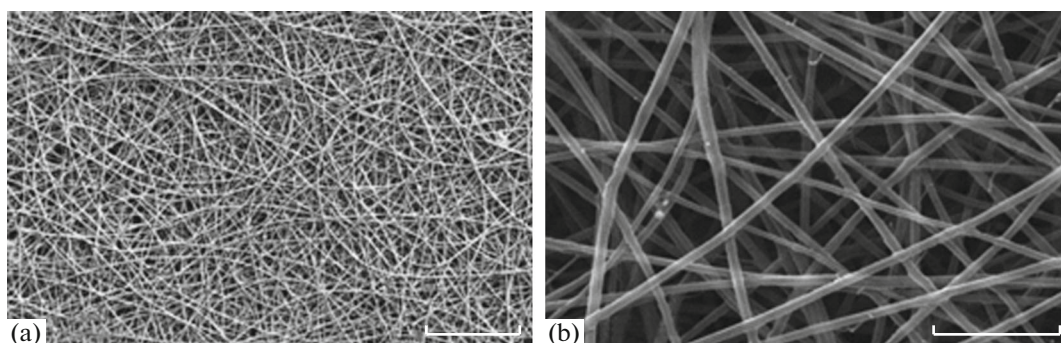

Fig. 6. SEM micrograph of the sample obtained from electrospinning of the blend solution composed of 1 : 2 mass ratio of PEO to MMW-chitosan when the primary solutions of PEO and chitosan were 3 and 1.5 wt % respectively. Scale bar represents 10 micrometer.

Fig. 7. SEM micrograph of the sample obtained from electrospinning of the blend solution composed of 1 : 3 mass ratio of PEO to LMW-chitosan. The primary solutions of PEO and chitosan were 3 and 2 wt % respectively. (a) 1000× and (b) 20 000× original magnification. Scale bars at (a) and (b) represent 10 and 2 micrometers, respectively.
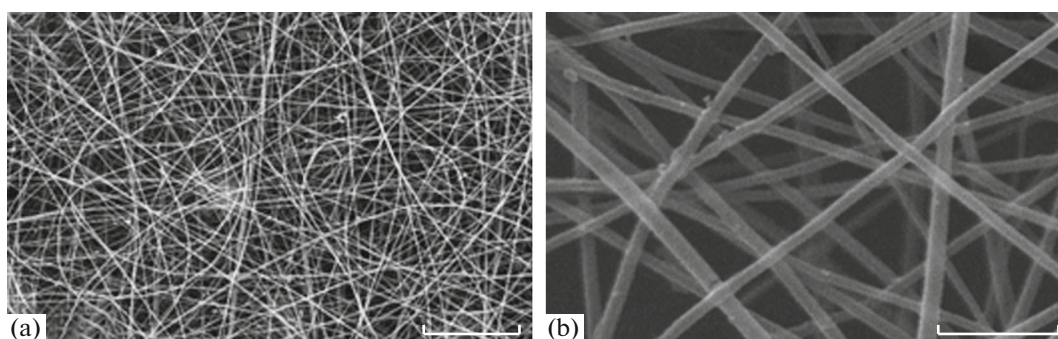

Fig. 8. SEM micrograph of the sample obtained from electrospinning of the blend solution composed of 1 : 9 mass ratio of PEO to LMW-chitosan. The primary solutions of PEO and chitosan were 3 and 2.5 wt %, respectively. (a) 5000× and (b) 50 000× original magnification. Scale bars at (a) and (b) represent 5 and 0.5 micrometers, respectively.

