Comparison of Structural Modification and Argon Plasma Treatment of Poly(lactide-*co*-glycolic acid) Nanofibrous Scaffolds for Cell Culture

Fatemeh Zamani*, Fatemeh Nadipour, Masoud Latifi, and Aliakbar Merati

***Abstract*- Since poly(lactide-*co*-glycolic acid) (PLGA), as a biodegradable material, is a hydrophobic polymer which might lead to the incoherence of optimal growth of cells on the scaffold, the scaffold surface modification can promote the cell growth and proliferation. In this study, two methods including structural modification and plasma treatment were employed to improve the surface properties and epithelial kidney cells (Vero) culture efficiency for the PLGA nanofibrous scaffolds. Moreover, the physical, and chemical properties of the modified scaffolds were characterized. Plasma treatment enhances surface hydrophilicity and structural modification improves physical properties of surface such as fiber diameter, surface porosity and alignment index. It was found that the plasma-treated scaffold is more hydrophilic compared to the structurally-modified and non-treated scaffolds. From the ATR-FTIR spectra of the samples, it was observed that the extent of C=O and C-O groups was increased in the plasma-treated samples in comparison with the other groups. Furthermore, *in-vitro* studies demonstrated that, despite the greater hydrophilicity of the plasma-treated scaffold, both of modified scaffolds enhanced the cell growth and proliferation of Vero cells. In conclusion, the structurally-modified scaffolds have shown a promising potential to improve the cell proliferation as compared with the plasma-modified scaffolds.**

***Keywords*: electrospun nanofibrous scaffold, hydrophilicity, surface modification, structural modification, plasma-treated scaffold**

I. INTRODUCTION

Quite recently, the majority of investigations on the treatment of damaged tissue has been devoted to the methods based on tissue engineering. Despite the fact that the tissue graft method has led to the best results in the treatment of those damages, but due to the substantial issues including lack of donor tissue availability and the high risk of infectious complications, an alternative method for the treatment of damaged issue has been the subject of many studies lately. Use of scaffolds is regarded as one of the best methods since the production of scaffolds from both natural and synthetic polymers with desired properties has been progressively reported in the literature [1,2]. A diverse range of methods has been suggested for production of nanofibrous scaffolds from which the electrospinning technique is of great importance due to its simplicity, good adaptability, ability to produce nanoscale fibers, controllability, repeatability, and also cost-effectiveness [3,4].

Biocompatible and biodegradable polymers are required for the manufacture of scaffolds. According to the previous studies, poly(lactide-*co*-glycolic acid) (PLGA), being a synthetic polymer, is not sufficiently hydrophilic to promote the cell attachment and growth as compared with the natural polymers. Various procedures have been proposed to enhance the hydrophilicity of the nanofibers, and consequently, improve the cell adhesion such as coating with hydrophilic particles, mixing with hydrophilic non-ionic surfactants, induction of surface roughness, enhancement of layer porosity, plasma, and etc. [3-8].

Plasma, being one of the four fundamental states of matter, is a gas-state matrix composed of the opposite electric charges but its overall electric charge is zero. In addition to the charged particles, plasma also contains inert molecules and atoms, excited molecules and atoms, radicals and photons. Therefore, plasma is a mixture of active and energetic components, whose coexistence in a charge-stable state is one of its major characteristics [9,10]. The surface modification using plasma processes significantly alters the physical and chemical properties of the materials surfaces. This method can produce a new surface which is anti-coagulation, biocompatible, and similar to the biocompatible surfaces. Using the plasma process, one could easily impart any desirable modification with no influences on the bulk properties of materials. The plasma method could clean the surface from any possible

F. Zamani
Hazrat-e Masoumeh University, 371951179, Qom, Iran.

F. Nadipour, M. Latifi, and A. Merati
Department of Textile Engineering, Amirkabir University of Technology, 15875-4413, Tehran, Iran.

Correspondence should be addressed to F. Zamani
e-mail: fzamani@hmu.ac.ir

contaminants, improve the initial adhesion of the cells to the surface, increase the surface free energy, improve the biocompatibility, reduce the surface frictional drag, and functionalize the surface [9-14].

