Colloids and Surfaces B: Biointerfaces 142 (2016) 165–172

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Superelastic, superabsorbent and 3D nanofiber-assembled scaffold for tissue engineering

Weiming Chen[a,1], Jun Ma[b,1], Lei Zhu[c], Yosry Morsi[d], Hany EI-Hamshary[e,f], Salem S. Al-Deyab[e], Xiumei Mo[a,g,*]

[a] College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China
[b] Department of Orthopaedics, Changzheng Hospital affiliated with Second Military Medical University, 415 Fengyang Road, Shanghai 200003, China
[c] State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Materials Science, Donghua University, Shanghai 201620, China
[d] Faculty of Sciences, Engineering and Technology, Hawthorn, Victoria 3122, Australia
[e] Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[f] Department of Chemistry, Faculty of Science, Tanta University, Tanta 31527, Egypt
[g] Shandong International Biotechnology Park Development Co., Ltd., China

ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form 20 February 2016
Accepted 23 February 2016
Available online 27 February 2016

Keywords:
Electrospinning
Scaffold
Nanofiber
Tissue engineering
Superelastic

ABSTRACT

Fabrication of 3D scaffold to mimic the nanofibrous structure of the nature extracellular matrix (ECM) with appropriate mechanical properties and excellent biocompatibility, remain an important technical challenge in tissue engineering. The present study reports the strategy to fabricate a 3D nanofibrous scaffold with similar structure to collagen in ECM by combining electrospinning and freeze-drying technique. With the technique reported here, a nanofibrous structure scaffold with hydrophilic and superabsorbent properties can be readily prepared by Gelatin and Polylactic acid (PLA). In wet state the scaffold also shows a super-elastic property, which could bear a compressive strain as high as 80% and recovers its original shape afterwards. Moreover, after 6 days of culture, L-929 cells grow, proliferate and infiltrated into the scaffold. The results suggest that this 3D nanofibrous scaffold would be promising for varied field of tissue engineering application.

1. Introduction

Scaffold plays a critical role in tissue engineering, as it provides mechanical support and offers a proper microenvironment for cells growth. An ideal scaffold should mimic not only the organized structure but also the physiological function of a normal tissue, which is essential for successful tissue repair [1]. In recent decades, many researchers have focused their attention on creating ideal scaffold for usage in tissue regeneration. The main obstacle of tissue engineering is the design of porous 3D scaffolds that can mimic the structure and biological functions of the nature extracellular matrix (ECM), for providing mechanical support, and regulating cellular physiological activities [2]. Scaffolds began to be fabricated with nanofibrous features, as the main protein of the ECM (collagen) regulates cell behavior through its nanofibrous architecture [1,3]. To get 3D porous scaffold, various fabrication approaches have been explored such as microembossing [4], fiber felts, solvent casting and particulate leaching, fiber bonding, and 3D printing etc. [5–7]. However, it is still difficult using the above mentioned technique to fabricate an ideal scaffold with the appropriate nanofibrous architecture [8].

Currently, there are mainly three major approaches to produce nanofibrous scaffolds: self-assembly [9–13], phase separation [14–18], and electrospinning [19–23]. Nanofibrous scaffold fabricated by self-assembly can be constructed in situ which is useful for clinical settings [24]. However, most self-assembled scaffolds reported were made from gels which lack mechanical strength and not appropriate for structural tissues [1]. Phase separation is a simple and versatile technique to create 3D scaffold as it is simple to control the shape of the scaffold base on the formed mold, and easy for cells entrapped. However, only few materials such as PLA [14] and gelatin [25] could be fabricated into nanofibrous scaffold by thermally induced phase separation. Gelatin [25] can form nanofibrous scaffold by phase separation, but it lack mechanical strength. Compared with other nanofiber fabrication, eletrospinning is regarded as versatile and superior approach for

* Corresponding author at: College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China.
E-mail address: xmm@dhu.edu.cn (X. Mo).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.colsurfb.2016.02.050
0927-7765/

fabricating uniform ultrafine fibers in a continuous process. Electrospun nanofibers are structurally similar to collagen in ECM. The scaffold constructed by electrospinning has many advantages such as controlling of porosity, fiber size, and morphology. However, electrospinning fabrication technique most commonly produces relatively 2D mats and the construction of scalable and controllable assembly of nanofibers for 3D structure is still a major challenge [8].

In addition, there are several requirements in the design of scaffold for tissue engineering such as mechanical properties, porosity, pore size, and hydrophilic, etc. [5,26,27]. Scaffold should have the mechanical strength needed for implantation and the appropriate strength that can influence the biostability of implants [5]. It is also shown that the porosity and pore size of supporting 3D scaffold are important for tissue regeneration [27]. Large surface area favors cell attachment and growth. In addition, highly porous scaffolds are desirable for diffusion of nutrients and waste products from the implant [5,8]. Hydrophilic is also an important factor which can influence the 3D scaffold for implantation as hydrophilic scaffold will enhance cell growth and proliferation [26]. Therefore, a nanofibers 3D scaffold with the appropriate mechanical integrity, pore size, and hydrophilic property will provide a great potential for tissue engineering.

Recently, novel pathways of fabricating nanofibrous scaffolds have been reported in literature. By electrospinning and freeze-drying technique, Polyacrylonitrile (PAN) [28] was fabricated into nanofiber-assembled cellular aerogels with super-recyclable compressibility and multifunctionality. Similarly, Poly(MA-co-MMA-co-MABP) [29] was also fabricated into scaffold for tissue engineering. Though using combined electrospinning and freeze-drying to construct a nanofibrous scaffold is considered to be innovative and novel. However, PAN and Poly(MA-co-MMA-co-MABP) were hydrophobic and non-biodegradable. Hydrophilic and biodegradable materials are better choice for many tissue engineering applications. In this paper we report a robust methodology for creating superelastic scaffold with cellular structure that consists of nanofibers. Gelatin and PLA were chosen as materials since they were all widely used biomaterials for various biomedical applications. Firstly, gelatin/PLA nanofibers membranes were prepared by electrospinning; secondly, nanofibers membranes were cut into small pieces and were dispersed in *tert*-butanol by homogenizer; thirdly, the dispersions were frozen and freeze dried. Finally the scaffold was crosslinked by glutaraldehyde and washed by water. Subsequently a superelastic, superabsorbent and 3D nanofiber-assembled cellular scaffold was fabricated. The scaffold serves as an ECM template and offers a proper microenvironment for cells growth and proliferation.

