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ORIGINAL ARTICLES

Electrospun Gelatin Nanofibers: Effect of Gelatin Concentration on Morphology and Fiber Diameters

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ABSTRACT

Gelatin, a naturally – occurring biopolymer, was electrospun. It has been recognized that although gelatin can be easily dissolved in water the gelatin / water solution was unable to be electrospun into ultra-fine fibers. Gelatin solutions were prepared in a single solvent system [glacial acetic acid].The electrospinning was carried out under fixed electrostatic field strength of 20 kV/18 cm and the polarity of the emitting electrode was positive. The effects of gelatin concentration on morphology and/or size of the electrospun materials were observed by scanning electron microscopy (SEM). Electrospinning of 30-50% w/v gelatin solutions in acetic acid produced beads, or smooth fibers, depending on the concentration range. Only smooth fibers were observed at the concentration range of 40 – 50 % w/v, with their diameter ranging between 69 – 138 nm and 98 – 345 nm respectively. Further lower or higher concentration was inapplicable in electrospinning at ambient conditions. The gelatin fiber mats were further cross-linked with moist glutaraldehyde vapor to improve their stability in an aqueous medium.

Key words: Electrospinning; Gelatin; Nanofibers.

Introduction

Electrospinning is a unique method to prepare electrospun fibers with diameters in the range from micrometers to nanometers that depends on the kinds of polymer and processing conditions. Electrospinning technique has been recognized as an efficient processing method to manufacture nanoscle fibrous structures for a number of applications [Huang *et al*, 2003]. Gelatin is a natural biopolymer with a wide range of applications in medical, pharmaceutical and foodindustries. In the last years, gelatin based scaffolds prepared by electrospinning were intensely investigated because their three-dimensional (3D) structure that mimics very well the extracellular matrix which makes them veryattractive for tissue engineering applications [Li *et al*, 2005; Cole, 2000; Hule and Pochan, 2007].The first important step in electrospinning a natural polymer is the preparation of an electrospinnable solution using a proper solvent. Water is a good solvent of gelatin because it breaks very easy the inter chain links and produces stable solutions at 50°C, without gelatin degradation. However, an aqueous gelatin solution changes into a gel in the syringe needle at room temperature and the electrospinning process becomes impossible. Moreover, water has a slow evaporation rate, making the transformation of the solution filament into a dry nanofiber impossible during the travel between needle and collector. Nevertheless, the gelatin solution dissolved in water is unable to be electrospun into ultra-fine fibers even under heated and non-gelation condition. This may be probably attributed to its polyelectrolyte characteristics. Unlike synthetic polymers that are generally nonionic and can be dissolved in organic solvents through nonionic interactions between solute and solvent, gelatin is a kind of polyelectrolyte polymers which possesses many ionizable groups. Its amine and carboxylic functional groups can be ionized by acidic agents or hydrolyzed to carry positive or negative charges. In aqueous solution, such ionization (pH dependent) gives rise to a polyion bearing many charges, accompanied by small counter ions as shown below:

Therefore, although water is commonly used to make gelatin solution, searching for an alternative organic solvent plays a key role in successfully electrospinning this biopolymer.Gelatin is a biopolymer with strong polarity. There are very few high polarity organic solvents available for dissolving this biopolymer. It has been known that fluorinated alcohols, such as trifluoroethanol (TFE) hexafluoroisopropanol (HFIP) and acetic acid are good solvents for polypeptide biopolymers [Huang *et al*, 2004].The second important problem in gelatin

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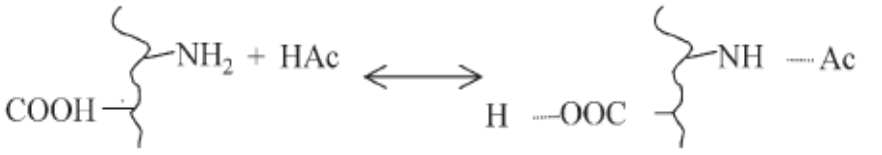
electrospinning is the gelatin concentration of the electrospinnable solution. In the present work, we investigated the effect of the gelatin concentration of electrospinnablesolutions by using acetic acid (AA) on morphology and fiber diameters.Glutraldhyde (GTA) vapor was used to improve the stability of the fiber mats in a moist environment.