Plasma method can be implemented for many types of materials and is environmentally friendly as well. The only drawback of using plasma in surface modification is the fact that the induced changes on the material might be temporary. To retain the plasma-induced changes, one could place the material in a humid and low-temperature environment [12,13]. In the previous studies, the oxygen plasma has been majorly used for surface modification. For instance, Hasirci *et al.* reduced the water contact angle of polymeric films, PLGA (50/50), from 67° to 38° using the plasma method. In their research, the fibroblast cells were more adhered on the surface-modified samples [15]. In another work, Khorasani *et al.* reported similar results regarding the increased hydrophilicity and improved cell adhesion [16]. Park *et al.* have compared the chemical surface modification methods with the air plasma modification on the PLGA surfaces. The plasma method exhibited the best results in improvement of hydrophilic behavior, and the water contact angle reduced from 73° to 56° [17]. Safinia *et al.* also investigated the effect of air plasma on the surface properties of PLGA and reported rather similar results [18].

On the other hand, there have been reported several researches that enhanced the hydrophilicity and cell attachment via alteration of surface physical properties such as porosity, roughness, and orientation of nanofibers [2,5,6,19-23]. Zamani *et al.* in their researches demonstrated that the oriented fibers can change other physical properties of a scaffold, simultaneously. Also they confirmed that the nanoroughness of scaffolds can change surface properties and increase the cell growing rate to 50% in comparison with conventional electrospun web [5,23].

In the current research, an attempt was made to propose a procedure for increasing the level of cell growth and proliferation of electrospun nanofibrous scaffolds, made of PLGA, by means of two methods including plasma and modification of physical structure, which could lead to an enhanced hydrophilic behavior of scaffolds. Eventually, a comparison of the used methods will be given as well.

II. EXPERIMENRAL

A. Materials and Methods

A.1. Materials

Poly(lactide-*co*-glycolic acid) (85/15) with an inherent viscosity of 5.9 $dL.g^{-1}$ was supplied by Boehringer Ingelheim, Germany. Chloroform, dimethylformamide (DMF), ethanol, glutaraldehyde, MTT solution (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) and trypsin were all supplied by Sigma-Aldrich, USA. 2-Propanol was purchased from Merck, Germany. Dulbecco modified eagle's medium (DMEM), phosphate buffered saline (PBS) and fetal bovine serum (FBS) were purchased from Gibco, UK.

A.2. Preparation of Polymer Solutions and Electrospinning Setting

Two types of solvents including pure chloroform and chloroform:DMF (80:20) were used for electrospinning the PLGA solution at the concentration of 3 w/v%. To make a homogeneous solution, both of solutions were stirred for 12 h prior to the electrospinning process at room temperature. The solutions were then fed by a 1 mL plastic syringe with 0.5 mm needle diameter, at a feeding rate of 0.25 $mL.h^{-1}$, electrospun at the voltage of 20 kV and were collected on 20 mm round cover slips wrapped over the rotating drum placed at the distance of 10 cm from the needle tip. To remove the residual solvent, all the electrospun scaffolds were put in a vacuum oven at 20 mbar and room temperature for 48 h.

A.3. Preparation of Modified Scaffolds

A.3.1. Structural Modification

In structural modification, the physical properties of scaffolds were promoted by controlling the rate of fiber collection without any post-processing. Non-treated scaffolds with random construction were collected on the drum with a linear speed of 0.2 $m.s^{-1}$. To obtain the modified scaffolds with more oriented and porous structure and less fiber diameter, the drum with a linear rate of 2.4 $m.s^{-1}$ was employed.

A.3.2. Plasma Modification

A laboratory plasma equipment (Plasma Fanavar Amin, Iran) was employed for the plasma surface treatment of the electrospun PLGA nanofibrous scaffolds. The electrospun membranes were maintained on the sample stage and their surfaces were placed above the inner electrode. The pressure inside the plasma chamber was kept at 0.1-0.2 torr prior to be filled with gas. Finally, the PLGA nanofibrous samples were treated with plasma under argon gas in 1 torr and 30 °C for a time period of 2 min.

A.4. Characterization of Scaffolds

The morphology of fibrous mats before and after cell culturing was investigated by scanning electron microscopy (SEM, Philips, XL30, and Netherlands) at an accelerating voltage of 20 kV. Prior to SEM observation, the vacuum-dried scaffolds were gold sputtered using

a sputter coater (Hummer II) for 70 s. By analyzing the SEM images with image analysis software (Image J, National Institutes of Health), the average fiber diameter was measured, and according to the following equation, the porosity of scaffolds was calculated considering that ρPLGA=1.3 g.cm^{-3}:

$$\varepsilon = (1 - \rho_{scaffold} / \rho_{material}) \times 100 \quad (1)$$

$$\rho_{scaffold} = \text{mass} / (\text{base area} \times \text{thickness}) \quad (2)$$

The alignment index represents the percentage of fibers that are oriented in a certain direction and is calculated by an image processing tool (IP.1). The hydrophilicity of electrospun scaffolds was studied by the water contact angle (WCA) measurement through a video contact angle system (VCA Optima, AST Products). Five seconds after placing the deionized water droplet (5 μL) on the surface of the samples, the video measured the contact angle of the droplets. Five scaffolds were used for each test.