2. Materials and methods

2.1. Materials

Gelatin was purchased from MP Biomedicals, LLC. PLLA was provided by Medprin Regenerative Medical Technologies Co., Ltd. Glutaraldehyde and *tert*-butanol were purchased from Sinopharm Chemical Reagent Co., Ltd. L-929 cell lines were supplied by Institute of Biochemistry and Cell Biology (the Chinese Academy of Sciences, Shanghai, China). All other chemicals were of analytical grades and commercially available.

2.2. Fabrication of gelatin/PLA nanofibers

Gelatin/PLA nanofibers were prepared by electrospinning. Briefly, 1.2 g gelatin and 0.8 g PLA were separately dissolved in 10 mL 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP), then the precursor solution was prepared by blending 10 mL gelatin solution and 3 mL PLA solution. The mass ratio of PLA and gelatin was 1:5. The solution was loaded into a syringe capped with a 9-G metal needle with a controllable fed rate of 5 mL per hour. A high voltage of 15 kv was applied to the need trip. The resultant nanofibers were deposited onto aluminum foil, the spinneret-collector distance was 15 cm.

2.3. Fabrication of 3D nanofiber-assembled cellular scaffold

1 g, 2 g, 3 g, and 4 g gelatin/PLA nanofibers membranes were separately cut into small pieces (1 cm × 1 cm), and were dispersed in 100 mL *tert*-butanol by homogenizing the mixture for 15 min at 12,000 rpm using an IKA T18 homogenizing. Then the uniform nanofibers dispersions were poured into mould, frozen in −80 °C for 2 h, and then freeze dried for 24 h to obtain the unstable scaffold [28]. Glutaraldehyde was added into ethanol (the concentration of glutaraldehyde was 5%) as crosslinker. The unstable scaffolds were crosslinked by glutaraldehyde for 10 min. By controlling the concentration of nanofibers dispersions, different density of scaffolds could be obtained. Four scaffolds (3DS) were prepared. 3DS-1, 3DS-2, 3DS-3, and 3DS-4 were respectively fabricated by 1 g, 2 g, 3 g, and 4 g gelatin/PLA nanofibers dispersions. To improve the biocompatibility of crosslinked scaffold, it was immersed in 5% solution of L-glutamic acid in 0.5 M HCl for 48 h [30]. Finally, the scaffolds were washed by water 5 times before use.

2.4. Characterization

2.4.1. Morphology of nanofibers

Electrospun nanofibers were sputter coated with gold, and their morphologies were observed by scanning electron microscopy (SEM, Hitachi TM-1000, Japan) at an accelerating voltage of 5 kV. Visualization software Image-J (National Institutes of Health, USA) was used to measure the diameters of electrospun nanofibers. Average nanofiber diameters and diameter distributions were determined by measuring at least 100 random fibers from the SEM micrographs. Homogenized nanofibers were dispersed in ethanol and observed by optical microscope. Fibers length distribution of the homogenized short nanofibers was calculated by measuring at least 50 random fibers from the optical microscope micrographs.

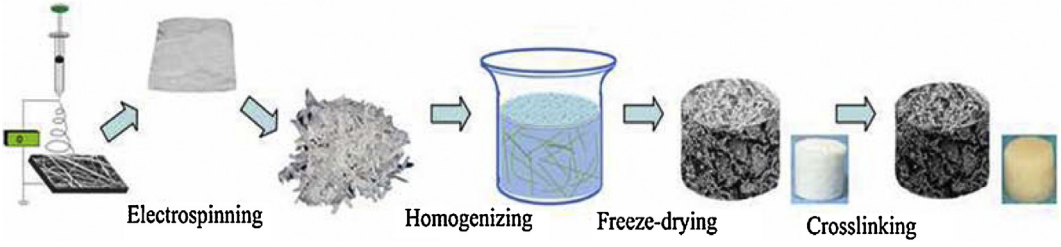

Fig. 1. Schematic illustration of the process for 3D scaffold.

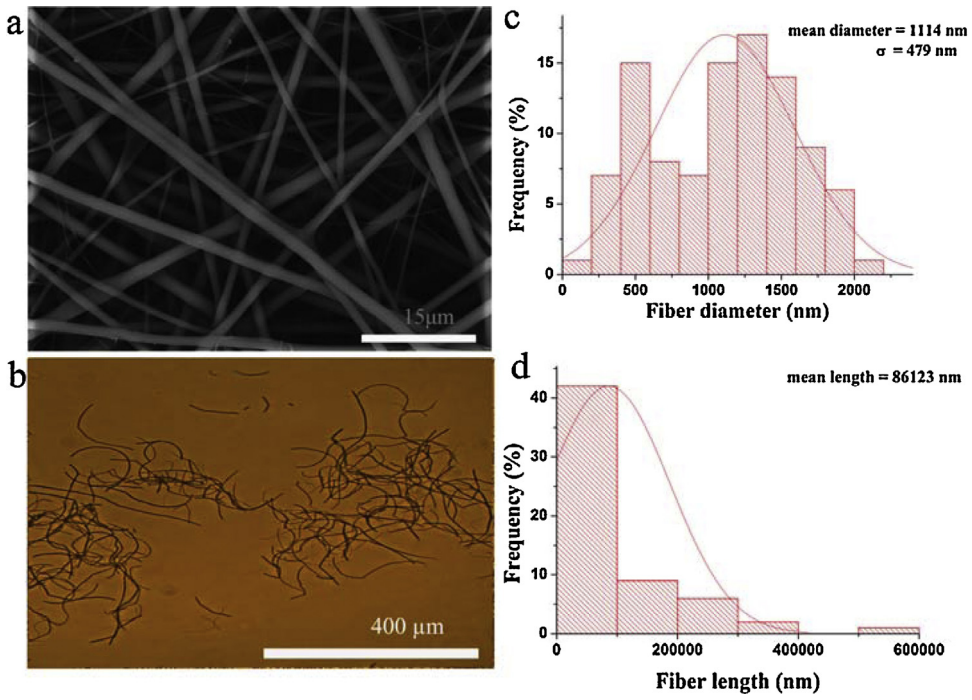

Fig. 2. Structure of nanofibers and short nanofibers. (a) SEM image of electrospun nanofibers. (b) Optical microscope image of the homogenized short nanofibers. (c) Diameter distribution of electrospun nanofibers. (d) Fibers length distribution of the homogenized short nanofibers.

