Research Article Adv. Mater. Lett. 2016, **7**(2), 163-169 Advanced Materials *Letters*

www.amlett.com, www.vbripress.com/aml, DOI: 10.5185/amlett.2016.6003 Published online by the VBRI Press in 2016

Synthesis, physico-chemical characteristics and cellular behavior of poly (lactic-co-glycolic acid)/ gelatin nanofibrous scaffolds for engineering soft connective tissues

Farnaz Ghorbani[1], Hanieh Nojehdehyan[2], Ali Zamanian[3*], Mazaher Gholipourmalekabadi[4], Masoud Mozafari[3]**

[1]*Department of Biomedical Engineering, Tehran Science and Research Branch, Azad University, P.O. Box: 4515/775, Tehran, Iran*
[2]*Department of Dental Materials, School of Dentistry, Shahid Beheshti University of Medical Sciences, P.O. Box: 1983963113, Tehran, Iran*
[3]*Bioengineering Research Group, Nanotechnology and Advanced Materials Department, Materials and Energy Research Center (MERC), P.O. Box 14155-4777, Tehran, Iran*
[4]*Biotechnology Department, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

*Corresponding author. Tel. (+98) 912-3211180; Fax: (+98) 263-6201818; E-mail: a-zamanian@merc.ac.ir (A. Zamanian)
**Co-corresponding author. Tel. (+98) 912-6490679; Fax: (+98) 263-6280033; Email: mozafari.masoud@gmail.com (M. Mozafari)

Received: 24 May 2015, Revised: 22 November 2015 and Accepted: 05 December 2015

ABSTRACT

There have been several attempts to synthesis biodegradable polymeric constructs with adequate porous structures for soft connective tissues. In this study, randomly-oriented PLGA-gelatin nanofibrous scaffolds were synthesized by electrospinning method. We offered an appropriate solvent (2, 2, 2-trifluoroethanol) to dissolve both polymers for achieving a homogenous solution without inducing any toxic effects. The results confirmed the formation of high porous and bead free scaffolds, in which an increase in the injection rate slightly decreased the mechanical, swelling ratio and biodegradation behaviors. The modulus and tensile strength for the scaffolds with the injection rate of 0.2 ml/hr were 0.72 ±0.02 and 2.70 ±0.33, respectively. In addition, the evaluation of cell proliferation demonstrated that L929 fibroblast cells spread well on the scaffolds, indicating that they are able to support cell attachment. A possible chemical bond formation has been also suggested for the blending mixture of PLGA and gelatin molecules. Copyright © 2016 VBRI Press.

Keywords: Nanofibrous; scaffold; electrospinning; tissue engineering; cellular behavior.

Introduction

In recent years, there have been a large number of patients who suffer from the tissue defects. Tissue engineering developed when investigators observed that biodegradable scaffolds promote regeneration [**1, 2**]. The fundamental goal of tissue engineering is to evolve biological substitutes that restore, maintain or improve diseased, injured or congenitally absent tissues or organs [**3**]. Scaffolds are porous structure similar to extra-cellular matrix, so they are appropriate for the adhesion, growth, proliferation, migration and differentiation of cells [**4**]. Nano scale scaffolds can mimic the structure of extra cellular matrix (ECM), which can provide a suitable environment to support and cell regeneration [**5–8**]. There are many different techniques to fabricate tissue engineering scaffolds; for instance, freeze casting [**6,7, 9–14**], freeze drying [**12**], electrospinning [**15,16**], solvent casting [**17**], gas foaming [**18**], phase separation [**19–21**] and the like are common ones. Among existing methods, electrospinning is a simple, cost effective and convenient technique to produce highly porous nano fibers. Electrospinning essentially consists of the creation of an electric field between a target being usually grounded and a positively charged capillary filled with a polymer solution. When the electrostatic force related to charges overcomes the surface tension of the polymer solution at the capillary tip, a polymer jet is created. After traveling to the grounded target, submicron to nanoscale fibers collected. It is known that many various factors, starting from chemical composition, topography, porosity, fiber diameter, fiber

alignment, mechanical properties affect the bio-activity of scaffolds as cellular supports [**15**]. The electrospun scaffolds possess reticular structure with high specific surface area, high porosity and interconnect pores [**22**].

Different synthetic and natural materials have been used for fabrication of nanofibrous scaffolds [**23–26**]; for example, PLGA, PLLA, PCL, PHB, gelatin, collagen, chitosan, hyaluronic acid, alginate and the like are popular materials. Even though, the degradation rate, mechanical properties and porosity of synthetic materials can be controlled [**27**], the biological scaffolds can provide better cell adhesion. PLGA is a biodegradable polymer that increases compression modulus so improve the mechanical properties. However, the cell surface adherence on PLGA is poor due to the fact that PLGA lacks of surface cell discrimination points and has poor hydrophilicity and cellular affinity [**28–38**]. Gelatin is a natural biopolymer derived from the collagen which is the main component of the natural extracellular matrix in human body [**30, 32, 33, 36–38**]. Although natural polymers like gelatin have negative effects on mechanical properties, owing to improvement of cell adhesion and hydrophilic structure have been well known [**39, 40**]. Sharma *et al*. increased cellular proliferation (L929 Fibroblast) on the surface of PANI–CNT composite nanofibers can be attributed due to their conductivity [**41**]. Additionally, Levenberg *et al.* differentiated human stem cell on PLLA and PLGA scaffolds [**42**]. Besides, Tiwari *et al*.understood microfabric poly(*N*-isopropylacylamide)-CNT-polyaniline scaffold which was fabricated by electrospinning technique, provided an excellent surface for cell growth and proliferation [**43**]. Furthermore, Yang *et al*. used PLA electrospinning nanofibers to improve cell attachment [**8**]. Moreover, Zamani *et al*. found that structure of nanofibers influence nerve cell culture and cells on the random scaffold are irregularly formed [**32**].

