# Nanofibrous Scaffold Prepared by Electrospinning of Poly(vinyl alcohol)/Gelatin Aqueous Solutions

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**ABSTRACT:** A series of nanofibrous scaffolds were prepared by electrospinning of poly(vinyl alcohol) (PVA)/gelatin aqueous solution. PVA and gelatin was dissolved in pure water and blended in full range, then being electrospun to prepared nanofibers, followed by being crosslinked with glutaraldehyde vapor and heat treatment to form nanofibrous scaffold. Field emission scanning electron microscope (FESEM) images of the nanofibers manifested that the fiber average diameters decreased from 290 to 90 nm with the increasing of gelatin. *In vitro* degradation rates of the nanofibers were also correlated with the composition and physical properties of electrospinning solutions. Cytocompatibility of the scaffolds was evaluated by cells morphology and MTT assay. The FESEM images revealed that NIH 3T3 fibroblasts spread and elongated actively on the scaffolds with spindle-like and star-type shape. The results of cell attachment and proliferation on the nanofibrous scaffolds suggested that the cytotoxicity of all samples are grade 1 or grade 0, indicating that the material had sound biosafety as biomaterials. Compared with pure PVA and gelatin scaffolds, the hybrid ones possess improved biocompatibility and controllability. These results indicate that the PVA/gelatin nanofibrous have potential as skin scaffolds or wound dressing. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 3047–3055, 2011

**Key words:** electrospinning; PVA; gelatin; nanofiber; scaffold

## INTRODUCTION

In recent years, electrospun nanofibers and their potential as biomaterials have been intensively investigated. On account of being similar to natural extracellular matrix (ECM) in architecture, nanofibrous scaffolds could provide suitable and beneficial surroundings for cell attachment and proliferation.[1,2] Plenty of studies revealed that electrospun scaffolds possessed enhanced engraftment rate, optimal cellular organization, and reduced wound contraction than porous scaffolds with micron pores on wound healing test.[3–5]

Selection of materials is a key factor for fabrication of scaffolds. Synthetic biopolymers such as poly (vinyl alcohol) (PVA), poly(ethyl oxide), poly(ε-caprolactone), poly(lactic-*co*-glycolic acid), and polylactic acid and natural ECM analogs such as collagen, gelatin, hyaluronic acid, and chitosan are frequently used to fabricate nanofibrous scaffolds for dermal replacement or wound dressing and other biomedical applications.[4–7] However, their impedimental biodegradation rate may limit their application in soft tissue engineering. ECM analogs such as collagen and gelatin favor tissue regeneration. However, cytotoxic solvents, such as 2,2,2-trifluoroethanol, 1,1,1,3,3,3-hexafluoro-2-propanol, trifluoroacetic acid, formic acid, and dimethylformamide, are generally used for their electrospinning. What's more, the mechanical properties of the electrospun scaffolds are not satisfied.[8–11]

Among these synthetic polymers, PVA is one of the few cases that can be successfully electrospun in aqueous solution to obtain nanofibrous mates with sound mechanical properties. It is a biocompatible and biodegradable polymer, which has been widely used in biomedical fields such as wound dressing and biodegradable scaffolds.[12] However, owing to its insufficient cell recognition sites, the bioactivity

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of PVA is substantially restricted. On the contrary, gelatin is a natural polymer derived from collagen by controlling hydrolysis, which possess many integrin-binding sites for adhesion and differentiation.[5] To combine the advantages of PVA and gelatin, the PVA/gelatin hybrid scaffolds are expected to own sound mechanical properties and biocompatibility and meanwhile acquire promising cell adhesion and biodegradation properties. In addition, the possibility to fabricate PVA/gelatin nanofibers from aqueous solution will significantly reduce the risk of material cytotoxicity. Furthermore, pure gelatin nanofibrous scaffolds were too fragile to be handled in practical application.[10] Therefore, the hybrid of PVA with gelatin will significantly enhance the flexibility of the gelatin scaffolds.

Some previous studies on PVA/gelatin materials and PVA/gelatin nanofibers have been reported. Kunal et al. prepared the PVA/gelatin hydrogel membranes by esterification. The high hemocompatibility of hydrogel have been evaluated, and it indicated that the PVA/gelatin hydrogel have potential for various biomedical applications, such as moist wound dressing.[13] Linh et al. fabricated PVA/gelatin electrospinning nanofiber in water-acetic acid solvent, and Huang et al. fabricated PVA/gelatin in deionized water solvent. Both of them studied the effects of instrument parameters on the physics properties of nanofibers. However, the biological evaluation of PVA/gelatin nanofibers was absent.[14,15]

In this study, PVA/gelatin nanofibrous scaffolds were prepared by electrospinning of PVA/gelatin aqueous solution and glutaraldehyde crosslinking. The morphology and the molecular structure of the scaffolds were studied by SEM and Fourier transform infrared (FTIR). Biodegradability of the scaffolds was investigated by *in vitro* degradation in phosphate buffer solution (PBS). NIH 3T3 fibroblast was cultured on the scaffolds to evaluate the cytotoxicity and cell viability of the scaffolds. The results will support these materials for their application as wound dressing or scaffolds for soft tissue.

