Fabrication and characterization of chitosan/gelatin/thermoplastic polyurethane blend nanofibers

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Abstract
Polymer blending is a method to provide nanocomposite nanofibers with improved strength and minimal defects. Chitosan exhibits biocompatibility, biodegradability, antimicrobial activity, and wound healing properties. A combination of gelatin and thermoplastic polyurethane (TPU) blends was explored as a means to improve the morphological deficiencies of chitosan nanofibers and facilitate its electrospinnability. The morphology of the electrospun chitosan, chitosan/gelatin, and chitosan/gelatin/TPU blend nanofibers were characterized using scanning electron microscopy (SEM), while the miscibility and thermal behavior of the blends were determined using differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy/attenuated total reflectance (FTIR/ATR). The optimum results were achieved in blend with 3 wt% chitosan, 8 wt% gelatin, and 5 wt% TPU, which resulted nanofibers with a mean diameter of 100.6 nm ± 17.83 nm.

Keywords
Chitosan, gelatin, thermoplastic polyurethane, nanofibers, electrospinning

Introduction
Nanofibers from biocompatible polymers have demonstrated prospective applications in biomedicine, including wound dressings,¹ drug delivery,² tissue engineering scaffolds,³ and many more. The unique properties of nanofibers, such as high surface to volume ratio,⁴ insignificantly small pore size,⁵ high oxygen-permeable porosity,⁶,⁷ and ease of fabrication,⁸ made them such practical materials.

Several approaches have been developed to construct nanofibers, such as template, self-assembly, phase separation, melt-blown, and electrospinning. Electrospinning is one of the mostly utilized processes for the fabrication of nanofibers as it provides a simple and cost-effective method for the production of long and uniform fibers from most polymers with control over direction.⁹ Electrospinning has been used for the fabrication of nanofibrous structures for diverse applications.¹⁰ So far, electrospinning has been performed by biomaterials, such as gelatin, chitosan, and other natural and synthetic polymers.¹¹

Chitosan is a linear, semicrystalline polysaccharide derived from the deacetylation of chitin, which is the most abundant biodegradable natural polymer, after cellulose.¹² The content of free amino groups in polysaccharide, defined as the degree of deacetylation (DD), can be employed to differentiate between chitin and chitosan.¹³ Chitosan also contains free amino groups, which makes it a positively charged polyelectrolyte when the pH is between 2 and 6; this contributes to its higher solubility in comparison with chitin. However, this property makes chitosan solutions highly viscous complicating its electrospinnability.¹⁴ The dynamic interactions between polymer chains, such as entanglement, hydrophobic interaction, and hydrogen bonding, are essential for reducing the fiber diameter to the nanoscale level and maintaining continuous fiber formation.

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Due to its biocompatibility, nontoxicity, and biodegradability, chitosan is used in medical fields such as tissue engineering and wound dressing. To overcome chitosan electrospinnability challenges, polymer blending is suggested more specifically with a polyelectrolyte for its ability to be negatively charged due to pH conditions.\textsuperscript{15–17} Hence, gelatin, a natural biopolymer produced by hydrolytic extraction of animal’s collagen, can be considered an alternative for blending with chitosan. Collagen molecules are mainly stabilized by intra and interchain hydrogen bonding.\textsuperscript{18} When collagen is heated above its denaturation temperature, hydrogen bonds that stabilize the adjacent polypeptide chains are destroyed. Individual $z$-chains and $\beta$-chains are produced from the intact trimers ($\gamma$-chains), and thus, the rigid triple helical collagen state is transformed into a single-stranded, random-coil state (helix-coil transition) resulting in the production of gelatin.\textsuperscript{19} Gelatin is also known to have no antigenicity, and it is more economical than collagen, making it an attractive component for the fabrication of prostheses and incorporation into drug delivery systems and wound healing materials.\textsuperscript{20–22}

Several research studies on electrospinning of gelatin and chitosan blend nanofibers indicate that both chitosan and gelatin possess good biocompatibility, biodegradability, and commercial availability.\textsuperscript{23–27} However, these two materials are too fragile to provide sufficient mechanical strength in aqueous environments. Thus, thermoplastic polyurethane (TPU) could be a good candidate for reinforcement. As a thermoplastic elastomer, TPU has been widely used as coatings for breast implants, catheters, and prosthetic heart valve leaflets because of its mechanical properties.\textsuperscript{28,29} Merging all chitosan, gelatin, and TPU properties together, the final nanofibers could be suggested as a great candidate to be used in nerve tissue engineering and drug delivery applications. Hence, the main objective of this work was to study the fabrication possibility of gelatin/chitosan/TPU blend nanofibers.