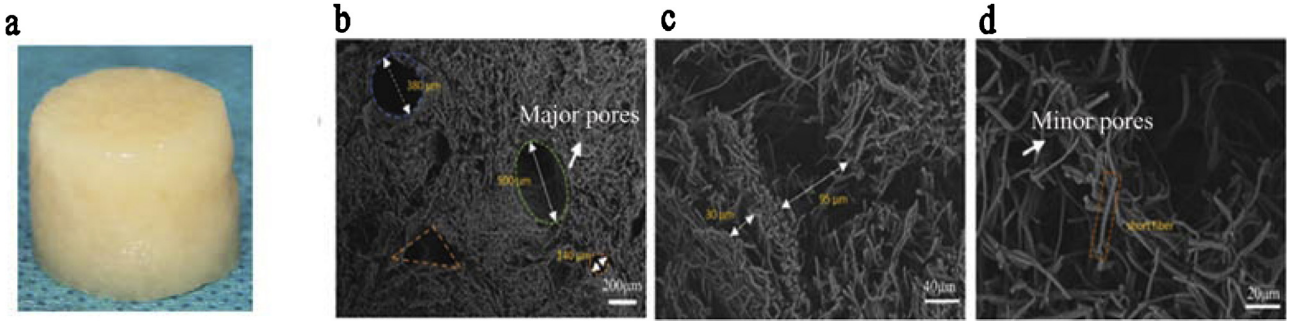

Fig. 3. (a) The digital camera of 3D scaffold (3DS-4), (b–d) SEM images of 3D scaffold (3DS-4) with different magnification.

2.4.2. Densities and pore size of 3D scaffold

The densities (ρ) of 3D scaffold were calculated by the following equation [29]:

$$P = m/v = (4m)/(\pi d^2 h) \quad (1)$$

Where m and v were the weight and volume of the scaffold, d and h were the diameter and height of the scaffold.

The pore sizes were measured from SEM image. The average value of densities was obtained from three parallel samples.

2.4.3. Water absorption capacity

The water absorption capacity of 3D scaffolds was tested following the reported method [31]. Firstly, 3D scaffolds were weighted and recorded as the dry weight. Secondly, 3D scaffolds were placed in tubes containing distilled water, and were removed after 5, 15, 30, 60, and 120 min. The wet 3D scaffolds were placed on a paper towel, and the excess water was allowed to drain off. The 3D scaffolds were then weighed and measured as wet weight. The water absorption percentage (w) of the 3D scaffolds was then calculated with the following equations:

$$w = (w_1 - w_0)/w_0 \times 100\% \quad (2)$$

w_0 and w_1 were the weight of dry and wet 3D scaffolds, respectively. The average value was obtained from three parallel samples.

2.4.4. Mechanical properties

Compression tests were carried out by Instron 5969 testing system with a 500-N loaded sensor. The compression strain-stress curves with compression strain (60% and 80%) were measured at strain rate of 5 mm/min at room temperature. A 100 cycle loading-unloading fatigue test was performed by measuring compression strain (ε = 60%) at a compressing speed of 30 mm/min. Cylinder-shaped scaffolds with height of 10 mm and diameter of 9 mm were used for the tests. It should be noted that the mechanical properties were tested in wet state.

2.4.5. Cell proliferation on scaffold

L-929 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco) which contain 10% FBS, 100 μg/mL streptomycin and 100 U/mL penicillin. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Before cell seeding, the scaffold was immersed in 75% ethanol for 3 h and washed by phosphate-buffered saline (PBS) four times. 200 μL L-929 cells suspension with a cell number of 1×10^4 were seeded into different