To investigate the surface chemistry of electrospun membranes and the effect of plasma treatment on the surfaces of nanofibrous scaffolds after plasma treatment, attenuated total reflection–Fourier transform infrared (ATR–FTIR) spectrophotometry (Thermo Nicolet Nexus 670, USA) was performed on samples for a spectral range from 400 cm^{-1} to 4000 cm^{-1}. All the measurements were made at room temperature.

A.5. Cell Culture

Epithelial kidney/Vero cells were cultured in DMEM supplemented with 10% FBS and were placed in an incubator (37 °C, 5% CO_2, and 95% humidity). After detaching the Vero cells from the flasks by trypsinization with 1% trypsin solution, the viable cells were counted by trypan blue assay. The Vero cells were then seeded on the sterilized nanofibrous scaffolds inside a 12-well plate and tissue culture polystyrene (TCP as control) for 48 h. After two days of cell seeding, the scaffolds were washed with PBS solution, and cells were fixed on the scaffolds by 2.5% glutaraldehyde for about 30 min. Afterwards, the specimens were rinsed with PBS and dehydrated in ethanol with sequential concentrations of 25, 50, 75, 90, and 100% for 10 min. The dried cellular samples were coated with gold and observed through SEM at an accelerating voltage of 20 kV.

A.6. Cell Viability and Proliferation Studies

MTT is a colorimetric assay used to determine the cell viability and proliferation. After two days of cell seeding, the scaffolds were removed from the culture medium and incubated in MTT assay solution (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (5 g.L^{-1} in PBS) for 4 h. Then, the medium was replaced with 2-propanol for 30 min. The resulting color solution was pipetted into a 96-well plate to measure their absorbance at 560 nm using a spectrophotometric plate reader.

A.7. Statistical Analysis

The resulting data were analyzed using single-factor analysis of variance (ANOVA). A value of $P<0.05$ was considered statistically significant. Moreover, all data were presented as mean ± standard deviation.

III. RESULTS AND DISCUSSION

A. Studying the Electrospun Scaffolds

Two types of solvents including pure chloroform and chloroform/DMF were used for electrospinning the PLGA solution with the concentration of 3 w/v%. As mentioned in the methods section, two methods were also used for their modification. The SEM images of scaffolds, formed by using chloroform as solvent, and structurally modified scaffolds are displayed in Figs. 1A and 1B as CR and CA, respectively. Moreover, the SEM images of scaffolds, formed by using the mixture of chloroform/DMF as solvent, and modified by drum rotating are shown in Figs. 1C and 1D as DR and DA, respectively.

SEM images of the non-treated and plasma-modified scaffolds are quite similar exhibiting no pronounced differences.

The electrospinning conditions and the properties of fibrous scaffolds are also reported in Table I.

B. Structural Properties of Scaffolds

As seen in Fig. 2, the fiber diameter for the samples

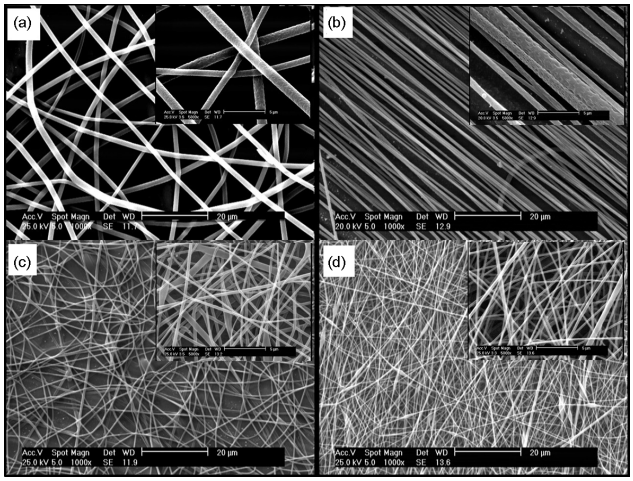

Fig. 1. SEM micrographs of scaffolds: (a) CR, (b) CA, (c) DR, and (d) DA (magnifications: 1000x, 5000x).

