Polymer 50 (2009) 3762–3769

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Chemical crosslinking and biophysical properties of electrospun hyaluronic acid based ultra-thin fibrous membranes

Shanshan Xu [a], Junxing Li [a], Aihua He [a,b,*], Wenwen Liu [c], Xingyu Jiang [c], Jianfen Zheng [a], Charles C. Han [a,*], Benjamin S. Hsiao [d,e], Benjamin Chu [d,e], Dufei Fang [e]

[a] State Key Laboratory of Polymer Physics and Chemistry, Joint Laboratory of Polymer Science and Materials, Beijing National Laboratory for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China
[b] Key Laboratory of Rubber-Plastics (Ministry of Education), College of Polymer Science and Engineering, Qingdao University of Science and Technology, Qingdao 266042, China
[c] National Center for NanoScience and Technology, Beijing 100190, China
[d] Department of Chemistry, Stony Brook University, Stony Brook, NY 11794-3400, USA
[e] Stonybrook Technology and Applied Research (STAR), Stony Brook, NY 11794-3400, USA

ARTICLE INFO

Article history:
Received 29 April 2009
Received in revised form
30 May 2009
Accepted 4 June 2009
Available online 11 June 2009

Keywords:
Hyaluronic acid
Electrospinning
Crosslinking

ABSTRACT

Hyaluronic acid (HA) ultra-thin fibrous membranes by electrospinning technology had been fabricated successfully by our group. However, these HA fibrous membranes dissolve and decompose rapidly in water. In this study, chemical crosslinking of HA and HA based ultra-thin fibrous membranes was studied in detail. It was found that the chemically crosslinked pure HA ultra-thin fibrous membranes with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) as the crosslinking agent did not show an obvious improvement in the water-resistance. However, by simply adding and changing the gelatin content, the degradation time of the HA based fibrous membranes could be effectively adjusted from one week to several months. That is with the increase in gelatin content, the water-resistance of the HA/gelatin bi-component membranes could be increased greatly. The crosslinked HA/gelatin bi-component membranes were evaluated in vitro by seeding with mouse embryonic fibroblasts (3T6-Swiss Albino). The results showed that the HA/gelatin fibrous membranes were cytocompatible and suitable for cell viability. However, cells were hard to attach and grow on the HA based membranes. Thus, the HA/gelatin fibrous membranes with controllable degradation rate could have good potential applications in the anti-adhesion and wound dressing fields.

1. Introduction

HA, as a component of extracellular matrix, has been used in biomedical field extensively [1–4]. However, few studies on the application of electrospun HA fibers have been reported due to its poor electrospinning processability and strong water absorption property [5,6]. In our former work, HA nanofibrous membranes from its water/DMF solution were successfully fabricated by electrospinning technology [7]. Due to its lightweight, soft texture and specific fibrous surface, HA fibrous membranes would have interesting applications in biomedical field. Normally, for the in vivo application, material with controllable degradation rate is necessary. For example, materials with a degradation time of 25-day is suitable for acute wound healing (burn and skin excision), and with a degradation time of 8-week is suitable for chronic wound healing (diabetic ulcer, pressure ulcer) [8–10]. However, the water-resistance of HA fibrous membranes was rather poor, and the HA fibrous membranes disappeared rapidly once on contact with water [11]. Therefore, study on crosslinking of HA nanofibrous membranes become an important issue [12].

Lots of attention has been focused on the crosslinking of HA products. General crosslinking agents for HA chains usually include carbodiimides, hydrazides, aldehydes, sulfides, and polyfunctional epoxides [13–16]. Among these crosslinking agents, carbodiimide is more preferred due to its biocompatibility and non-cytotoxicity. What is important is that carbodiimide will not be incorporated in the cross-linked structure, which is pretty accepted for the biomedical application [12,15]. Furthermore, the crosslinking reaction with water-soluble carbodiimide is gentle and easy to controlled. In the previous literature, the in-vitro degradation rate of the crosslinked HA casting film by carbodiimide was studied, and the crosslinked HA casting film could loss 80% weight in only 5 days

* Corresponding authors. State Key Laboratory of Polymer Physics and Chemistry, Joint Laboratory of Polymer Science and Materials, Beijing National Laboratory for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China. Tel.: +86 10 82618089; fax: +86 10 62521519.
E-mail addresses: aihuahe@iccas.ac.cn (A. He), c.c.han@iccas.ac.cn (C.C. Han).

0032-3861/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2009.06.009

[15]. Considering the ultra high surface area, crosslinking of the HA nanofibrous membranes will be a more difficult case. Chu et al. [11] studied the physical crosslinking of HA fibrous membranes and found its water-resistance was not improved significantly.

In this study, chemical crosslinking and water-resistance of HA based nanofibrous membranes were studied in detail with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) as the crosslinking agent. In order to achieve the improvement and control of the water-resistance of HA membranes, another natural polymer gelatin was introduced into the HA solution and HA/gelatin bi-component fibrous membranes were fabricated [7]. As a natural biopolymer derived from collagens by controlled denaturation, gelatin contains many functional groups including hydroxyl, carboxyl and amino groups [17]. It was expected that gelatin could not only improve the water-resistance of the HA based fibrous membranes by forming relatively stable peptide bonds, but also endow the HA based membranes with a protein character, which might enhance or improve the cell attachment and proliferation on the materials.

It was well known that HA showed specific interactions with cell surface molecules. The hydrophilic, polyanionic surfaces of HA biomaterials do not thermodynamically favor attachment to the anionic cell surfaces [18]. In addition, high molecular weight HA showed less adhesion than did lower molecular weight HA [19,20]. As a result, the HA-based biomaterials are widely employed to prevent protein absorption, inhibit cell attachment, and prevent post-surgical adhesions [21–24]. In this study, the crosslinked HA based membranes, containing a main component of HA with high molecular weight in the form of nanofibers and a small quantity of gelatin which may adjust or affect the cell compatibility and cell adhesion property of membranes were evaluated by cell culture experiment. We hope that the crosslinked HA/gelatin membranes with high surface area, controllable degradation behavior, and good cell biocompatibility, will have the great potential applications as implant devices, especially as anti-adhesion membranes.