Consequently, we focused on PLGA-gelatin composite solution to fabricate nanofibrous scaffolds and evaluate cellular behavior on the scaffolds to regenerate defects. Since Aqueous and organic solvents are suitable for gelatin and PLGA, respectively. Therefore, 2, 2, 2-trifluoroethanol dissolve both polymers at the same time to achieve homogeneous composite solution and fabricate scaffolds by electrospinning method. Hence, finding a common solvent for both PLGA and gelatin could be one of the strength of our study. Obtained results showed that PLGA-gelatin nanofibrous scaffolds have both suitable structure and properties to support regeneration of defects.

Experimental

Materials

Nanocrystalline Poly lactic-co-glycolic acid (PLGA, LA/GA 50/50, p2191, M_w= 40000-75000 gr/mol) was purchased from Sigma Co. Ltd. (USA). Gelatin (M_w 40-50 kDa) was purchased from Merck Co. Ltd. (Germany). 2,2,2-trifluoroethanol (TFE, M_w 100.04 gr/mol) was selected as the solvent and was purchased from Alpha Aesar Co. Ltd. (USA). All chemicals were used directly without further purification. Aqueous solutions were prepared with doubly distilled water.

Preparation of electrospinning scaffolds

The experimental polymer composite solutions were prepared by dissolving PLGA and gelatin with the weight ratio 8:2 and concentration of 15 % w/v in TFE. The mixture was stirred for 12 hours at room temperature to ensure a complete dissolution and eventually obtained homogeneous composite solution for electrospinning.

Electrospinning operations were done by electrospun machine produced by ANST Co. Ltd. (Iran). 5 ml of composite solution was placed in a 5 ml plastic syringe fitted with a stainless steel blunt needle of 0.5 mm in diameter and an injection rate of 0.1 and 0.2 ml/hr using an infusion pump. The needle tip of the syringe was connected with the high voltage power supply with the applied voltage of 16 kV. Nanofibers were simply collected by a rotating drum with 50 mm diameter at a rotation speed of 1000 rpm and linear speed of 3 ml/sec and wrapped with aluminum foil which was kept at a distance of 12 cm from the needle tip. All samples were dried overnight under vacuum at room temperature.

Characterization of electrospinning scaffolds

Scanning Electron Microscope (SEM)**:** The morphological and microstructural study of the electrospun PLGA-gelatin nanofibrous scaffolds were observed by scanning electron microscopy (SEM, Stereoscan S 360-Leica, UK) at an accelerating voltage of 20 kV. All samples were coated with a thin layer of gold in double 30 sec consecutive cycle at 45 mA to reduce charging and produce conductive surface.

Fiber Diameter**:** To determine fiber diameters, 5 images from different parts of samples were prepared, and at least 25 measurements were performed. Finally, diameters of nanofibers were analyzed by using software Image J.

Porosity**:** The apparent density of the electrospun scaffolds was accurately measured by using density bottle method. An average of three measurements was taken for each sample. The porosity of electrospinning nanofibrous scaffolds was calculated by using the equation 1 [**44**], where $\rho_{scaffold}$ is the density of the electrospun scaffold, ρ_{solid} is the density of the bulk polymer:

$$\text{Porosity } (\%) = 1-(\rho\text{scaffold} / \rho\text{solid})*100 \quad (1)$$

Fourier Transform Infrared (FTIR)**:** Chemical characteristics of the electrospun PLGA-gelatin nanofibrous scaffolds were evaluated by the Fourier transform infrared spectrophotometer (FTIR, Nicolet Is10, USA). The spectra were obtained in the range of 400-4000 cm^{-1} with a resolution of 4.0 cm^{-1} and 8 scans.

Mechanical Analysis**:** Mechanical properties of different nanofibers were determined by a comparison strength test system (Santam, STM 20, Iran) with electronic data evaluation and by using a 50N load cell under a crosshead speed of 10 mm/min.

Swelling Ratio**:** The water adsorption capacity was determined by swelling ratio. Thus, the samples were cut

and dipped in deionized water for 1, 3 and 7 hours. Then the water on the specimen surface was removed with filter paper and the specimen was weighed in wet condition [**45**]. The swelling ratio was calculated according to the equation 2 [**46, 47**], where W_0 is the initial weight and W is the wet weight of sample. Each swelling experiments were repeated five times.

$$\text{Swelling ratio (\%)} = [(W-W_0) / W_0]*100 \quad (2)$$

Biodegradation**:** To determine biodegradation rate of samples; first, the scaffolds were weighed respectively to get the initial weight (W_0), and then cut them into two pieces, respectively. The pieces was immersed in an ampoule with 10 ml phosphate buffer saline (PBS); then, the ampoule was placed in thermo shaker (LS 500, Germany) with 50 rpm and constant temperature at 37±0.5 ◦C for 1 month. During the 1 month experiment period, the pieces were taken out to get their wet weight and dry weight as well as observe their appearance at each sampling time point, while the PBS solution was updated weekly. The hydrolytic biodegration rate was calculated according to the equation 3 [**47, 48**], where W_0 is the initial weight and W is the dry weight of samples. Each biodegradation experiments were repeated five times.

