Influence of substrate temperature parameter on electrospinning process: example of application to the formation of gelatin fibers

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Abstract

The substrate temperature was investigated to broaden the applicability of controlling the morphology of polymeric fibers produced during the electrospinning process. A laboratory electrospinning setup was designed using a substrate heated in a temperature range of 25 °C to 100 °C. A gelatin polymer was used as an example to obtain beads-free gelatin fibers by fixing the main electrospinning parameters. Based on XRD, FTIR, and DSC techniques, the electrospun gelatin fibers did not show any change in their chemical composition up to 100 °C. Heating the substrate at 50 °C may be the best selection factor to obtain gelatin fibers; the fiber diameters experienced a significant decrease from 680 ± 140 nm to 420 ± 120 nm with increasing substrate temperature from 25 to 50 °C, respectively. They showed stability of the diameter at 380 ± 130 nm and 390 ± 130 nm when increasing substrate temperatures from 75 to 100 °C, respectively, with a significant variation in their diameter distribution. Therefore, this ability to control the electrospinning process using a heated substrate makes it promising for fabricating electrospun beads-free fibers of biopolymers such as gelatin for tissue engineering and drug delivery carriers.

Keywords Electrospinning · Substrate temperature · Gelatin · Nanofibers

Introduction

Biopolymer nanofibers and their fabrication methods include self-assembly, wet spinning, electrospinning, and solution blowing, which have become of great interest for their specific biomedical applications such as drug carriers and tissue engineering [1–6]. Electrospinning is interesting nanotechnology for producing non-woven polymer fibers in the micron to nanometer range with a large surface area

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per mass ratio and high porosity [7–9]. Recently, electrospinning is used to design medical scaffolds for tissue engineering and tissue regeneration requiring controlled morphology [10–13]. The main feature of the electrospinning technique is its simple setup; its setup consists of three simple components: a high-voltage source, a capillary tube, and a grounded collector [14]. The electrospinning process is achieved by subjecting the dissolved polymers to high electrostatic forces to generate electric jets through a spinneret by drawing them until they evaporate and form continuous polymer filaments to be deposited on a grounded metal substrate [15, 16]. Several forces control the fiber formation process include viscoelastic force, surface tension, electrostatic force, and gravitational force [17, 18]. To improve the electrospinning process, the various parameters affecting the morphology of the electrospun fibers are classified into three categories: polymer solution parameters such as molecular weight, surface tension, and viscosity; parameters of processing conditions such as applied voltage, flow rate, needle diameter, and distance between tip and collector; and ambient parameters such as humidity, type of atmosphere, and external pressure [19–21]. However, all these many parameters, which can influence the electrospinning process, can be reduced to a limited number of independent parameters. For example, the non-independent parameters such as viscosity, evaporation rate, and conductivity depend on independent parameters such as polymer concentration, polymer type, and ambient parameters [22, 23]. Although most electrospinning parameters have been studied in all the literature, none of these works investigated the effect of substrate temperature. Here, gelatin was chosen as a biopolymer for electrospinning because it is a commonly used biopolymer in many medical applications such as tissue engineering and drug delivery due to its biocompatible, bioactivity, and biodegradability [24, 25]. Unfortunately, the gelatin electrospinning process is subject to some challenges because aqueous gelatin solutions are not electrospinnable, and the morphology of the electrospun gelatin depends on the concentration [26, 27]. Herein, this study aims to improve the effectiveness of a heated substrate on electrospun fiber. Besides, there is still a need to enhance the electrospinnability of gelatin fibers to produce fiber mats with suitable physicochemical properties for biomedical applications. Therefore, this study aimed to investigate the effect of substrate temperature on the morphology and composition of electrospun gelatin fibers. Gelatin was electrospun at different substrate temperatures under control of key electrospinning parameters such as the applied voltage, needle-collector distance, setup orientation, polymer concentration, and solution feeding rates. Conventional techniques are used to characterize gelatin fibrous such as X-ray diffraction (XRD), Fourier transformed spectroscopy (FTIR), differential scanning calorimetry (DSC), optical microscope, and scanning electron microscopy (SEM).