2. Experimental

2.1. Materials

Hyaluronic acid (HA) (sodium salt, $M_w = 2{,}000{,}000$) was purchased from Dali Co. (Nanning, China). Polymers of gelatin Type A (Approx. 220 Bloom, $M_n = 80{,}000$), extracted from porcine skin by acidic process, were purchased from Sanhesheng Gelatin Co. (Wenzhou, China). *N*,*N*-dimethylformamide (DMF) and ethanol were obtained from Beijing Chem. Co. (Beijing, China). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) powder were purchased from Fluka. The pH of phosphate-buffered saline (PBS) was fixed at 7.4. All the materials were used without further purification.

2.2. Preparation of spinning solution

1.5 w/v% (w in gram and v in milliliter) HA solution in DMF/water (volume ratio = 1) was prepared to produce pure HA fibers. HA/gelatin blend solutions were prepared as followings: 1.875 w/v% HA solution in DMF/water (volume ratio = 1) was prepared using the same procedure as above. 0.375 w/v%, 1.5 w/v% and 6 w/v% gelatin solutions in water were prepared at 40 °C under gentle stirring for 20 min. The gelatin solutions were added into the HA solutions with specific volume ratio to obtain the HA/gelatin solutions, and the concentration of HA in each solution was fixed at 1.5 w/v%. The weight ratios of HA to gelatin in the blended solutions were set as 100/5, 100/20 and 100/80, respectively.

2.3. Electrospinning setup

The temperatures of all electrospinning solutions, spinneret and the environment were controlled at 40 ± 3 °C, while the relative humidity of the electrospinning environment was also considered and controlled at around 40%. The electrospinning solution was placed into a 5 ml syringe with a capillary tip with an inner diameter of 0.3 mm. A syringe pump was used to feed the polymer solution and the feeding rate was fixed at 60 μl/min. A DC high-voltage generator (The Beijing machinery & electricity institute, China) was applied to produce voltages ranging from 0 to 50 kV. The applied voltage was fixed at 22 kV in this experiment and the tip-to-collector distance was fixed at 15 cm.

2.4. Crosslinking

The crosslinking reaction was carried out in 5 ml crosslinking medium (volume ratio of ethanol to water is 8/2). The HA and HA/gelatin fibrous membranes (1.5 cm × 1.5 cm) were weighed (around 10 mg) and immersed into the crosslinking medium containing crosslinking agent EDC (50 mM) or ECD/NHS (50 mM/50 mM) at 4 °C and kept for 24 h to fulfill the crosslinking reactions, respectively. After crosslinking, the crosslinked membranes were washed with water and ethanol for three times, respectively, and then were dried in vacuum at room temperature for three days to remove the residual solvents.

2.5. In vitro degradation of the crosslinked HA and HA/gelatin fibrous membranes in PBS

To investigate the degradation behavior of crosslinked HA and HA/gelatin (100/5, 100/20, 100/80) fibrous membranes in vitro, the crosslinked HA and HA/gelatin fibrous membranes (1.5 cm × 1.5 cm, about 10 mg, respectively) were immersed in 5 ml PBS (pH = 7.4) at 37 °C for different time intervals. The degradation rate was evaluated through the weight changes before and after immersion according to Equation (1).

$$\text{Weight remaining}\,(\%) = (W_r/W_d) \times 100 \quad (1)$$

where W_r is the remained weight (after dried) of membranes after immersion in PBS and W_d is the original weight of the same membranes before immersion.

2.6. Characterization of the fibrous membranes

The morphologies of electrospun HA and HA/gelatin fibrous membranes before and after degradation tests were observed with scanning electron microscope (SEM, JSM 6700F, JEOL). Each sample was sputter-coated with gold for analysis.

FT-IR spectra of the electro-spun membranes before and after cross-linking were measured using a Nicolet 760 spectrometer.

2.7. Cell culture and cell proliferation

3T6-Swiss Albino (Mouse embryonic fibroblasts) were provided by the Institute of Biophysics, Chinese Academy of Sciences. Growth medium consists of 1640 (Gibco) containing 10% (v/v) fetal bovine serum (FBS, Gibco), 1% (v/v) penicillin/streptomycin (Hyclone) and 1% L-glutamine (Hyclone). Cell cultures were maintained in a 37 °C incubator containing 5% CO_2 in air and the medium was replaced once in two days. Cells were transferred at 70% confluence using a standard trypsin and versene protocol.

Crosslinked HA/gelatin fibrous membranes and glass substrates were sterilized in 75% (v/v) ethanol solution for 30 min and then

washed 3 times in sterile phosphate buffered saline (PBS) to remove traces of ethanol. Cells were seeded on the film or glass substrate with the same initial density. Before harvest, they were cultured for 1, 3, 5, and 7 days, respectively.

For cell proliferation experiments, specimens were transferred to a new tissue culture plate and washed by PBS three times. After that, 500 μl trypsin was added into the plate, the specimens were incubated at 37 °C for 2 min to ensure a complete detachment of cells from the film. The cell number was calculated by a hemocytometer and the areas of the membranes were measured using a microscope (Leica DMI6000B). The cell proliferation was assessed by the increase of cell density.

2.8. Cell morphology analysis

Cell seeded membranes were rinsed in PBS, fixed in 2.5% (v/v) glutaraldehyde solution for 30 min and permeated with 0.3%(v/v) Triton X-100 for 5 min. Then specimens were incubated with Rhodamine–phalloidin and DAPI for 40 min under dark condition. Cells were observed by a fluorescence microscope (Leica DMI6000B).

For SEM observation, the cell seeded membranes were dehydrated in ethanol solution of varying concentration (30%, 50%, 70%, 90% and 100%, respectively) for 5 min each. Later, the specimens were dried in air to remove the ethanol.

