

# Structure and wettability relationship of coelectrospun poly (L-lactic acid)/gelatin composite fibrous mats

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Coelectrospun polylactide(PLA)/gelatin (GE) composite fibrous matrixes have been identified to exhibit much improved performances compared to the respective components; however, the reasons for their water contact angles decreasing to zero at proper PLA/GE ratios remain unclear. To get a deep understanding of the phenomenon, PLA and GE were coelectrospun with different PLA/GE ratios in this study. Although the resulting composite fibers were homogeneous in appearance, they were detected different microscopic structures by transmission electron microscope (TEM) and via morphological observations after selective removal of either PLA or GE component. Together with the results of degradation study in phosphate buffered solution, a kind of cocontinuous phase separation microstructure could be identified for the PLA(50 wt%)/GE(50 wt%) composite fibers, which also showed the water contact angle of 0°. This value was far lower than those of electrospun PLA (~123°) and GE (~42°) fibrous matrixes. The X-ray photoelectron spectrometry (XPS) data revealed that the polar side groups of protein macromolecules have moved toward composite fiber surface with solvent evaporation during electrospinning, due to the hydrophobic interaction between PLA and GE. Then the excellent hydrophilicity of PLA(50 wt%)/GE(50 wt%) composite fibers could be suggested as the consequence of: (1) the cocontinuous phase separation structure could provide more interface and void for water molecules penetrating; and (2) the accumulation of polar groups on composite fiber surface significantly increased the surface wettability. Copyright © 2010 John Wiley & Sons, Ltd.

**Keywords:** electrospinning; gelatin; poly (L-lactic acid); phase separation

## INTRODUCTION

Many synthetic (e.g. aliphatic polyester) and natural polymers (e.g. proteins and polysaccharides) have been reported to possess the tissue regenerative potentials.<sup>[1,2]</sup> However, the innate concerns associated with synthetic polymers are their poor cell affinity,<sup>[3,4]</sup> while biopolymers are rarely considered as scaffold materials for tissue engineering applications without any special treatment (e.g. cross-linking, hydrophobic modification).<sup>[5–8]</sup> Mixing synthetic and natural polymers is a feasible approach to theoretically circumvent the shortcomings of the individual materials and produce new biomaterials with good performances for tissue engineering applications. Published data have demonstrated polylactide(PLA)/gelatin (GE) and poly(lactide-co-glycolide) (PLGA)/dextran mixtures exhibiting desired cell behaviors and degradation properties depending on components, blending ratio, and processing means.<sup>[9–11]</sup>

Recent developments in tissue engineering have highlighted that nanofibrous scaffolds served as suitable environment for cell attachment and proliferation, due to their mimetic structure of natural extracellular matrix. This increased interest toward employing electrospinning for scaffold fabrication.<sup>[12–14]</sup> Jiang *et al.*<sup>[15]</sup> firstly reported the preparation of coelectrospun composite membranes composed of PLGA and dextran by dissolving them in DMSO/DMF (50/50, v/v) cosolvent. After that, different synthetic/natural polymer pairs were coelectrospun,

among which, PLA, PLGA, or polycaprolactone (PCL) combined with collagen or GE were mostly studied.<sup>[16–23]</sup> Contact-angle measurement and tensile tests indicated that the composite

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Contract/grant sponsor: National Natural Science of China; contract/grant number: 50873012.

Contract/grant sponsor: National High Technology Research and Development Program of China; contract/grant number: 2007AA03Z351.

Contract/grant sponsor: International Science and Technology Cooperation Program 2007; contract/grant number: DFA30690.

Contract/grant sponsor: Program of New Century Excellent Talents (NCET) of Universities in China.

fibrous membrane displayed improved mechanical properties as well as more favorable wettability than that obtained from either component alone. In view of these merits, this kind of nanofibrous scaffold has been proved to have potentials in reconstruction,<sup>[18,21,23]</sup> wound healing,<sup>[24]</sup> and tissue engineering.<sup>[17,25]</sup>

An intriguing but confusing phenomenon discovered in the electrospun PCL or PLA (50 wt%)/GE (50 wt%) composite nanofibrous membranes was that, their water contact angles were lowered to 0° in comparison with the 70–80° of pure GE nanofibrous membranes.<sup>[16,17]</sup> This was thought to be the consequence of a kind of microstructure forming in the fast fiber solidification during electrospinning due to the phase separation between the two components. Zhang *et al.* observed the GE/PCL (1:1 in weight) composite fibers showing an alternative array of GE and PCL segments via optical microscope.<sup>[16]</sup> However, it was a confusion to contribute the zero water contact angle of the GE/PCL (1:1 in weight) composite fibrous sheets to such a kind of microstructure, since the pure GE nanofibrous membranes showed water contact angle around 76.5° as stated by the same author. Nevertheless, no more proofs could be found in published literatures to correlate this interesting phenomenon with the microstructure of the composite fibers.

Electrospun water-soluble collagen and GE nanofibrous matrix themselves should be crosslinked before use; however, some disadvantages, such as the cytotoxicity of crosslinking agents, deformation of fiber morphology, and pore structure, limit their applications as tissue engineering scaffolds.<sup>[6,7]</sup> Among the reports of coelectrospun synthetic/natural polymer composite nanofibers, a majority of authors stated that the composite fibers could retain form without crosslinking treatment because the presence of hydrophobic synthetic polymer was able to prevent the dissolution of biopolymers.<sup>[16–21]</sup> A few deemed that uncrosslinked biopolymer would dissolve into medium in cell culture gradually within short time.<sup>[22,23]</sup>

Hence, two questions remain unclear in the coelectrospun of synthetic/natural polymer composite nanofibers until now. One is why the hydrophilicity of electrospun PLA/GE composite fibrous membrane is significantly improved. The other is if the GE component in the composite fiber needed to be crosslinked before cell culture.