$$\text{Biodegradation ratio (\%)} = [(W-W_0) / W_0]*100 \quad (3)$$

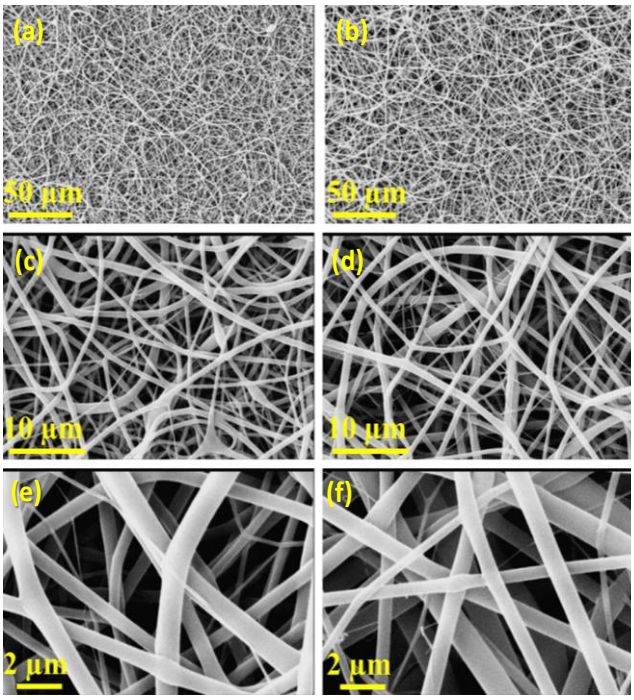

Fig. 1. The morphology of randomly-oriented electrospun nanofibrous PLGA-gelatin scaffolds. The injection ratio of 0.1(a, c, e) and 0.2(b, d, f) ml/hr in different magnifications.

In vitro cellular response

Cell culture: The responses of L929 cells to the samples were evaluated by MTT, Lactate Dehydrogenase specific activity assay (LDH) and Scanning Electron Microscope (SEM). For these purposes, 5×10^5 cells were seeded within the each sample (1 cm^2) and left in Dulbecco's modified Eagle medium (DMEM) supplemented with 15 % fetal bovine serum, 1 % pen/strep, and 1 % nonessential amino acids (all from Gibco-BRL, Life Technologies, Grand Island, NY) for various incubation time points (1-7 days) at 37 °C, 5 % CO_2 and 95 % humidity.

Cell viability and cytotoxicity**:** The cell viability and cytotoxicity of the specimens were investigated by MTT test and LDH assay, respectively, as described in our previously published work [**49, 50**]. For MTT test, the cells were exposed to the samples for 1, 2, 3, 5 and 7 days as described above. After each predetermined incubation time, the DMEM was removed and replaced with fresh medium containing 10% MTT solution (3-(4, 5-Dimethyl-2-thiazolyl) 2, 5-diphenyl-2Htetrazolium bromide) and left for 2 hours at 37 ˚C. The cells were then treated with dimethyl sulfoxide DMSO for 30 minutes. The optical density (OD) of the samples, as a cell viability indicator, was measured by ELISA (enzyme-linked immunosorbent assay) reader at a wavelength of 590 nm with a reference filter of 620 nm. The cells cultured in medium without ES served as control (100% cell viability). For LDH specific assay, the medium in which the cells were exposed to the samples for 1, 2, 3, 5 and 7 days (DMEM with 1% FBS) was collected and centrifuged to discard the remaining cells. The LDH level in supernatant for each time point was measured using a colorimetric plate-based enzymatic assay kit (ZistShimi kits, Iran).

It has been found that the damaged or dead cells release lactate dehydrogenase into the medium. Measurement of such enzyme has found to be an accurate way for assessing the cytotoxicity effect of the materials [**51, 52**]. The data was normalized for the total amount of LDH released from 10^6 cells after freeze-thawing. The cells cultured in medium without ES served as control.

Cell-scaffold interaction**:** The cell-scaffold interaction was investigated after 3 and 7 days of cell culture, as described above. After removal of the culture medium, the L929-loaded specimens were prepared for taking SEM micrographs by a protocol described in our previously published article [**50**]. In brief, the samples were rinsed with PBS twice and the cells were then fixed with 3% glutaraldehyde solution in PBS. After 30 minutes, the specimens were rinsed again with PBS and kept in PBS at 40 °C. The specimens were then fixed with 1% Osmium tetroxide (Polyscience, Warmington, PA, USA) followed by dehydration through ethanol solutions of ascending concentrations (i.e., 30, 50, 70, 90, and 100%) for about 20 minute at each concentration. The specimens were then let dry in air. After being dried completely, the specimens were mounted on copper stubs, coated with gold, and observed by SEM (Philips XL30, Netherland) at an acceleration voltage of 15 kV.

Statistical analysis

Data were processed using Microsoft Excel 2007 software and the results were presented as mean ± standard deviation of at least 3 experiments. Significance between the mean values was calculated using standard software program (SPSS GmbH, Munich, Germany) and $p\leq0.05$ was considered significant**.**

Results and discussion

Physicochemical characterizations

Morphology Observation**:** **Fig. 1 (a-f)** shows the morphology of randomly-oriented electrospun nanofibrous PLGA-gelatin scaffolds. It can be seen that the nanofibers possessed smooth surface without the occurrence of bead defects. In this research, concentration of 15% PLGA-gelatin solution was useful for electrospinning. Moreover, the randomly-oriented nanofibrous scaffolds depicted the interconnected structure with high surface to volume ratio for the cells and nutrients [**43**], in which the mean fiber diameters were 924 nm and 777 nm corresponding to the injection ratio of 0.1 and 0.2 ml/hr, respectively. As can be seen in **Fig. 2**, increasing the injection rate of solution resulted in reducing fibers diameter, so it can decrease strength of scaffolds; nonetheless, differences in diameter are not significant when all the parameters are stable. In addition, smaller fibers cause better cell adhesion. As a consequence, nanofibers with injection rate of 0.2 ml/hr were selected for cell culture due to the fact that they have lower fiber diameter. Besides, by referring to similar reports gelatin decreased diameter of nanofibers, which caused decreasing porosity of scaffolds. As a matter of fact, a possible reason is that the addition of gelatin increased the charge density of the solution which improved the stretching force and self-repulsion of the jet, and thus decrease the fiber diameter [**53, 54**]. Porosiy is an important and noteworthy parameter in order to selecting the scaffold for the cell culture experiment, so 90% porosity of scaffolds indicates that they were highly porous and were beneficial for the adhesion and proliferation of the cells [**40**].