3. Results and discussions

Compared with HA products in other forms, the unique properties of electrospun fibrous membranes such as high specific surface area, high porosity and low density make the nanofibrous products more suitable for biomedical use especially as implantable materials. However, the fibrous morphologies of water-soluble HA fibrous membranes disappear in water rapidly due to its fast dissolution [6,11]. Therefore, the crosslinking of those electrospun HA membranes became necessary for many applications. The crosslinking agent EDC, as a safe and effective crosslinking agent for polymers with –COOH and –OH or $–NH_2$ groups was used in this study and the crosslinking behavior of those HA fibrous products was investigated. It is expected that the water resistance of the HA fibrous membranes will be increased by esterification reactions initiated by the EDC crosslinking agent.

3.1. Crosslinking and in vitro degradation of HA fibrous membranes

The chemical crosslinking structures of the pure HA fibers could be characterized by the FTIR spectra (Fig. 1). Compared with non-crosslinked HA fibers and HA powders, the most prominent difference in the crosslinked HA fibers is the noticeable new peak at a wave number of 1700 cm^{-1}, which is assigned to the carbonyl group in the newly formed ester bond. At the same time, it was found that the absorption peaks at 1412 cm^{-1} and 1618 cm^{-1} of crosslinked HA fibers, which are assigned to the symmetric and asymmetric stretching vibration bands of carboxyl groups, decreased greatly. To quantify the ester bond formation and avoid the influence of membrane thickness on the IR analysis, peak at 1050 cm^{-1} was selected as a control, since this peak is assigned to inert C–O–C group of the glucose ring and remains unchanged during the crosslinking reaction. The intensity ratio of the absorbance at 1412 cm^{-1} to that at 1050 cm^{-1}, A_{1412}/A_{1050}, evaluated from Fig. 1, decreased from 0.79 of HA fibers to 0.72 of crosslinked HA fibers. The same result of A_{1618}/A_{1050} was from 0.98 to 0.89. These results indicated that the –COOH groups took part in the crosslinking reactions initiated by EDC and the ester bonds were finally formed.

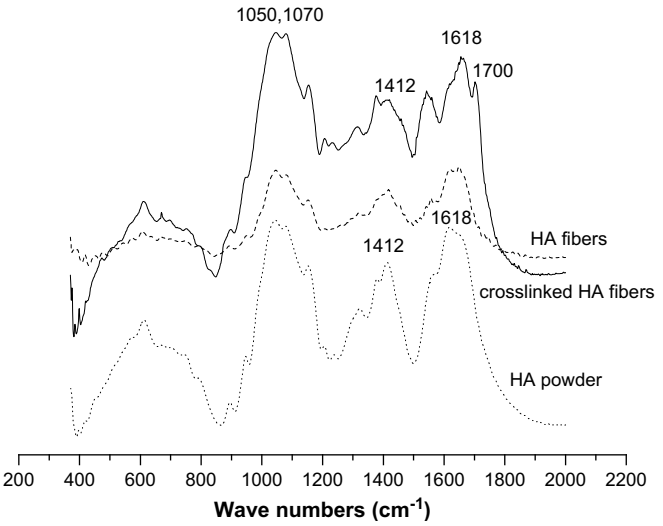

Fig. 1. FTIR spectra of HA powders, electrospun HA fibers and crosslinked HA fibers.

The crosslinked HA fibrous products were immersed in 37 °C water and PBS, respectively, to investigate the in vitro degradation behaviors. The results are shown in Figs. 2 and 3.

It can be seen from Fig. 2 that crosslinked HA fibrous membranes exhibited a totally different degradation profile in water and PBS. The HA products still possessed about 70% of its initial weight after a 3-day degradation in water. However, nearly 100% weight lost occurred after only a 1-day immersion in PBS. This might be caused by the fast hydrolysis process of the ester bonds in PBS. In strong ionic environments of PBS, the ester groups formed by the esterification reactions were more inclined to hydrolyze when compared with those in water.

Fig. 3 shows morphologies of crosslinked HA fibrous membranes for different degradation time periods in water and PBS. It can be seen from Fig. 3b that the fibrous morphology of HA membrane was still maintained and only small degree of swelling and adhesion between fibers were observed after 6-h degradation in PBS. The average fiber diameter became larger when compared with that of the original fibers (Fig. 3a). The fibrous structure almost disappeared after 16-h degradation in PBS (Fig. 3d), and only few fibrous frames

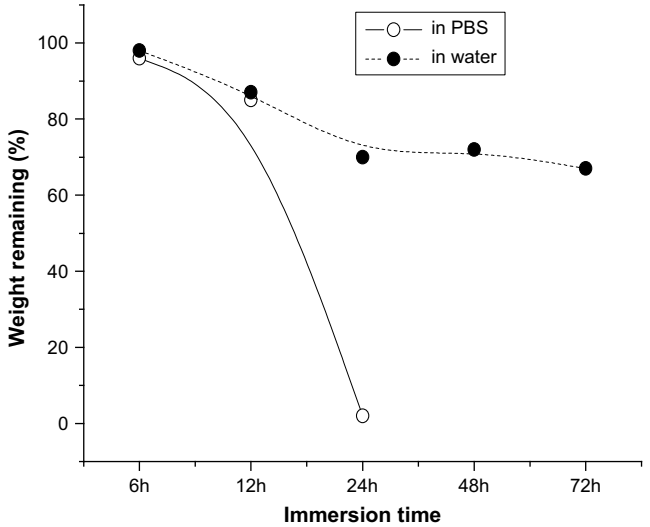

Fig. 2. Degradation behaviors of crosslinked HA fibrous membranes in PBS and water at 37 °C.