Experimental sections

Materials

Polymers of gelatin Type B from bovine skin (Approx. 225 Bloom) and glacial acetic acid (98%, Al-Nasr, Egypt) were used without further treatment or

purification. A gelatin solution (300 mg/mL) was prepared using acetic acid (50% v/v) as a solvent. The gelatin solution was dissolved at 40 °C for 40 min and then was set to cool at room temperature.

Aluminum plate substrate design

A typical electrospinning setup coupled with heating and temperature maintenance accessories (Fig. 1) is used to maintain the substrate temperature at a fixed temperature. The substrate consists of a square aluminum plate of length 30 cm. The heating element is made of wrapped nickel chrome wire that is clamped and homogeneously distributed under the substrate between two mica sheets (30×30 cm). A calibrated digital thermocouple, stability ± 0.5 to 1 °C, was attached to the substrate to regulate and measure temperatures. Before the electrospinning process, the substrate was heated for 10 min at different temperatures 25 °C, 50 °C, 75 °C, and 100 °C.

Electrospinning process

Figure 1 shows a schematic diagram of the laboratory-designed electrospinning setup and the heated substrate. The electrospinning setup consists of a high-DC voltage adjustable power supply (Goldmann 40K), a syringe pump (KD Scientific 100 series), a polyethylene syringe, a stainless-steel needle connected to a positive supply, and a grounded aluminum plate substrate. The gelatin solution, 300 mg/mL, was inserted into a 10-mL syringe connected to a rubber tube (long: 50 cm, internal diameter: 2 mm) ended with a metal needle placed vertically to the collecting substrate plate. The main parameters values were selected to produce consistent electrospun beads-free gelatin fibers: applied voltage at 15 kV, the distance between needle and collector at 12 cm, and the solution flow rate at 1.2 mL/hr. The substrate temperatures were set at 25 °C, 50 °C, 75 °C, and 100 °C.

Fig. 1 Schematic diagram of the electrospinning setup with a heated substrate

Characterization methods

To confirm the best selecting of the previous independent electrospinning parameters, a typical optical microscope (OPTIKA with Digital Imaging, Italy) was used to image the number of electrospun gelatin fibers for 5 and 25 s on a glass slide. The crystallization structure of the electrospun gelatin fibers was characterized by X-ray diffraction (XRD) analysis using a GNR-APD 20000 pro, H423-vertical diffractometer. The chemical structure and functional groups of electrospun gelatin were confirmed by Fourier Transform Infrared Spectroscopy (FTIR) using a Perkin-Elmer-1600 spectrophotometer. FTIR spectra were recorded in the range from 400 to 4000 cm^{-1} . Differential scanning calorimetry (DSC: TA Instruments—SDT Q600) was used to analyze the thermal properties of the electrospun gelatin fibers. All samples were heated from 25 to 200 °C at a heating rate of 15 °C/min under a constant flow of a nitrogen atmosphere. The morphology and microstructures of the electrospun gelatin fibers samples were examined by scanning electron microscopy (SEM) using a JEOL 6400 electron microscope. The average fiber diameter, distribution, and standard deviation for 50 arbitrary measured fibers were obtained by processing SEM images with the Image J software.

Results and discussion

Selection of electrospinning parameters

Obtaining optimal electrospun gelatin fibers has been influenced by several parameters that should be adjusted and fixed in this study to obtain beads-free fibers. The electrospinning process was carried out as described in previous sections, with a gelatin solution fed to a vertical needle and the application of a direct electric field. At room temperature, before changing the substrate temperatures, the independent electrospinning parameters had to be optimized. Optimizing these parameters is a crucial step to confirm the production of consistent electrospun beads-free gelatin fibers. As aforementioned, the independent electrospinning parameters were set as outlined in Table 1.