lized in our previous examinations. According to the SEM imaging, homogeneous bead-free nanofibers was formed when the mass ratio of PEO : LMW-chitosan was 1 : 3 (Fig. 7). At the next step, concentration
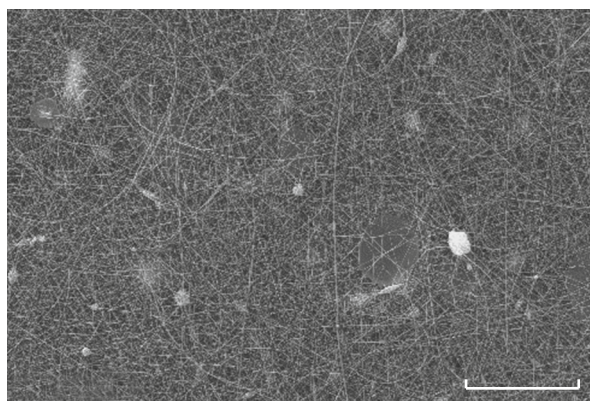

Fig. 9. SEM micrograph of the sample depicted in figure 8 with 1000× original magnification. Scale bar represents 20 μm.

of primary chitosan solution was elevated to 2.5 wt %. Interestingly decrease in PEO : chitosan ratio to 1 : 9 was still sufficient to produce nanofibers (Fig. 8) with ignorable amounts of beads detected only by low magnification imaging in few areas of the whole sample as illustrated in Fig. 9. Feasibility of increasing the concentration of primary chitosan solution to 2.5 wt % without intolerable promotion in viscosity was arose from existence of shorter polymer strands within the LMW-chitosan sample as discussed above. Advantageously, this elevated