Accordingly, PLA/GE composite fibers were coelectrospun from their mixed solutions in trifluoroethanol with varied weight ratios of PLA to GE in this study, for the attempt to interpret the questions. Several techniques, including contact-angle measurement, selective removal of one component, scanning electron microscope (SEM) and transmission electron microscope (TEM) observations, as well as degradation study had been applied to investigate the microphase separation between PLA and GE in composite fibers. Additionally, morphology and weight loss of the as-spun and carbodiimide crosslinked PLA/GE composite fibrous membranes were studied. With all these efforts, the structure and wettability relationship of coelectrospun PLA/GE composite fibrous mats were discussed.

## MATERIALS AND METHODS

### Materials

Poly(L-lactic acid) (PLLA,  $M_w = 100\,000$ ), GE (type B, from bovine, bloom number 240–270), and 2, 2, 2-trifluoroethanol (TFE, 99%) were purchased from Sigma-Aldrich for electrospinning, which

were used without any treatment or further purification. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (EDC, 97%) and *N*-Hydroxysuccinimide (NHS, 97%) were purchased from Aldrich Chemical (Milwaukee, WI) and used without any further treatment. 2,2-Bis-[4-(methacryloxypropoxy)-phenyl]-propane (Bis-GMA, 95%) resin, tri-(ethylene glycol) dimethacrylate (TEGDMA, 95%), camphorquinone (CQ, 97%), and 2-(dimethylamino)ethyl methacrylate (DMAEMA, 98%) were supplied by Aldrich Chemical Co. All other reagents and solvents used were of analytical grade and supplied by Beijing Chemical Reagent Co., Ltd. (Beijing, China).

### Fabrication of PLLA/GE composite fibers

The polymer solution with concentration of 10 wt% was prepared by dissolving PLLA and GE with a weight ratio of 1:0, 3:1, 1:1, 1:3, and 0:1 in TFE. The solutions were stirred for 24 hr at room temperature and subsequently electrospun from a 10 ml syringe with an inner needle diameter of 0.37 mm and a mass flow rate of 0.4 ml hr<sup>-1</sup>. A high voltage (12 kV) was applied to the tip of the needle attached to the syringe when a fluid jet was ejected. A flat aluminum plate was used as the collector. The distance between the needle and the collector was 20 cm. The as-spun fibrous membranes were recorded as PLLA, PG31, PG11, PG13, and GE, respectively. Taking PG11 as the example, "P" represented PLLA, "G" represented GE, and the number represented the weight ratio of PLLA to GE. These fibrous membranes were then crosslinked by EDC and NHS at 4°C in ethanol/deionized water (the volume ratio of ethanol/deionized water was 95:5) for 24 hr, followed by being washed three times with deionized water and freeze-dried for 24 hr. Accordingly, the crosslinked samples were named as PG31crosslinked, PG11crosslinked, PG13crosslinked, and GEcrosslinked, respectively.

### Selective removal of PLLA or GE

**Selective removal of PLLA:** The PLLA/GE composite fibers were weighed ( $W_1$ , the weight of each sample was over 50 mg.) and immersed into dichloromethane (The ratio of solvent volume to specimen mass was about 0.001 g ml<sup>-1</sup>.) and ultrasonically agitated at room temperature to selectively extract PLLA. The residual samples were vacuum dried and weighed again ( $W_2$ ). The weight loss of the removed PLLA was calculated with respect to its original amount in the composite: weight loss of PLLA (%) =  $[(W_1 - W_2)/W_1A] \times 100$ , where  $A$  is the initial percentage of PLLA in the composite.

**Selective removal of GE:** The weighted PLLA/GE composite fibers ( $W_1$ ) were immersed into deionized water (The ratio of solvent volume to specimen mass was about 0.001 g ml<sup>-1</sup>.) and ultrasonically agitated at 40°C for overnight to selectively extract GE. The residual samples were freeze-dried and weighed again ( $W_3$ ). The weight loss of the removed GE was calculated with respect to its original amount in the composite: weight loss of GE (%) =  $[(W_1 - W_3)/W_1A] \times 100$ , where  $A$  is the initial percentage of GE in the composite.

To evaluate the weight loss of PLLA/GE composite fibers with and without crosslinking treatment in the condition of cell culture, the composite samples (PG11 and PG31) were also doused in deionized water and ultrasonic agitated at  $37 \pm 1^\circ\text{C}$  to investigate the dissolution of GE.

### Degradation study of PLLA/GE composite fibers

Crosslinked PG31, PG11, and PG13 composite fibrous membranes ( $59.3 \pm 19.1 \mu\text{m}$  in thickness) were cut into specimens with the

size of 20 mm × 30 mm and immersed in a phosphate buffer solution (PBS; pH 7.4; 20 ml PBS per mg sample) at 37 ± 1 °C. The PBS solution (0.1 M) was prepared by dissolving NaCl, KCl, Na<sub>2</sub>HPO<sub>4</sub>, and KH<sub>2</sub>PO<sub>4</sub> in distilled deionized water. pH value of solution was adjusted to 7.4 by HCl. During the six-week degradation period, the PBS was refreshed by every other day. At each predetermined time point, two parallel samples were taken out and freeze-dried for morphological observation.

### Characterization of scaffolds

The morphologies of all the samples were studied by scanning electron microscopy (FESEM, Hitachi S-4700, Japan) at an accelerating voltage of 10 kV. The samples were sputter coated with a thin layer of platinum under the metallization conditions of 2 mA and 0.001 Pa to allow for better electrical conduction (Polaron E5600, USA). Based on the SEM photos, fiber diameters and interfiber distances were obtained by using the image visualization software Image J (National Institutes of Health, USA). Interfiber distance was calculated by measuring the distance between a fiber and the closest adjacent fiber within the same plane. A minimum of 80 fibers was calculated for each group.

For the purpose of assessing the wettability of the obtained fibrous membranes, water contact angles of the samples were monitored with a video contact-angle instrument (JC2000C1, Shanghai Glory Numeral Technique & Device Co., Ltd., China). As 2 μl of deionized water was automatically dropped onto the membrane, the contact angle was determined within 10 sec. Five samples were used for each test.

