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Electrospinning of Gelatin Nanofibers: Effect of gelatin concentration on chemical, morphological and degradation characteristics

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Keywords
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ABSTRACT

Electrospinning is a well-known technique that produces polymeric nanofibers using an electrically driven jet of a polymer solution. Due to unique properties such as high surface area, porosity, tensile strength and extensibility of the materials produced by electrospinning, several applications of them in protective clothing, space technology, filtration and tissue engineering have been proposed and investigated. In this study; we prepared gelatin nanofibrous scaffolds by using the electrospinning method for tissue engineering applications. The beads-free, smooth and uniform gelatin nanofibers were successfully fabricated. The blend solutions at different weight ratios were prepared by dissolving gelatin in a solvent mixture containing formic acid, dichloromethane and acetic acid. The fabricated nanofibers were chemically crosslinked by glutaraldehyde vapor. The crosslinked nanofibrous scaffolds were characterized by chemical and morphological analysis. The morphology and size distribution curves of nanofibers were determined by Scanning electron microscopy (SEM). The chemical structure of nanofibers was investigated by Fourier transform infrared spectroscopy (FTIR) analysis. The strategy based on electrospinning of gelatin nanofibers can be used to develop new biomimetic materials for tissue engineering applications.

1. INTRODUCTION

Tissue engineering/regenerative medicine is an emerging and interdisciplinary field that aims to maintain, improve or restore damaged tissues or whole organs using a combination of cells, scaffolds and bioactive molecules with the principles of biology, materials science and engineering (Langer and Vacanti 1993). The tissue engineering strategy includes the use of three-dimensional (3D) scaffold materials to provide a suitable microenvironment for the regeneration of tissues and organs (O'Brien 2011). The scaffolds seeded with cells assist a 3D support for cell migration, attachment and proliferation by mimicking the features of native extracellular matrix (ECM) architecture (Wang et al. 2013). The ECM in tissues and organs provides a physicochemical environment for cells and bioactive agents required for tissue morphogenesis, differentiation and homeostasis (Wu et al. 2014). Hence, the ideal scaffold should possess a similar structure to ECM.

Scaffolds can be manufactured from metals, polymers, ceramics or composite biomaterials to simulate the properties of tissues and organs. In addition, different techniques have been used to fabricate various types of scaffolds such as electrospinning (Chahal et al. 2015), freeze-drying (Al-Munajjed et al. 2009), casting/solvent evaporation (Liao and Ho 2010), foam replication technique (Reiter et al. 2019) and 3D printing (Soundrapandian et al. 2010). Among them, electrospinning is a well-known, attractive and simple technique for processing polymers into fibers with diameters ranging from several nanometers to a few micrometers (Demir et al. 2018). Electrospun nanofibrous 3D scaffolds have attracted great interest in the field of tissue engineering mainly due to their large surface area-to-volume ratio, high porosity, mechanical properties and morphology similar to the ECM of natural tissues.

A variety of natural and synthetic polymers has been used to fabricate the electrospun scaffolds. In this study, the composition of the electrospun scaffold is designed to

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simulate the natural tissue. Thus, gelatin, as a natural polymer, which is a denatured form of collagen, was selected for the preparation of nanofibers (Echave et al. 2017). Gelatin is an excellent material for tissue engineering applications due to its unique features such as biodegradability, biocompatibility, promoting cell adhesion and proliferation, and low immunogenicity (Tondera et al. 2016; Echave et al. 2017). Therefore, the use of gelatin as a natural polymer in tissue engineering has attracted many interests and is also widely used in clinics as wound dressings, pharmaceuticals and adhesives (Maleknia and Majdi 2014). Electrospinning is an attractive option for fabricating gelatin nanofibrous scaffolds in high surface area, physically stable and controllable thickness in different forms including thick nanofiber sheet (Huang et al. 2004) and tubular structure (Shalumon et al. 2015). Previous studies showed that electrospun gelatin scaffolds were biocompatible and have been used in a variety of biomedical applications such as bone tissue engineering (Meng et al. 2013), wound healing (Yao et al. 2017) and drug delivery (Kamble et al. 2018).