TABLE I
ELECTROSPINNING CONDITIONS AND MODIFICATIONS (APPLIED VOLTAGE, ELECTROSPINNING DISTANCE, AND FEED RATE FOR ALL THE SAMPLES ARE 20 KV, 10 CM AND 0.25 ML.H^{-1}, RESPECTIVELY)

Scaffold	Electrospinning conditions and modifications			
	Property of fibers	Solvent	Linear speed of collector (m.s^{-1})	Plasma treatment
CR	Non-treated	Chloroform	0.2	---
CA	Structural modified	Chloroform	2.4	---
CP	Plasma modified	Chloroform	0.2	✓
DR	Non-treated	Chloroform/DMF	0.2	---
DA	Structural modified	Chloroform/DMF	2.4	---
DP	Plasma modified	Chloroform/DMF	0.2	✓

TABLE II
STRUCTURAL PROPERTIES OF SIX TYPES OF PLGA ELECTROSPUN SCAFFOLDS

Scaffold	Fiber diameter (nm), n=120		Alignment index, n=30		Porosity (%), n=10		WCA (º), n=5	
	Ave.	SD	Ave.	SD	Ave.	SD	Ave.	SD
CR	1352.17	300.12	83.19	5.02	98.62	0.11	107.01	4.55
CA	870.97	290.06	98.62	3.17	98.86	0.12	53.16	1.97
CP	1352.17	300.12	83.19	5.02	98.62	0.11	10.80	1.02
DR	478.25	146.02	78.31	5.81	98.50	0.14	93.85	3.05
DA	367.51	98.06	88.52	3.71	98.75	0.10	83.06	2.31
DP	478.25	146.03	78.31	5.81	98.50	0.14	14.64	2.89

prepared by the use of chloroform/DMF as solvent is smaller than that of the other samples. The diameters of the fibers, prepared with chloroform and chloroform/DMF as solvents, were in the diameter range of 870-1352 nm and 367-478 nm, respectively (Fig. 1 and Table II). Since DMF has a higher dielectric constant (36.71) than that of chloroform (4.8), and also, considering the fact that the bending instability of the electrospinning jet increases with increasing the dielectric constant, the jet length is increased in the electrospining process which might lead to the reduction of the fiber diameter of the scaffolds [24]. Moreover, since chloroform is more volatile than DMF, it leads to faster solvent evaporation, more polymer concentration and viscosity during electrospinning and finally larger fiber diameters.

In the case of structurally-modified sample, the collection speed of fibers is increased to 2.4 m.s^{-1}. The rate of fiber collection was found to affect the porosity and hydrophilicity of the scaffolds via changing their physical properties such as diameter and orientation [23].

The data presented in Table II reveal that the increment of the collection rate could change the structure of scaffolds by varying the orientation of fibers together with the reduction of their diameters. The diameters of the electrospun nanofibers, prepared with higher collection speed (CA, DA), were in the range of 367-870 nm. According to the alignment index of samples, measured based on the fibers angle representing the percentage of fiber orientation, the scaffolds prepared with chloroform as solvent exhibit a higher number of aligned nanofibers as compared to the membranes electrospun by chloroform/DMF solvents, albeit not significantly. However, a significant difference could be observed between the alignment indices of the structurally-modified scaffolds and other groups. Increasing the fiber orientation can be due to the increase in the speed of collector from 0.2 m.s^{-1} to 2.4 m.s^{-1} and approaching the speed of fiber collection to

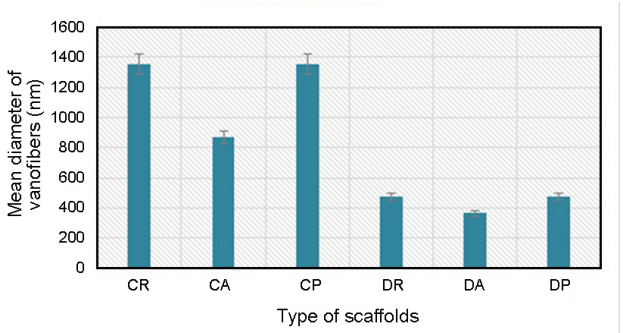

Fig. 2. Mean diameter of non-treated, structurally-modified, and plasma-treated nanofibers in two groups.