## MATERIALS AND METHOD

### Materials

PVA (degree of hydrolysis 88 ± 2%, $M_w$ = 88,000) was obtained from Acros Organics. Gelatin of type A (300 bloom) from porcine skin was purchased from Sigma Chemicals. Glutaraldehyde solution (50%) was supplied by Beijing Chemical Reagents. All other chemicals were analytical reagent and used as received.

### Preparation and characteristics of the polymer solution

The 8 wt % PVA and 8 wt % gelatin aqueous solutions were prepared at 40°C independently. Then, the 8 wt % PVA solution was mixed with 8 wt % gelatin solution with different weight ratios (PVA/gelatin 100/0, 80/20, 60/40, 40/60, 20/80, 0/100), by continuously stirring for 2 h to form homogeneous solutions.

The shear viscosities of the mixed solutions were measured by rotational viscometer (Dial Reading-Viscometer, Brookfield Engineering Laboratories, Massachusetts, USA). The conductivity of the solutions was determined by electric conductivity meter (DDSJ-308A, Shanghai Precision & Scientific Instrument, China). Surface tension of the solutions was measured by surface tensionmeter (BZY-1, Shanghai Hengping Instrument, China) at 40°C.

### Fabrication and crosslinking of the nanofibrous scaffolds

The nanofibrous scaffolds were fabricated using a electrospinning equipment.[14,15] Briefly, the homogenous hybrid solutions were preserved in 40°C for 2 h, transferred to a 5-mL syringe equipped a blunt needle (diameter 0.4 mm) and then connected with a high voltage generator (Model SL 60, 0-50 kV, Spellman High Voltage Electronics). Polished titanium plate was used as collector, and the distance between the spinneret and collector is 10 cm. The solution feed was driven by gravity and the electrostatic force. The electrospinning procedure was performed at 40°C. The scaffolds were dried at 120°C for 2 h and then crosslinked with glutaraldehyde vapor for 8 h at 40°C, followed by heating at 120°C for another 12 h to remove residual glutaraldehyde and enhance the crosslinking density.

### Morphology

Morphology of the nanofibrous scaffolds was studied by field emission scanning electron microscope (FESEM; S-4800, Hitachi, Japan). Before the observation, surface of the scaffold was sputter-coated with gold.

Fiber diameters were calculated from the SEM images using Adobe Photoshop 7.0 software. Average diameter was determined by measuring the diameters of 20 random selected fibers.

### FTIR analysis

FTIR spectra were recorded by Nicolet Magna-IR 750 with Nicolet NicPlan IR microscope attachment. The crosslinked samples were scanned at the range of 4000–600 $cm^{-1}$ with the resolution of 2 $cm^{-1.}$

### *In vitro* degradation in phosphate buffer solution

Biodegradability of the scaffolds was evaluated by monitoring the changes of the morphology of the fibers caused by *in vitro* degradation. Samples collected on the titanium plate were incubated in 5-mL PBS at 37°C. The scaffolds were taken out and dehydrated in a series of graded ethanol (50, 70, 90, 99, and 100%) after incubated for 1 and 5days, respectively.

### Cell culture, attachment, and proliferation

#### Cell culture

NIH 3T3 fibroblasts were cultured in medium that consists of Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) fetal bovine serum containing 50 U/mL penicillin/streptomycin (p/s: GIBCO: Grand Island, NY) and incubated at 37°C, 5% $CO_2$. The medium was refreshed every 2 days, and the cells were harvested with PBS containing 0.25% (w/v) trypsinase and 5 mmol/L ethylene diamine tetraacetic acid, centrifuged, and resuspended in the fresh DMEM medium.

#### Cell adhesion

Before cell seeding, the samples were sterilized by UV light for 30 min and then immersed in PBS for 10 h to remove the sol parts and residue glutaraldehyde on the fibers. After that samples were placed in a 24-well plate. NIH 3T3 fibroblasts at a density of $2 \times 10^6$ cells/well were seeded onto the surface of the scaffolds, and the cells were incubated for 6 h as described above. Each sample was assayed in triplicate, and the tissue culture plate was used as control.

Cell adhesion on the surface of the scaffolds was assessed by MTT assay.[16] Before adding MTT solution, the samples were rinsed with PBS for three times to remove the nonattached cells. Tissue culture polystyrene containing only the cells and the culture medium were served as negative controls. The cell adhesion was defined as:

$$\text{Cell adhesion} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

#### Cell proliferation

Cells were seeded onto the surface of the scaffolds in 24-well plate at a density of $5 \times 10^4$ cells/well. The culture medium was refreshed every 2 days. Cells cultured on the tissue culture plate were used as control group. After different time intervals (1, 2, 3, and 4 days) of culturing, the number of viable cells attached on the surface of the scaffolds was determined by MTT assay.