In this study, we produced nanofibrous scaffolds with different weight ratios of gelatin via electrospinning and revealed the effect of gelatin concentration on chemical, morphological and degradation properties. Gelatin scaffolds prepared by electrospinning were crosslinked by glutaraldehyde vapor for stability in aqueous media and mechanical strength. The morphological observations of nanofibers before and after crosslinking were studied in detail using Scanning electron microscopy (SEM). The fiber size distributions and average fiber diameters of nanofibers were determined. The chemical composition and molecular bonds of the scaffolds were analyzed by Fourier Transform Infrared Spectroscopy (FTIR). Degradation of electrospun scaffolds was studied gravimetrically. The findings of this study showed that morphological structure and degradation profile of gelatin nanofibers can be adjusted by changing the polymer concentration in the initial electrospinning solution. The nanofibrous scaffolds could be used as biomaterials for potential tissue engineering applications.

2. MATERIALS AND METHODS

2.1. Reagents

Gelatin as a natural polymer and glutaraldehyde solution (25%, v/v) as a crosslinking agent were received from Merck, Germany. Glacial acetic acid (100%, v/v) as a solvent was obtained from Sigma-Aldrich, USA. Formic acid and dichloromethane as solvents were purchased from PanReac (Spain) and Carlo Erba Reagents (France), respectively. All the chemicals were used as received.

2.2. Electrospinning of gelatin nanofibers

The electrospinning apparatus consists of a high voltage power supply (Gamma High Voltage, ES40P, USA), a syringe pump (New Era, NE-300), a 2.5 ml of a plastic syringe, a stainless steel needle connected to the power supply electrode and a grounded collector. A vertically positioned metal sheet covered with aluminum foil was used as the collector. Solutions with concentrations of 15, 20, 25 and 30% (w/v) (15% Gel, 20% Gel, 25%Gel, 30% Gel) were prepared by dissolving gelatin in a solvent mixture containing formic acid, dichloromethane and acetic acid with a volumetric ratio of 70:20:10, respectively. The prepared solutions were loaded into a 2.5 ml syringe with a stainless steel needle and 13 kV voltage was applied to the solutions. The tip-to-collector distance was 10 cm. The feeding rate of the gelatin solutions was 0.3 ml/h. The schematic illustration of electrospinning set-up is demonstrated in Figure 1A.

2.3. Crosslinking of Electrospun Nanofibers

To prevent the dissolution of the electrospun nanofibers, the prepared scaffolds were crosslinked under glutaraldehyde vapor in a glass chamber for 3 days as illustrated in Figure 1B. The crosslinked electrospun nanofibrous materials were called 15% Gel-GA, 20% Gel-GA, 25% Gel-GA and 30% Gel-GA. The reaction scheme between gelatin and glutaraldehyde is also reported in Figure 1B.

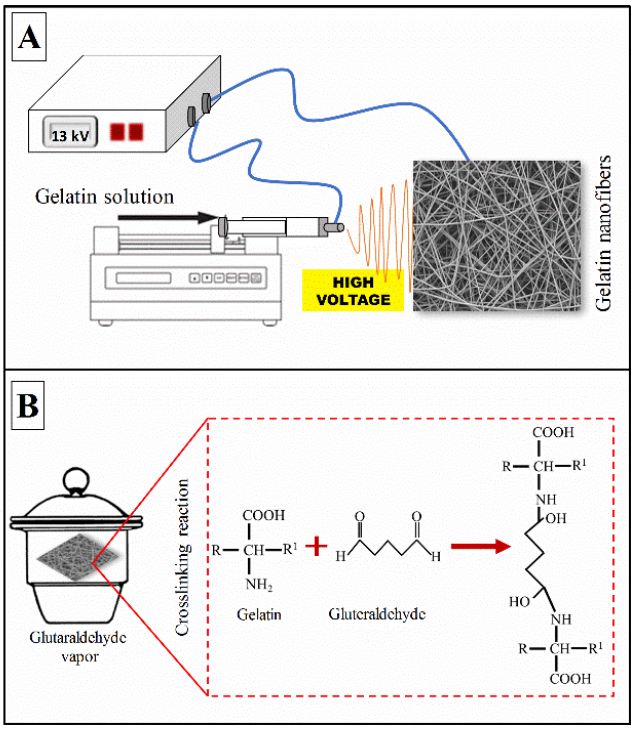

Figure 1. A) The schematic illustration of the electrospinning set-up and B) reaction mechanism of crosslinking between gelatin and glutaraldehyde

2.4. Characterization studies of the electrospun nanofibers

FTIR spectrometer (Perkin-Elmer Spectrum 1000, USA) was used to determine the chemical compositions of the produced materials. The spectra in the range of 475-4000 cm^{-1} with automatic signal gain were collected in 10 scans at a resolution of 4 cm^{-1}.