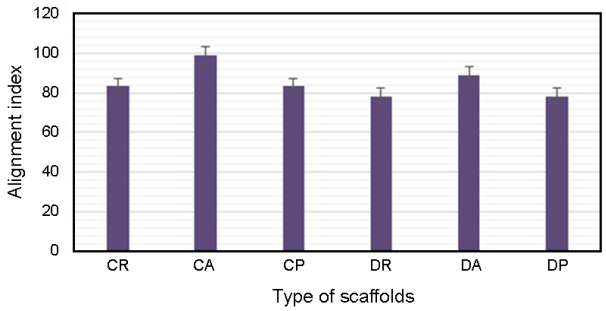

Fig. 3. Alignment index for the non-treated, structurally-modified, and plasma-treated scaffolds in two groups; there is no significant difference between the alignment indices for two groups of scaffolds (C and D).

the fiber production rate.

There is a significant difference between the alignment indices of the CA and DA. To explain this, although the collection speed of CA and DA is similar to each other, their production speed is different from each other. Production speed of fibers formed by using the mixture of chloroform/DMF is more than production speed of fibers formed by using chloroform. If the production rate is equal to the collection rate, the fibers will be aligned. If the production rate is higher than the collection rate, the alignment will decrease.

As shown in Table II, the porosity of samples prepared by chloroform (CA/CR) is more than those made by chloroform/DMF (DA/DR). The porosity of structurally-modified scaffolds with oriented construction (CA: 98.86±0.12) is significantly more than that of the non-treated scaffolds with random construction (CR: 98.62±0.11). The same trend is seen about the other samples (DA: 98.75±0.10 and DR: 98.50±0.14). The groups were statistically analyzed using SPSS software. Also according to our previous research, the best structure with optimized porosity of scaffolds, was obtained at the collector linear speed of 2.4 m.s^{-1} [23]. Figs. 3 and 4 illustrate the diagrams for the alignment index and the porosity of scaffolds, respectively.

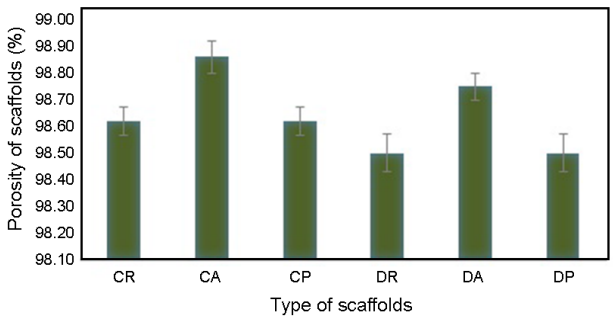

Fig. 4. Porosity of non-treated, structurally-modified, and plasma-treated scaffolds in two groups; there is significant difference between the porosities of two groups of scaffolds (C and D).

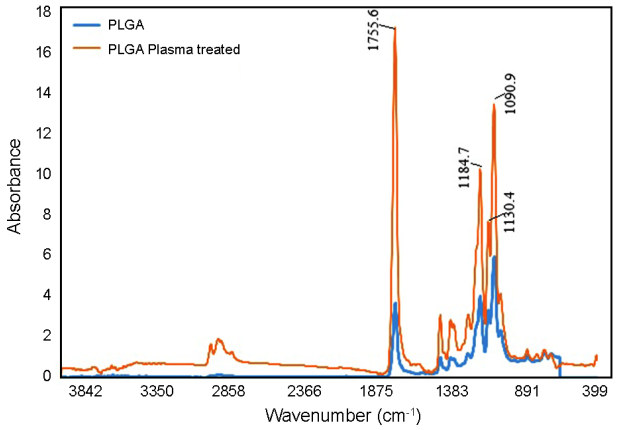

Fig. 5. ATIR–FTIR spectra for non-treated PLGA and argon plasma treated PLGA.

C. ATR-FTIR Analysis

The spectra were first normalized to the wavelength of 1450 cm^{-1}, which is the reference peak of the PLGA. The vibrations in the range 1660–1820 and 1000–1300 cm^{-1} correspond to (C=O) and (C-O) bonds, respectively [25,26]. To evaluate the chemical structure of non-treated and plasma-treated scaffolds, the ATR–FTIR spectra in Fig. 5 demonstrate that for the PLGA plasma-treated scaffold, the absorption peak at 1755 cm^{-1} increased significantly due to the higher extent of (C=O) bonds. Moreover, the absorbance values at 1090, 1130, and 1184 cm^{-1} increased upon the plasma treatment which could be attributed to the formation of a higher number of (C-O) bonds. The enhanced number of (C=O) and (C-O) bonds, as functional groups on PLGA nanofibers, might lead to an improvement in the wettability of scaffolds [24].