concentration of chitosan not only inhibited drop in total concentration of polymers in the blend solution with the 1 : 9 mass ratio of PEO to chitosan, but raised it compared to the prior solution. As was expected according to the former reports [37], fibers diameters were decreased in this sample due to increment of chitosan concentration as well as decrement of PEO proportion. The distribution of the nanofibers diameters is shown by the histogram diagrams in the Figs. 10 and 11. All the results mentioned herein are briefly illustrated below.
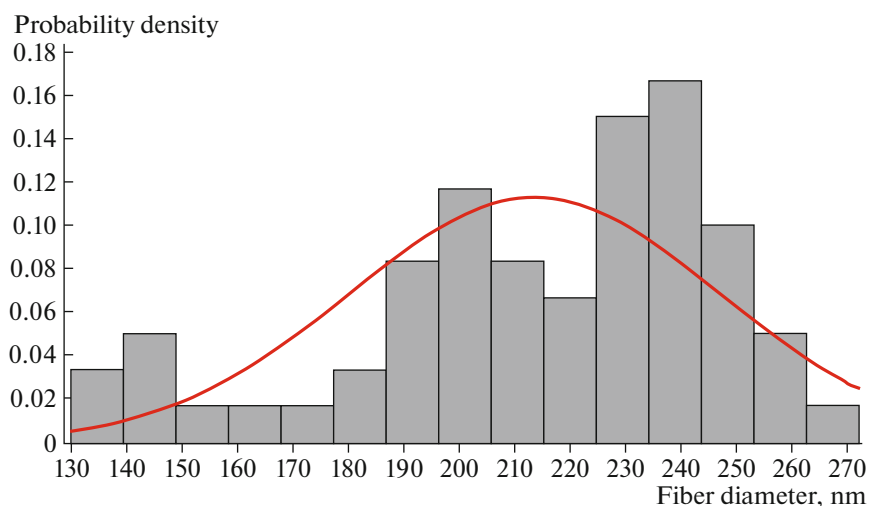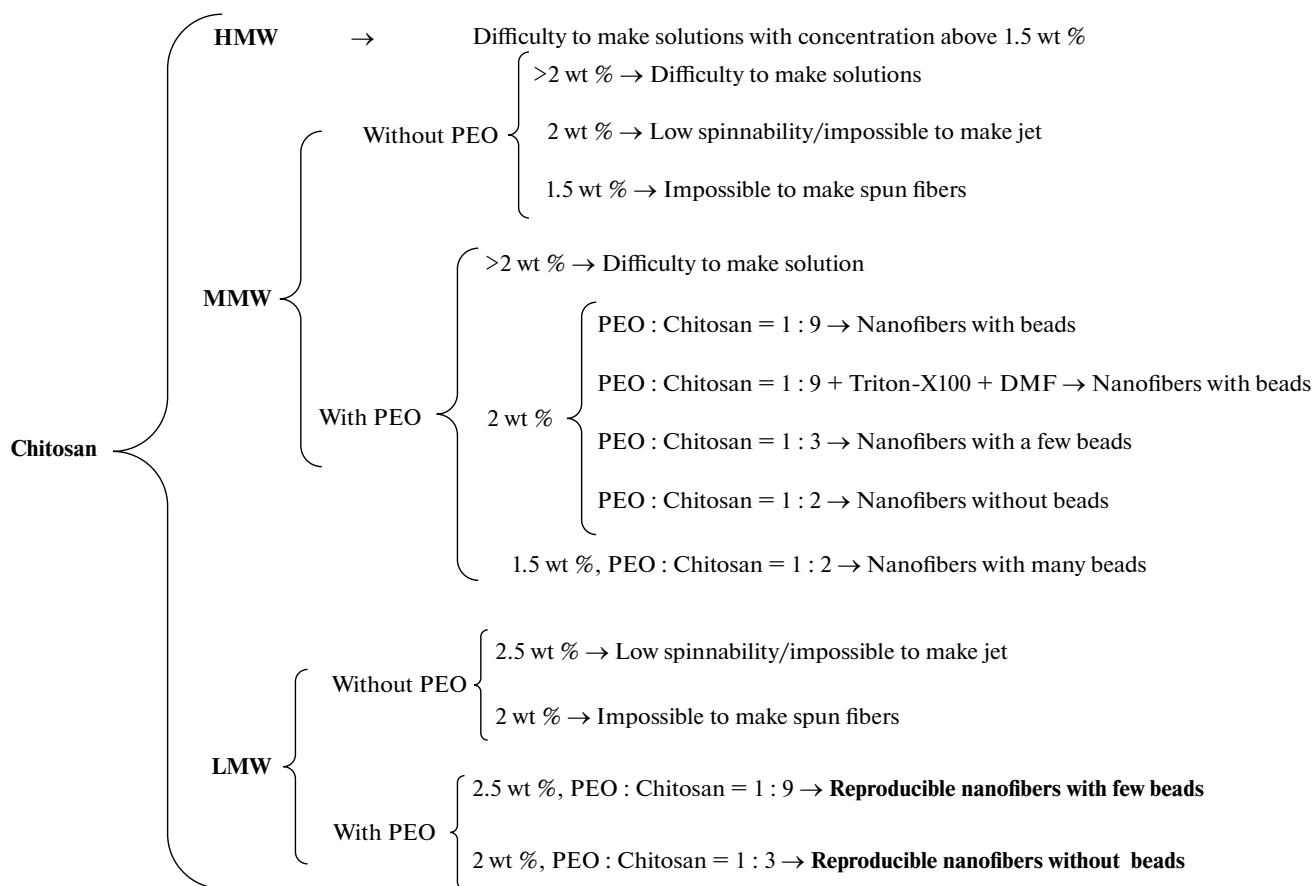
Fig. 10. (Color online) Histogram of fibers diameter distribution resulted from the analysis of SEM photographs. Electrospun nanofibers obtained from the blend solution composed of 1 : 3 mass ratio of PEO to LMW-chitosan. The average diameter ± standard deviation (SD) was 213 ± 33 nm (%CV = 15.5%).