PLLA/GE composite fibrous membranes were observed under a transmission electron microscope (JEM-3010 TEM, JEOL Japan Inc.). After stained with 1% osmium tetroxide aqueous solution for 1 hr, the membranes were fixed with photocurable resin (Bis-GMA/TEGDMA (50/50 wt%) resin with 0.5 wt% of CQ as photo initiator and 1 wt% of DMAEMA as coinitiator) using curing light (QHL 75 Densply International, York, USA) in the yellow-light room to avoid the premature curing. The cured samples were cut into ultrathin slides with ~70 nm, mounted on imaging plates and then stained with osmium tetroxide again.

Surface compositions of all the electrospun fibrous sheets were determined by an X-ray photoelectron spectrometry (XPS, Thermo V6 ESCALAB 250, Britain.), and the incidence angle of Al K<sub>α</sub> ray was 90°.

### Cell culture

The PLLA, PG11, and PG11 crosslinked scaffolds were sterilized by <sup>60</sup>Co irradiation and placed in a 24-well cell culture plate and then MG-63 cells were seeded at a density of 1 × 10<sup>4</sup> cell/well onto the samples in Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 10% fetal bovine serum (FBS, Gibco) and incubated at 37 °C in a 5% CO<sub>2</sub> incubator (Sanyo, Japan). After 1 day and 3 days of culture, the specimens were rinsed with PBS, and fixed in 2.5% glutaraldehyde for 24 hr at 4 °C. The cells were dehydrated through a graded series of alcohols (30, 50, 70, 80, 90, 100%, v/v) for 10 min each at room temperature. After being freeze-dried, the specimens were sputter coated with platinum for observation of cell morphology under SEM. The spreading area of single cell was measured and averaged basing on SEM photos with software Image J and at least 50 cells were counted.

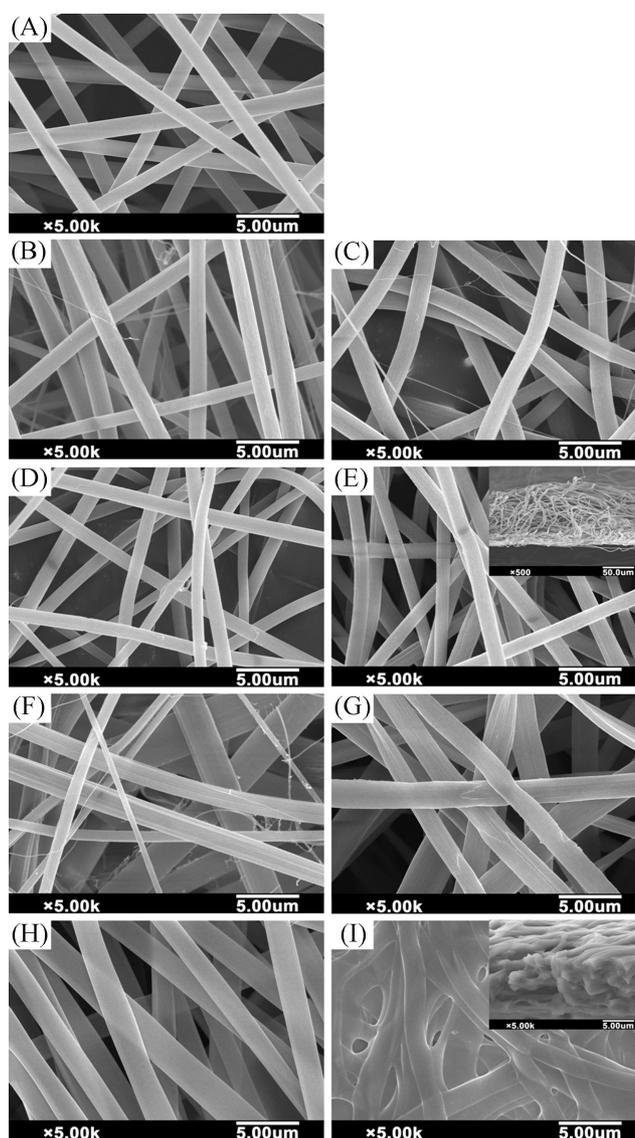
### Statistical analysis

All data presented were expressed as mean standard deviation (SD). Statistical analysis was carried out using single-factor analysis of variance (ANOVA). A value of  $p \leq 0.05$  was considered to be statistically significant.

## RESULTS

### Fiber morphology

Figure 1 shows the SEM micrographs of electrospun fibers of PLLA blended with GE at five different weight ratios. As it could be seen from Fig. 1A, B, D, F, H, all the as-spun fibers were bead-free strings with smooth surfaces. Analyzed with Image J (Table 1.), their average diameters varied from 1 to 2 μm depending on fiber compositions, and PG11 fibers had the smallest fiber diameter.



**Figure 1.** SEM micrographs of as-electrospun and crosslinked PLLA/gelatin composite fibers with different compositions: (A) PLLA; (B) PG31; (C) PG31 crosslinked; (D) PG11; (E) PG11 crosslinked; (F) PG13; (G) PG13 crosslinked; (H) GE; (I) GE crosslinked.

**Table 1.** Fiber diameter and interfiber distance of all the electrospun fibrous sheets in this study

|                     | Fiber diameter ( $\mu\text{m}$ ) | Interfiber distance ( $\mu\text{m}$ ) |
|---------------------|----------------------------------|---------------------------------------|
| As-spun PLLA        | $1.39 \pm 0.12$                  | $6.17 \pm 2.77$                       |
| As-spun PG31        | $1.57 \pm 0.20$                  | $6.06 \pm 2.20$                       |
| PG31 crosslinked    | $1.44 \pm 0.19$                  | $5.87 \pm 2.83$                       |
| As-spun PG11        | $1.01 \pm 0.19$                  | $5.24 \pm 2.33$                       |
| PG11 crosslinked    | $1.23 \pm 0.24$                  | $5.88 \pm 2.96$                       |
| As-spun PG13        | $1.68 \pm 0.62$                  | $6.08 \pm 2.68$                       |
| PG13 crosslinked    | $1.96 \pm 0.38$                  | $5.64 \pm 2.85$                       |
| As-spun gelatin     | $1.95 \pm 0.25$                  | $5.70 \pm 2.77$                       |
| Gelatin crosslinked | —                                | —                                     |

However, the interfiber distances were similar for all the as-spun and crosslinked fibers except pure GE fibrous sheet. After being crosslinked, severe morphological deformation and conglutination had occurred to GE fibers which caused the damage of porous structure (Fig. 1). The three crosslinked PLLA/GE composite fibers maintained their original patterns with little change in interfiber distances (Fig. 1C, E, G). The difference could be seen more clearly from the inserted cross-section images of PG11 and GE.