Gelatin macromolecule Ionized Gelatin macromolecule

Materials and Methods

2.1. Materials:

Gelatin (from porcine skin, type A) was purchased from Sigma Aldrich.Acetic acid (96%) ($C_2H_4O_2$) with a molecular weight of 60.05 was purchased from (Adwic) El-Nasr Pharmaceutical Chemicals Co.Glutaraldehyde, (GTA; 25 wt. % solution in water) [$HCO(CH_2)_3CHO$] was purchased fromSigma Aldrich. Glycine pure lab. Chemical (99%) with a molecular weight of 75.07 was purchased from (Adwic) El-Nasr Pharmaceutical Chemicals Co. All chemicals were of analytical reagent grade and were used without further purification.

2.2. Preparation and characterization of spinning solutions:

Gelatin solutions were prepared in acetic acid in various compositional ratios. The compositional ratio of acetic acid in water was 70:30 v/v. The solutions of gelatin in acetic acid were prepared in various concentrations ranging from 30 to 50% w/v. Prior to electrospinning, all of the as-prepared solutions were measured for their shear viscosity and conductivity by a Brookfield DV-III ultra-programmable rheometer and a professional portable conductivity meter HC3010, trans-instruments, respectively.

2.3. Preparation of e-spun gelatin fiber mats:

2.3.1.Electrospinning Process:

Electrospinning was carried out byfirst stocking each of the as-prepared gelatin solutions in a 3-mL plastic syringe.The open end of which was attached to a needle, which was used as the nozzle. A piece of aluminum (Al) sheet was placed below the capillary tip as the ground collector. Capillary was connected with a high voltage power supply (Model Glassman high voltage, NJ08829), which could generate positive DC voltages. A High-Voltage DC powersupply was used to charge the solution by attaching the emitting electrode of positive polarity to the nozzle, and the grounding one to the collecting device. An electrical potential of 20 kV was applied across a distance of 18 cm between the tip of the needle and the outer surface of the collection device (i.e., collection distance).

2.3.2. Crosslinking:

Crosslinking of the neat gelatin fiber mats was carried out by soaking the fiber mat samples in 2wt% glutaricdialdehyde (GTA / ethanol) for different time intervals. After exposure, the samples were washed with 0.2M glycine in order to remove the unreacted GTA.

2.3.3. Characterization:

The gelatin fiber mats were examined for the size individual fibers using a Quanta FEG 250 FEI, Holandascanning electron microscope (SEM), the average diameters of the fibers were determined from SEM images. FT-IR measurements were performed in a FT-IR Spectrometer 1760x (Perkin-Elmer) to verify the composition of fibers for functional groups.

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3. Results and discussion

3.1. Viscosity, and conductivity of the gelatin solutions

Figure 1and figure 2 show that the solution concentration of gelatin solution as 50 (w/v%) showed the highest viscosity. It was presumed that both the viscosity and the conductivity of the solutions were found to increase with increasing solution concentration. The monotonous increase in the solution viscosity with increasing solution concentration was obviously a result of the increased molecular entanglements; also, less polymer weights in solutions contribute to lower viscosity of solutions, while the monotonous increase in the solution conductivity with increasing solution concentration could be a result of the increase in the number of charged species resulting from the dissolution of gelatin in the acidic condition of the solutions [Choktaweesap *et al*, 2007].