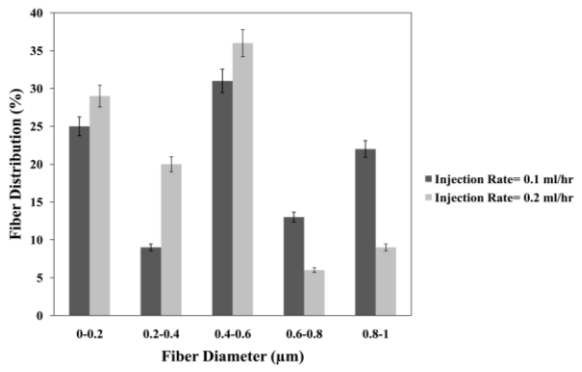

Fig. 2. Fiber size distribution of the synthesized PLGA-gelatin scaffolds.

Fourier Transform Infrared Spectrum**:** **Fig. 3 (a)** shows the FT-IR spectrum of the electrospun PLGA-gelatin nanofibers. For pure PLGA, the strong characteristic absorption bands at about 1761 cm^{-1} attributes to the stretching vibration of C-O bond, and the bands at 1188 cm^{-1} can be assigned to the C–O–C ether group stretching, and the bands at 1089 cm^{-1} and 1451 cm^{-1} arise from C–O bond and methyl group C–H bond of PLGA respectively [**55**]. These characteristic absorption bands were also observed in the FT-IR spectra of PLGA-gelatin nanofibers. Additionally, two absorption peaks appeared at 1650 cm^{-1} and 1538 cm^{-1} corresponded to amide I band and II band of the gelatin, respectively [**56**]. Among them, the amide I band is caused by C-O stretching vibrations of peptide linkages in the backbone of protein and the amide II band is caused by the combination of N–H in plane bending and C–N stretching vibrations. Moreover, the absorption at about 3400 cm^{-1} was observed in the FT-IR spectra, which attributed to the N–H and OH-O stretching vibration and the intermolecular hydrogen bonding. **Fig. 3 (b)** shows the possible chemical structure between PLGA and gelatin in the scaffolds.

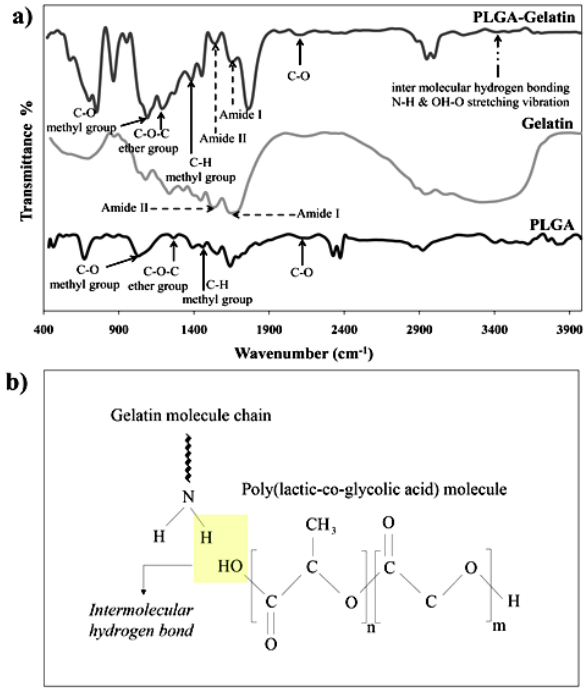

Fig. 3. a) FTIR spectra of pure PLGA, gelatin and the synthesized PLGA-gelatin scaffolds. b) Possible chemical structure of the PLGA-gelatin scaffolds.

Mechanical Analysis**:** Mechanical property is one of the significant properties of tissue engineering scaffolds. Scaffolds should be strong enough to resist forces from body movement or outer environment [**40**]. The modulus and tensile strength of electrospun fibers with the injection rate of 0.2 ml/hr were 0.72 ±0.02 and 2.70 ±0.33, respectively. These amounts showed that Scaffolds are capable of withstanding mechanical loads after placement in the body or surgical procedures. In comparison with similar report, the addition of the gelatin decreased the mechanical properties of PLGA-gelatin composite nanofibrous scaffolds [**29**]. In other words, the tensile strength and modulus of the PLGA-gelatin scaffolds increased slightly by adding gelatin.

Swelling Ratio**:** The characteristic of hydrophilicity is an important parameter to tissue engineering scaffolds, which would affect surface properties, enhance the cellular viability and proliferation and control stability of the polymeric scaffolds [**45**]. **Fig. 4 (a)** illustrates swelling ratio of the nanofibers. It can be concluded that owing to the fact that the addition of gelatin causes hydrophilic structure, it increases water absorption and improves the cellular adhesion [**25, 29, 33, 38**]. This event attributed to the amine and carboxylic functional groups in the gelatin structure.

The relatively high water absorption of the scaffolds causes appropriate cell interactions; thus, better cellular attachments and migrations occur.