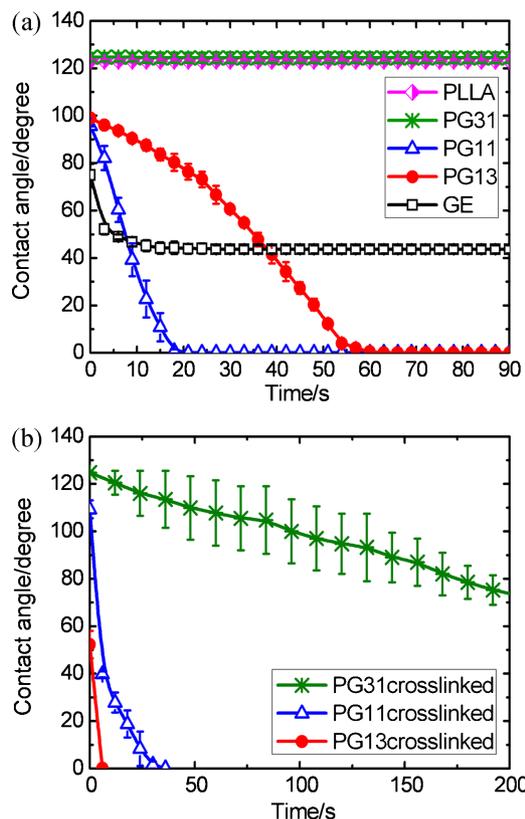
### Dynamic water contact angle and surface composition

The wettability of all the five as-spun fibrous sheets was characterized by dynamic contact-angle measurement (Fig. 2a). Both pure PLLA and PG31 composite fibrous sheets demonstrated surface contact angles around  $123^\circ$  during the whole monitoring procedure, while pure GE, PG11, and PG13 composite fibrous membranes exhibited much improved hydrophilicity. Although the initial water contact angles of PG11 and PG13 fibrous matrixes were near  $100^\circ$ , they decreased to zero within tens of seconds. However, the contact angle of pure GE fibrous membrane leveled off at  $\sim 42^\circ$ . On the other hand, crosslinking treatment was found having significant effect on the wettability of fibrous membranes depending on fiber compositions (Fig. 2b). From Fig. 2b, crosslinked PG11 and PG13 fibrous membranes could be seen exhibiting accelerated soaking rates, and even hydrophobic PG31 fibrous membrane turned to be some hydrophilic after being crosslinked with EDC.

The surface compositions of as-spun PLLA, PG11, and GE fibrous sheets were detected with XPS analysis. From the spectra and data shown in Fig. 3, it could be seen that the molar ratio of oxygen to carbon atom of PG11 composite fibers was between those of PLLA and GE, while the molar ratio of nitrogen to carbon atom of PG11 was far below that of GE fibers. On the other hand, the molar ratios of group C–O (N) and C=O to group C–C of PG11 were similar to that of pure GE fibers, which were higher than that of PLLA fibers; and the molar ratio of group C–O (N) to group C=O also showed the same trend.

### Selective removal of PLLA or GE component

Water and dichloromethane were used to dissolve GE or PLLA from the as-spun composite fibers, respectively. The morphologies of



**Figure 2.** Curves of soaking speed of as-electrospun and crosslinked composite fibrous membranes containing PLLA and GE at different weight ratios. This figure is available in colour online at [wileyonlinelibrary.com/journal/pat](http://wileyonlinelibrary.com/journal/pat)

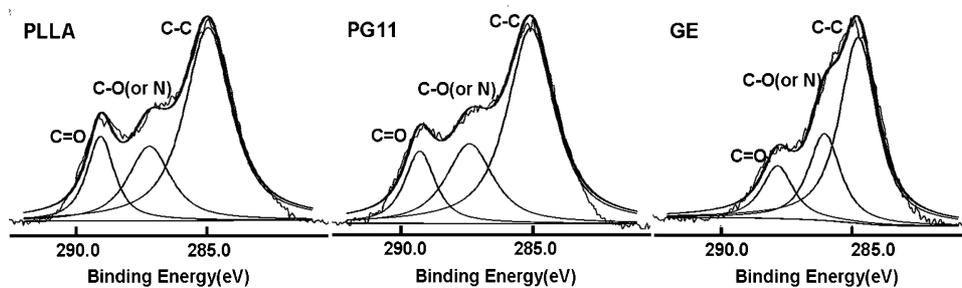
residual samples were observed with SEM (Fig. 4) and the weight losses were monitored (Fig. 5). By removing GE component with deionized water at  $40^\circ\text{C}$ , little change could be seen in the residual PG31 fibers (Fig. 4B), but the surface of residual PG11 fibers was rough (Fig. 4E). However, the residual PG31 fibers became fibrillar-like (Fig. 4C) and porous structure formed in the residual PG11 fibers (Fig. 4F) with the removal of PLLA component. In the case of PG13 fibers, split structures were detected for both samples by selectively removing either PLLA or GE component (Fig. 4H and I).

From Fig. 5a, it was found that the PLLA component could be completely removed from the composite fiber with dichloromethane for all the samples, however, the GE component could not dissolve into hot water ( $40^\circ\text{C}$ ) thoroughly depending on the PLLA/GE ratios even without crosslinking treatment. With the increase in PLLA content, more GE had been entrapped in the PLLA/GE composite fiber after water extraction at  $40^\circ\text{C}$ , at which GE was easy to dissolve.