The morphology of viable cells growing on the scaffolds was investigated after 72 h of incubation. Samples were first fixed with 10% (v/v) formalin for 20 min and then dehydrated in an ethanol series (75, 80, 90, 99, and 100%) for 20 min, respectively. After that the samples were lyophilized for SEM observation.

### Statistical analysis

All data were expressed as mean ± standard deviations (SD). Statistical significance of differences between means was determined by one-way analysis of variance (ANOVA). *P*-values less than 0.05 denote statistical significance.

## RESULTS AND DISCUSSION

### Preparation and characterization of PVA/gelatin nanofibrous scaffolds

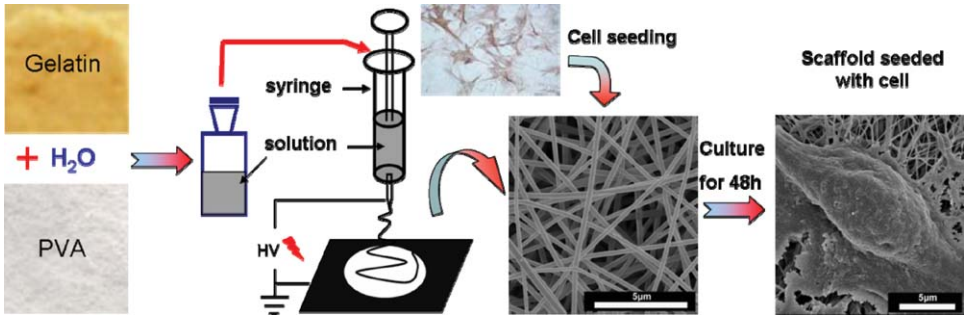
#### Preparation routine of the scaffolds

Natural and synthetic materials are widely used to fabricate nanofibrous scaffolds. Synthetic materials such as PVA have low cell affinity due to the lack of surface cell-recognization sites. Meanwhile, the demerits of some natural materials such as gelatin and collagen are their weak physical properties and processability. Therefore, the composite scaffolds containing both synthetic and natural materials are expected to provide desired properties for the application of biomedical purpose.

Scheme 1 manifests the preparation procedure of PVA/gelatin nanofibrous scaffolds. Gelatin was blended with PVA to fabricate nanofibrous scaffolds with sound biocompatibility and processability. To keep lower cytotoxicity of the scaffolds, gelatin and PVA were both dissolved in distilled water for electrospinning. After that the mats were crosslinked by glutaraldehyde vapor for 8 h at 40°C and heated at 120°C for 18 h to remove the residual glutaraldehyde and enhance the crosslinking density. To evaluate the cytocompatibility and potential of the scaffolds for skin tissue regeneration, mouse NIH 3T3 fibroblasts were seeded onto the nanofibrous mats.

#### Morphology of fiber and impact of the solution on electrospinning procedure

Some physical properties of the electrospinning solutions, such as temperature, composition, viscosity, and surface tension, which significantly influence the electrospinning processability and morphology of the resulting nanofiber, were measured.[17–20] As




**Scheme 1** Illustration of the procedure for preparing PVA/gelatin nanofibrous scaffolds. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

shown in Table I, with increasing the amount of gelatin in the blend, the viscosity significantly decreased from 193 cP (PVA) to 12.5 cP (gelatin), meanwhile the conductivity increased significantly and the surface tension slightly increased.

Gelatin is usually dissolved in organic solvents instead of water for electrospinning due to the low evaporability of water. Their high viscosity in room temperature, conductivity, and surface tension also caused the low processability. Only a few reports showed that aqueous solution of gelatin can be electrospun at elevated temperature.[21] In this study, the ambient temperature was set to 40°C during the electrospinning. PVA/gelatin blend solutions in full range ratios could be successfully electrospun to smooth and thin fibers (Fig. 1). With the increasing of gelatin, the average diameter of fiber decreased from about 290 nm (pure PVA) to about 90 nm (pure gelatin; Fig. 2). Correspondingly, the diameter distribution of the fibers narrowed down slightly with the increase of gelatin.

Polymer solutions with higher viscosity tend to form smooth fibers because the longer stress relaxation times could prevent the fracturing of ejected jets during electrospinning.[21] However, when the viscosity exceeds a critical point, lower viscosity will result in smaller fiber diameter.[18,19,21] Therefore, the PVA-rich nanofibers were smooth and straight, and with the increased amount of gelatin, spindles, and defects frequently appeared; at the same time, the diameters of the fibers decreased from about 290 nm (pure PVA) to about 90 nm (pure gelatin). On the other hand, the higher amount of gelatin will, to some extent, enhance the bioactivity of the scaffold.