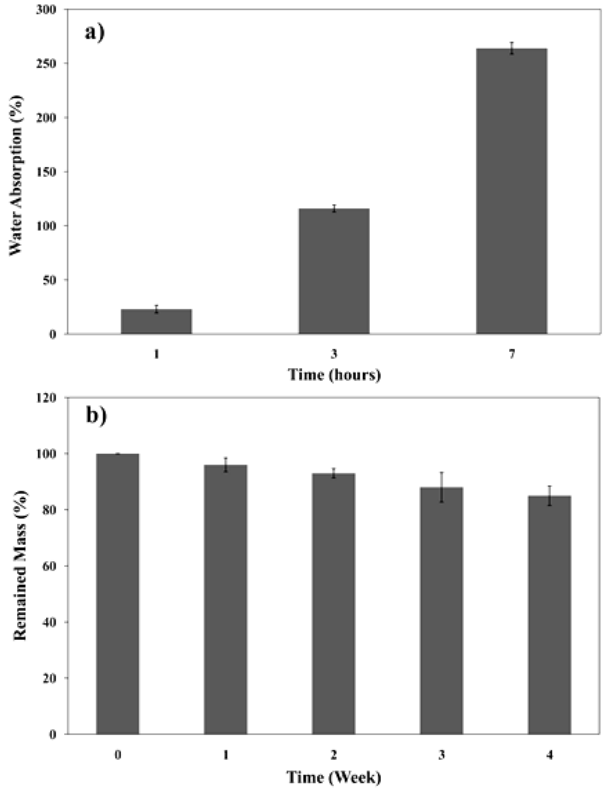

Fig. 4. a) Swelling ratio of the synthesized PLGA-gelatin scaffolds at different time intervals. b) Biodegradation behavior of the synthesized PLGA-gelatin scaffolds at different time intervals.

Biodegradation**:** Biodegradation rate of polymeric scaffolds is one of the most important characteristics to find an appropriate biomedical applications [**57**]. **Fig. 4 (b)** shows the biodegradation of scaffolds with injection rate of 0.2 ml/hr during 1 month. Not only does synthetic polymer, PLGA, increase the Properties and mechanical strength without any effects on cellular adhesion, but also it can control the rate of degradation; as a consequence, the scaffolds are able to keep their efficiency and integrity during neo-tissue formation. Furthermore, it has been proved by scientists, amorphous polymers degrade faster than crystal polymers in aqueous environments [**40**]. Moreover, owing to the fact that hydrophilic nature of gelatin increases water absorption, biodegradation rate became faster with adding this material in scaffolds **[25, 29, 33, 38]**.

Cellular responses**:** The cellular responses of the scaffolds were evaluated by culturing the L929 fibroblasts on the scaffolds for various incubation periods (1-7 days). The cell viability, cytotoxicity and cell-scaffold interaction were then studied by MTT, LDH and SEM, respectively. In this study, the nanofibers with the injection rate of 0.2 ml/hr (with lower fiber diameters) were selected for the following cell culture tests.

Cell viability and cytotoxicity**:** The cell viability test was assayed based on ability of living cells in reduction of MTT salt by their mitochondria. The MTT result is shown in **Fig 5 (a)**. For this purpose, the cells were exposed to the ES samples for 1, 2, 3, 5 and 7 days and then their viability was normalized to those in the control samples, as 100% cell viability. As can be seen, the ES samples did not affect the viability of the cells in all time points (independent sample *t-test, p<0.05*) compared to control.

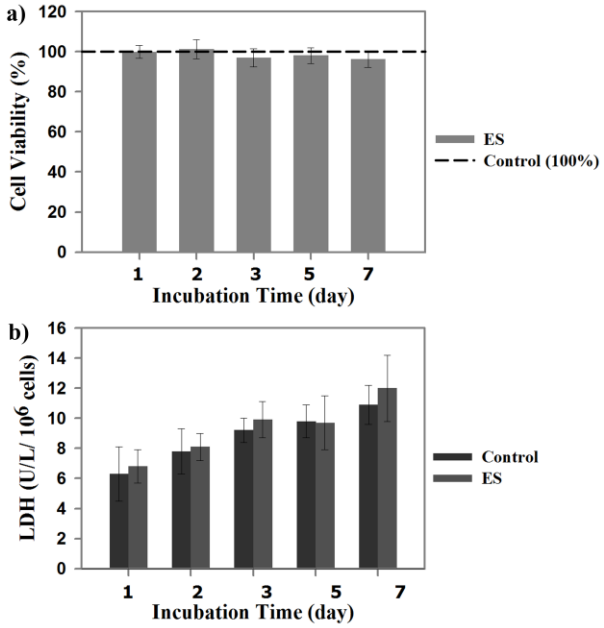

Fig. 5. The cell viability and cytotoxicity of the scaffolds were assayed by MTT (a) and LDH specific activity (b) tests, respectively. There was no significant difference between MTT and LDH results of the scaffold containing samples in comparison to control (independent sample *t*-test, $p<0.05$).

Lactate Dehydrogenase (LDH) is an enzyme that converts pyruvate to lactate using NADH as a coenzyme. This enzyme is found in almost all of the cells. It has been reported that LDH is released from the cells upon cell damage. Elevated level of LDH in medium indicates cells' death or damage [**51, 52**]. In the current study, we have investigated whether the ES samples had cytotoxicity effects on the L929 cells through measuring the LDH level in the medium in which the cells were exposed to the scaffolds for 1, 2, 3, 5 and 7 days incubation periods at 37 ˚C. As shown in **Fig. 5 (b)**, there was no significant difference between the LDH levels of the samples in comparison to control (independent sample *t-test, p<0.05*), indicating no cytotoxicity activity of the ES samples against the tested cells. The data obtained from MTT and LDH are in consistent with other studies that showed non cytotoxicity property of the PLGA and gelatin [**58, 59**].