Figure 2 shows the images obtained by the optical microscope for gelatin fibers electrospun on a glass slide for 5 s (Fig. 2a) and 25 s (Fig. 2b) before the study to

Table 1 Electrospinning parameters of electrospun beads-free gelatin fibers

Parameter	Value	References
Setup orientation	Vertical	[28–34]
Substrate temperature	25 °C	
Voltage	15 kV	
Needle-collector separation	12 cm	
Concentration of gelatin solution	300 mg/mL	
Polymer feed rates	1.2 mL/h

Fig. 2 Optical microscope images (magnification: 100×) of the number of gelatin fibers electrospun on a glass slide for **a** 5 s and **b** 25 s

test the selected optimizing parameters. The results confirmed the formation of uniform electrospun fiber morphology with beads-free.

Structural analysis

XRD analysis was used to investigate the effect of substrate heating on the crystalline structure of electrospun gelatin fibers fabricated at substrate temperatures of 25, 50, 75, and 100 °C, respectively. As apparent from Fig. 3, the diffraction patterns showed an amorphous nature of the electrospun gelatin fibers with the appearance of two broadened humps around 2-theta equals 4° and 19°. The formation of these crystalline structures is due to the secondary structure of gelatin, triple helix, and polypeptide strands [18, 35]. Samples at temperatures up to 100 °C had similar patterns. By increasing the substrate temperatures up to 100 °C, the broadened humps

Fig. 3 SEM images (magnification: 3500×) and size distribution of electrospun gelatin fibers at different substrate temperatures: **a** 25 °C, **b** 50 °C, **c** 75 °C, and **d** 100 °C

around $2\theta=4^\circ$ and 19° showed no difference or sharpening. The XRD results revealed that the polymorphic behavior of electrospun gelatin fibers is not subject to variation when the substrate temperatures are changed.

FTIR Spectra of gelatin fibers

FTIR analysis was used to investigate the functional groups and structural modifications of electrospun gelatin fibers fabricated at substrate temperatures of 25, 50, 75, and 100 °C, respectively. The FTIR spectra (Fig. 4) showed that all samples had exhibited the identical characteristic vibrational bands belonging to the electrospun gelatin fibers: amide I, amide II, amide III, and amide B. The peak that appeared at 1645 cm^{-1} can be assigned to stretching vibration of $\text{C}=\text{O}$ belonging to amide I, while those at 1540 cm^{-1} can be assigned to $\text{N}-\text{H}$ bending vibration of amide II [10]. The peaks that appeared at 2940 and 3280 cm^{-1} are assigned to the $\text{C}-\text{H}$ stretching vibration of amide B and $\text{N}-\text{H}$ stretching vibration of amide A, respectively [13, 25]. For the $\text{N}-\text{H}$ bending vibration of the amide III, the spectra showed a peak at 1240 cm^{-1} [26]. The results indicated that increasing the substrate temperatures to 100 °C did not affect the absorption bands, confirming the absence of chemical structural modifications of the electrospun gelatin fibers.

Thermal properties of gelatin fibers

Figure 5 shows the DSC thermogram of the electrospun gelatin fibers fabricated at substrate temperatures of 25, 50, 75, and 100 °C, respectively. The samples exhibited a single endothermic peak above 100 °C that attributed to the melting temperature of gelatin [35]. This peak results from the breakage of hydrogen bonds of

Fig. 4 XRD patterns of electrospun gelatin fibers at different substrate temperatures: **a** 25 °C, **b** 50 °C, **c** 75 °C, and **d** 100 °C