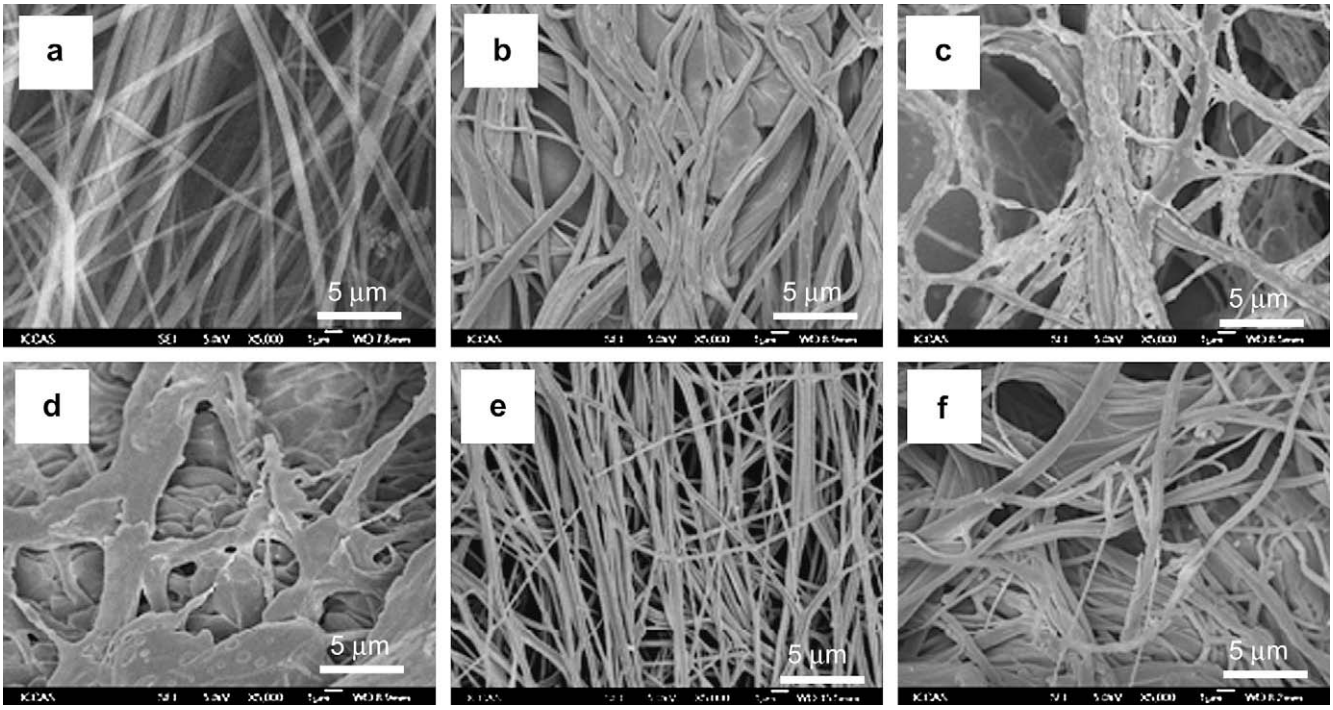

Fig. 3. SEM images of crosslinked HA fibrous membranes after immersion in water and PBS for different time periods at 37 °C. (a) Before immersion in PBS; (b) After immersion in PBS for 6 h; (c) After immersion in PBS for 12 h; (d) After immersion in PBS for 16 h; (e) After immersion in water for 24 h; (f) After immersion in water for 72 h. Crosslinking reaction was carried out with EDC at 4 °C for 24 h.

remained and the whole fibrous membrane became a compact film. However, the morphologies were kept very well and little adhesion was observed even after 3-day degradations in water (Fig. 3f). These results indicated that pure HA fibrous membranes responded with different water-resistance and different degradability in different medium. In another word, unlike the un-crosslinked membrane, the water-resistance of chemically crosslinked HA fibrous membranes were different in water and in PBS. However, in order to further improve the stability of the HA fibrous membranes in PBS, the chemical structure of the crosslinkages should be considered carefully.

3.2. In vitro degradability of crosslinked HA/gelatin blend fibrous membranes in PBS

It is generally accepted that amide bond (–CONH–) was much more stable than the ester bonds (–COO–). In order to introduce $–NH_2$ group into the HA fibers, another natural polymer, gelatin, containing free $–NH_2$ groups was used to blend with HA. In addition, it has been reported that HA/gelatin blends in films or sponges showed better cell attachment and proliferation behavior, and exhibited great potential for applications in tissue scaffolds, artificial skins and wound dressings [8,13]. Therefore, it was expected that the introduction of gelatin could not only improve the controllability in the degradation of HA based fibrous membranes but also contribute to the biomedical properties of the membranes. In our previous study [7], we have successfully fabricated HA/gelatin blends fibrous membranes with different compositions. We found that the addition of gelatin could improve the HA processability due to the decrease in surface tension of the spinning solutions [7]. In this study, HA/gelatin fibrous membranes with different compositions (100/5, 100/20, 100/80) were fabricated, and the crosslinking conditions and in vitro biodegradation behavior of the bi-component HA/gelatin fibrous membranes were investigated in detail.

The crosslinking reaction of the HA/gelatin (100/5, 100/20, 100/80) fibrous membranes was primarily carried out with EDC as crosslinking agent. After crosslinking at 4 °C for 24 h, the crosslinked HA/gelatin fibrous membranes were immersed into PBS at 37 °C for the in vitro degradation test. However, it was observed that the fibrous morphology with high gelatin content (weight ratio of HA to gelatin = 100/80) was lost in only 24 h (Fig. 5a). This implied the crosslinking bonds were mainly ester bonds which could be easily damaged in PBS, and little –NH–CO– bonds were formed initiated by EDC due to the low crosslinking efficiency between –COOH and $–NH_2$ groups [25] It has been reported that EDC/NHS system is much more effective in initiating the crosslinking reactions between –COOH and $–NH_2$ groups [25]. Therefore, in order to improve the biodegradability of HA based fibrous membranes, EDC/NHS was used for chemical crosslinking of HA/gelatin materials. Compared with pure HA fibrous membranes, the degradation time of HA/gelatin bi-component fibrous membrane prolonged greatly from hours to days, as shown in Fig. 4. When the weight ratio of HA to gelatin was only 100/5, the degradation of the blend membrane started to be inhibited in comparison with pure HA fibrous membrane. When the weight ratio of HA to gelatin was 100/80, the crosslinked fibrous membranes were much more stable in PBS, and about 80% of the initial weight remained after 1 month in vitro degradation. This indicated that the degradation time can be regulated precisely by simply changing the gelatin content in the HA based fibers.