To overcome the difficulties of chitosan electrospinning, gelatin was used to facilitate the process. However, electrospinning of neat chitosan has been a challenge.\textsuperscript{24} It has been found that when a chitosan and gelatin blend is used for electrospinning, during the formation of polyelectrolyte complexes, the oppositely charged polyelectrolytes electrostatically interact with each other, and it is gelatin that facilitates the electrospinning process; meanwhile, preparing a blend of chitosan/gelatin nanofibers resulted in deterioration of morphological and thermal properties of chitosan. In this research, for the first time, chitosan/gelatin/TPU blend nanofibers were electrospun to investigate the possibility and changes of nanofiber formation.\textsuperscript{23} Hence, understanding the behavior of such nanofibers in this new process is of utmost significance, and this work examines the morphology and miscibility and thermal behavior of nanofibers using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy/attenuated total reflectance (FTIR/ATR), and differential scanning calorimetry (DSC) techniques.

**Materials**

All the polymers used in this research are biodegradable and include medium-molecular-weight chitosan powder with the DD of 75–85% obtained from Aldrich (USA), TPU 85A desmopan from Bayer (Germany), and gelatin (Aldrich, USA). Solvents used are glacial acetic acid (AcOH), dimethylformamide (DMF), and tetrahydrofuran (THF) and purchased from Merck (Germany).

**Electrospinning of chitosan and gelatin blend solution**

To obtain a well-defined final blend solution of chitosan, gelatin, and TPU with 3, 8, and 5 wt%, a series of chitosan with 3 and 5 wt%, gelatin with 80 and 20 wt%, and TPU with 5 and 10 wt% were examined to determine the most appropriate ratio of pure polymers. Afterward, chitosan and gelatin with weight ratios 80/20 and 50/50, and chitosan, gelatin, and TPU with 3, 8, and 5 wt% were prepared by dissolving chitosan and gelatin in 99% acetic acid, and DMF/THF in the ratio of 60/40 was used to dissolve TPU. Pure chitosan (3 wt%) and gelatin (8 wt%) were dissolved separately and mixed to prepare the series of chitosan/gelatin blend solutions at 60°C. Then, TPU was added to 50/50 blend of chitosan and gelatin, to obtain 40/40/20 blend of chitosan, gelatin, and TPU, respectively. Polymers were placed into a syringe and needle gaged 18 was used as nozzle in electrospinning unit (Kato Tech. Co., Ltd, Japan) with a rotating collector. The applied voltage was 20 kV, and tip-to-collector distances and flow rate were fixed at 100 mm and 0.08 mm min\textsuperscript{-1}, respectively. Figure 1 shows the steps for preparing polymer solutions and electrospinning of nanofibers.

**Fourier transform infrared spectroscopy/attenuated total reflectance**

The characterization of chemical structure of the nanofiber samples was done using the FTIR/ATR technique (Tensor 27, Bruker). All data were recorded by means of a zinc selenide internal reflective element in the range of 500–4000 cm\textsuperscript{-1}.

**Scanning electron microscopy**

The morphology of the electrospun webs was observed using a Bal-Tec SCD 005 SEM, with accelerated voltages of 26–29 kV and magnification of 2500 ×. Prior to observation under SEM, all the samples were sputter coated with a gold layer. The diameters of the 10 fibers of each sample were determined by analyzing the SEM images with SnapShot software.
Differential scanning calorimetry

A differential scanning calorimeter (TA Instruments 2010, New Castle, Delaware, USA) was used to evaluate the thermal properties of nanofiber webs. All samples were first dried and about 4 mg of each sample was loaded in an aluminum pan and sealed, and then heated at a flow rate of 10°C min⁻¹ from −50°C to +200°C under nitrogen atmosphere (one reverse cycle).

Results and discussion

Fourier transform infrared spectroscopy/attenuated total reflectance

Figure 2(a) to (c) shows the FTIR/ATR spectra of pure chitosan, gelatin, and a blend of chitosan/gelatin. Because of common functional groups found in chitosan and gelatin, there are many similar peaks observed in FTIR/ATR spectra. For instance, all the samples exhibited a broad band around 3350 cm⁻¹ and a weak peak at 2922 cm⁻¹, which are assigned to the groups of O–H and CH₂, respectively. However, there are some peculiar peaks that appear for pure chitosan and gelatin to explain their special properties. Appearance of signal around 1740 cm⁻¹ and a peak at 1555 cm⁻¹ confirms the existence of amide and amine groups. However, the absorbance peak at 1066 cm⁻¹ is prominent in pure chitosan and corresponds to the −C–O–C– bonds in Figure 2(a). As it can be seen in the spectra of the gelatin sample, the bands at 1640, 1540, and 1240 cm⁻¹ were corresponded to the stretching of amide I, N–H bending of amide II, and N–H bending of amide III, respectively.