D. Hydrophilicity

The surface hydrophilicity can be influenced by many factors including the surface physical properties and chemical functionality. Fig. 6 and the data presented in Table II show that the hydrophilicity for both the modified groups were much higher than that for the non-treated scaffold. In particular, the plasma treatment was found to rapidly decrease the contact angle of the scaffolds. Therefore, the imparted modifications on the scaffolds reduced the water contact angles of CA and CP as high as 50 and 90%, respectively, as compared with CR. Moreover, the reduction percentage of water contact angles was found to be 11 and 84% for DA and DP in comparison with DR electrospun scaffolds, respectively.

Since the hydrophilicity has great correlation with the collector speed [27], the hydrophilicity for structurally-modified scaffold is higher than that for the non-treated scaffold. Also the cause of improved hydrophilicity in

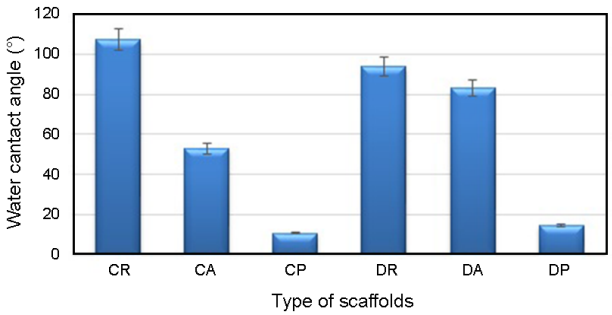

Fig. 6. Water contact angle values for the non-treated, structurally-modified, and plasma-treated scaffolds in two groups; there is no significant difference between the hydrophilicity of the two groups of scaffolds (C and D).

plasma-treated scaffold is the increase of functional groups of (C=O) and (C-O).

Statistical analysis of water contact angles of the samples prepared by chloroform (C) and chloroform/DMF (D) show that there exist no significant differences between the WCA values of "C" and "D" groups.

In the case of plasma modification, although no apparent differences were observed between the treated and non-treated scaffolds, the nature of chemical functional groups is changed. Fig. 5 depicts the ATR–FTIR spectra for the PLGA and PLGA plasma-treated scaffolds, further corroborating the wettability results.

E. In Vitro Study

The *in vitro* studies were carried out using Vero cells which were cultured on six types of electrospun scaffolds to compare the extent of cell attachment and proliferation.

The level of cell attachment on both the modified scaffolds was found to be slightly higher comparing with the random scaffolds. The viability of cells on the scaffolds was calculated according to the following equation where OD_{sample} and $OD_{control}$ are the mean optical density of cells on the scaffolds and control plates, respectively. In the MTT assay, the optical density is directly proportional to the viability of cells [28]:

$$\text{Viability} = \frac{OD_{sample}}{OD_{control}} \times 100 \quad (3)$$

Fig. 7 demonstrates the viability of cells on different scaffolds. The results obtained from MTT assay indicate that the two modified groups, with no significant difference, are more suitable scaffolds rather than the non-treated scaffolds in terms of cell growth and proliferation. Data reported in Tables II and III show that although the plasma-treated scaffolds are more hydrophilic than the structurally-modified group, the cell proliferation on both of them is rather the same. This interesting

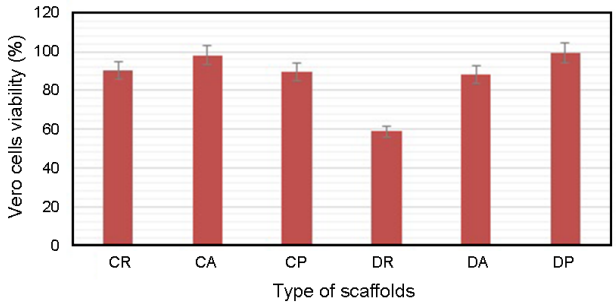

Fig. 7. Viability of Vero cells on different samples.

TABLE III
VIABILITY OF VERO CELLS ON SCAFFOLDS

Scaffolds	CR	CA	CP	DR	DA	DP
Viability	90.52	98.32	89.41	58.78	88.34	99.37
SD	3.21	3.11	1.90	2.41	2.70	3.01

observation can be attributed to the proper physical structure of these specimens such as smaller diameter, greater porosity and higher alignment index.

It can be concluded that although the hydrophilicity improves the cell adhesion and proliferation, modifying the structural properties of the scaffold, namely, porosity of scaffold, fiber diameter, and alignment, could simultaneously impose a significant effect on the cells' behavior.