Yang et al.[9] electrospun gelatin/PVA nanofibers using formic acid solution as solvent. It was found that tensile strength and elongation at break of gelatin/PVA nanofibers decreased as the ratio of gelatin increased, and pure gelatin nanofibrous mat was too brittle to be handled and measured. Zhang et al.[22] electrospun gelatin nanofibers in aqueous solution. Although the tensile strength and elongation at break in dry status could be determined (2.44 Mpa and 1.3%), the rather low elongation at break implied its brittleness.

In this study, similar phenomenon was observed, that is, pure gelatin could not be peeled off from the collector due to its weak mechanical properties. However, the PVA/gelatin fibrous scaffolds possess certain flexibility owning to the incorporation of PVA.

### FTIR analysis

Figure 3 displays the FTIR spectra of PVA, gelatin, and PVA/gelatin fibrous scaffolds. In PVA spectrum, the broad band in the range of 3000–3500 $cm^{-1}$, and the distinct absorption peak at 2945 $cm^{-1}$ was attributed to the O—H stretching vibration, and the 2914 $cm^{-1}$, which represents C—H stretching vibration was merged in 2945 $cm^{-1}$ peak. The strong peak at 1735 $cm^{-1}$ was ascribed to C=O stretching vibration of the residue carboxyl group during hydrolysis reaction of PVA material.

The spectra of gelatin showed characteristic peaks in the range of 3100–3500 $cm^{-1}$ due to N—H stretching of secondary amide, C—H stretching at 2945 $cm^{-1}$, C=O stretching between 1680 and 1620 $cm^{-1}$, and N—H bending between 1550 $cm^{-1}$ and 1500 $cm^{-1}$.[6,23] The broad N—H bending and C=O

**TABLE I**
**Physical Properties of PVA/Gelatin Electrospun Solutions**

| PVA/gelatin (wt %/wt %) | 100/0 | 80/20 | 60/40 | 40/60 | 20/80 | 0/100 |
|---|---|---|---|---|---|---|
| Conductivity (μs/cm) | 336.5 ± 2.6 | 487.5 ± 5.1 | 662.3 ± 5.5 | 829.2 ± 7.4 | 1010.1 ± 3.9 | 1153.6 ± 15.6 |
| Viscosity (mPa s) | 193.3 ± 6.3 | 172.5 ± 2.2 | 47.5 ± 0.0 | 38.7 ± 1.0 | 24.6 ± 0.4 | 12.5 ± 0.5 |
| Surface Tension (mN/m) | 29.4 ± 1.2 | 30.1 ± 0.7 | 29.0 ± 0.6 | 29.8 ± 0.9 | 30.4 ± 0.4 | 32.4 ± 0.6 |

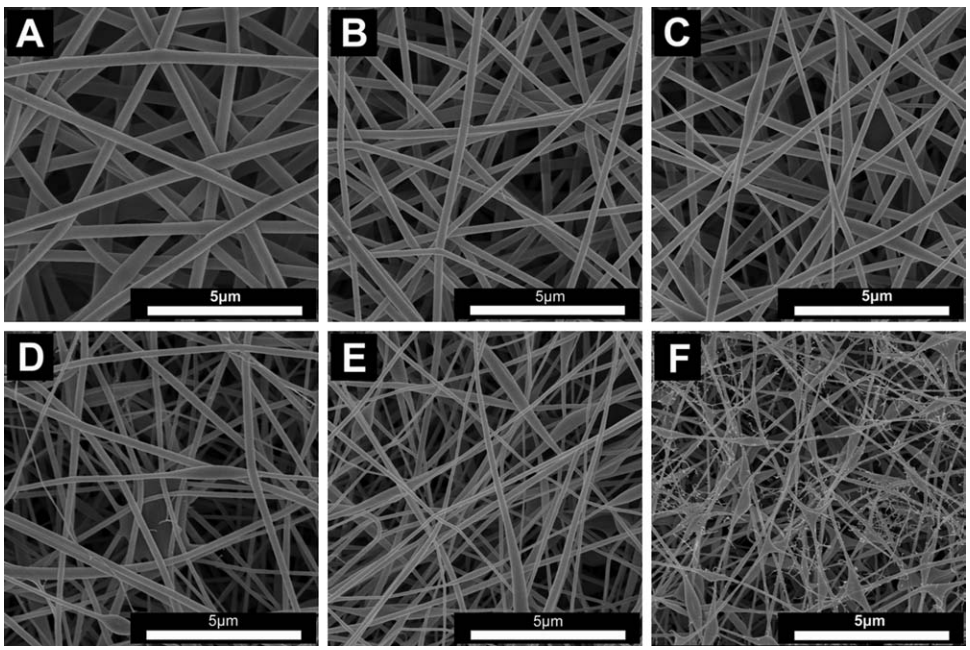



**Figure 1** SEM images of PVA/gelatin blended fibers (A) PVA/gelatin = 100/0, (B) PVA/gelatin = 80/20, (C) PVA/gelatin = 60/40, (D) PVA/gelatin = 40/60, (E) PVA/gelatin = 20/80, (F) PVA/gelatin = 0/100.