The morphology of the nanofibrous structure of the scaffolds before and after crosslinking was investigated by SEM (SEM, Quanta 400F Field Emission, Supra 55, Zeiss) at an accelerating voltage of 5 kV. The specimens were coated with platinum using a sputter coater before SEM analysis.

The fiber diameters of the nanofibers were identified from SEM images by using Lucia 32G image analysis software. The average nanofiber diameter was calculated by measuring the diameter of 80 nanofibers. The fiber size distribution curve of scaffolds was created using a histogram graph in OriginPro.

The *in-vitro* degradation studies of electrospun nanofibrous scaffolds after crosslinking with glutaraldehyde were performed by first recording the initial dry weight of the scaffolds. Then the samples were transferred to the falcon tubes filled with distilled water. The tubes were placed into a shaking water bath (Daihan Scientific Co. Ltd., WiseBath WB-22, Korea) at 37°C. At pre-determined time intervals (1, 6, 12, 18 and 21 days), samples were withdrawn, dried and weighed. Each value was the average of the result of three parallel measurements. The percentage of degradation (D, %) was calculated by the following Eq. (1):

$$D, \% = \frac{(Mi - Mt)}{Mi} \times 100 \qquad (1)$$

where Mi is the initial dry weight of samples, Mt is the final dry weight at pre-determined time intervals.

The degradation data were analyzed by using analysis of variance, ANOVA, by Origin Pro 2016 software and expressed as mean value and standard deviation, compared using the Tukey test. Differences were considered statistically significant at ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1. Chemical structure of the electrospun nanofibers before and after crosslinking

The produced gelatin nanofiber scaffolds can easily dissolve or lose their fibrous structure when coming into contact with an aqueous medium or exposure to high ambient humidity because of the water solubility of gelatin (Oraby et al. 2013). The crosslinking of these nanofibers is necessary to extend their use in various applications. Glutaraldehyde is the most frequently used crosslinking agent which is a bifunctional, water-soluble and economical crosslinker to resist both enzymatic degradation and hydrolysis of gelatin nanofibers (Lee et al. 2017). Therefore, the samples were chemically crosslinked under vapor of glutaraldehyde solution (Figure 1B). The crosslinking mechanism between gelatin and glutaraldehyde can be explained by the reaction of the aldehyde groups of glutaraldehyde with nonprotonated ε-amino groups (-NH_2) of lysine or hydroxylysine amino acids present in gelatin (Farris et al. 2010).

To compare the change in the chemical structure before and after crosslinking, the FTIR spectrum of nanofibers was provided. The FTIR spectra of gelatin nanofibers fabricated at 15, 20, 25 and 30% concentration (labeled as 15% Gel, 20% Gel, 25% Gel and 30% Gel, respectively) is shown in Figure 2A. After crosslinking, the samples were named 15% Gel-GA, 20% Gel-GA, 25% Gel-GA and 30% Gel-GA, respectively and their spectra is presented in Figure 2B.

As can be seen, all spectra are similar and exhibit the characteristic peaks of gelatin. The typical bands of gelatin are Amides I (C-O stretching at 1650 cm-1), Amides II (N=H bending at 1540 cm-1) and Amides III (N=H bending at 1235 cm-1) and their intensity were observed relatively decreasing after crosslinking (Erencia et al. 2015).