Fig. 5b–f shows morphology changes of the HA based membranes with different compositions in different degradation time periods in PBS at 37 °C. Obviously, the morphologies of HA/gelatin blends had longer persistence than pure HA fibrous membranes. This might be attributed to the amide bonds which were more stable than ester bonds in PBS. FT-IR spectra were used to characterize the crosslinking structure of the HA/gelatin membranes. It can be seen from Fig. 6 that no obvious peak around

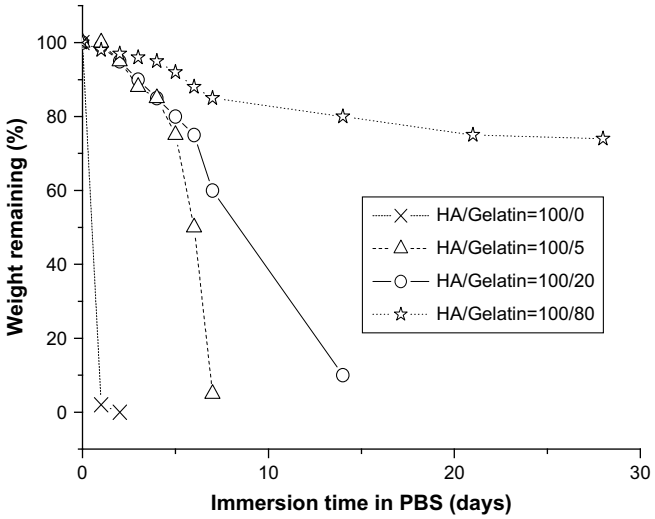

Fig. 4. Weight remaining of HA/gelatin electrospun products after immersion in PBS at 37 °C. Crosslinking reaction was carried out with EDC/NHS at 4 °C for 24 h.

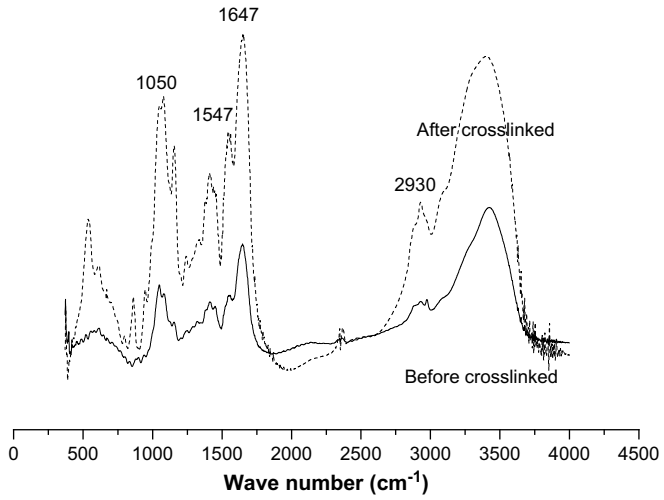

Fig. 6. FTIR of HA/gelatin (100/80) fibers before and after crosslinking with EDC/NHS.

1700 cm^{-1} assigned to ester group was observed. However, the absorption bands corresponding to C–N–H stretching vibration at about 1547 cm^{-1} and the absorption band corresponding to C═O stretching vibration at 1647 cm^{-1} became more intense in the spectra of crosslinked fibers. The intensity ratio of the absorbance at 1547 cm^{-1} to that at 1050 cm^{-1}, A_{1547}/A_{1050}, evaluated from Fig. 6, increased from 0.84 of HA/gelatin fibers to 0.90 of crosslinked HA/gelatin fibers. Both peaks at 1547 cm^{-1} and 1647 cm^{-1} can be assigned to the amide groups, and this indicated the formation of the new crosslinking bonds (–CONH–).

It can be concluded that the fibrous morphologies were kept much longer after introducing gelatin into the HA fibrous membranes and the weight loss in PBS became controllable through changing gelatin content in the blends. This indicated that HA/gelatin fibrous membranes could be produced into novel materials with different degradation time according to different requirements in biomedical applications, especially in implantable materials. Therefore, the adhesion and proliferation of cells on these fibrous materials are a focus of ongoing experiments.

3.3. Cell culture and cell adhesion study

The composition of the extracellular matrix (ECM) is proposed as selective key element in providing specific adhesion characteristics. Different composition and morphology of the biomaterials could regulate cell growth, cell proliferation, and cell differentiation [26,27]. HA possesses inherent bioactivity that it may impart to the