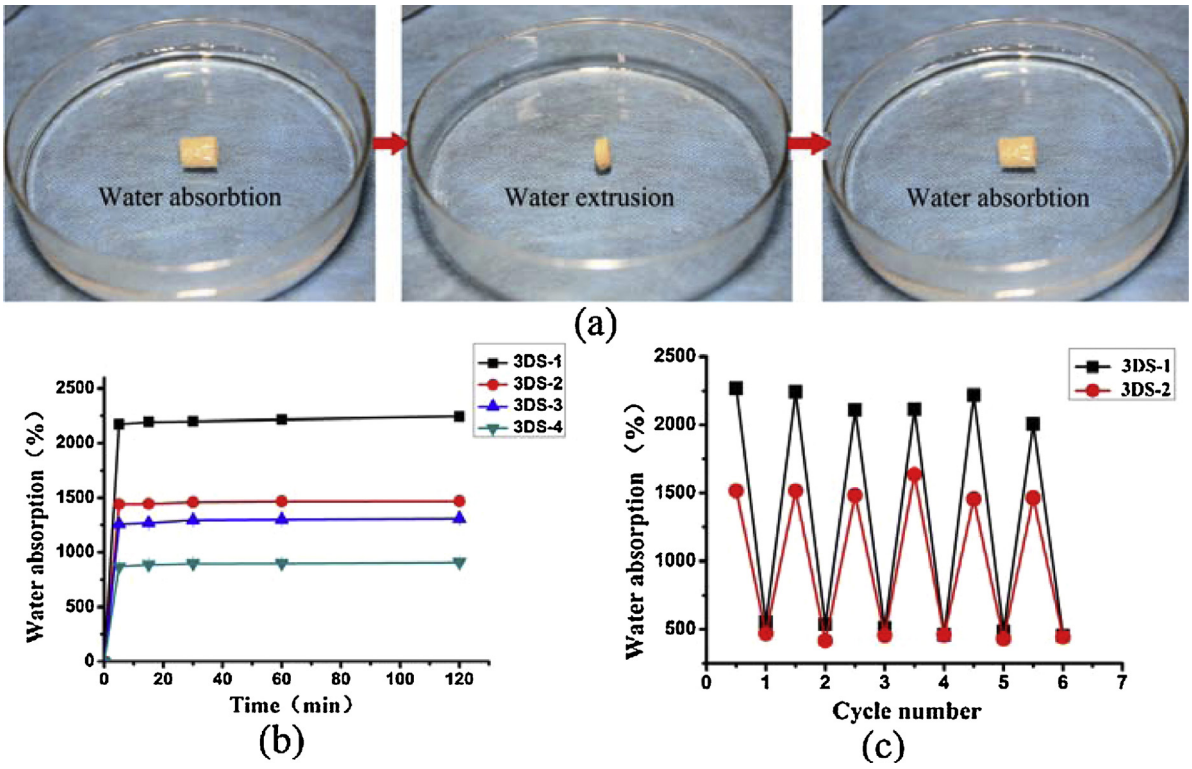

Fig. 4. Water absorption property of the scaffolds. (a) Macroscopic demonstration of the reversible water uptake and extrusion. The scaffold would absorb water after placed in water, and remove water after compressed by external force. (b) Water absorption rate of the scaffolds at different absorption time. (c) Water absorption ratio of scaffolds at different compression cycles.

scaffold (3DS-2, 3DS-3, and 3DS-4). 12 h after seeding, the scaffolds were transferred into a 96-well plate to remove the unattached cells. The proliferation of cell on the scaffold was evaluated with MTT assay after 1, 3, and 6 days of culture. Briefly, the cells were incubated in MTT solution at 37 °C for 4 h, the MTT solution was replaced with 200 μL dimethyl sulfoxide (DMSO) and the whole plate was shaken for 30 min. The absorbance was measured at 492 nm with a microplate reader.

The morphologies of L-929 cells seeded on scaffolds after 6 days were studied by SEM. The cell-seeded scaffolds were subsequently washed with PBS and fixed with 4% paraformaldehyde in PBS buffer for 2 h at 4 °C. After that, the fixed samples were dehydrated in an ascending series of ethanol for 8 min each and dried in a vacuum. Consequently the scaffolds were gold sputtered and observed under SEM.

2.4.6. Cell infiltration

Cell infiltration studies on the 3D scaffolds were performed using paraffin embedding hematoxylin-eosin (H&E) staining [32]. After 3 and 6 days post-seeding, cell scaffolds were removed from media, washed with PBS and fixed in 4% paraformaldehyde for 30 min at 4 °C. Cross sections of the cells scaffolds constructs were obtained after dehydration, clarification, infiltration and paraffin embedding. The specimens were then stained with H&E and observed under an optical microscope.

2.5. Statistical analysis

All experiments were conducted at least three times. Statistically significant differences were obtained by comparing data using one-way ANOVA. The criteria for statistical significance were $^{*}p < 0.05$.

3. Results and discussions

3.1. The preparation of scaffold

Fig. 1 illustrates the pathway for fabricating 3D scaffold. The challenge was how the nanofibers can be assembled into 3D scaffold with open-cell cellular architecture with the appropriate elastic interconnectivity [28]. To address these issues, the first step was breaking the originally lamellar deposited nanofiber membranes into homogeneously nanofiber dispersions. Then a high-speed homogenizer was used to break the closely packed and entangled electrospun nanofibers into short nanofibers. To make nanofibers assemble into 3D scaffold, nanofibers were homogenized in *tert*-butanol to form well dispersed nanofiber despersions and then freeze dried into an unstable scaffold. To make nanofibers form elastic interconnected networks rather than simply stack together, the unstable scaffold were crosslinked by glutaraldehyde to give a 3D scaffold, with stable bonded elastic fibrous networks and a cellular architecture.

3.2. Structure of nanofiber

Smooth structure of gelatin/PLA nanofibers could be observed as shown in Fig. 2a, the diameter of the nanofibers range from 179 nm to 2166 nm, and the average diameter was about 1114 nm (Fig. 2c). The dispersity of homogenized nanofibers were suspended in ethanol and observed by optical microscope. As shown in Fig. 2b, the short cut nanofibers were well dispersed. Moreover, Fig. 2d illustrates the fibers length ranged primarily from 4 μm to 600 μm, with an average length of 86 μm which were shorter than electrospun nanofibers before homogenized (10 mm), which indicated that closely packed and entangled electrospun nanofibers had been broken and homogenized by high-speed homogenizer.