Fig. 5 FTIR spectra of electrospun gelatin fibers at different substrate temperatures: **a** 25 °C, **b** 50 °C, **c** 75 °C, and **d** 100 °C

the triple-helix structure and its transition into the values coil-like structure [36]. DSC curves showed that the diameter of the electrospun gelatin fibers significantly influenced the endothermic peaks. The endothermic peak at 108.5 °C (fibers diameter ~680 nm spun at a substrate temperature of 25 °C) shifted to about 106 °C for spun fibers at substrate temperatures above 50 °C. Above 50 °C, the endothermic peaks did not differ significantly due to the stability of the electrospun gelatin fiber diameters which also indicate no effect on the electrospinnability of the gelatin working solutions [37, 38]. This behavior resulted from acetic acid diffusion in the microfiber that became more difficult for thicker ones. Also, acetic acid evaporation is responsible for the peak broadening between 40 and 130 °C [39]. The proximity of the melting temperature of gelatin, 105–108 °C, to the boiling point of acetic acid, 118 °C, can cause an overlapping between them due to the amount of acetic acid inside the samples [40]. The results showed a shift in the endothermic peaks to lower temperatures when the diameter of the fibers was smaller. This behavior for smaller fiber diameters can be explained by easily solvent evaporation due to the shorter diffusion paths. These results together with the results of X-ray and FTIR analysis confirm that increasing the substrate temperatures up to 100 °C did not affect the structural or chemical composition of the electrospun gelatin fibers, confirming the absence of chemical structural modifications on them.

Structural morphologies

SEM analysis was used to investigate the influence of substrate temperatures on the topological and morphological features of electrospun gelatin fibers fabricated at substrate temperatures of 25, 50, 75, and 100 °C, respectively. The electrospun fibers were collected in random orientations so that the diameters of electrospun fibers were measured in relative distributions. SEM images, Fig. 6,

Fig. 6 DSC curves of electrospun gelatin fibers at different substrate temperatures: **a** 25 °C, **b** 50 °C, **c** 75 °C, and **d** 100 °C

show that all electrospun gelatin fibers have a regular morphology free of beads defects. The increase in the substrate temperatures did not change the surface of the fibers; the fibers had smooth surfaces without the formation of bead defects. The results exhibited that heating the substrate induces a significant reduction in the diameter of electrospun gelatin fibers. Figure 4 shows the differences in the statistical results between the four samples. The results showed that increasing the substrate temperature from 25 °C, at room temperature, to 50 °C led to a significant decrease in the fiber diameter from 680 ± 140 nm to 420 ± 120 nm, respectively. However, increasing the substrate temperature above 50 °C did not affect the diameter of the electrospun gelatin fibers as the mean diameters were 380 ± 130 nm and 390 ± 130 nm for the substrate temperatures at 75 °C and 100 °C, respectively. The increase in the substrate temperatures from 50 to 100 °C did not significantly affect the diameter of the electrospinning gelatin fibers. However, the samples show a significant variation in their diameter distributions (Fig. 6e, f). The increase in the substrate temperatures from 50 to 100 °C had no effects on the Electrospinnability of the gelatin working solution, which can be explained by the ability of the heated substrate to cause a thermal zone surrounding it where the jet accelerated into this zone may undergo an increased viscosity and constant evaporation of the solvent.

Conclusion

This work investigated the influence of substrate temperatures, as an electrospinning parameter, on the structure and morphology of electrospun gelatin fibers. The substrate temperatures were varied in the temperature range of 25 °C to 100 °C to produce beads-free gelatin. The main electrospinning parameters were fixed; they include applied voltage, spinning distance, polymer concentration, polymer, and feed rates. Increasing the substrate temperatures up to 100 °C did not affect the composition of gelatin fibers. The results exhibited that the diameters of the fibers decrease from 680 ± 140 nm to 420 ± 120 nm with increasing substrate temperature from 25 °C to 50 °C, respectively. Raising the substrate temperatures from 50 °C to 100 °C did not affect the average diameter of the gelatin fibers, while it significantly affected their diameter distribution. The results showed that heating the substrate to 50 °C may be a good critical selection factor for obtaining microfibers. This attempt to optimize the effect of heating the substrate on the electrospun gelatin fibers provided satisfactory results. This possibility will make the approach of electrospinning gelatin with heated substrate promised for fabricating electrospun fibrous mats for tissue engineering and drug delivery carriers.

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Declarations

Conflict of interest There are no conflicts to declare in this study among authors.

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