Chitosan
- **HMW** → Difficulty to make solutions with concentration above 1.5 wt %
- **MMW**
  - Without PEO
    - >2 wt % → Difficulty to make solutions
    - 2 wt % → Low spinnability/impossible to make jet
    - 1.5 wt % → Impossible to make spun fibers
  - With PEO
    - >2 wt % → Difficulty to make solution
    - 2 wt %
      - PEO : Chitosan = 1 : 9 → Nanofibers with beads
      - PEO : Chitosan = 1 : 9 + Triton-X100 + DMF → Nanofibers with beads
      - PEO : Chitosan = 1 : 3 → Nanofibers with a few beads
      - PEO : Chitosan = 1 : 2 → Nanofibers without beads
    - 1.5 wt %, PEO : Chitosan = 1 : 2 → Nanofibers with many beads
- **LMW**
  - Without PEO
    - 2.5 wt % → Low spinnability/impossible to make jet
    - 2 wt % → Impossible to make spun fibers
  - With PEO
    - 2.5 wt %, PEO : Chitosan = 1 : 9 → **Reproducible nanofibers with few beads**
    - 2 wt %, PEO : Chitosan = 1 : 3 → **Reproducible nanofibers without beads**

*Analysis of Nanofibers
by the Atomic Force Microscopy (AFM)*

The nanofibrous mats obtained from electrospinning of the LMW-chitosan/PEO blend solutions were scanned by AFM in the tapping mode. Figures 12 and 13 represent the TOPO (height) images obtained from those samples without any pre-imaging preparation or post-imaging picture edition. The average diameters
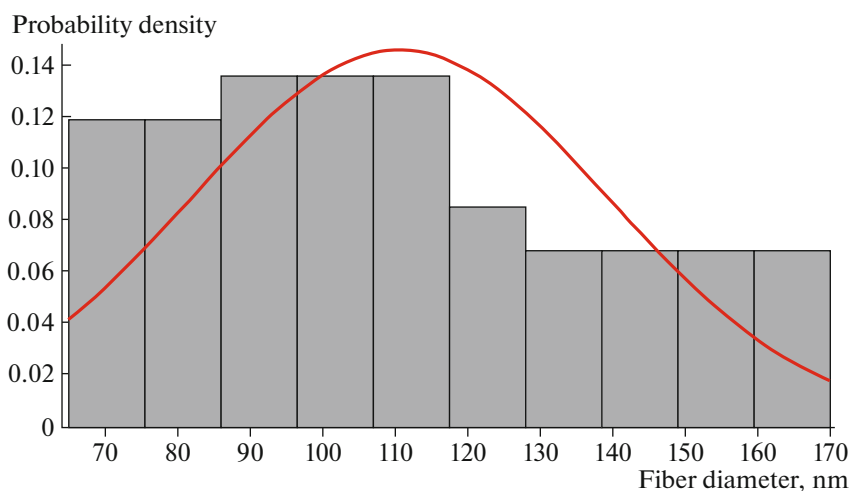
Fig. 11. (Color online) Histogram of fibers diameter distribution resulted from the analysis of SEM photographs. Electrospun nanofibers obtained from the blend solution composed of 1 : 9 mass ratio of PEO to LMW-chitosan. The average diameter ± standard deviation (SD) was 111 ± 29 nm (%CV = 26.13%).
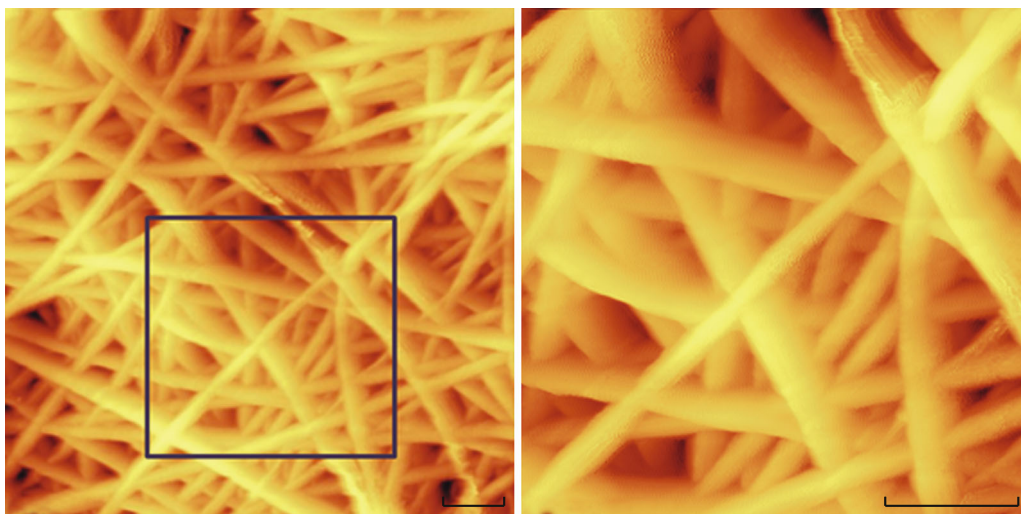