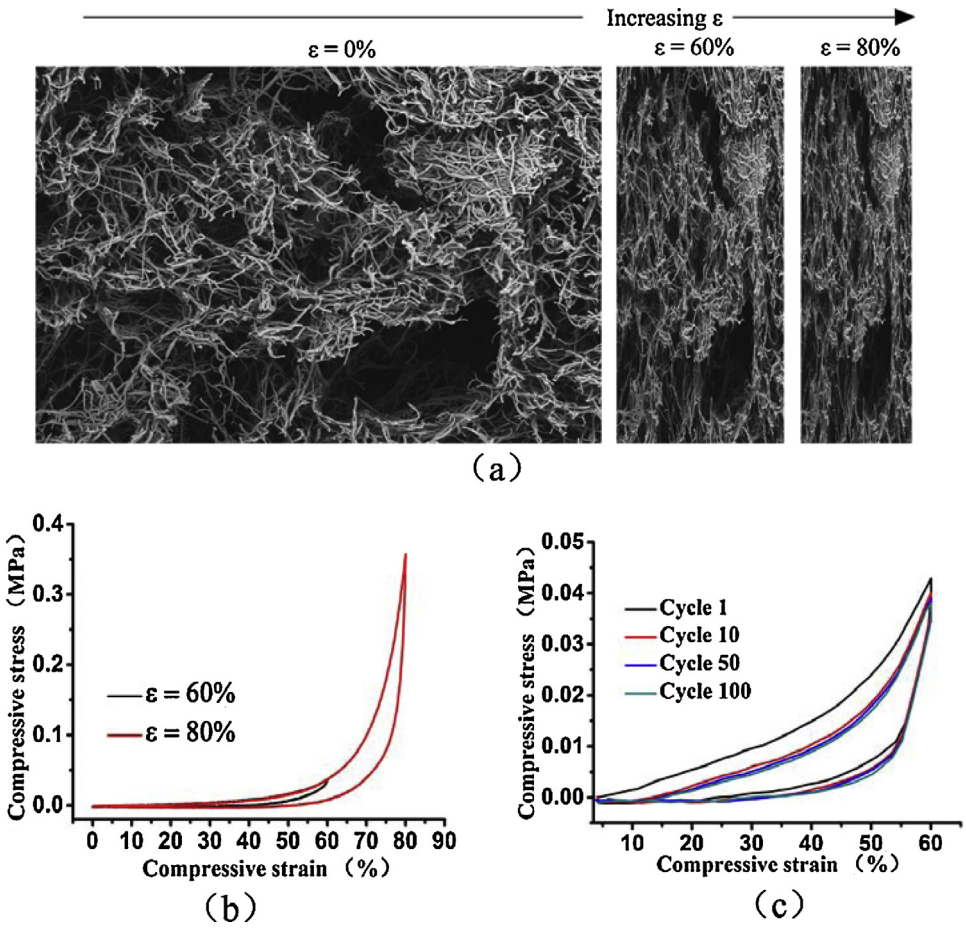

Fig. 5. Compressive mechanical property of the scaffold in wet state (3DS-4). (a) SEM image of the nanofiber structure by changing the compressive deformation. (b) Compressive stress-strain curves of the scaffold under two compressing and releasing cycles (ε = 60% and 80%). (c) Compressive stress–strain curves of the scaffold under different cycles in the compressive fatigue test (ε = 60%).

3.3. Structure and densities of nanofibrous scaffold

The densities of 3DS-1, 3DS-2, 3DS-3, and 3DS-4 were 76.6 mg/cm^3, 105.3 mg/cm^3, 131.1 mg/cm^3, and 138.8 mg/cm^3. An obvious cellular architecture could be observed from SEM images of scaffold (3DS-4) as shown in Fig. 3b–d. Various shapes of pore in the scaffold were observed, like circular, elliptical, triangle, and irregular shape (Fig. 3b). The diameter of pore was also non-uniform, as shown in Fig. 3b, the major pores with about 100–500 μm in diameter could be attributed to the formation of crystals of frozen solvent formed in the freeze-drying process. As shown in Fig. 3d, smaller pores with the size of 20 μm were observed in the scaffold. 3DS can mimic the structure of nature ECM, and the SEM pictures of nature ECM, 3DS-1, 3DS-2, 3DS-3, and 3DS-4 can be found in Supplementary information.

3.4. Water absorption study

As collagen nanofiber networks in nature tissue, the 3D scaffold showed superabsorbent property. The 3D scaffold would absorb water quickly after placed in water, and remove most absorbed water after compressed by external force. The scaffold would absorb water again after placed in water, which showed reversible sorption and desorption water property (Fig. 4a). It was observed that water absorbed was completed in less than 5 min after the scaffold placed in water (Fig. 4b). Generally, the water absorption amount of the scaffolds depends on their densities and ranged from 800% to 2200%, which indicated that pores filling was the mechanism for water absorption. However, the water absorption of our scaffold was different from other superabsorbent and 3D nanofiber-assembled cellular scaffold reported in literature [28,29], which were fabricated by hydrophobicity polymer and showed oil absorption and hydrophobic property. It indicated that the hydrophilic gelatin was important for water absorption property. Reversible sorption and desorption of water was observed (Fig. 4c), the procedure was repeated several times, and there was no loss for water absorption capacity.

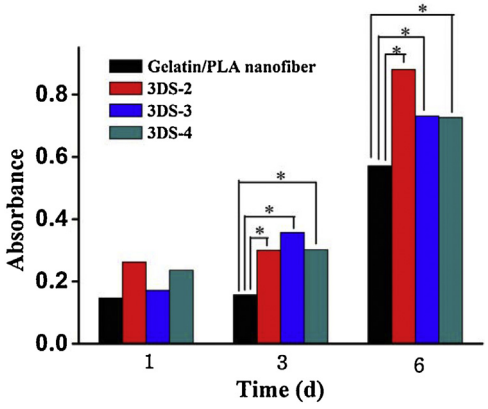

Fig. 6. L-929 cells proliferation test on 3D scaffold (Gelatin/PLA nanofiber, 3DS-2, 3DS-3, and 3DS-4). Error bars represent the mean ± SD (n = 3, *indicates statistically significant difference, $p < 0.05$).