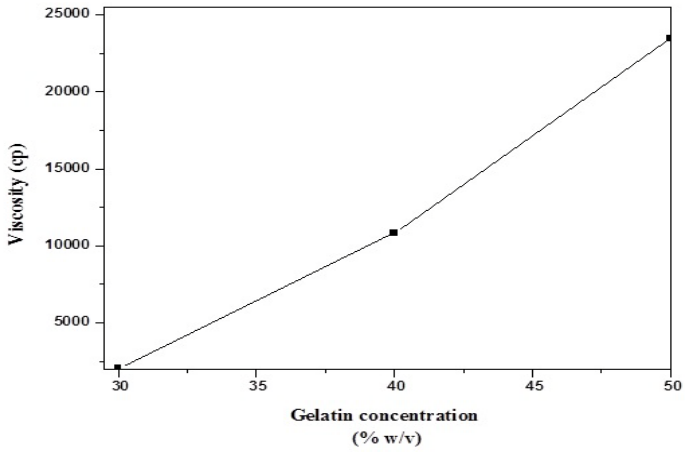

Fig. 1: Viscosity of gelatin solutions in acetic acid as a function of the solution concentration.

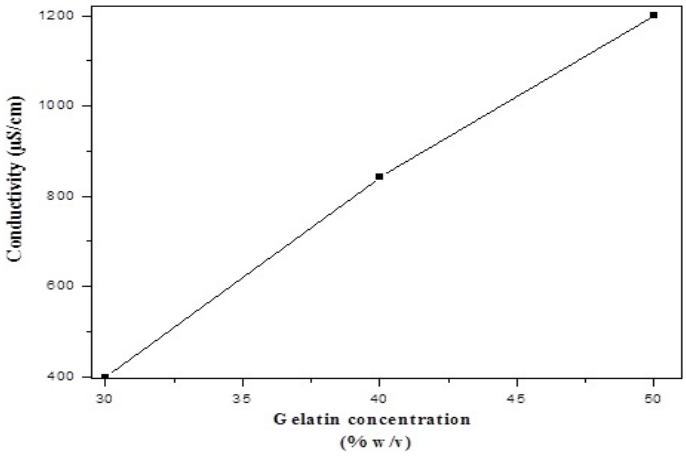

Fig. 2: Conductivity of gelatin solutions in acetic acid as a function of the solution concentration.

3.2. E-spun gelatin fiber mats:

3.2.1. Fourier-transform Infrared Spectroscopy (FT-IR):

In order to determine electrospun gelatin fiber structure before and after crosslinking, FT-IR was carried out on the electrospun fibers before and after crosslinking. Figure 3 reports the FT-IR spectra of gelatin fiber mats prepared from acetic acid/water solution, and from crosslinked gelatin together.

FTIR measurements were conducted on uncrosslinked gelatin and crosslinked gelatin in order to determine whether crosslinking of the electrospun gelatin has affected the gelatin structure or not (figure 3).

The uncrosslinked electrospun gelatin had an amide I peak (C = O stretch) at1636-1640 cm^{-1} , amide II peak (N-H bend and C-H stretch) at 1542-1544 cm^{-1} , amide III peak (C-N stretch plus N-H in phase bending) at 1240 cm^{-1} and amide A peak (N-H stretching vibration) at 3316 cm^{-1} , which are the distinguishing features of gelatin.

The spectra of the crosslinked gelatin is shown in detail in figure 2. In addition to the previously mentioned peaks, a strong peak at 1450 cm^{-1} was observed in the crosslinked gelatin due to aldimine absorption [Akin and Hasirci, 1995]. In comparison to uncrosslinked gelatin we found that the amide II peak was changed from smooth to several small peaks. Glutaraldehydes have an aldehyde group (-CHO) that reacts with the amino group of the lysine residues of proteins [Bigi and Cojazzi, 2001]. The uncrosslinked and crosslinked membranes were discriminated by a slow change in color from white to yellow. The color change occurred because the aldimine linkage (CH = N) reactions took place during the crosslinking process.