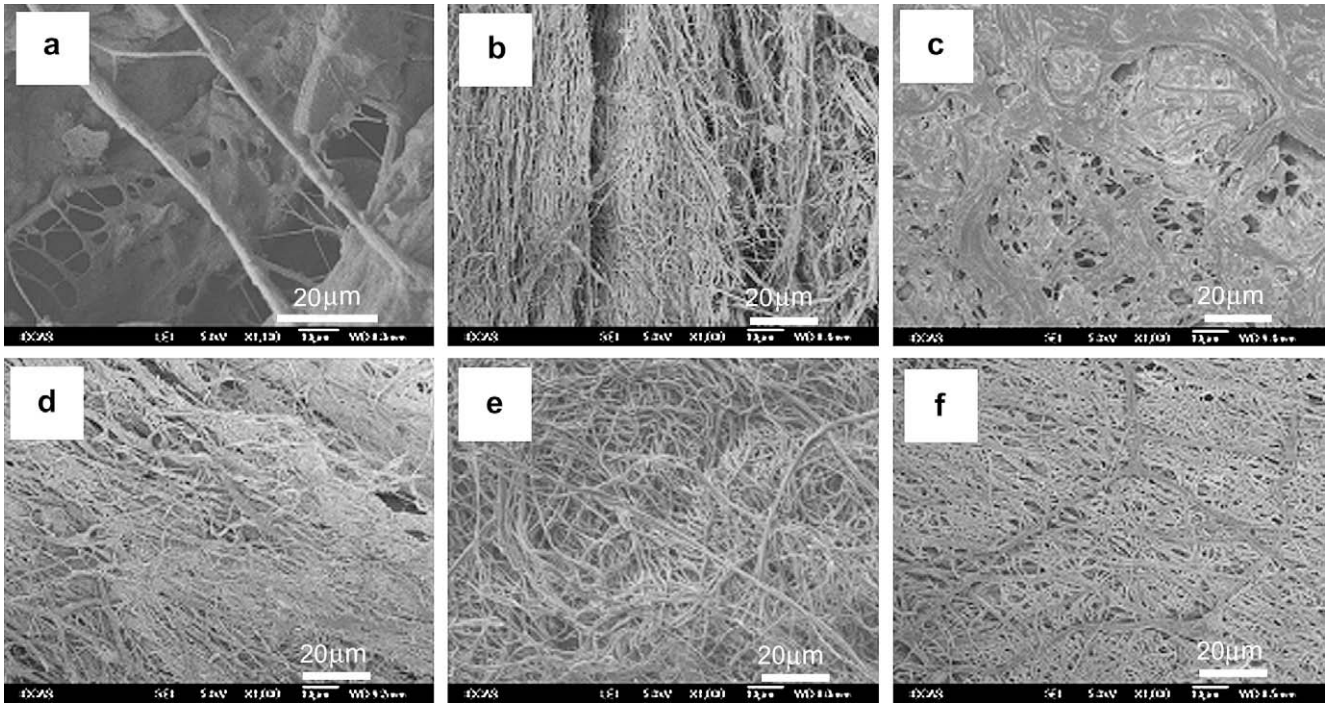

Fig. 5. SEM images of the fibrous morphologies of HA/gelatin (100/5,100/20,100/80, weight ratio) membranes after immersion in PBS for different time at 37 °C. (a) 100/80, 1 day; Crosslinking in 50 mM EDC; (b) 100/5, 1 day; (c) 100/5, 4 day; (d) 100/20, 5 day; (e) 100/80, 14 day; (f) 100/80, 28 day. (b–f) Crosslinking in 50/50 mM EDC/NHS.

material, which allows specific interactions with cells and other biomolecules. In this study, biocompatibility and cell adherence of the crosslinked HA/gelatin fibrous membranes were evaluated by culturing with 3T6 cells.

Fig. 7 shows fluorescence images of 3T6 cells cultured on a glass substrate and on the fibrous membranes for only 1 day. The actin microfilaments and nuclei of the cells were stained red and blue, respectively. It can be seen from Fig. 7a that all cells appeared to adhere and grow very well on the glass substrate and exhibit normal morphology. However, there was fewer cells adherence on the HA/gelatin fibrous membranes, as shown in Fig. 7b–d. The 3T6 cells on the HA based membranes showed round morphology, and appeared to aggregate on the HA based fibrous membranes. It seemed that the 3T6 cells would rather adhere to themselves than adhere on the HA/gelatin fibrous substrate. This result indicated that the 3T6 cell did not prefer the surface of the HA based fibrous membranes. Nevertheless, it can be found from Fig. 7b–d that volume of cell aggregations decreased and the number of aggregation increased accordingly with the increase in gelatin content, implying that gelatin could change the surface properties of the materials for cell adhesion.

The cell morphologies were further studied by SEM. The cells cultured on the glass substrate and HA/gelatin 100/80 fibrous membranes for 3 days were showed as examples in Fig. 8. The glass surface was completely covered by cells (Fig. 8a), and the high magnification SEM image (Fig. 8b) shows that the cells attach to the surfaces by discrete filopodia and have spreading and spindle shape. However, cell clusters were observed on the HA/gelatin fibrous surface (Fig. 8c). The high magnification SEM image (Fig. 8d) shows that several cells gather together, indicating that cells do not favor the HA/gelatin fibrous surfaces.

On the glass substrate, where the cells became crowded due to proliferation and should be passaged on day 3, while on the HA/gelatin fibrous membranes, an extremely low cell attachment was observed even on the HA/gelatin 100/80 membranes after cell culture for 7 days. The aggregation of the cells on the HA based membranes made it hard cell to proliferate. The cell number was calculated by a hemocytometer after a complete detachment of cells from the membranes. It can be seen from Fig. 9 that the cell proliferation on the HA/gelatin fibrous membranes presented much slower rate than that on the glass substrate. The cell density on the glass substrate was almost the same as those on the HA/gelatin fibrous membranes after 1-day culture. However, the difference between cell densities on the two types of surfaces became notable after 3 days culture. The cells on the glass substrate performed a higher proliferation rate than the HA based membranes after 3-day culture. In summary, the HA based fibrous membranes present good cell viability but bad cell proliferation. This phenomenon is possibly related to the intrinsic nature of HA reported in previous literature [23,28]. Although HA possesses good biological compatibility and non-toxicity, HA is not suitable for cell attachment and proliferation [28]. However, this feature of HA materials can be changed by increasing the gelatin content in HA/gelatin blend. Our further study will be published soon and provide an interesting result of the HA/gelatin bi-component membrane with enriched gelatin content.

It can be concluded that the HA/gelatin fibrous membranes with controllable degradation rate and specific cell adherence could have good potential applications in the biomedical field, especially in wound or burn dressings, anti-adhesion treatment, artificial skin and implantable materials.