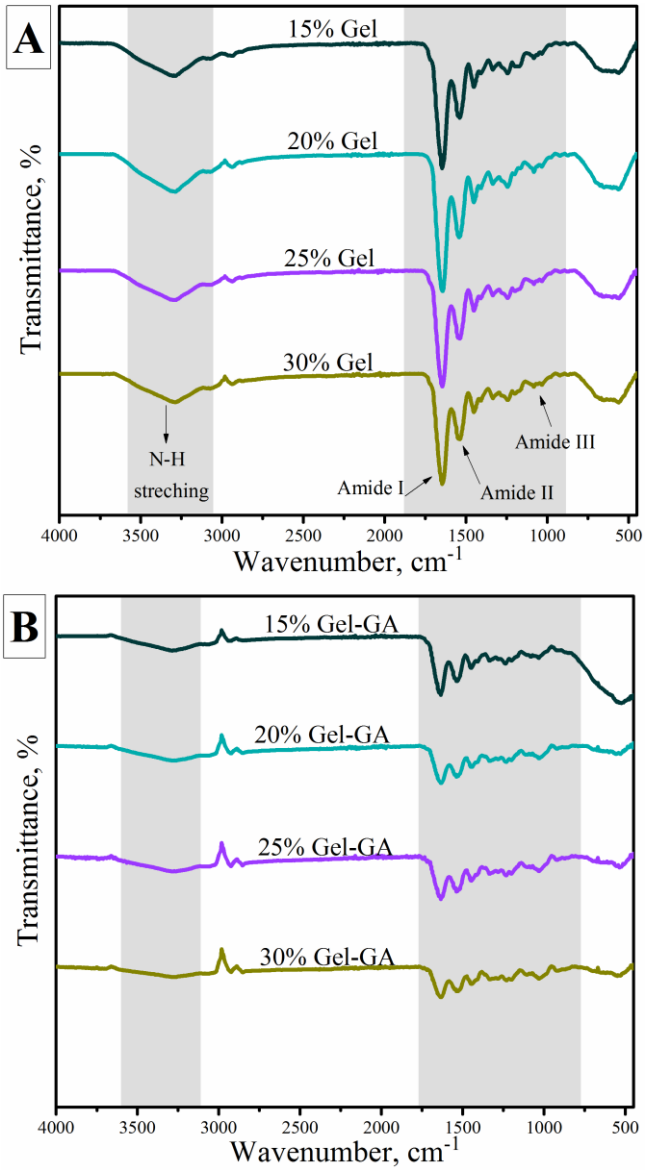

Figure 2. The FTIR spectrum of nanofibers before and after crosslinking with glutaraldehyde

3.2. Morphological observations of the electrospun nanofibers before and after crosslinking

Beads-free and smooth gelatin nanofibrous scaffolds were fabricated by electrospinning. The SEM images at different magnifications (700KX and 6000KX) and fiber size distribution curves of the electrospun scaffolds which were fabricated from different concentrations of gelatin solutions (15, 20, 25 and 30%, w/v) are presented in Figure 3 and Figure 4, respectively. From the SEM images in Figure 3, it is clearly observed that beads-free and uniform nanofibers were produced with an average diameter ranging from 142.47 to 451.64 nm. The average fiber diameters of the scaffolds were significantly

different from each other because of the increased gelatin concentration.

With increasing the polymer concentration, fibers with larger diameters were obtained due to the increase in viscosity of the gelatin solution (Soundrapandian et al. 2010). In addition to fiber diameter, the size of the pores between fibers has an important role in the ability of the cells to infiltrate into the electrospun scaffold. It was observed that the porosity of the electrospun gelatin scaffold changed depending on the fiber diameter. Lower pore size was obtained in scaffolds with smaller sized fiber diameters.

The effect of concentration on the diameter of gelatin nanofibers can also be examined from the fiber diameter distributions showed in Figure 4A, B, C and D.