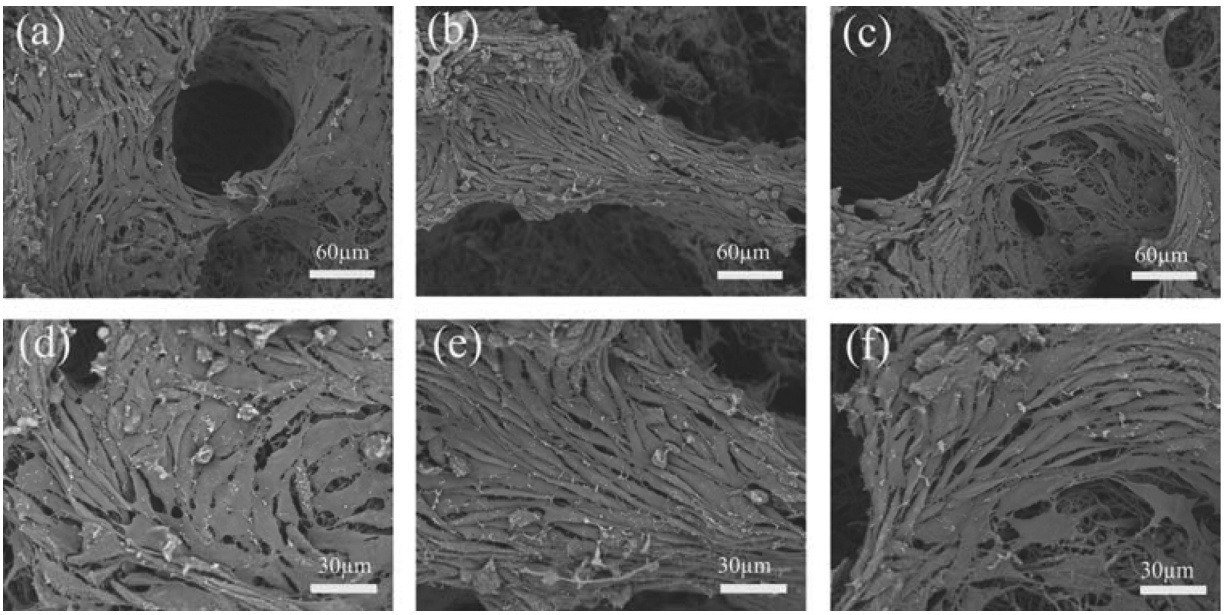

Fig. 7. SEM images of L-929 cells cultured on the 3D scaffolds 3D scaffolds 3DS-2 (a, d), 3DS-3 (b, e) and 3DS-4 (c, f) after 6 days.

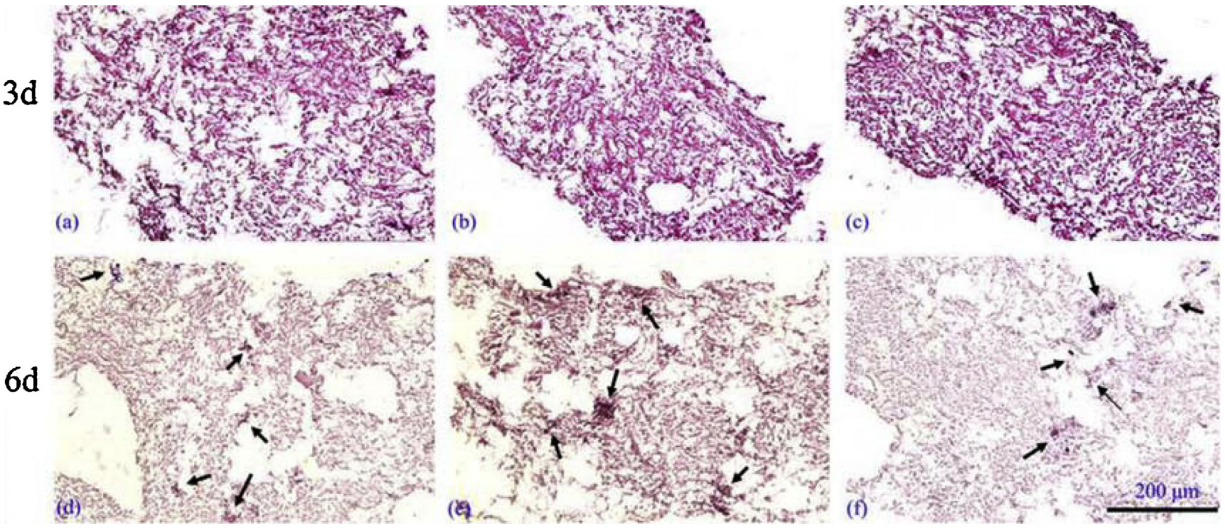

Fig. 8. HE-stained histology images of L-929 cells infiltration into the 3D scaffolds 3DS-2 (a, d), 3DS-3 (b, e) and 3DS-4 (c, f) at culture time of 3 days (a–c) and 6 days (d–f).

3.5. Compressive mechanical property

The scaffold structure would change by pressure (Fig. 5a). Fig. 5b shows the compressive stress-strain curves of the scaffold in wet state (ε = 60% and 80%), which indicated that the outstanding mechanical property was achieved. The scaffold could bear a compressive strain as high as 80% and could recover their original shape after the stress was released. Moreover, the scaffolds were subjected to a cyclic compression test with 100 loading-unloading fatigue cycles with 60% strain, which also exhibited excellent cycling performance as shown in Fig. 5c. After 100 cyclic compressions, no significant decrease in the strength was observed for the scaffold.

Preparing 3D scaffold by electrospun nanofibers still face a challenge, most of products reported in literature were cotton-like nanofibers deposits, which showed poor mechanical property and no elastic resilience [28]. Electrospun gelatin nanofibers and its composite nanofibers had been widely researched for tissue engineering application. However, most of reported nanofibrous gelatin scaffolds were 2-dimensional sheets [33–35]. Liu et al. fabricated gelatin nanofibers scaffold by thermally induced phase separation [25,36], but the elastic property of that scaffold was unknown. Our scaffold showed outstanding compressive mechanical properties. Freeze-drying technique make nanofibers be 3D structure and crosslink step make them be a scaffold with elastic property. Before crosslink, the nanofibers were just contact with each other, and hence the mechanical property of scaffold was weak. However, nanofibers with strong bonding were formed after crosslink [37]. The formation of molecular crosslink between gelatin fibers by glutaraldehyde was a key factor in the preparation of this scaffold. Moreover, the density of scaffold could influence the mechanical property, the compressive stress of scaffold was enhanced with the density was increased (Supplementary information (Fig. 2)).

3.6. Cell proliferation

Cell proliferation and biocompatibility of the composite scaffold was evaluated using MTT assay (Fig. 6). MTT assay was carried

out on days 1, 3, and 6 respectively after cell seeding. During this culture period, the cells proliferation was significant. MTT reading indicated that the number of cells on scaffold (3DS-2, 3DS-3, and 3DS-4) increased much more than those on gelatin/PLA nanofibers. Gelatin and PLA were all biocompatible polymer and efficient for cells growth. On the other side, the scaffold provided enough three dimensional spaces with porous structure for cells proliferation and migration. It indicated that the 3D scaffold showed ideal biocompatibility and provided desired microenvironment for cells growth and proliferation. However, no significant difference on proliferation was found among 3DS-2, 3DS-3, and 3DS-4, respectively.