The characteristic absorption of the aldimine groups occurred at 1450 cm^{-1}. Furthermore, additional peaks were observed at 1470-1570 cm^{-1} , which increased as the crosslinking reaction was proceeded (figure 2). To more conclusively determine if crosslinked gelatin was successfully fabricated, we expanded the FT-IR spectra from 1100-1750 cm^{-1} (figure 2). In this region of the FT-IR spectra, the amount of CH=N groups increased [Bigi and Cojazzi, 2001]. Thus, both uncrosslinked and crosslinkedelectrospun mats gelatin were successfully fabricated via the electrospinning method using acetic acid/water as shown in figure 3.

This technique proved that crosslinking of gelatin, using glutaraldehyde during the crosslinking process, which is an essential condition to limit the insolubility and increase the biocompatibility of gelatin for applications in the biomedical field [Bigi and Cojazzi, 2001].

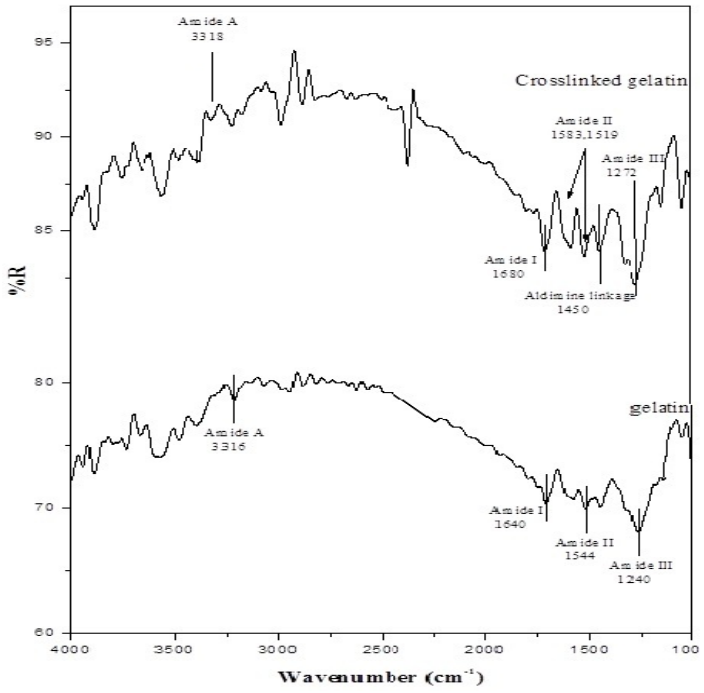

Fig. 3: FT-IR of uncrosslinked gelatin and crosslinked gelatin electrospun fibers.

3.2.2. Morphology and fiber diameters of electrospun gelatin fiber mats:

Gelatin electrospun fibers were obtained by electrospinning process from 30%, 40%, and 50% gelatin solution respectively in the following condition: 18 cm collection distance and 20 kV applied electrical potential.

Scanning electron micrographs of the electrospun gelatin fibers prepared in aqueous 70%v/v acetic acid at various concentrations of gelatin are shown in figure 4. The fibers diameters were decreased continuously at ratios ranging from 50% to 40% but there are no fibers formed at 30% (figure 4). And also the fibers were found bonded at their contact sites. This could be caused by incomplete evaporation of solvents (both distilled water and aqueous acetic acid).

Solution concentration and viscosity are two closely correlated factors, increase in solution concentration always results in increase in solution viscosity, and decrease in solution concentration always results in decrease in solution viscosity. Therefore, there are two factors were investigated. Increase the concentration and viscosity

favors the uniform fibers. The viscosity has an important role in fiber formation in the electrospinning process. Larrondo and Manley, 1981 showed that the viscosity was important when they electrospun fibers from the melt. The critical viscosities and applied electrical fields required for melt-electrospinning were much higher than those required when spinning from solution. It was seen that as the viscosity of the solution or melt increased, the fiber diameter increased exponentially.