In Fig. 5b, c the weight loss of PG11 and PG31 composite fibers in water at  $37^\circ\text{C}$  are presented. The as-spun PLLA/GE composite fibers lose their weight rapidly, while the crosslinked sample demonstrated little weight loss in an extended period.

### Microscopic observations of TEM

Microscopic observations of TEM are shown in Fig. 6, indicating that electrospun PG31, PG11, and PG13 fibers have quite different microstructures. In the TEM images, the black areas represent GE



| Samples         | Molar ratio of different atoms |       |         | Molar ratio of different groups |           |                     |                 |
|-----------------|--------------------------------|-------|---------|---------------------------------|-----------|---------------------|-----------------|
|                 | O/C                            | N/C   | (O+N)/C | C-O (or N) / C-C                | C=O / C-C | C-O(or N)+C=O / C-C | C-O(or N) / C=O |
| As-spun PLLA    | 0.478                          | /     | 0.478   | 0.353                           | 0.259     | 0.611               | 1.363           |
| As-spun PG11    | 0.446                          | 0.035 | 0.481   | 0.403                           | 0.238     | 0.640               | 1.695           |
| As-spun gelatin | 0.259                          | 0.177 | 0.436   | 0.403                           | 0.237     | 0.640               | 1.705           |

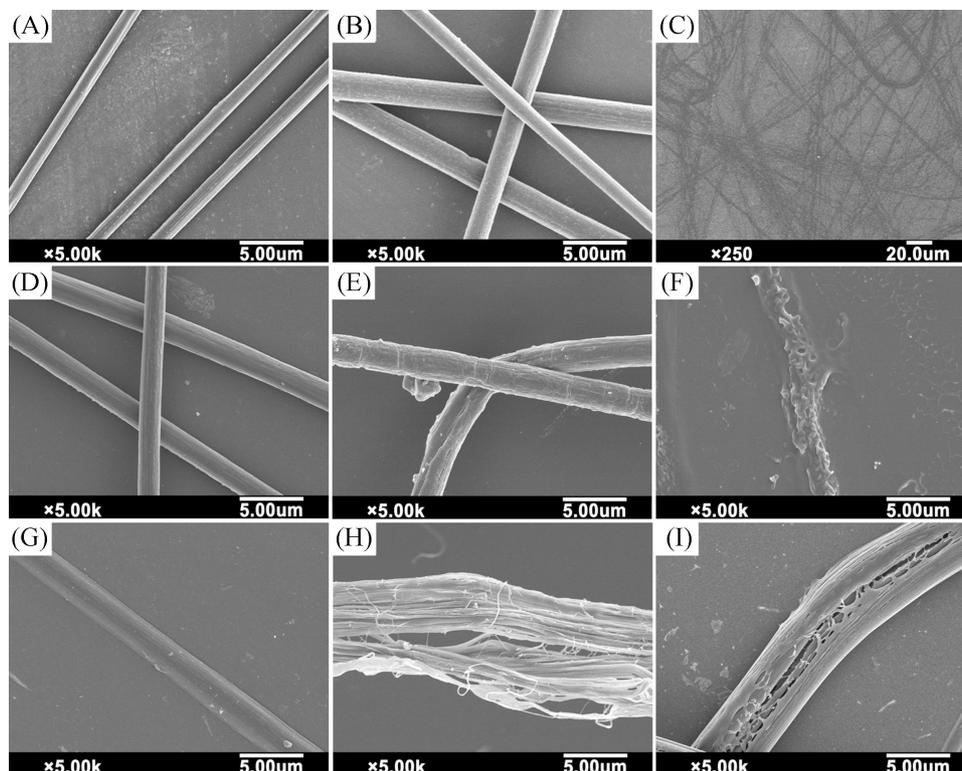
**Figure 3.** XPS spectra of C1s peaks of as-electrospun PLLA, PG11, and gelatin (GE) fibrous sheets, as well as the molar ratios of different atoms and groups calculated from the spectra.

component, which could be stained by osmium tetroxide while PLLA could not. By surveying the pictures, continuous black strips could be detected along the PG31 fibers, strips and dots coexisted along the PG11 fibers, while PG13 fibers showed large area of black domains with light strips. The cross-sectional TEM images of all the composite fibers clearly showed the microphase separation between the two components. In both PG31 and PG13

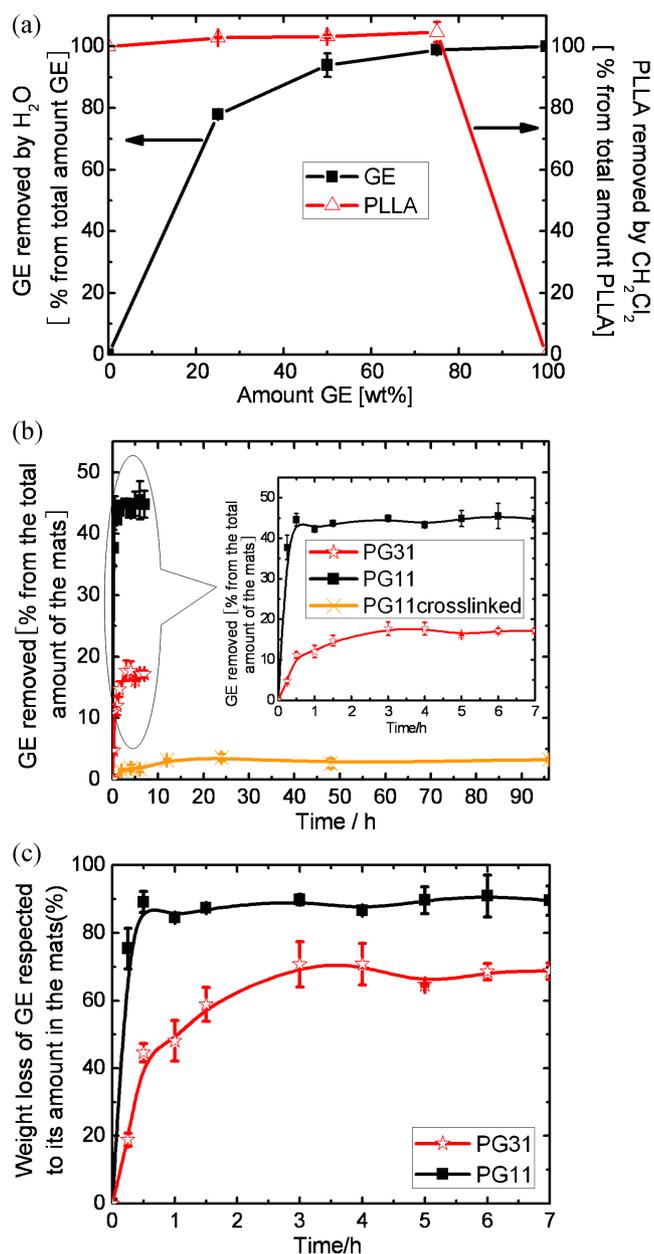
fibers, the component of low content (GE or PLLA) had been separated by the continuous phase (PLLA or GE).