The L-929 cells morphology and attachment on the 3D scaffolds after 6 days culture were observed by SEM. As shown in Fig. 7, firstly, cells attached and spread on the surface of scaffold, and the smaller pores in the network could facilitated cell infiltration into the scaffolds. Secondly, the cells adhered on the nanofibers and proliferated along the nanofibrous network, with a normal phenotypic and elongated morphology, indicating that nanofibers features can significantly influence the proliferation of cells.

3.7. Cell infiltration

To evaluate cell infiltration in the 3D scaffolds, the cell-seeded scaffold was examined by the HE-staining (Fig. 8). As expected that scaffold could enhance cell infiltration, as confirmed by the HE-stained images. After 6 days of culture, cells were found not only on the surface of scaffolds, but also deep inside them. L-929 cells had migrated to a depth of approximately 463 μm, 450 μm, and 363 μm in the 3DS-2 (Fig. 8d), 3DS-3 (Fig. 8e), and 3DS-4 (Fig. 8f), respectively. Cells in the 3DS-2 and 3DS-3 cells had almost moved from one side of scaffold to the other side. This phenomenon was attributed to the large pores and the high degree of porosity of the scaffold that positively enhanced cell infiltration. Previous studies [38,39] had indicated that the ability of cells to move through layers of electrospun nanofibers membranes was limited, due to the dense packing of nanofibers and the relatively small pore size. However, these limitations could be overcome through the use of the 3D scaffold. The nanofibers would provide spaces for cell growth, and the large pore sizes could benefit cells migration throughout the scaffold.

4. Conclusions

In this study, we successfully fabricated a 3D scaffold by combined the electrospining and freeze-drying technique. The scaffold presented porous structure and good water absorption capacity. Moreover, in wet state, the scaffold showed super recyclable compressibility and the cells seeded on it showed normal phenotypic morphology and excellent proliferation, suggesting that the scaffold has excellent biocompatibility. The nanofibers-based scaffold may have great potential for various tissue engineering applications.

Acknowledgements

This research was supported by National Natural Science Foundation of China (31470941, 31271035), Science and Technology Commission of Shanghai Municipality (15JC1490100, 15441905100), Ph.D. Programs Foundation of Ministry of Education of China (20130075110005) and light of textile project (J201404), the Fundamental Research Funds for the Central Universities (CUSF-DH-D-2016021), Yantai Double Hundred Talent Plan. The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the research group project no. RGP-201

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2016.02.050.

References

[1] J.M. Holzwarth, P.X. Ma, 3D nanofibrous scaffolds for tissue engineering, J. Mater. Chem. 21 (2011) 10243–10251.
[2] H. Xu, H. Li, Q. Ke, J. Chang, An anisotropically and heterogeneously aligned patterned electrospun scaffold with tailored mechanical property and improved bioactivity for vascular tissue engineering, ACS Appl. Mater. Interfaces 7 (2015) 8706–8718.
[3] W.J. Li, C.T. Laurencin, E.J. Caterson, R.S. Tuan, F.K. Ko, Electrospun nanofibrous structure: a novel scaffold for tissue engineering, J. Biomed. Mater. Res. 60 (2002) 613–621.
[4] Y. Yang, S. Basu, D.L. Tomasko, L.J. Lee, S.-T. Yang, Fabrication of well-defined PLGA scaffolds using novel microembossing and carbon dioxide bonding, Biomaterials 26 (2005) 2585–2594.
[5] S. Yang, K.-F. Leong, Z. Du, C.-K. Chua, The design of scaffolds for use in tissue engineering. Part I. Traditional factors, Tissue Eng. 7 (2001) 679–689.
[6] A.G. Mikos, Y. Bao, L.G. Cima, D.E. Ingber, J.P. Vacanti, R. Langer, Preparation of poly (glycolic acid) bonded fiber structures for cell attachment and transplantation, J. Biomed. Mater. Res. 27 (1993) 183–189.
[7] C.X.F. Lam, X. Mo, S.-H. Teoh, D. Hutmacher, Scaffold development using 3D printing with a starch-based polymer, Mater. Sci. Eng. C 20 (2002) 49–56.
[8] B. Sun, Y. Long, H. Zhang, M. Li, J. Duvail, X. Jiang, H. Yin, Advances in three-dimensional nanofibrous macrostructures via electrospinning, Prog. Polym. Sci. 39 (2014) 862–890.
[9] C.M. Yamazaki, S. Asada, K. Kitagawa, T. Koide, Artificial collagen gels via self-assembly of de novo designed peptides, Pept. Sci. 90 (2008) 816–823.
[10] E. Beniash, J.D. Hartgerink, H. Storrie, J.C. Stendahl, S.I. Stupp, Self-assembling peptide amphiphile nanofiber matrices for cell entrapment, Acta Biomater. 1 (2005) 387–397.
[11] E.L. Rexeisen, W. Fan, T.O. Pangburn, R.R. Taribagil, F.S. Bates, T.P. Lodge, M. Tsapatsis, E. Kokkoli, Self-assembly of fibronectin mimetic peptide-amphiphile nanofibers, Langmuir 26 (2009) 1953–1959.
[12] M. Zhou, A.M. Smith, A.K. Das, N.W. Hodson, R.F. Collins, R.V. Ulijn, J.E. Gough, Self-assembled peptide-based hydrogels as scaffolds for anchorage-dependent cells, Biomaterials 30 (2009) 2523–2530.
[13] A. Zhu, Y. Lu, Y. Pan, S. Dai, H. Wu, Self-assembly of N-maleoylchitosan in aqueous media, Colloids Surf. B 76 (2010) 221–225.
[14] P.X. Ma, R. Zhang, Synthetic nano-scale fibrous extracellular matrix, J. Biomed. Mater. Res. 46 (1999) 60–72.
[15] L. Xiaohua, P.X. Ma, The nanofibrous architecture of poly(L-lactic acid)-based functional copolymers, Biomaterials 31 (2010) 259–269.
[16] Y.-C. Wang, M.-C. Lin, D.-M. Wang, H.-J. Hsieh, Fabrication of a novel porous PGA-chitosan hybrid matrix for tissue engineering, Biomaterials 24 (2003) 1047–1057.
[17] R. Heijkants, R. van Calck, T. van Tienen, J. de Groot, A. Pennings, P. Buma, R. Veth, A. Schouten, Polyurethane scaffold formation via a combination of salt leaching and thermally induced phase separation, J. Biomed. Mater. Res. A 87 (2008) 921–932.
[18] A. Nieponice, L. Soletti, J. Guan, Y. Hong, B. Gharaibeh, T.M. Maul, J. Huard, W.R. Wagner, D.A. Vorp, In vivo assessment of a tissue-engineered vascular graft combining a biodegradable elastomeric scaffold and muscle-derived stem cells in a rat model, Tissue Eng. Part A 16 (2010) 1215–1223.
[19] S.A. Sell, M.J. McClure, K. Garg, P.S. Wolfe, G.L. Bowlin, Electrospinning of collagen/biopolymers for regenerative medicine and cardiovascular tissue engineering, Adv. Drug Deliv. Rev. 61 (2009) 1007–1019.
[20] Y. Zhang, H. Ouyang, C.T. Lim, S. Ramakrishna, Z.M. Huang, Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds, J. Biomed. Mater. Res. B Appl. Biomater. 72 (2005) 156–165.
[21] L. Buttafoco, N. Kolkman, P. Engbers-Buijtenhuijs, A. Poot, P. Dijkstra, I. Vermes, J. Feijen, Electrospinning of collagen and elastin for tissue engineering applications, Biomaterials 27 (2006) 724–734.
[22] W. Chen, D. Li, E.-S. Ahmed, M. El-Newehy, H.A. El-Hamshary, S.S. Al-Deyab, C. He, X. Mo, Dexamethasone loaded core?shell SF/PEO nanofibers via green electrospinning reduced endothelial cells inflammatory damage, Colloids Surf. B 126 (2015) 561–568.
[23] K. Zhang, H. Wang, C. Huang, Y. Su, X. Mo, Y. Ikada, Fabrication of silk fibroin blended P (LLA-CL) nanofibrous scaffolds for tissue engineering, J. Biomed. Mater. Res. A 93 (2010) 984–993.
[24] K.L. Niece, C. Czeisler, V. Sahni, V. Tysseling-Mattiace, E.T. Pashuck, J.A. Kessler, S.I. Stupp, Modification of gelation kinetics in bioactive peptide amphiphiles, Biomaterials 29 (2008) 4501–4509.
[25] X. Liu, P.X. Ma, Phase separation, pore structure, and properties of nanofibrous gelatin scaffolds, Biomaterials 30 (2009) 4094–4103.
[26] E.R. Fisher, Challenges in the characterization of plasma-processed three-dimensional polymeric scaffolds for biomedical applications, ACS Appl. Mater. Interfaces 5 (2013) 9312–9321.