At 30 wt.% gelatin concentration figure 4 (A,B), no fibers were observed. At 40 wt.% gelatin concentration figure 4 (C,D), the fibers were appeared as the fiber diameter from 69 –138 nm. At 50 wt. % gelatin concentration figure 4 (E, F, G), the diameter of the fibers increased to 98 – 345 nm. The average fiber diameter becomes large with increasing concentration, which is consistent with the result obtained by **Huang *et al*, 2004**.

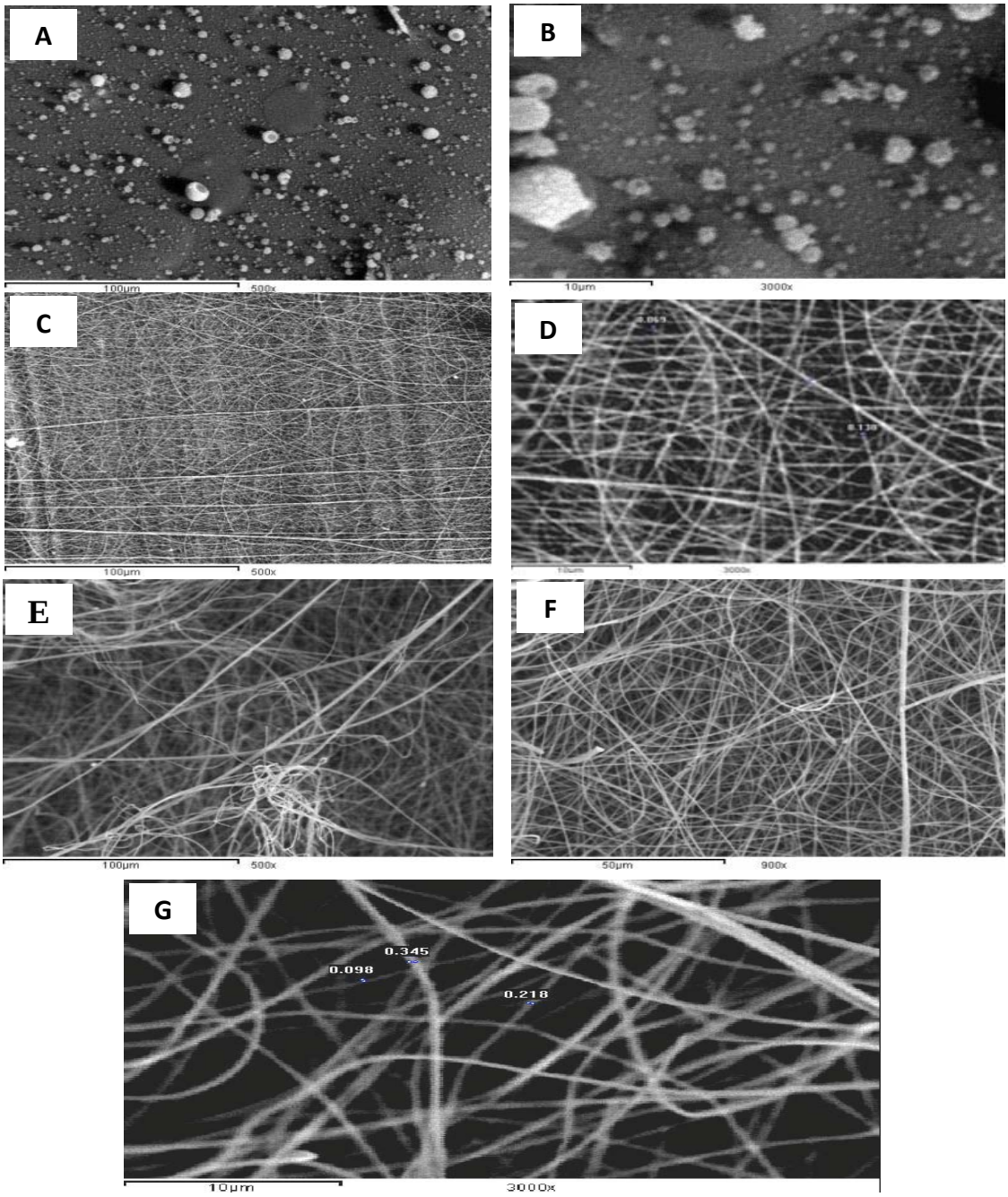

Fig. 4: Selected SEM images at different magnifications of fiber diameter of gelatin electrospun fiber mats at a voltage of 20 kV, collector distance 18 cm for different concentrations of the base gelatin (A, B) 30 wt. % gelatin at magnifications 500x, and 3000x respectively, (C, D) 40 wt. % gelatin at magnifications 500x, and 3000x respectively, and (E, F, G) 50 wt. % gelatin at magnifications 500x, 900x, and 3000x respectively.

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3.2.3. Morphology before and after crosslinking treatment:

Since gelatin is water-soluble, an e-spun gelatin fiber mat can easily dissolve either partially or completely, losing its fibrous structure when coming into contact with an aqueous medium. It may partially dissolve and loose its fibrous structure upon an amount of exposure to a high ambient humidity (e.g., 80–90%) for a certain period of time.

To extend the use of e-spun gelatin fiber mats in applications that require exposure to an aqueous medium or high humidity, further crosslinking is necessary. Among the various chemical systems used to crosslink an e-spun gelatin fiber mat.

GTA is seemingly the most suitable, as it is economical and does not compromise the fibrous structure of the e-spun membrane [Zhang *et al*, 2006].

Fig. 5 shows selected SEM images of the e-spun fiber mats for the neat gelatin solutions after having been crosslinked with GTA for either 2 or 4 hrs. Evidently, the neat after cross-linking, changed their color from white to yellow (for the fibers mats that had been cross-linked for 2hrs) and finally to brown (for the fiber mats that had been crosslinked for 4hrs). They also shrunk slightly from their original dimensions. The change in color of the gelatin upon crosslinking with GTA is caused by the formation of aldimine linkages (–CH=N–) between the free amino groups of lysine or hydroxylysine amino acid residues of the protein and the aldehyde groups of GTA [Akin and Hasirci, 1995; OldeDamink *et al*, 1995].