Cell-scaffold interaction**:** The randomly-oriented electrospun nanofibrous scaffolds provide a matrix for cell adhesion and proliferation owing to mimic ECM of native tissue [**15, 25, 43**]. In this study, we also investigated the effects of the randomly-oriented PLGA-gelatin nanofibrous scaffolds on the fibroblast (L929) cell adhesion and proliferation. Therefore, the cells were seeded on the nanofibrous scaffolds and left in cell culture incubator for 3 and 7 days. After the predetermined times, the morphology

of the cells cultured on the scaffolds was observed by SEM. The SEM micrographs of the cell-scaffold samples after 3 and 7 days are shown in **Fig. 6 (a, b)** and **6 (c, d)** respectively. It is apparently seen that the cells were grown, attached and spread actively on the surface of the electrospun scaffolds after 3 and 7 days incubation times. Taken together, all data obtained from MTT, LDH and SEM indicated high cyto-compatibility of the synthesized nanofibrous scaffolds *in vitro*.

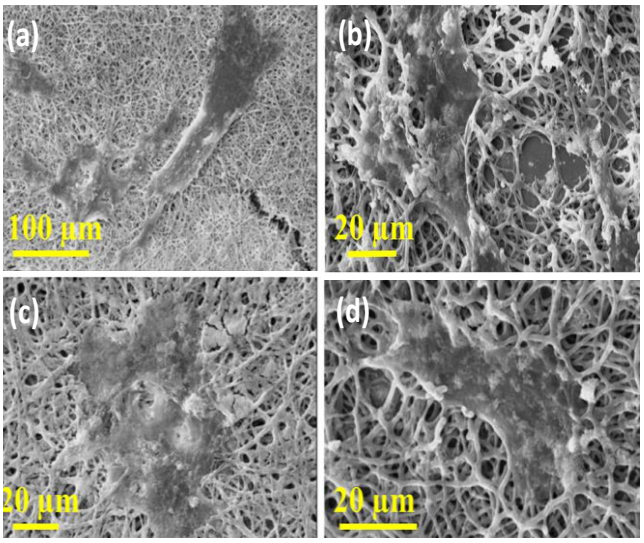

Fig. 6. SEM micrographs of the L292 cells cultured on the nanofibers for 3 (a, b) and 7 (c, d) days.

Conclusion

In this study, randomly-oriented PLGA-gelatin nanofibrous scaffolds with the weight ratio 80:20 were fabricated by electrospinning technique. Furthermore, one of the strength of this study is finding 2, 2, 2-trifluroethanol as a solvent to dissolve both synthetic and natural polymers with organic and aqueous basis, respectively. On the basis of SEM images, fiber diameters of nanofibers decreased by increasing the injection rate. Moreover, in spite of the fact that gelatin decreased the mechanical properties such as modulus and tensile strength, it increased swelling ratio and biodegradation; additionally, mechanical requirements were provided by PLGA. Besides, the scaffolds had about 90 percent porosities that help to cellular anchorage and proliferation. In brief, it seems that the prepared nanofibrous scaffolds are able to support cell attachment, to maintain the required structural integrity and to prevent the pores of the scaffolds from collapsing during neo-tissue formation. This research also demonstrated that the synthesized scaffolds are suitable for ongoing soft tissue engineering studies in animal models in vivo.