Therefore, since the same results for cell proliferation was attained in both the modified methods, and also considering the fact that the structural modifications are made during the electrospinning process without any post-processing, the structural modifications may be used to improve the cell proliferation on the scaffolds instead of the plasma modification. Plasma modification occurred after electrospinning with the need for some additional processes requiring more time and cost. However, through the use of structural modifications, some of the physical properties of a scaffold (fiber diameter, orientation, and consequent hydrophilicity and porosity) can be simultaneously tuned during the electrospinning process to achieve the desired level of cell attachment and growth.

Comparing the results of cell proliferation on the scaffolds prepared by chloroform (C) and those made by chloroform/DMF (D), one could infer that the group "C" exhibits a higher level of cell proliferation rather than the group "D". The most probable reason for this behavior could be the greater porosity of the group "C" as compared to the other group.

IV. CONCLUSION

In this study, PLGA nanofibrous scaffolds were used for cell culture and their hydrophilicity was improved

by means of plasma modification. From the ATR-FTIR analysis, it was confirmed that the C=O and C-O groups have been increased in the case of plasma-treated samples. Moreover, a structural modification was applied on PLGA nanofibrous scaffolds to improve scaffolds hydrophilicity and cells proliferation. The characterization results for the scaffolds further corroborate the proper physical structure of the structurally-modified scaffolds including smaller diameter, greater porosity and higher alignment index. *In vitro* studies demonstrated that two groups of the modified scaffolds could enhance the initial adhesion, growth and proliferations of Vero cells as compared to the non-treated scaffold via improving the hydrophilicity and physical properties of scaffolds.

REFERENCES

[1] S. Yang, K.F. Leong, Z. Du, and C.K. Chua, "The design of scaffolds for use in tissue engineering. Part I. Traditional factors", *Tissue Eng.*, vol. 7, pp. 679-689, 2001.

[2] Y. Ikada, *Tissue Engineering Fundamentals and Applications*, Netherland: Elsevier/Academic, 2008, pp. 173-188.

[3] F. Mokhtari, M. Salehi, F. Zamani, F. Hajiani, F. Zeighami, and M. Latifi, "Advances in electrospinning: the production and application of nanofibere and nanofibrous structures", *Text. Prog.*, vol. 48, no. 3, pp. 119-219, 2016.

[4] E. Andronescu and A.M. Grumezescu, *Nanostructures for Drug Delivery*, Amsterdam: Elsevier/Matthew Deans, 2017, pp. 239–270.

[5] F. Zamani, M. Amani-Tehran, M. Latifi, and M.A. Shokrgozar, "The influence of surface nanoroughness of electrospun PLGA nanofibrous scaffold on nerve cell adhesion and proliferation", *J. Mater. Sci. Mater. Med.*, vol. 24, pp. 1551-1560, 2013.

[6] F. Jahanmard, M. Amani-Tehran, F. Zamani, M. Nematollahi, L. Ghasemi, and M.H. Nasr-Esfahani, "Effect of nanoporous fibers on growth and proliferation of cells on electrospun poly(ϵ-caprolactone) scaffolds", *Int. J. Polym. Mater. Polym. Biomater.*, vol. 63, pp. 57-64, 2013.

[7] M.P. Prabhakaran, J. Venugopal, C.K. Chan, and S. Ramakrishna, "Surface modified electrospun nanofibrous scaffolds for nerve tissue engineering", *Nanotechnology*, vol. 19, no. 45, pp. 5102, 2008.

[8] S. Miroshnichenko, V. Timofeeva, E. Permykova, S. Ershov, P. Kiryukhantsev-Korneev, E. Dvorakov, D.V. Shtansky et al., "Plasma-coated polycaprolactone nanofibers with covalently bonded platelet-rich plasma enhance adhesion and growth of human fibroblasts", *Nanomaterials*, vol. 9, pp. 637-656, 2019.

[9] H. Rauscher, M. Perucca, and G. Buyle, *Plasma Technology For Hyperfunctional Surface*, Weinheim: Weily-VCH, 2010, pp. 63-78.

[10] R. Morent, N. De Geyter, T. Desmet, P. Dubruel, and C. Leys, "Plasma surface modification of biodegradable polymers: a review", *Plasma Proc. Polym.*, vol. 8, pp. 171-190, 2011.

[11] G.H. Ryu, W.S. Yang, H.W. Roh, I.S. Lee, J.K. Kim, G.H. Lee, D.H. Lee et al., "Plasma surface modification of poly(D, L-lactic-*co*-glycolic acid) (65/35) film for tissue engineering", *Surf. Coat. Technol.*, vol. 193, pp. 60-64, 2005.