stretching of gelatin implied that the carboxyl and amide group of gelatin tend to form hydrogen bond network. However, in Figure 3(B–D), the N—H bending peak at about 1534 $cm^{-1}$ and C=O stretching peak at about 1650 $cm^{-1}$ became narrow and incisive, which indicate the hydrogen bond formed inside gelatin was interrupted with the incorporation of PVA. At the same time, C=O stretching of carboxyl group at 1735 $cm^{-1}$ became weak when portion of gelatin increased. This result can be interpreted by the formation of intermolecular hydrogen bonds between residue carboxyl group of PVA and amide group of gelatin, which lead to the good compatibility of the two materials. Meanwhile, the interruption of intramolecular hydrogen bond by PVA resulted in the improved processability compared with pure gelatin. Finally, semi-interpenetrate-network was achieved after blending and crosslinking of PVA and gelatin

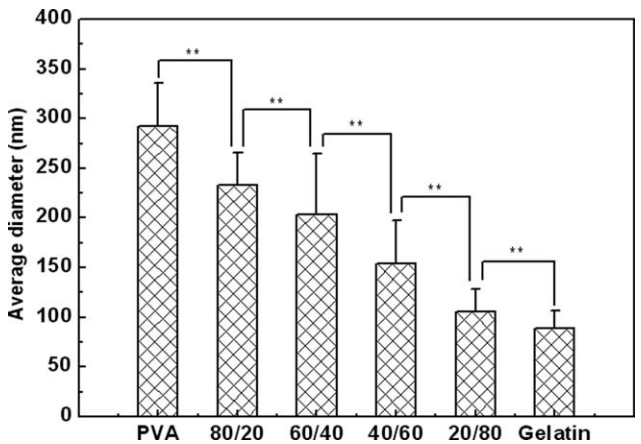



**Figure 2** Diameter distributions of nanofibers for different composition. All data are expressed as mean ± SD, n = 90, ** $P < 0.01$.

## Crosslinking of PVA/gelatin nanofibers and *in vitro* degradation

PVA and gelatin are both water-soluble. Therefore, the nanofibers were crosslinked by glutaraldehyde

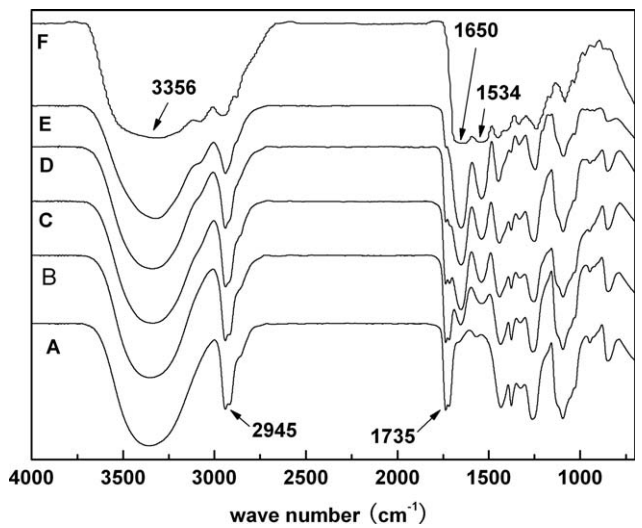



**Figure 3** FTIR spectra of electrospun PVA/gelatin blended fibers (A) PVA/gelatin = 100/0, (B) PVA/gelatin = 80/20, (C) PVA/gelatin = 60/40, (D) PVA/gelatin = 40/60, (E) PVA/gelatin = 20/80 (F) PVA/gelatin = 0/100.