Reference

1. Liang, X.; Cai, H.; Hao, Y.; Sun, G.; Song, Y.; Chen, W. *Neural Regeneration Research*. **2014**, *9*, 594. **DOI:** 10.4103/1673-5374.130099
2. Liu, B.; Cai, S.-X.; Ma, K.-W.; Xu, Z.-L.; Dai, X.-Z.; Yang, L.; Lin, C.; Fu, X.-B.; Sung, K. L. P.; Li, X.-K. *Journal of materials science. Materials in medicine*. **2008**, *19*, 1127. **DOI:** 10.1007/s10856-007-3224-1
3. Leor, J.; Amsalem, Y.; Cohen, S. *Pharmacology & therapeutics.* **2005**, *105*, 151. **DOI:** 10.1016/j.pharmthera.2004.10.003
4. Drury, J. L.; Mooney, D. J. Biomaterials. **2003**, *24*, 4337. **DOI:** 10.1016/S0142-9612(03)00340-5
5. Zamanian, a; Moztarzadeh, F.; Kordestani, S.; Hesaraki, S.; Tahriri, M. R. *Advances in Applied Ceramics*. **2010**, *109*, 440. **DOI:** 10.1179/174367610X12804792635107
6. Ghazanfari, S. M. H.; Zamanian, A. *Ceramics International.* **2013**, *39*, 9835. **DOI:** 10.1016/j.ceramint.2013.05.096
7. Pourhaghgouy, M.; Zamanian, A.; Shahrezaee, M.; Masouleh, M. P. *Materials Science and Engineering: C.* **2016,** *58*, 180. **DOI:** 10.1016/j.msec.2015.07.065
8. Vasita, R.; Katti, D. S. *International Journal of Nanomedicine.* **2006**, *1*, 15. **DOI:** 10.2147/nano.2006.1.1.15
9. Zamanian, A.; Farhangdoust, S.; Yasaei, M.; Khorami, M.; Abbasabadi, M. *Key Engineering Materials.* **2012**, *529*, 133. **DOI:** 10.4028/www.scientific.net/KEM.529-530.133
10. Farhangdoust, S.; Zamanian, a; Yasaei, M.; Khorami, M. Materials science & engineering. C, *Materials for biological applications.* **2013**, *33*, 453. **DOI:** 10.1016/j.msec.2012.09.013
11. Zamanian, A.; Farhangdoust, S.; Yasaei, M.; Khorami, M.; Hafezi, M. *International Journal of Applied Ceramic Technology.* **2014**, *11*, 12. **DOI:** 10.1111/ijac.12031
12. Zamanian, A.; Ghorbani, F.; Nojehdehian, H. *Applied Mechanics and Materials.* **2013**, *467*, 108. **DOI:** 10.4028/www.scientific.net/AMM.467.108
13. Pourhaghgouy, M.; Zamanian, A. In 2014 21th Iranian Conference on Biomedical Engineering (ICBME). **2014**, 48. **DOI:** 10.1109/ICBME.2014.7043892
14. Arabi, N.; Zamanian, A. *Biotechnology and applied biochemistry*. **2013**, *60*, 573. **DOI:** 10.1002/bab.1120
15. Denis, P.; Dulnik, J.; Sajkiewicz, P. *International Journal of Polymeric Materials and Polymeric Biomaterials*. **2014**, *64*, 354. **DOI:** 10.1080/00914037.2014.945208
16. Son, W. K.; Youk, J. H.; Lee, T. S.; Park, W. H. *Journal of Polymer Science Part B: Polymer Physics*. **2004**, *42*, 5. **DOI:** 10.1002/polb.10668
17. Cai, Q.; Shi, G.; Bei, J.; Wang, S. *Biomaterial.* **2003**, *24*, 629. **DOI:** 10.1016/S0142-9612(02)00377-0
18. Yoon, J. J.; Kim, J. H.; Park, T. G. *Biomaterials.* **2003**, *24*, 2323. **DOI:** 10.1016/S0142-9612(03)00024-3
19. Liu, X.; Ma, P. X. Biomaterials. **2009**, *30*, 4094. **DOI:** 10.1016/j.biomaterials.2009.04.024
20. Asefnejad, A.; Khorasani, M. T.; Behnamghader, A.; Farsadzadeh, B.; Bonakdar, S. *International journal of nanomedicine.* **2011**, *6*, 2375. **DOI:** 10.2147/IJN.S15586
21. Rowlands, a S.; Lim, S. a; Martin, D.; Cooper-White, J. *J. Biomaterials.* **2007**, *28*, 2109. **DOI:** 10.1016/j.biomaterials.2006.12.032
22. Hutmacher, D. W. *Biomaterials.* **2000**, *21*, 2529. **DOI:** 10.1016/S0142-9612(00)00121-6
23. Meng, Z. X.; Xu, X. X.; Zheng, W.; Zhou, H. M.; Li, L.; Zheng, Y. F.; Lou, X. *Colloids and surfaces. B, Biointerfaces*. **2011**, *84*, 97. **DOI:** 10.1016/j.colsurfb.2010.12.022
24. Nojehdehian, H.; Moztarzadeh, F.; Baharvand, H.; Nazarian, H.; Tahriri, M. *Colloids and surfaces. B, Biointerfaces.* **2009**, *73*, 23. **DOI:** 10.1016/j.colsurfb.2009.04.029
25. Meng, Z. X.; Wang, Y. S.; Ma, C.; Zheng, W.; Li, L.; Zheng, Y. F. *Materials Science and Engineering: C*. **2010**, *30*, 1204. **DOI:** 10.1016/j.msec.2010.06.018
26. Nandagiri, V. K.; Gentile, P.; Chiono, V.; Tonda-Turo, C.; Matsiko, A.; Ramtoola, Z.; Montevecchi, F. M.; Ciardelli, G. *Journal of the mechanical behavior of biomedical materials.* **2011**, *4*, 1318. **DOI:** 10.1016/j.jmbbm.2011.04.019
27. Wang, G.; Hu, X.; Lin, W.; Dong, C.; Wu, H. *In vitro cellular & developmental biology. Animal.* **2011**, *47*, 234. **DOI:** 10.1007/s11626-010-9381-4
28. Evans, G. R. .; Brandt, K.; Katz, S.; Chauvin, P.; Otto, L.; Bogle, M.; Wang, B.; Meszlenyi, R. K.; Lu, L.; Mikos, A. G.; Patrick, C. W. *Biomaterials.* **2002**, *23*, 841. **DOI:** 10.1016/S0142-9612(01)00190-9