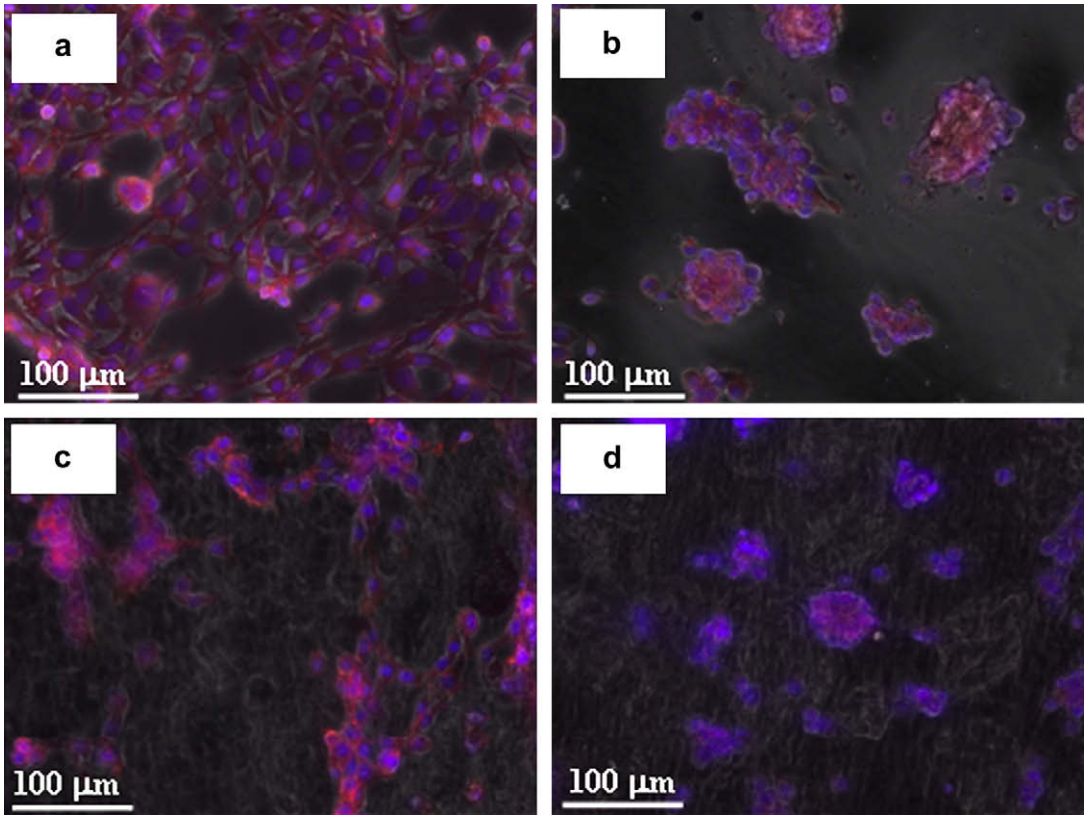

Fig. 7. Fluorescence micro-images of 3T6 cells cultured on HA/gelatin fibrous membranes and glass substrates after 1 day: (a) glass substrates; (b) HA/gelatin 100/5; (c) HA/gelatin 100/20; (d) HA/gelatin 100/80.

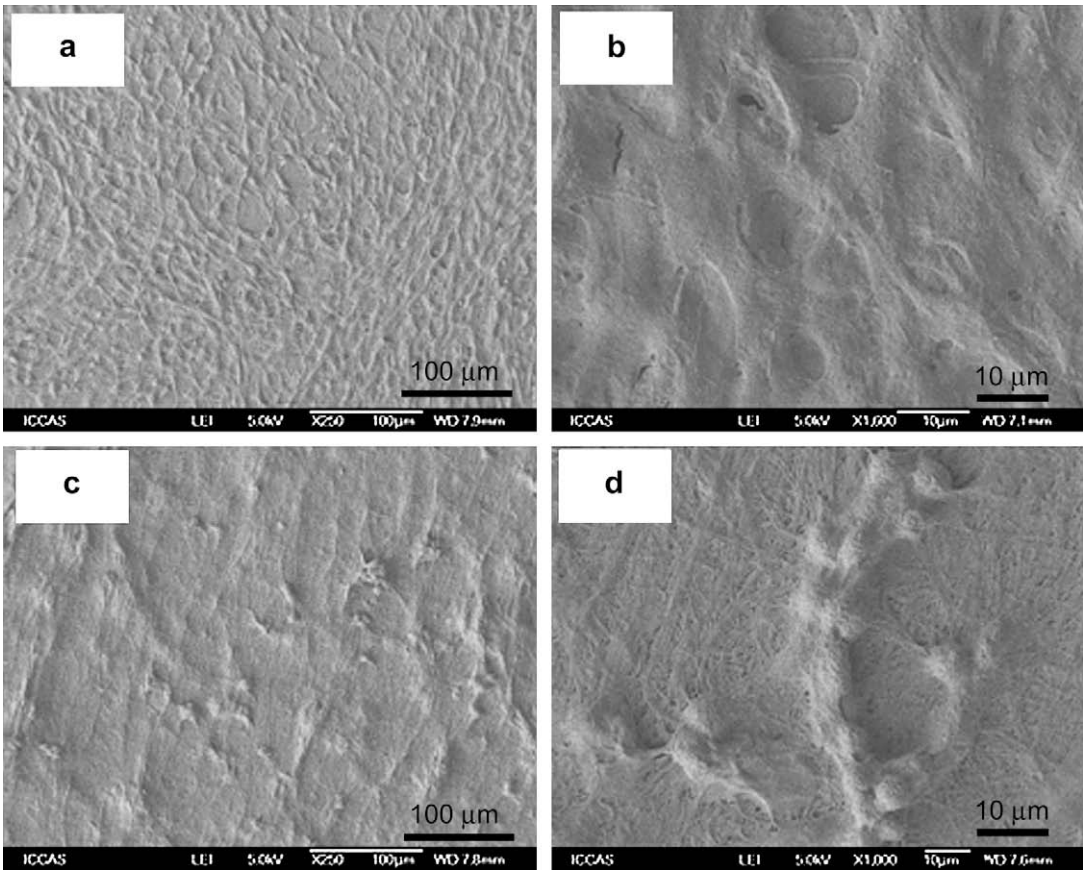

Fig. 8. SEM images with different magnification of 3T6 cells on HA/gelatin fibrous membranes and glass substrate after cells are cultured for 3 days. (a–b) Glass substrate; (c–d) HA/gelatin 100/80.