Fig. 12. (Color online) AFM TOPO (height) images from the electrospun nanofibers with 1 : 3 mass ratio of PEO to LMW-chitosan. Higher magnification was generated by re-scanning the marked square. Scale bars represent 1 micrometer.
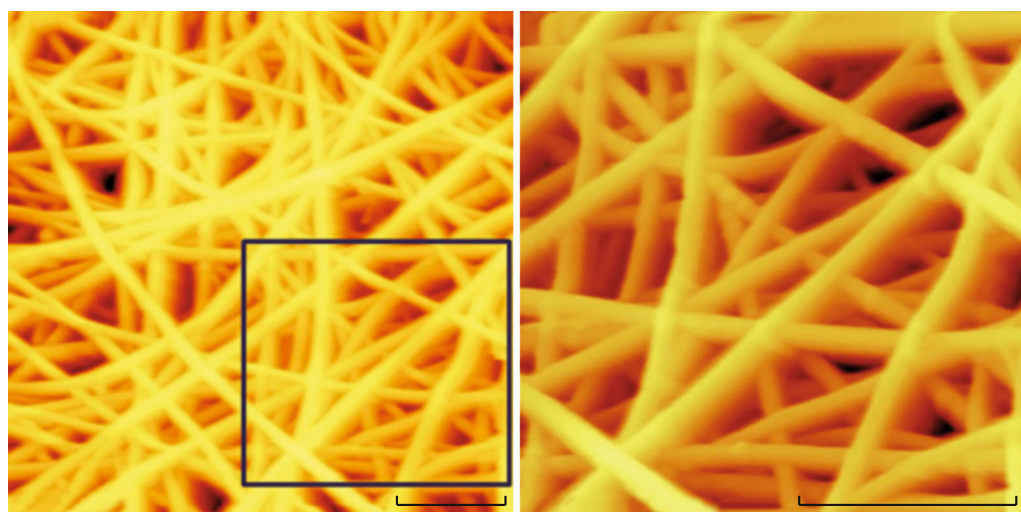

Fig. 13. (Color online) AFM TOPO (height) images from the electrospun nanofibers with 1 : 9 mass ratio of PEO to LMW-chitosan. Higher magnification was generated by re-scanning the marked square. Scale bars represent 1 micrometer.
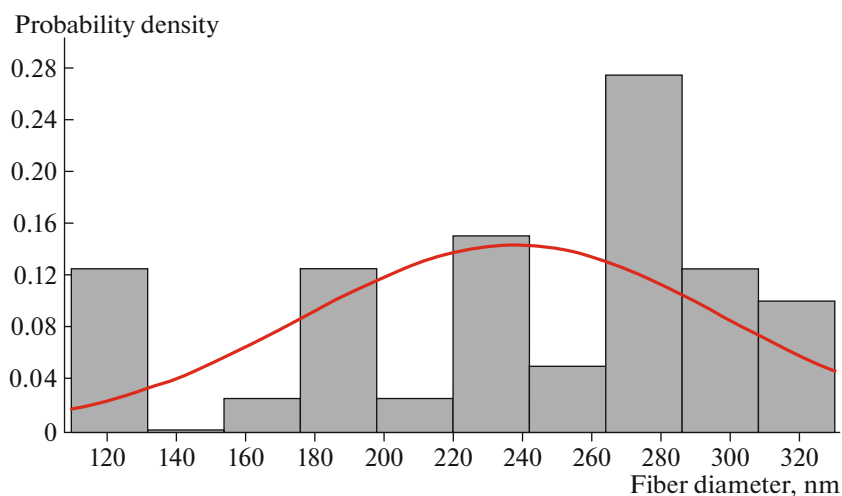
Fig. 14. (Color online) Histogram of fibers diameter distribution resulted from the analysis of AFM images. Electrospun nanofibers obtained from the blend solution composed of 1 : 3 mass ratio of PEO to LMW-chitosan. The average diameter ± standard deviation (SD) was 238 ± 61 nm (%CV = 25.63%).
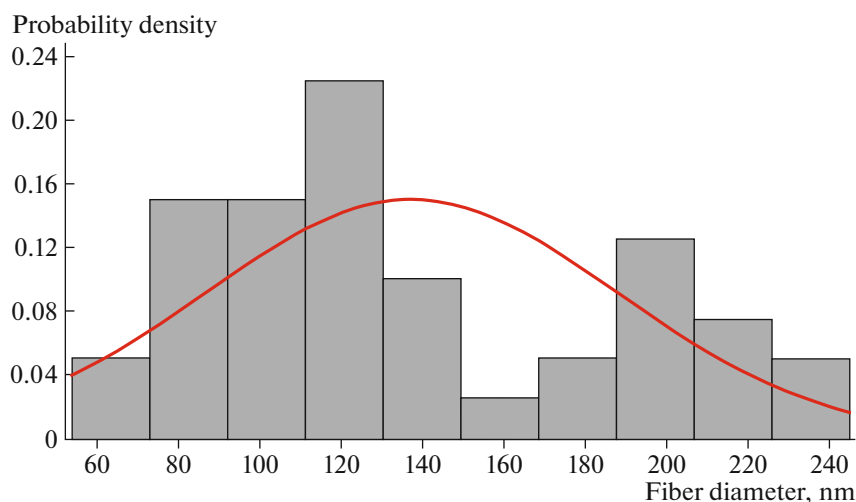