#### Degradation study

After being crosslinked and immersed in PBS for several weeks, the residual samples were retrieved and submitted to SEM



**Figure 4.** SEM images of electrospun PLLA/GE composite fibers with different weight ratios and selective removal of one component with water at 40°C or dichloromethane at R.T.: (A) as-spun PG31; (B) PG31 with removal of gelatin; (C) PG31 with removal of PLLA; (D) as-spun PG11; (E) PG11 with removal of gelatin; (F) PG11 with removal of PLLA; (G) as-spun PG13; (H) PG13 with removal of gelatin; (I) PG13 with removal of PLLA.



**Figure 5.** Selective removal of gelatin or PLLA component from PLLA/gelatin composite fibers with different ratios (a), and the effect of crosslinking treatment on the dissolution of gelatin component in composite fibers (b and c). This figure is available in colour online at [wileyonlinelibrary.com/journal/pat](http://wileyonlinelibrary.com/journal/pat)

observation. The results (Fig. 7) demonstrated that PG31 and PG11 fibers still kept intact appearance after three-weeks degradation; on the contrary, the residual PG13 fibers had changed into fibrillar-like form. At the sixth week, PG13 samples could no longer be recovered for observation, and significant weight loss ( $22 \pm 3\%$  and  $47 \pm 5\%$ , respectively) was detected for both PG31 and PG11 to result in the formation of porous structures in the residual fibers. Microtunnels could be observed inside the degraded PG31 fibers with relatively intact fiber surface, while PG11 fibers had degraded into fragments with pores and grooves both inside and on the surface. Since PLLA is

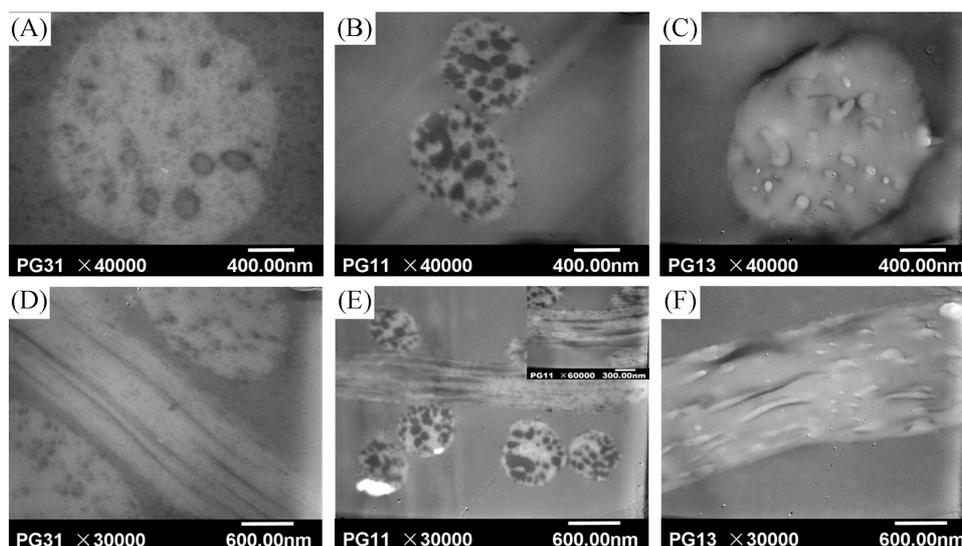
more stable than GE, it could be inferred that the pores on the fibers were the consequence of preferential degradation of GE.

### Cell culture

MG-63 cells were seeded onto pure PLLA, as-spun PG11, and crosslinked PG11 fibrous membranes, and cultured for 1 and 3 days. The cell morphologies were observed with SEM and shown in Fig. 8. At the first day, more cells had attached and better cell morphology could be seen on GE-contained membrane than those on pure PLLA matrix. Cells were found in spindle shape on PLLA matrix while in ellipse shape on both PG11 and PG11 crosslinked scaffolds. The average spreading areas of single cell were  $175.23 \pm 77.42$ ,  $323.47 \pm 122.12$ , and  $646.66 \pm 234.73 \mu\text{m}^2$ , respectively, for cells adhering to pure PLLA, as-spun PG11, and crosslinked PG11 fibrous sheets at this stage. However, the cell morphology turned to be alike on both pure PLLA and as-spun PG11 fibrous matrix at the third day, while MG-63 cells spread further extensively on the crosslinked PG11 fibrous membrane.