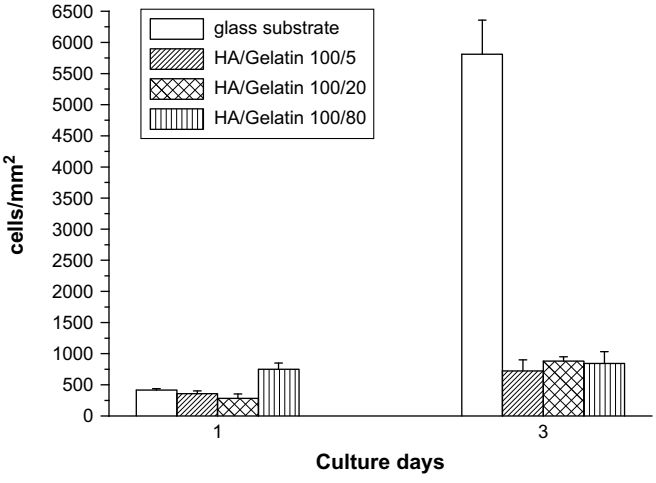

Fig. 9. Cell proliferation results on the HA/gelatin fibrous membranes and the glass substrate. The result was assessed by the cell density.

4. Conclusions

In this study, HA and HA/gelatin fibrous membranes were crosslinked by EDC and EDC/NHS, respectively. The crosslinked HA fibrous membranes showed better stability in water than in PBS. However, the degradation time of crosslinked HA membrane in PBS was less than 1 day. HA/gelatin blends exhibited inhibited degradation rate due to the forming of CO–NH linkages, and the blended fibers were more stable in PBS with increasing gelatin content. Therefore the HA based fibrous membranes with controllable degradation time from 1 week to 1 month could be fabricated. The cell culture experiments showed that HA based fibrous membranes exhibited excellent cell viability and specific cell adherence. The fibrous materials from natural biopolymer HA and gelatin could have many biomedical applications.

Acknowledgements

This work was financially supported by the National Nature Science Foundation of China (Funds No. 50503023, 50821062).

References

[1] Yoo HS, Lee EA, Yoon JJ, Park TG. Biomaterials 2005;26:1925–33.
[2] Ito T, Iida-Tanaka N, Niidome T, Kawano T, Kubo K, Yoshikawa K, et al. J Control Release 2006;112:382–8.
[3] Grigolo B, Franceschi LD, Roseti L, Cattini L, Facchini A. Biomaterials 2005;26:5668–76.
[4] Bulpitt P, Aeschlimann D. J Biomed Mater Res 1999;47:152–67.
[5] Ji Y, Ghosh K, Shu XZ, Li BQ, Sokolov CJ, Prestwich GD, et al. Biomaterials 2006;27:3782–92.
[6] Um IC, Fang D, Hsiao BS, Okamoto A, Chu B. Biomacromolecules 2004;5:1428–36.
[7] Li J, He A, Han CC, Fang D, Hsiao BS, Chu B. Macromol Rapid Commun 2006;27:114–20.
[8] Choi YS, Hong SR, Lee YM, Song KW, Park MH, Nam YS. J Biomed Mater Res 1999;48:631–9.
[9] Collins MN, Birkinshaw C. J Appl Polym Sci 2007;104:3183–91.
[10] Masters KS, Shah DN, Leinwand LA, Anseth KS. Biomaterials 2005;26:2517–25.
[11] Wang X, Um IC, Fang D, Okamoto A, Hsiao BS, Chu B. Polymer 2005;46:4853–67.
[12] Tomihata K, Ikada Y. J Biomed Mater Res 1997;37:243–51.
[13] Ramires PA, Milella E. J Mater Sci Mater Med 2002;13:119–23.
[14] Djagny KB, Wang Z, Xu S. Crit Rev Food Sci Nutr 2001;41:481–92.

[15] Adekogbe I, Ghanem A. Biomaterials 2005;26:7241–50.
[16] Yannas V, Burke JF. J Biomed Mater Res 1980;14:65–81.
[17] Galassi G, Brun P, Radice M, Cortivo R, Zanon GF, Genovese P, et al. Biomaterials 2000;1:2183–91.
[18] Coelho AM, Berumen L, Avrameas S. J Cell Physiol 1974;83:379–88.
[19] Saettone MF, Chetoni P, Torracca MT, Burgalassi S, Giannaccini B. Int J Pharm 1988;51:203–12.
[20] Pritchard K, Lansley AB, Martin GP, Helliwell M, Marriott C, Benedetti LM. Int J Pharm 1996;129:137–45.
[21] Shu X, Liu Y, Palumbo F, Prestwich GD. Biomaterials 2003;24:3825–34.
[22] Vercruysse KP, Prestwich GD. Crit Rev Ther Drug Car Systems 1998;15:513–55.
[23] Cho MK, Lee GH, Park EH, Kim SG. Tissue & Cell 2004;36:293–305.
[24] Zhang J, Sengera B, Vautiera D, Picarta C, Schaafa P, Voegela J, et al. Biomaterials 2005;26:3353–61.
[25] Liang H, Chang W, Liang H, Lee MH, Sung HW. J Appl Polym Sci 2004;91:4017–26.
[26] Moore KA, Lemischka IR. Science 2006;311:1880–5.
[27] Yaniv I, Stein J, Farkas DL, Askenasy N. Stem Cells Develop 2006;15:4–16.
[28] Xu FM, Nacker JC, Crone WC, Masters KS. Biomaterials 2008;29:150–60.