Thus, it is shown in Figure 2(b) that similar absorption bands of 1640, 1534, and 1234 cm⁻¹ appeared for the same functional groups.²³⁻²⁷

Although there are some similar peaks in these polymers, the blends (Figure 2(c)) should contain both notable peaks of chitosan at 1066 cm⁻¹ and gelatin at 1640 and 1534 cm⁻¹, to demonstrate the existence of both gelatin and chitosan on the surface. Because FTIR/ATR customized the surface of the web, the existence of these three peaks for chitosan and gelatin is obvious apparent evidence for a perfect combination of these two polymers and ionic interaction between positively charged chitosan (see Figure 3) and negatively charged gelatin. In other words, FTIR/ATR verifies the chemical structure and proves the
possibility of miscibility and unaffected structures of chitosan and gelatin after electrospinning process.

Figure 3 shows that in the spectrum of chitosan/gelatin, C=O groups of gelatin adsorbed with N–H groups of chitosan resulting in strong hydrogen bonds, leading to a far more miscible chitosan/gelatin component.\textsuperscript{24}

As shown in Figure 4(b), the characteristic absorption bands of gelatin were observed at 1640 cm\(^{-1}\) (amide I), 1534 cm\(^{-1}\) (amide II), and 1234 cm\(^{-1}\) (amide III), respectively, while the peak at 1130 cm\(^{-1}\) was assigned to chitosan for its saccharine structure. Moreover, as shown in Figure 4(a), TPU has characteristic absorption bands at 3349 cm\(^{-1}\) (N–H), 2926 cm\(^{-1}\) (C–H), 1725 cm\(^{-1}\) (C=O), 1217 cm\(^{-1}\) (C–O), and 1528 cm\(^{-1}\) assigned to the characteristic absorption of benzene.\textsuperscript{28} Meanwhile, due to the spectrum shown in Figure 3(b) (chitosan/gelatin blend), the characteristic absorption peaks of TPU at 1528 cm\(^{-1}\) (benzene), 3349 cm\(^{-1}\) (N–H), and 1217 cm\(^{-1}\) (C–O) overlapped with the characteristic bond of the blend, including peak at 1529, 3350, and 1240 cm\(^{-1}\). Thus, as shown in Figure 4(c), there are characteristic bonds of TPU and chitosan in the range of 3500–2500 cm\(^{-1}\), while the peaks between 2000 cm\(^{-1}\) and 1000 cm\(^{-1}\) overlapped with strengthened related peaks of TPU in the final layer, confirming the existence of TPU and gelatin (Figure 4(c)).\textsuperscript{28,30}

**Scanning electron microscopy**

As it can be seen in Figure 5, both (8 and 20 wt\%) gelatin nanofibers illustrate almost uniform and beadless structure. However, when gelatin concentration is 20 wt\%, as shown in Figure 5(b), thick and branched electrospun fibers with corroded microstructure have been provided, while 8 wt\% gelatin, shown in Figure 5(a), led to fabricate fine fiber. Because solvent evaporates quickly from fine fiber, any corrosion related to acid detention in fiber disappears. Therefore, the higher the concentration of gelatin used, the thicker the appearance of fibers will be.
As shown in Figure 6, 8 wt% gelatin had far more monotonous microstructure compared with 20 wt% gelatin in which the nanofiber diameters vary from 250–350 nm to 50–1050 nm. The main objective of this work was to produce the most oriented and thin nanostructures with least amount of failure, including beads, branches, and corroded structure; results confirm the existence of uniform nanofibers that would be an appropriate candidate for biomedical applications such as regenerative medicine and cell culture.3,31 Thus, 8 wt% gelatin could be the optimum concentration.