29. Li, X.-K.; Cai, S.-X.; Liu, B.; Xu, Z.-L.; Dai, X.-Z.; Ma, K.-W.; Lin, S.-Q.; Li, S.-Q.; Yang, L.; Sung, K. L. P.; Fu, X.-B. *Colloids and surfaces. B, Biointerfaces.* **2007**, *57*, 198. **DOI:** 10.1016/j.colsurfb.2007.02.010
30. Qian, L.; Zhang, H. *Journal of Chemical Technology & Biotechnology.* **2011**, *86*, 172. **DOI:** 10.1002/jctb.2495
31. Vaz, C. M.; van Tuijl, S.; Bouten, C. V. C.; Baaijens, F. P. T. *Acta biomaterialia.* **2005**, *1*, 575. **DOI:** 10.1016/j.actbio.2005.06.006
32. Zamani, F.; Latifi, M.; Amani-Tehran, M.; Shokrgozar, M. A. *Fibers and Polymers.* **2013**, *14*, 698. **DOI:** 10.1007/s12221-013-0698-y
33. Gu, S.-Y.; Wang, Z.-M.; Ren, J.; Zhang, C. Y. *Materials Science and Engineering: C.* **2009**, *29*, 1822. **DOI:** 10.1016/j.msec.2009.02.010
34. Jose, M. V.; Thomas, V.; Dean, D. R.; Nyairo, E. *Polymer.* **2009**, *50*, 3778. **DOI:** 10.1016/j.polymer.2009.05.035
35. Sell, S.; Vol. 1. Totowa, New Jersey: Humana Press. **2004**. **DOI:** 10.1007/978-1-4614-7696-2
36. Hurtado, A.; Moon, L. D. F.; Maquet, V.; Blits, B.; Jérôme, R.; Oudega, M. *Biomaterials.* **2006**, *27*, 430. **DOI:** 10.1016/j.biomaterials.2005.07.014
37. Luís, A. L.; Rodrigues, J. M.; Geuna, S.; Amado, S.; Shirosaki, Y.; Lee, J. M.; Fregnan, F.; Lopes, M. a; Veloso, A. P.; Ferreira, A. J.; Santos, J. D.; Armada-Da-silva, P. a S.; Varejão, A. S. P.; Maurício, A. C. *Tissue engineering. Part A.* **2008**, *14*, 979. **DOI:** 10.1089/ten.tea.2007.0273
38. Mehrasa, M; *International journal of biological macromolecules.* **2015**, *79*, 687. **DOI:** 10.1016/j.ijbiomac.2015.05.050
39. Gupta, D.; Venugopal, J.; Mitra, S.; Giri Dev, V. R.; Ramakrishna, S. *Biomaterials.* **2009**, *30*, 2085. **DOI:** 10.1016/j.biomaterials.2008.12.079
40. Aghdam, R. M.; Shakhesi, S.; Najarian, S.; Mohammadi, M. M.; Ahmadi Tafti, S. H.; Mirzadeh, H. *International Journal of Polymeric Materials and Polymeric Biomaterials.* **2013**, *63*, 229. **DOI:** 10.1080/00914037.2013.800983
41. Sharma, Y.; Tiwari, A.; Hattori, S.; Terada, D.; Sharma, A. K.; Ramalingam, M.; Kobayashi, H. *International journal of biological macromolecules.* **2012**, *51*, 627. **DOI:** 10.1016/j.ijbiomac.2012.06.014
42. Atala, A. *Fertility and sterility.* **2012**, *98*, 21. **DOI:** 10.1016/j.fertnstert.2012.05.038
43. Tiwari, A.; Sharma, Y.; Hattori, S.; Terada, D.; Sharma, A. K.; Turner, A. P. F.; Kobayashi, H. *Biopolymers.* **2013**, *99*, 334. **DOI:** 10.1002/bip.22170
44. Webb, P. A. Micromeritics Instrument Corp **2001**.
45. Tiwari, A.; Grailer, J. J.; Pilla, S.; Steeber, D. a; Gong, S. *Acta biomaterialia.* **2009**, *5*, 3441. **DOI:** 10.1016/j.actbio.2009.06.001
46. Arnal-Pastor, M.; *Journal of colloid and interface science.* **2011**, *361*, 361. **DOI:** 10.1016/j.jcis.2011.05.013
47. Sharma, K.; Kumar, V.; Kaith, B. S.; Som, S.; Kumar, V.; Pandey, A.; Kalia, S.; Swart, H. C. *Industrial & Engineering Chemistry Research.* **2015**, *54*, 1982. **DOI:** 10.1021/ie5044743
48. Sharma, K.; Kumar, V.; Kaith, B. S.; Kumar, V.; Som, S.; Kalia, S.; Swart, H. C. *Polymer Degradation and Stability.* **2015**, *111*, 20. **DOI:** 10.1016/j.polymdegradstab.2014.10.012
49. Gholipourmalekabadi, M.; Mozafari, M.; Salehi, M.; Seifalian, A.; Bandehpour, M.; Ghanbarian, H.; Urbanska, A. M.; Sameni, M.; Samadikuchaksaraei, A.; Seifalian, A. M. *Advanced healthcare materials.* **2015**, *4*, 918. **DOI:** 10.1002/adhm.201400704
50. Gholipourmalekabadi, M.; Mozafari, M.; Gholipourmalekabadi, M.; Nazm Bojnordi, M.; Hashemi-Soteh, M. B.; Salimi, M.; Rezaei, N.; Sameni, M.; Samadikuchaksaraei, A.; Ghasemi Hamidabadi, H. *Biotechnology and applied biochemistry.* **2015**, *62*, 441. **DOI:** 10.1002/bab.1285
51. Shachar, M.; Tsur-Gang, O.; Dvir, T.; Leor, J.; Cohen, S. *Acta biomaterialia.* **2011**, *7*, 152. **DOI:** 10.1016/j.actbio.2010.07.034
52. Koh, J. Y.; Choi, D. W. *Journal of Neuroscience Methods.* **1987**, *20*, 83. **DOI:** 10.1016/0165-0270(87)90041-0
53. Ngiam, M.; Liao, S.; Patil, A. J.; Cheng, Z.; Chan, C. K.; Ramakrishna, S. *Bone.* **2009**, *45*, 4. **DOI:** 10.1016/j.bone.2009.03.674.
54. Pham, Q. P.; Sharma, U.; Mikos, A. G. *Tissue engineering.* **2006**, *12*, 1197. **DOI:** 10.1089/ten.2006.12.1197
55. Robey, P.; Bianco, P. *Adult and Fetal Stem Cells.* **2013**, *2*, 965. **DOI:** 10.1016/B978-0-12-385942-6.00079-2
56. Vaz; C. M.; Tuijl, S.; Bouten, C. V.; Baaijens, F. P. *Acta Biomater.* **2005**, *1*, 575. **DOI:** 10.1016/j.actbio.2005.06.006
57. Sharma, K.; Kumar, V.; Kaith, B. S.; Kumar, V.; Som, S.; Kalia, S.; Swart, H. C. *RSC Advances.* **2014**, *4*, 25637. **DOI:** 10.1039/c4ra03765k.
58. Tan, H.; Wu, J.; Lao, L.; Gao, C. *Acta Biomaterialia.* **2009**, *5*, 328. **DOI:** 10.1016/j.actbio.2008.07.030
59. Sarem, M.; Moztarzadeh, F.; Mozafari, M.; Shastri, V. P. *Materials science & engineering. C, Materials for biological applications.* **2013**, *33*, 4777. **DOI:** 10.1016/j.msec.2013.07.036