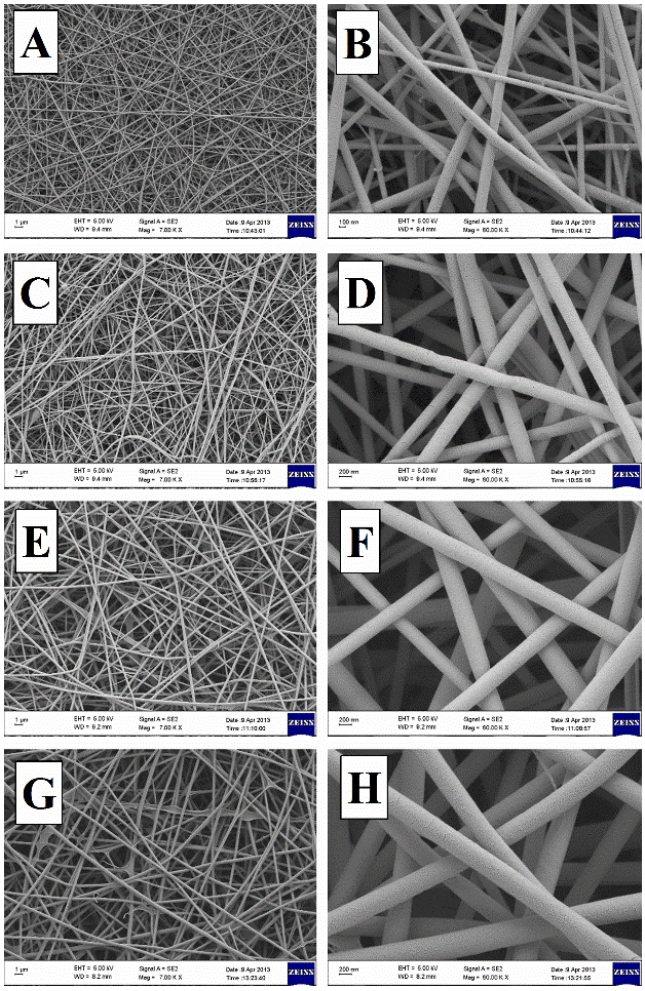

Figure 3. SEM microphotographs of electrospun gelatin scaffolds before crosslinking with glutaraldehyde. A, B: 15% Gel; C, D: 20% Gel; E, F: 25% Gel and G, H: 30% Gel

The morphology of the crosslinked nanofibers at different gelatin concentrations with a constant amount of glutaraldehyde are shown in Figure 5. Compared with Figure 3, although the nanofibrous form had been completely preserved for all samples, the pore size of the scaffolds formed between nanofibers was changed due to the junctions at connection points of nanofibers. This can be explained by the partial dissolution of gelatin fiber segments due to the interaction of water molecules in glutaraldehyde vapor (Laha et al. 2016).

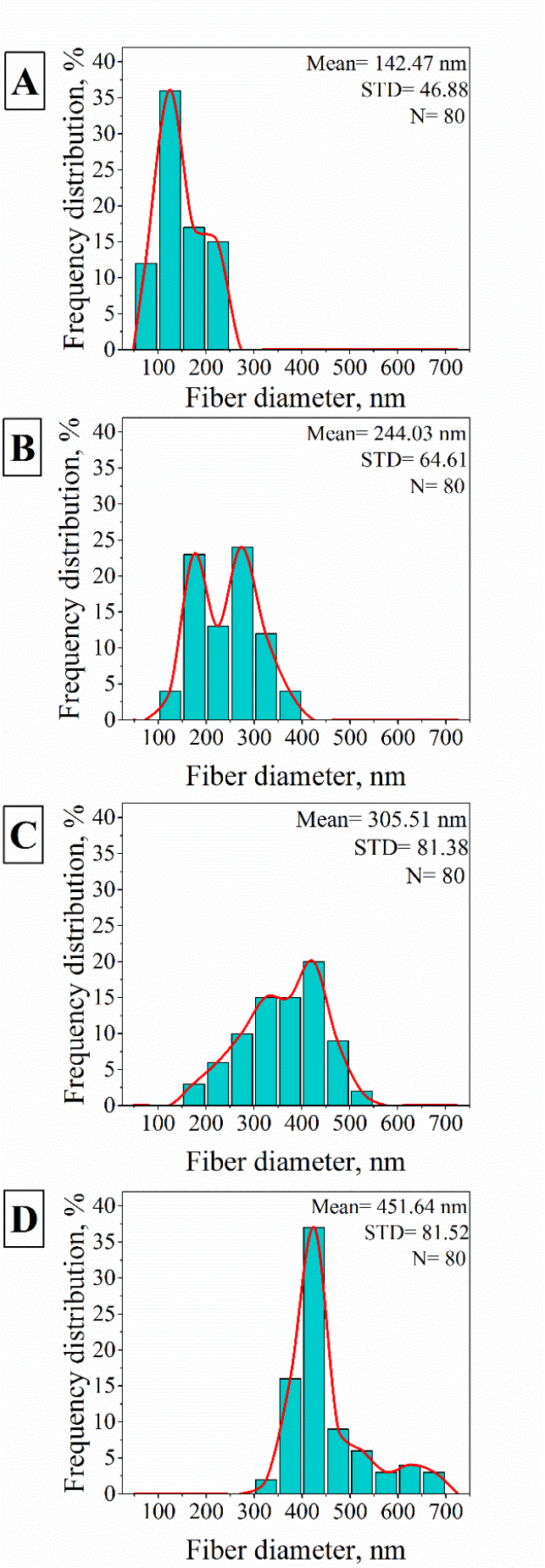

Figure 4. Fiber size distribution curves of electrospun gelatin scaffolds before crosslinking with glutaraldehyde. A: 15% Gel; B: 20% Gel; C: 25% Gel and D: 30% Gel