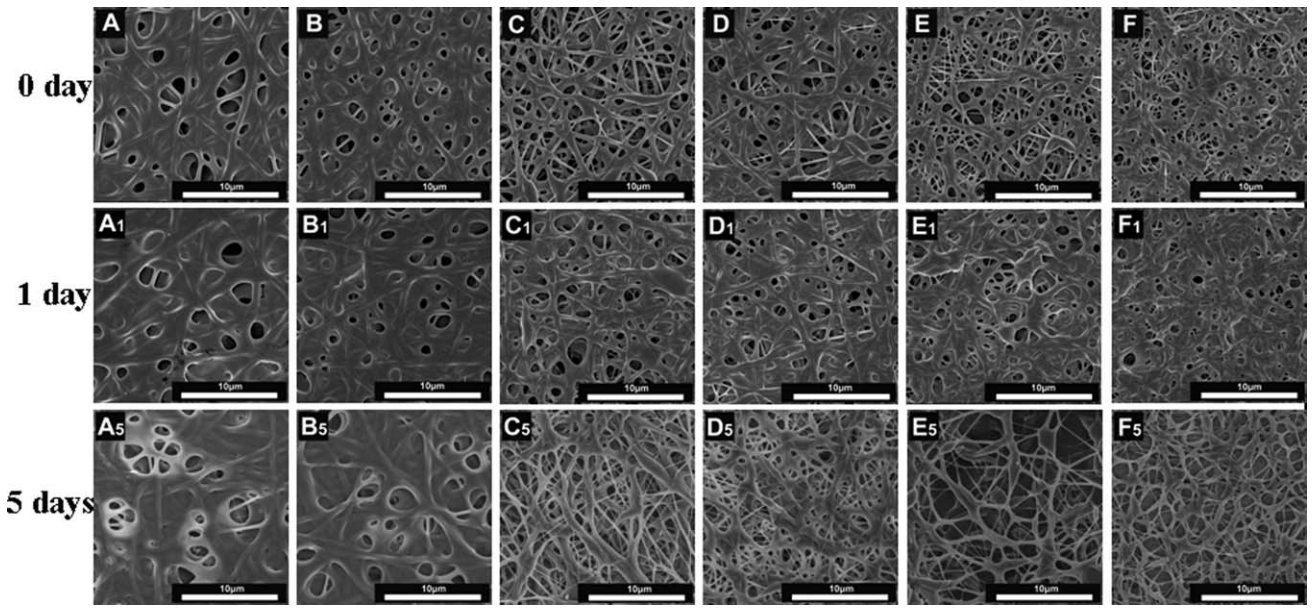



**Figure 4** SEM images of the PVA/gelatin nanofibers before and after degradation in PBS at 37°C for 1 and 5 days.

vapor, and the after-heated procedure not only got rid of residue glutaraldehyde but also increased the crosslinking density. Figure 4 presents the result of *in vitro* degradation of PVA/gelatin nanofiber mats in PBS solution for 1–5 days. The FESEM images of crosslinked nanofiber mats manifested that the crosslinking was efficient, the scaffolds was steady after immersing in PBS solution after 5 days, and the morphology of scaffold when immersed after 10 days was similar (the data was not shown), meaning it had a sound stability against degradation up to 10 days. It indicated that the scaffolds would retain stability during first several days if being applied as wound dressing, which could match the healing rate of wound. It should be noted that this study has examined only *in vitro* degradation, and *in vivo* studies will be carried out in our further work.

Figure 4 also displayed morphological alterations of the PVA/gelatin nanofibers with full range ratios during incubated in PBS at 37°C after a period of 1 and 5 days. It is clear that the fibrous network experienced significant swelling after 24 h immersing in PBS solution. After 5 days, the weakly crosslinked part of the gelatin-rich scaffolds deteriorated more significantly than the strongly crosslinked part, and the remaining network was connected by the fiber joint knots. The new generated vacancy during degradation would improve the blood and oxygen permeation for conveying nutrient for the proliferation of cells; what's more, the strong network still have sound mechanical properties to serve as cells scaffold.

## Cell culture, attachment, and proliferation

### Morphology of NIH 3T3 fibroblasts on the scaffold

The morphology of cells attaching on surface of the materials reveals the cytocompatibility of the scaffolds. When cells contact with materials, they undergo morphological changes to adapt to the cell-material surface.[24] FESEM image of NIH 3T3 fibroblasts on the scaffolds after 72 h culturing manifested that the cells spread well and attached firmly on the scaffold surface (Fig. 5). In Figure 5 (B–E), cells grew better than other groups. These cells have spread with spindle and flatten morphology extensively and randomly, indicating that the nanofibrous scaffolds fabricated in this work have excellent biocompatibility and nontoxic to the growth of NIH 3T3 fibroblasts.

### Cell attachment and proliferation

Cell attachment and proliferation on biomaterials are essential to evaluate their potential as scaffolds. The properties of matrix, such as hydrophobicity/hydrophilicity, surface morphology, mechanical strength, stiffness, molecular structure, and surface functional groups, will affect the cell attachment and proliferation on scaffolds.[25] PVA has excellent processability; although gelatin could enhance the attachment and proliferation of cells by providing cell recognition sites.[5,26] Therefore, the PVA/gelatin hybrid scaffolding should have certain cell-material interaction and cell motility.[27]

The cell adhesion efficiency was quantified by MTT assay of NIH 3T3 fibroblast on the nanofibrous scaffolds after incubation for 6 h. Figure 6 shows