As shown in Figure 7(b), when chitosan concentration is 5 wt%, droplets and beads were distributed on the surface, and fibers were hardly distinguishable. With decrease in chitosan concentration to 3 wt%, more fibers were visible among beads in Figure 7(a). A blend of 8 wt% gelatin and 3 wt% chitosan was prepared to investigate the optimum content of chitosan/gelatin blend for electrospinning; hence, 50/50 and 80/20 weight ratios of chitosan/gelatin were used. As shown in Figure 7(c), nanofibers appeared in polymer blends, electrospinning of nanofibers could be more difficult.23–25

Based on the found results, the chitosan/gelatin ratio of 50/50 mix resulted in the formation of uniform and thin fibers with minimal numbers of beads. The SEM images in Figure 8(a) and (b) show 10 and 5 wt% of TPU, respectively, in which 10 wt% of TPU led to ribbon-like nanostructure, as shown in Figure 8(b). In fact, higher concentration of TPU led to the formation of thick nanofibers, which would need more time to evaporate solutions from nanofibers. Therefore, collecting wet nanofiber could form ribbon-like structures. Meanwhile, nanostructure of 5 wt% TPU, Figure 8(a), has far more uniform and thin structure.32,33 In addition, Figure 8(c) and (d) shows the effect of two different contents of TPU in chitosan/gelatin
blend. Chitosan, gelatin, and TPU with 3, 8, and 5 wt%, Figure 8(c), in contrast to chitosan, gelatin, and TPU with 3, 8, and 10 wt%, Figure 8(d), illustrated more orientated, dense, and ultrafine nanofibers and the least amount of bead and interrupted fibers in comparison with other nanofiber samples.

As it can be seen in Figures 8 and 9 (average diameter of nanofibers), solution concentration of TPU was found to be a crucial factor in order to control the morphology of the chitosan/gelatin/TPU blend in the electrospinning process. Hence, as shown in Figure 8, increase in TPU concentration resulted in combination of both droplets and nanofibers. Hence, 5 wt% TPU could be considered as an optimum concentration, which led to uniform and miscible nanofibers.

**Effect of electrospinning parameters**

In order to investigate the optimum conditions and the least failures for nanofabrication, chitosan/gelatin/TPU nanofibers with 3, 8, and 5 wt% and weight ratio of 40/40/20 were fabricated by different device parameters. As shown in Figure 10(a) and (b), the rotating effect of collector with an Revolutions per minute (RPM) from 0 cm s\(^{-1}\) to 5 cm s\(^{-1}\) has been investigated. However, as it can be seen in Figure 10(b), the speed was not high enough to improve the orientation of nanofibers, and the rotation of the collector resulted in combination of both droplets and nanofibers. Furthermore, increasing tip-to-collector distance from 10 cm to 15 cm (Figure 10(c) and (d)) would cause the formation of ultrathin nanofibers with more drop and disruption of solution fluid and nanofibers. The results were confirmed in several works as well.\(^\text{14–16,34}\)

The effect of increase in voltage from 20 kV to 30 kV on nanofibers structures is illustrated in Figure 10(e) and (f). As it can be seen, increase in the applied voltage results in the formation of more beaded, interrupted fibers.

**Differential scanning calorimetry**

DSC is found to be an appropriate tool that is exclusively used to investigate the thermal and morphological properties of nanocomposite nanofibers. As it is shown in the thermal analysis of chitosan (Figure 11(a)) and a blend of chitosan and gelatin (Figure 11(b)), the \(T_{\text{onset}}\) position shifted from 45.27°C to 26.40°C as the content of chitosan decreased. Hydrogen bonding can appear due to interactions of amid bonds related to chitosan with peptide bonds of gelatin, which will cause miscibility of blend polymers.

Moreover, theoretically, a completely miscible blend should result in the formation of a single-phase transition between the transition temperatures of the chitosan and gelatin. Indeed, the ionic interaction and hydrogen bonding between the –COOH group of gelatin and the –NH\(_2\) group of chitosan lead to acceptable miscibility, which is related to the electrostatic interactions and intermolecular hydrogen bonds between oppositely charged polyelectrolytes, that is, chitosan with positive charge and gelatin with negative charge.\(^\text{23,27}\)

As shown in Figure 12, the three polymers revealed a typical endothermic peak in a blend sample. In fact, no phase segregation or secondary peaks are visible, suggesting good miscibility and homogenous structure of the blend sample.\(^\text{35}\) The phase transition temperatures of the blend nanofiber was close to the mean value of the transition
improve electrospinnability and morphological properties of the gelatin/chitosan blend. The effect of blend weight ratio and solution concentration on morphology of nanofibers was also investigated using SEM, FTIR/ATR, and DSC. Optimum condition was identified as 40/40/20 weight ratio of chitosan/gelatin/TPU with 3, 8, 5 wt% solution concentration that resulted in decrease of the fiber diameter to 85.81 nm ± 30.489 nm. Furthermore, voltage of 20 kV and tip-to-collector distance of 10 cm were recognized as the optimum electrospinning parameters.

Declaration of conflicting interests

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