Fig. 15. (Color online) Histogram of fibers diameter distribution resulted from the analysis of AFM images. Electrospun nanofibers obtained from the blend solution composed of 1 : 9 mass ratio of PEO to LMW-chitosan. The average diameter ± standard deviation (SD) was 137 ± 51 nm (%CV = 37.22%).

of nanofibers were estimated herein by measurement the fibers thicknesses toward the z-axis instead of xy-plane to avoid the tip convolution effect as previously described [50, 51]. Figures 14 and 15 demonstrate diameter distribution histogram of nanofibers. The average diameters of fibers calculated herein approximate those obtained by SEM images analysis. In some cases in which the nanofibrous sample is vacuum sensitive and/or is vulnerable to electric current or metal sputter coating [52], AFM probing [53, 54] may be an appropriate alternative rather than SEM imaging with some undeniable limitations especially confined scan size and difficulty in measurement of fibers diameter. Table 1 represents roughness parameters (Ra and Rq)

Table 1. Roughness parameters resulted from the surface analysis of electrospun nanofibers

Sample	Ra, nm	Rq (RMS), nm
1 : 3 mass ratio of PEO to LMW-chitosan	22.3	139.2
1 : 9 mass ratio of PEO to LMW-chitosan	15.9	103.0

obtained by the surface analysis of electrospun nano-fibers via atomic force microscopy. This information implies more surface roughness in the sample containing thicker nanofibers. It is worth noting that surface roughness is one of the most significant nano topographical parameters which affects the cell response to its microenvironment including cell adhesion, expansion, migration and etc. [55, 56].

CONCLUSIONS

Among the works reporting the production of spun chitosan/PEO nanofibers, those implying the significant role of chitosan molecular weight are scarce. Furthermore, to the best of our knowledge, there are no researches which have synchronously investigated this parameter with PEO : chitosan proportion at the blend solutions. In this work, three principle factors (chitosan molecular weight, chitosan solution concentration and PEO to chitosan ratio) impressing electrospinning of chitosan/PEO blend solutions were studied to find the optimum circumstances for construction of defect-free nanofibers. According to our results, chitosan with the low molecular weight is suitable for reproducible fabrication of bead-free and homogenous spun nanofiber even when mass ratio of PEO to chitosan is very low. Furthermore when using low molecular weight chitosan, it is possible to make polymer solutions with more concentration and convenient viscosity to get electrospun successfully.

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