### DISCUSSION

The results of water contact-angle measurement suggested that different microstructures had formed in composite fibers with different ratios of PLLA to GE (Fig. 2a). With regard to PG31 composite fibrous membrane, the fact that its water contact angle was identical to that of pure PLLA, indicated the embedment of GE component in the continuous PLLA phase. Therefore, with the removal of PLLA component by dichloromethane extraction, remnant GE component was in fibrillar-like appearance (Fig. 4C); and with the preferential degradation of GE in PBS, the residual PLLA-rich PG31 fibers demonstrated many channels inside the fibers with intact surface (Fig. 7D). This microstructure was illustrated in TEM images as black strips along fibers and black dots on the cross-section of fibers (Fig. 6A, D). In contrast, the PG13 composite fibers were suggested having a reverse microstructure to PG31 composite fibers that PLLA component was separated and embedded in the continuous GE phase, for it containing more amount of GE component. This hypothesis was strongly supported by the removal of GE component by either warm water extraction or preferential degradation in PBS, in which, the residual PLLA component was detected fibrillar-like form in both cases (Figs 4E and 7C). As for PG11 composite fibers, the experimental facts in this study revealed the formation of a kind of cocontinuous microstructure. As shown in Fig. 4F, porous structure could be seen clearly in the residual PG11 fibers after PLLA component had been thoroughly removed by dichloromethane. In Fig. 7E, it was detected that the residual PLLA fragments had pores and grooves both inside and on the surface with the preferential degradation of GE. In the TEM images of PG11 composite fibers (Fig. 6B and E), strips and dots could be seen coexisting along the fibers which implied GE component interlocking with PLLA component. This kind of cocontinuous microstructure seemed common in coelectrospinning the blend of synthetic and natural polymers, such as coelectrospun cellulose acetate/polyurethane<sup>[26]</sup> and PLGA/dextran,<sup>[27]</sup> when the mixing ratio of the two components was equal. Because such a phase separation microstructure could generate many interfaces and voids between the two phases, which favored the water penetration into the matrix,<sup>[28]</sup> it could be suggested that the much improved wettability of

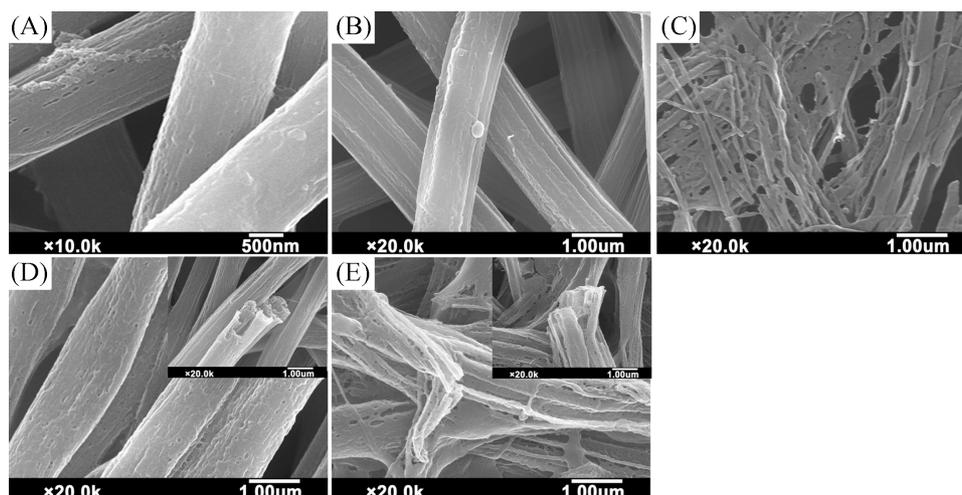


**Figure 6.** TEM images of electrospun fibrous membranes made of PLLA blended with 25% (PG31, A and D), 50% (PG11, B and E), and 75% (PG13, C and F) of gelatin.

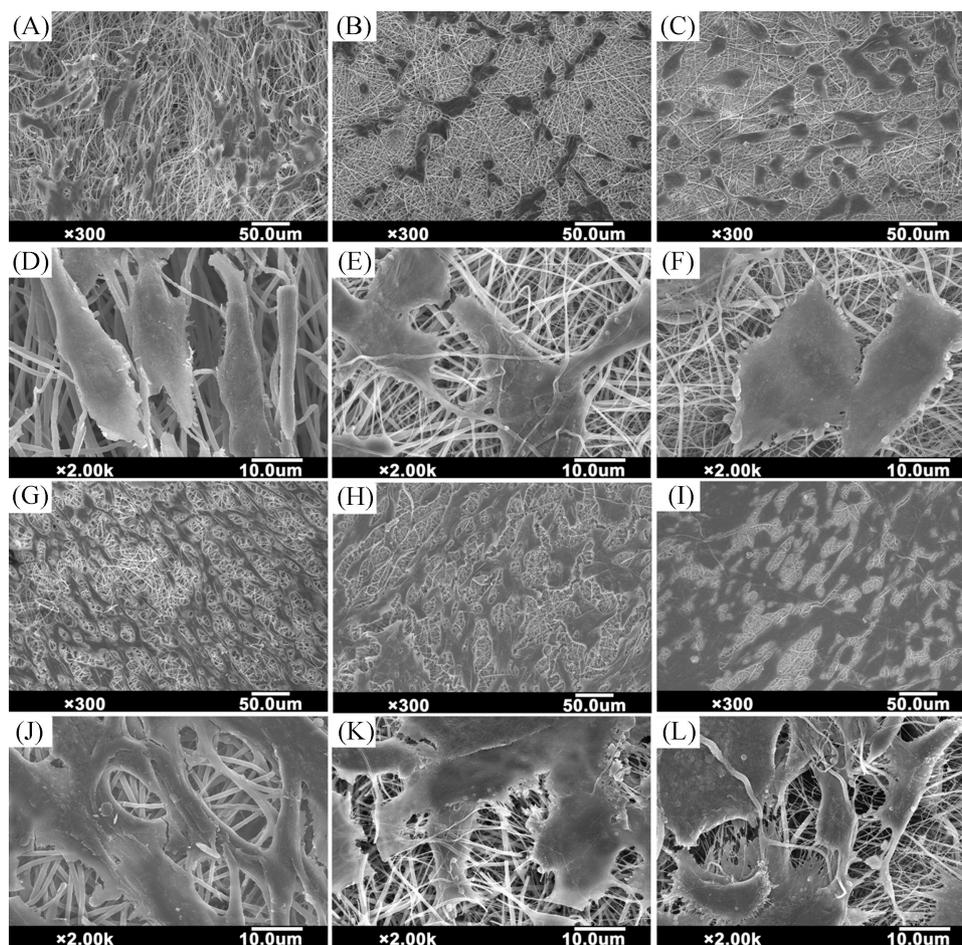
PLLA(50 wt%)/GE(50 wt%) was partly contributed by the cocontinuous microstructure.