[27] L. Cima, J. Vacanti, C. Vacanti, D. Ingber, D. Mooney, R. Langer, Tissue engineering by cell transplantation using degradable polymer substrates, J. Biomech. Eng. 113 (1991) 143–151.
[28] Y. Si, J. Yu, X. Tang, J. Ge, B. Ding, Ultralight nanofibre-assembled cellular aerogels with superelasticity and multifunctionality, Nat. Commun. 5 (2014) 5802.
[29] G. Duan, S. Jiang, V. Jérôme, J.H. Wendorff, A. Fathi, J. Uhm, V. Altstädt, M. Herling, J. Breu, R. Freitag, Ultralight, soft polymer sponges by self-Assembly of short electrospun fibers in colloidal dispersions, Adv. Funct. Mater. 25 (2015) 2850–2856.
[30] J.E. Gough, C.A. Scotchford, S. Downes, Cytotoxicity of glutaraldehyde crosslinked collagen/poly (vinyl alcohol) films is by the mechanism of apoptosis, J. Biomed. Mater. Res. 61 (2002) 121–130.
[31] S.K. Ramadass, S. Perumal, A. Gopinath, A. Nisal, S. Subramanian, B. Madhan, Sol–Gel assisted fabrication of collagen hydrolysate composite scaffold: a novel therapeutic alternative to the traditional collagen scaffold, ACS Appl. Mater. Interfaces 6 (2014) 15015–15025.
[32] J. Wu, C. Huang, W. Liu, A. Yin, W. Chen, C. He, H. Wang, S. Liu, C. Fan, G.L. Bowlin, Cell infiltration and vascularization in porous nanoyarn scaffolds prepared by dynamic liquid electrospinning, J. Biomed. Nanotechnol. 10 (2014) 603–614.
[33] L. Ghasemi-Mobarakeh, M.P. Prabhakaran, M. Morshed, M.-H. Nasr-Esfahani, S. Ramakrishna, Electrospun poly (ε-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering, Biomaterials 29 (2008) 4532–4539.
[34] Z.-M. Huang, Y. Zhang, S. Ramakrishna, C. Lim, Electrospinning and mechanical characterization of gelatin nanofibers, Polymer 45 (2004) 5361–5368.
[35] Y. Zhang, H. Ouyang, C.T. Lim, S. Ramakrishna, Z.M. Huang, Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds, J. Biomed. Mater. Res. B Appl. Biomater. 72 (2005) 156–165.
[36] X. Liu, L.A. Smith, J. Hu, P.X. Ma, Biomimetic nanofibrous gelatin/apatite composite scaffolds for bone tissue engineering, Biomaterials 30 (2009) 2252–2258.
[37] H.C. Liang, W.H. Chang, H.F. Liang, M.H. Lee, H.W. Sung, Crosslinking structures of gelatin hydrogels crosslinked with genipin or a water-soluble carbodiimide, J. Appl. Polym. Sci. 91 (2004) 4017–4026.
[38] C. Yang, G. Deng, W. Chen, X. Ye, X. Mo, A novel electrospun-aligned nanoyarn-reinforced nanofibrous scaffold for tendon tissue engineering, Colloids Surf. B 122 (2014) 270–276.
[39] N.L. Nerurkar, S. Sen, B.M. Baker, D.M. Elliott, R.L. Mauck, Dynamic culture enhances stem cell infiltration and modulates extracellular matrix production on aligned electrospun nanofibrous scaffolds, Acta Biomater. 7 (2011) 485–491.