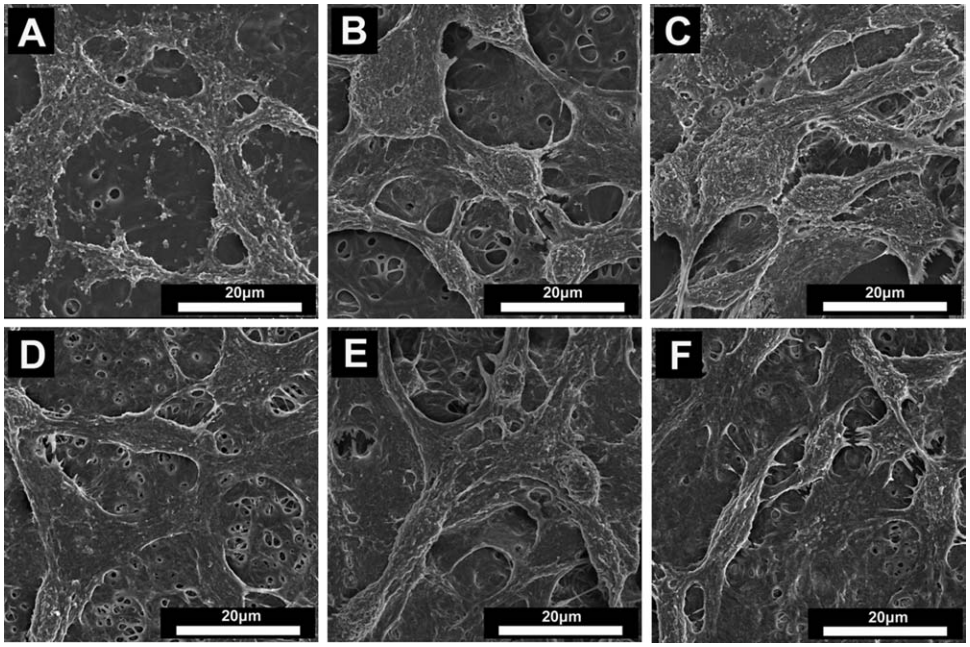



**Figure 5** SEM images of NIH 3T3 fibroblast cells on the nanofibrous scaffolds after culturing for 72h. (A) PVA/gelatin = 0/100 (B) PVA/gelatin = 80/20 (C) PVA/gelatin = 60/40 (D) PVA/gelatin = 40/60 (E) PVA/gelatin = 20/80 (F) PVA/gelatin = 0/100.

that the adhesion rate of all samples was around 100%, indicating that the material was noncytotoxic (toxicity 0 grade). At the initial stage of cell incubation (6 h), the cells adhere to the scaffold, and the adhesion rate of PVA scaffolds was higher than other groups. The higher adhesion rate in PVA scaffold could be ascribed to the higher hydrophilicity and smoothness of the fiber. On the contrary, pure gelatin fibers seemed to be disfavored by cells, probably for sake of its irregular fiber structure (i.e., the presence of deficiencies and beads) and insufficient mechanical properties.

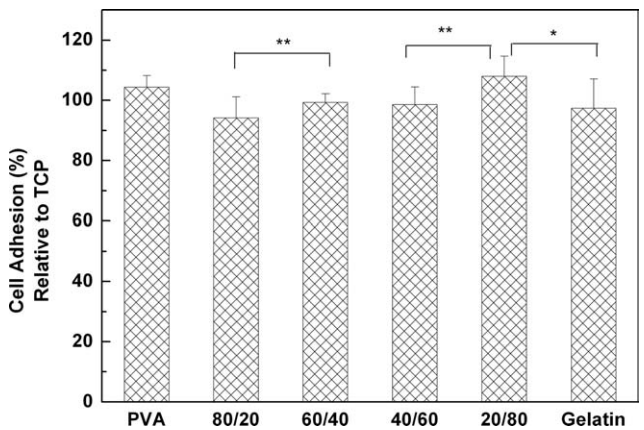



**Figure 6** Quantitative analysis of NIH 3T3 fibroblast cell attached on various scaffold after incubation for 6 h, which was determined by MTT assay from triplicate samples. Results were compared with cells growing on TCP and data were expressed as mean ± SD, $*P < 0.05$, $**P < 0.01$.

Figure 7 presents the proliferation result by MTT assay after culturing for 1, 2, 3, and 4 days on the scaffolds. With the progress of cell incubation (1–4 days), the cells began to proliferate on the scaffold, and the population of NIH 3T3 fibroblasts growing on the scaffold surfaces significantly increased in a period of 4 days. Viability of cells on the scaffold was nearly the same at the first day and on the second day PVA60/gelatin40 became higher. However, on the fourth day,

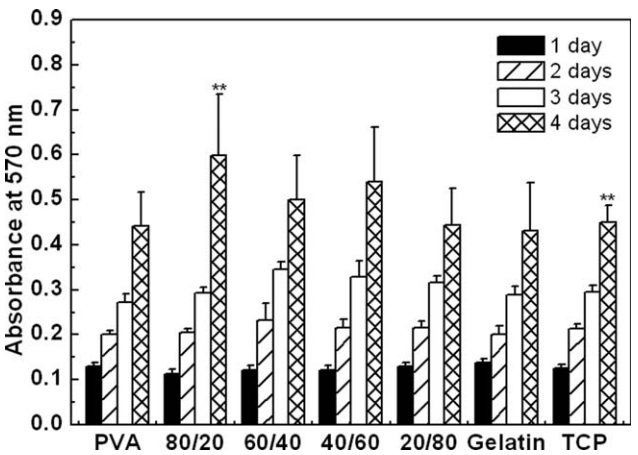



**Figure 7** Proliferation of NIH 3T3 fibroblast cells seeded on the nanofibrous scaffolds. Cells were cultured on the electrospun PVA/gelatin composite fibers with weight proportions of 100/0; 80/20; 60/40; 40/60; 20/80, 0/100 and TCP substrates over 4 days. The degree of proliferation was measured by MTT assay. All data are expressed as mean ± SD, n = 9, $*P < 0.05$, represent that values are significantly different from the previous groups.