On the second consideration, however, the fact that PLLA (50 wt%)/GE(50 wt%) composite fibers showed even higher hydrophilicity than pure GE fibers suggested that there should be other reasons in addition to the microstructure. The water contact angles were related to the surface roughness and it has been well known that thinner fibers should result in rougher and more non-wetting surface. All the electrospun samples had similar interfiber distances, while PG11 composite fibers were the thinnest fibers among them (Table 1). Thus, the decrease of water contact angle of PG11 fibrous membrane to  $0^\circ$  should not be caused by the surface roughness. XPS analysis was used to detect and compare the surface compositions of PLLA, PG11, and GE mats. Since the cocontinuous structure of PG11 composite fibers had been identified as above, the surface molar ratios of polar atoms to carbon atoms, and the molar ratios of polar groups (C–O

(or N), C=O) to C–C were theoretically between the two pure samples. However, all these values of PG11 sample were high as identified by the XPS data (Fig. 3). Then, it could be deduced that PG11 composite fibers should contain more polar atoms or groups on surface than pure GE fibers, which would further cause the much improved hydrophilicity of the composite. The reason to cause this phenomenon was supposed that hydrophobic interactions had occurred between hydrophobic PLLA and hydrophobic segments in GE, which could affect the formation of hydrogen bonds between GE macromolecules. As a consequence, the polar side groups of GE could migrate to the fiber surface with the evaporation of trifluoroethanol during electrospinning, owing to the hydrogen bond interaction between them. The occurrence of hydrophobic interaction between PLLA and GE could be proven by the fact that the GE component could not be completely removed from uncrosslinked PLLA/GE composite fibers with warm water (Fig. 5a). But for the pure GE



**Figure 7.** SEM micrographs of fibrous morphology of PLLA/gelatin composite fibers with degradation in PBS: (A) PG31 crosslinked, 3 W; (B) PG11 crosslinked, 3 W; (C) PG13 crosslinked, 3 W; (D) PG31 crosslinked, 6 W; (E) PG11 crosslinked, 6 W. Magnification of inserted graphs in D and E is  $\times 20.0k$ .



**Figure 8.** SEM images of MG-63 cells cultured on PLLA, as-electrospun and crosslinked PG11 fibrous membranes for 1 and 3 days: (A, D) PLLA, 1 day, (G, J) PLLA, 3 days, (B, E) PG11, 1 day, (H, K) PG11, 3 days, (C, F) PG11 crosslinked, 1 day, (I, L) PG11 crosslinked, 3 days.

fibers, the strong hydrogen bonds between protein macromolecules via their polar side groups would somewhat prevent the migration of polar groups toward fiber surface during fiber solidification. Therefore, the water contact angle of pure GE fibrous membrane was found leveling off at  $\sim 42^\circ$ . The wettability of PG13 composite fibrous sheets could be interpreted similarly.

The trend in the change of fiber diameter from pure PLLA to PG11 and to pure GE (Table 1) could also indicate the occurrence of interaction between PLLA and GE. The average fiber diameter decreased as the content of GE in the coelectrospun composite fiber increasing from 0 to 50 wt%, because the ionic properties of GE increased the conductivity of the mixture solutions, as well as the hydrophobic interaction between PLLA and GE components interfered the formation of hydrogen bonds between GE chains. When the content of GE exceeded 50wt%, however, the high solution viscosity resulting from the hydrogen bonds between GE macromolecules led the fiber diameter increasing.

Although the hydrophobic interaction between GE and PLLA could prevent the complete dissolution of GE from the composite, crosslinking treatment was still necessary before the PLLA/GE composite fibrous membranes were used for cell culture. Figure 5 shows that the uncrosslinked GE component was liable to dissolve into medium from the composite within short period. The culture of MG-63 cells, which are a human osteosarcoma cell line usually serving as a model for testing methods for tissue engineering, revealed that the cell behaviors became alike on pure PLLA and

uncrosslinked PG11 composite fibrous membranes with the elongated culture time due to the loss of GE component, while the cells grew the best on the crosslinked PG11 fibrous membranes (Fig. 8). Superior to pure GE fibers, which coagulated remarkably during crosslinking treatment, PLLA/GE composite fibers could sustain the crosslinking procedure and preserve their original morphologies. (Fig. 1). Because the presence of hydrophobic PLLA component and its hydrophobic interaction with GE could prevent the fiber swelling and coagulation in contact with water. The crosslinked PG11 fibrous matrix was proven good substrate for cell culture compared to pure PLLA and GE, since its morphology was improved, and the incorporated GE component could provide reorganization sites for cell adhesion, as well as the hydrophilic property of PG11 could favor the penetration of culture medium and cell proliferation. As shown in Fig. 8, it could be seen that cells adhered and spread out on PG11 crosslinked mats, while spindle cell shapes appeared on pure PLLA for its lacking reorganization sites. This result was similar to many other cell culture on coelectrospun PLA(or PCL)/GE (or collagen) composite fibrous matrixes.

## CONCLUSION

Coelectrospinning of blends of synthetic and natural polymers has become a trend in making tissue engineering scaffolds. The

PLLA/GE composite fibers prepared in this study exhibited different microstructures and surface wettability, relating to fiber compositions closely. Among which, the fibrous matrix composed of 50wt% PLLA and 50wt% GE exhibited a cocontinuous structure on account of the phase separation occurring in fiber solidification. Hydrophobic interactions were identified between PLLA and GE, which had led to the remarkably increasing hydrophilicity and lowered GE solubility. After being crosslinked, the PLLA/GE composite fibrous matrixes were more preferred for tissue engineering applications than matrixes from either pure PLLA or GE, for its significantly improved performance.

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