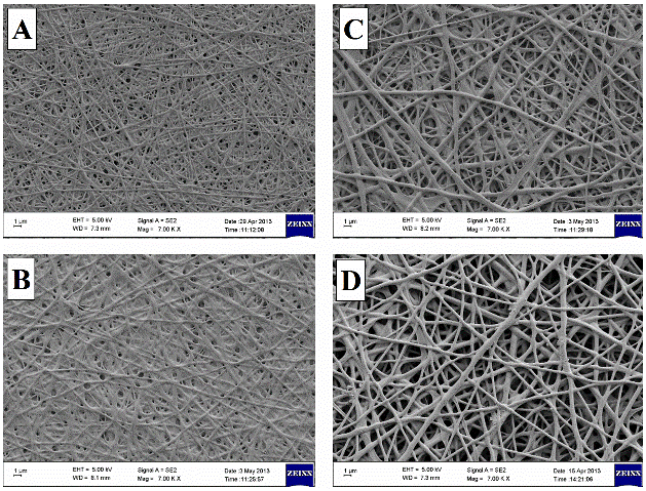

Figure 5. SEM microphotographs of electrospun gelatin scaffolds after crosslinking with glutaraldehyde. A: 15% Gel-GA; B: 20% Gel-GA; C: 25% Gel-GA and D: 30% Gel-GA

3.3. Degradation studies of nanofibers after crosslinking with glutaraldehyde

The degradation profile of the scaffolds is a crucial factor for tissue engineering applications. The degradation of the electrospun gelatin scaffolds after crosslinking was determined by gravimetric analysis as seen in Figure 6. These studies were performed in order to simulate degradation behavior of the fibrous scaffolds *in-vitro*. According to the examined degradation results, it is observed that as the gelatin concentration increases, the degradation decreases. 30% Gel-GA nanofibrous scaffold incubated for 21 days did not lose much mass, indicating minimal solubilization of the nanofibers in the distilled water over the time of the experiment. This can be explained by the increasing fiber diameter with increasing gelatin concentration. As the nanofiber diameter increased, the total surface area decreased and as a result, the degradation also decreased. When nanofibers prepared in low gelatin concentration are examined, it is seen that the degradation increases with decreasing gelatin concentration. The significant differences are found in the degradation between the low (15% Gel) and high (30% Gel) concentrations of nanofibrous scaffolds ($p<0.05$). The reason for the fast degradation of 15% Gel scaffold could be that the thin nanofibers have a higher ratio of surface-to-volume than fibers with larger dimensions. Similarly, Jeong et al. 2005 showed that the degradation of electrospun poly (butylene succinate) fibers was faster for ultrafine fiber diameters. In addition, the weight loss of the scaffolds increased with increasing incubation time.

4. CONCLUSIONS

Electrospinning is an efficient method to fabricate nano-sized fibers from both natural and synthetic polymers for tissue engineering applications. In this study, we electrospun nanofibrous scaffolds from different amounts of gelatin as a natural polymer. The beads free and smooth nanofibers were successfully fabricated. The nanofibers were then crosslinked with glutaraldehyde vapor to prevent the dissolution of scaffolds in aqueous media. The fabricated scaffolds before and after crosslinking were characterized by chemical and morphological analyzes. The morphological structure of the nanofibers changed depending on the gelatin ratio. The fabricated gelatin scaffolds have the potential to have wide applicability in a large number of tissue engineering applications due to their high surface area.

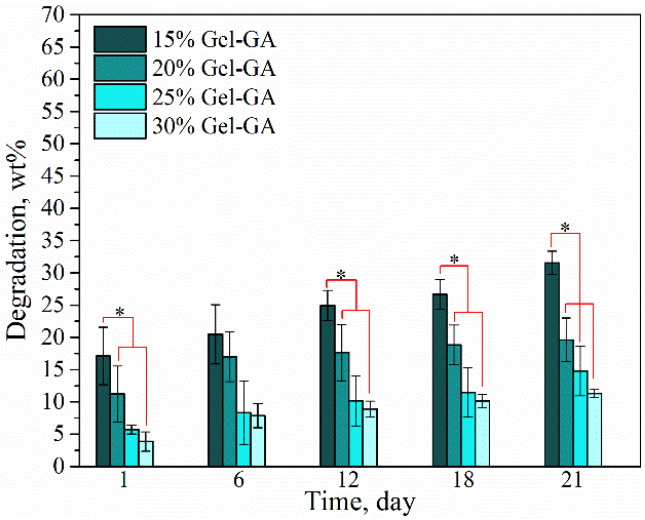

Figure 6. The degradation profile of the crosslinked gelatin nanofibers. (n=3). Data are presented as mean=SD. *$p<0.05$ compared to cryogels by days with an increase in gelatin concentration

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