[12] A. Solouk, B.G. Cousins, H. Mirzadeh, and A.M. Seifalian, "Application of plasma surface modification techniques to improve hemocompatibility of vascular grafts: a review", *Biotechnol. Appl. Biochem.*,vol. 58, pp. 311-327, 2011.

[13] A. Solouk, B.G. Cousins, H. Mirzadeh, M. Solati-Hashtjin, S. Najarian, and A.M. Seifalian, "Surface modification of POSS-nanocomposite biomaterials using reactive oxygen plasma treatment for cardiovascular surgical implant applications", *Biotechnol. Appl. Biochem.*, vol. 58, pp. 147-161, 2011.

[14] Z. Liu, L. Jia, Z. Yan, and L. Bai, "Plasma-treated electrospun nanofibers as a template for the electrostatic assembly of silver nanoparticles", *New J. Chem.*, vol. 42, no. 13, pp. 1-7 , 2018.

[15] N. Hasirci, T. Endogan, E. Vardar, A. Kiziltay, and V. Hasirci, "Effect of oxygen plasma on surface properties and biocompatibility of PLGA films", *Surf. Interface Anal.*, vol. 42, pp. 486-491, 2010.

[16] M. Khorasani, H. Mirzadeh, and S. Irani, "Plasma surface modification of poly(L-lactic acid) and poly(lactic-*co*-glycolic acid) films for improvement of nerve cells adhesion", *Radiat. Phys. Chem.*, vol. 77, pp. 280-287, 2008.

[17] K.E. Park, K.Y. Lee, S.J. Lee, and W.H. Park, "Surface characteristics of plasma-treated PLGA nanofibers", *Macromol. Symp.*, pp. 103-108, 2007.

[18] L. Safinia, K. Wilson, A. Mantalaris, and A. Bismarck, "Through-thickness plasma modification of biodegradable and nonbiodegradable porous polymer constructs", *J. Biomed. Mater. Res.*, vol. 87A, pp. 632-642, 2008.

[19] H. Cao, T. Liu, and S. Chew, "The application of nanofibrous scaffolds in neural tissue engineering", *Adv. Drug Deliver. Rev.*, vol. 61, pp. 1055, 2009.

[20] G. Kim, J. Park, and S. Park, "Surface-treated and multilayered poly(e-caprolactone) nanofiber webs

exhibiting enhanced hydrophilicity, *J. Polym. Sci: Polym. Phys.*, vol. 45B, pp. 2038-2045, 2007.

[21] L. Huang, J.T. Arena, S.S. Manickam, X. Jiang, B.G. Willis, and J.R. McCutcheon, "Improved mechanical properties and hydrophilicity of electrospun nanofiber membranes for filtration applications by dopamine modification, *J. Membrane Sci.*, vol. 460, pp. 241-249, 2014.

[22] F. Zamani, M. Amani-Tehran, A. Zaminy, and M.A. Shokrgozar, "Conductive 3D Structure nanofibrous scaffolds for spinal cord regeneration", *Fiber. Polym.,* vol. 18, pp. 1874-1881, 2017.

[23] F. Zamani, M. Latifi, M. Amani-Tehran, and M.A. Shokrgozar, "Effects of PLGA nanofibrous scaffolds structure on nerve cell directional proliferation and morphology", *Fiber. Polym.*, vol. 14, pp. 698-702, 2013.

[24] S. Ramakrishna, K. Fujihara, W.E. Teo, T.C. Lim, and Z. Ma, An Introduction to Electrospinning and Nanofibers, World scientific, Singapore, 2005, pp. 90-155.

[25] L. Safinia, N. Datan, M. Hohse, A. Mantalaris and A. Bismarck, "Towards a methodology for the effective surface modification of porous polymer scaffold", *Biomaterials*, vol. 26, pp. 7537-7547, 2005.

[26] D.L. Pavia, G. Lampman, and G.S. Kriz, *Introduction to Spectroscopy*, 5rd ed, Cengage Learning, Washington, 2013, pp. 14-106.

[27] F. Zamani, "Engineering of structural properties of PLGA nanofbrous scaffold for neural cell culture", Ph.D Dissertation, Dept. Text. Eng., Amirkabir University of Technology, Tehran, Iran, 2013.

[28] GE. Adams, A. Breccia, EM. Fielden, and P. Wardman, *Selective Activation of Drugs by Redox Processes,* New York: Plenum, 1990, pp. 200-210.