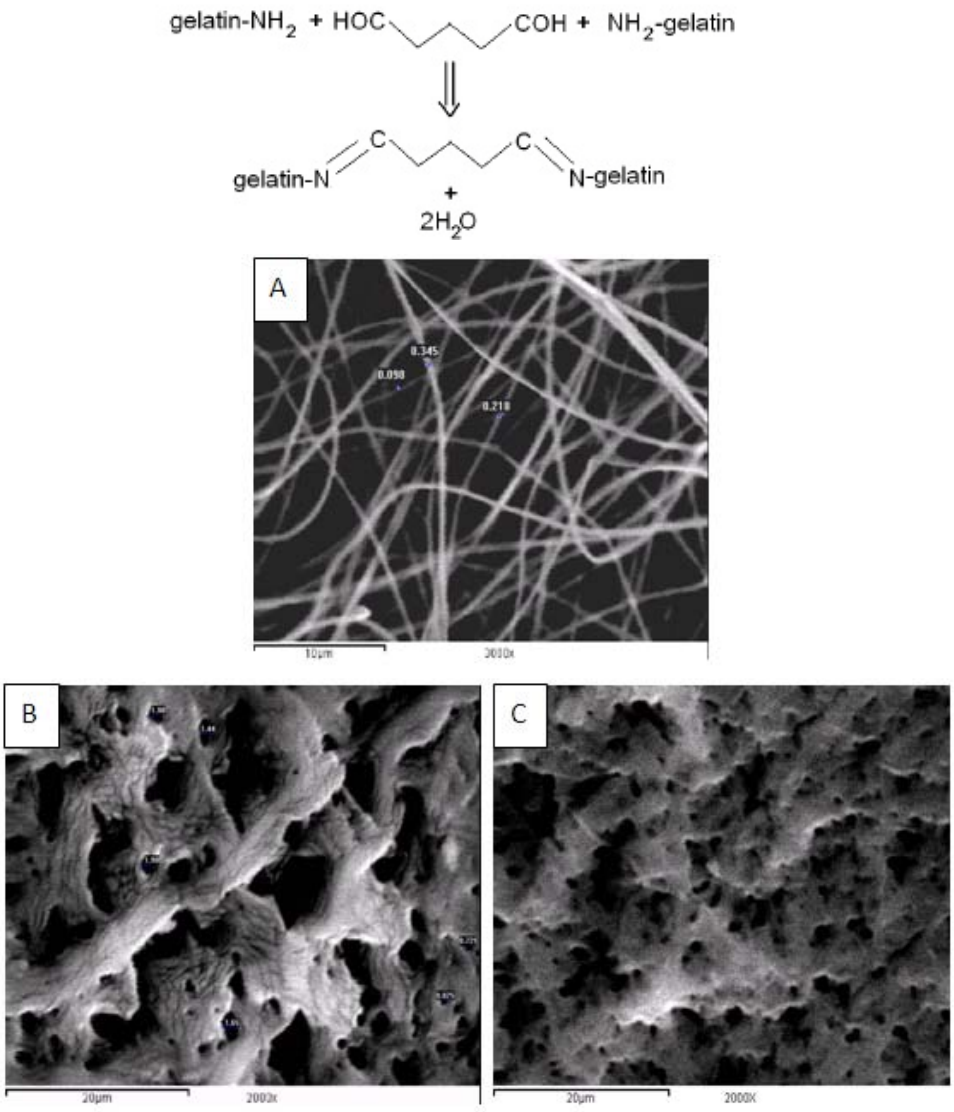

Fig. 5: Morphology of the electrospun fiber mates from the 50 wt.% gelatin solution (A) the base gelatin solution (before crosslinking) (magnification 3000x), (B), (C) the neat gelatin solution that had been crosslinked with glutraldhyde (GTA) for 2 hrs, and 4hrs (magnification 2000x) respectively.

Moreover, the shrinkage of the fiber mats is responsible for the observed decrease in the size of inter-fibrous pores, as well as the observed decrease in the thickness of the fiber mats and the observed increase in the diameters of the individual fibers.

Figure 5 shows the fiber morphologies of the samples before and after crosslinking. The fibrous form had been grossly preserved, however, due to the nanoscale size of the gelatin fibers: the coexistence of glutraldhyde (GTA) during crosslinking treatment had affected the fiber morphology to some extent. This is reflected by the fact that fibers at junctions were fused together forming bondings (the inset image of fig. 5 (B)). It is clear that the range of diameters of the pores decreased with increasing the time of crosslinking (the inset image of fig. 5 (C)).

Conclusions:

Electrospinning of a natural biopolymer,gelatin, was investigated. Although this biopolymer can be well dissolved in water, the resulting solution is recognized unspinnable though an electrospinning technique. Electrospinning of gelatin solutions prepared with acetic acid as a solvent, nanofiber membranes being successfully prepared from these solutions using the same electrospinning parameters. Gelatin nanofibers were successfully prepared by the electrospinning of gelatin solutions with gelatin concentrations of 40% and 50% (w/v). Increasing the gelatin concentration caused the increase of the gelatin nanofiber with diameter ranging between 69 – 138 nm and 98 – 345 nm. The neat gelatin fiber mats were further crosslinked, to improve their stability in an aqueous medium or a high humidity atmosphere,by 2% glutaraldehyde (GTA)aqueous solution. Crosslinking not only caused the color of the crosslinked gelatin fibers to change, but also was responsible for the fusing and shrinking of the crosslinked fiber mats. Electrospinning of high concentration gelatinallows a fast deposition of gelatin non-woven mats of enough thickness required in biomedical applications.

Acknowledgments

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