the number of cells on the PVA80/gelatin20 scaffold was much higher than other groups. Generally speaking, cells proliferated better on scaffolds with PVA/gelatin composition of 80/20, 60/40, 40/60 than the others. It suggests that proper composition and stiffness are beneficial to cell growth.

Both the SEM and MTT assay demonstrated that the cells could attach and proliferate well on the scaffolds. According to the results of MTT assay, the relative growth rates of all the samples were higher than 90%, which was classified to grade 1 or grade 0 by USP standard for material cytotoxicity. The slight cytotoxicity suggests that PVA/gelatin nanofibrous scaffolds have sound biosafety as biomaterials. The higher adhesion rate in PVA scaffold could be ascribed to the higher hydrophilicity, and smoothness of the fiber; although the higher proliferation of gelatin-rich fibers than PVA scaffold could be ascribed to the large amount of cell recognition sites of gelatin. Other factors, such as fiber diameters, should also impact the cell adhesion and proliferation. Aiming at improving the material bioactivity, many researches focused on preparation of nanofibers with smaller diameters to increase the surface area. However, some research indicated that the dense nanofiber mesh (with fiber diameters less than 100 nm) would lost its advantage of three-dimensional material and essentially behave as a two-dimensional sheet. The cells are able to migrate along the surface and hardly infiltrate the two-dimensonal scaffold.[28] Therefore, optimized fiber or pore diameters will benefit for the cell growth, and the optimal sizes vary for different kinds of cells. In this study, the lower cell growth rate on pure gelatin scaffold might be caused by the low fiber diameter, which would limit the ability of cells to infiltrate the scaffold material. Thus, the pure gelatin fibers, with the diameters of 90 nm, formed more dense networks than the others [Fig. 2(F)], which might influence the proliferation and infiltration of cells. For the other composition, although the diameter varied with the change of compositions, the impact of composition might be more significant than the diameter, and therefore, the correlation between diameter and cell growth is ambiguous.

Besides the promising composition, the biosafe fabrication procedure is another factor for the excellent cell proliferation of the scaffolds prepared in this work. Zhang et al.[6] prepared nanofibrous scaffold by dissolving gelatin in 2,2,2-trifluoroethanol and then crosslinked by glutaraldehyde vapor. Their results displayed that the scaffold was cytotoxic and negative to the cell growth compared with the control groups, which was ascribed to the residual crosslinker of glutaraldehyde vapor. In this work, the scaffolds were crosslinked with glutaraldehyde vapor as well; however, the cell proliferation is satisfying after the heat treatment at 120°C and thoroughly rinsing by PBS, which effectively removed the residual glutaraldehyde from the scaffold. Furthermore, pure water was used to prepare solutions for electrospinning, which played significant role in the good biocompatibility of PVA/gelatin nanofibrous scaffold. As a result, besides the nature of materials, the selection of a green fabrication approach is also remarkably important for the design of scaffold for tissue engineering.

In this work, we studied the preparation and characterization of PVA/gelatin nanofibrous mats, and a preliminary evaluation of the material cytotoxicity was also presented by MTT assay. The results indicated that PVA/gelatin nanofibrous mats had potential as wound dressing. We have planed the animal experiment to further evaluate the feasibility of PVA/gelatin nanofibrous mats as wound healing materials. The results will come out in our future publication.

## CONCLUSIONS

The PVA/gelatin hybrid nanofibrous scaffolds were fabricated by electrospinning. To achieve biomaterials with promising biocompatibility, pure water was used as solvent instead of organic solvents. Insoluble three-dimensional network microstructure of the scaffold was achieved by crosslinking with glutaraldehyde. NIH 3T3 fibroblasts were used to seed on the scaffolds to evaluate the biological properties that affecting the cell attachment and proliferation. The PVA/gelatin hybrid scaffolds were superior to pure PVA and gelatin scaffold in terms of their biocompatibility and handleability. Compared with the control group, NIH 3T3 fibroblasts preferred to adhere and proliferate on the surface of the scaffold, and the cytotoxicity of all scaffolds are grade 1 or grade 0, indicating that PVA/gelatin nanofibers had sound biosafety as biomaterials. Furthermore, cells proliferated better in PVA/gelatin scaffolds with the fraction of 80/20, 60/40, and 40/60 than the others. Hence, the excellent cell growth and proliferation on electrospun PVA/gelatin hybrid nanofibrous scaffolds suggested their potential to be used as soft tissue scaffolds and wound dressing.

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