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Original Article

Adipose-derived Stem Cells Growth and Proliferation Enhancement Using Poly (lactic-co-glycolic acid) (PLGA)/Fibrin Nanofiber Mats

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Abstract

Introduction: A Synthetic biomaterial, such as Poly Lactic-co-Glycolic Acid (PLGA) with superior mechanical properties, along with a natural polymer such as fibrin, which facilitates cell attachment and enhances biocompatibility, can be used in the production of novel composite tissue engineering scaffolds.

Materials and Methods: To carry out this study, 10% polymer solutions with different ratios of PLGA: Fibrin, including 10:0, 9:1, 8:2, and 7:3, were prepared and used in the production of aligned and unaligned electrospun nanofiber scaffolds. Human Adipose-Derived Stem Cells (h-ADSCs) were cultured on the scaffolds, and they were characterized using Fourier Transform Infrared Spectroscopy (FTIR), Energy-dispersive X-ray spectroscopy (EDX), Scanning Electron Microscopy (SEM), mechanical, hydrophilic, degradation, water absorption and biocompatibility tests.

Results: The obtained scaffolds consisted of homogeneous fibers, without any beads and water droplets. The percentage of porosities and internal correlation of the cavities were not significantly different between aligned and unaligned electrospun scaffolds ($p>0.05$) and by adding fibrin, these properties improved, while tensile strength and elasticity decreased. All the scaffolds were hydrophobic and the highest and lowest swelling rates belonged to PLGA/30% Fibrin scaffolds and pure PLGA scaffolds (more than 90% and less than 45%, respectively). There is a significant difference in degradation rates between fibrin-contained scaffolds and pure PLGA scaffolds. Moreover, compared to the aligned electrospun scaffolds, the highest degradation rate of unaligned ones was observed.

Conclusions: Considering the results of SEM and bio-compatibility experiments, the aligned electrospun PLGA/10% Fibrin scaffold with numerous spindle shape h-ADSCs and unaligned electrospun PLGA/20% Fibrin scaffold with many spindle shape cells together with round shape cells are introduced as optimal options.

Keywords: Poly Lactic-co-Glycolic Acid, Fibrin, Composite Fibers, Adipose-derived Stem Cells, Tissue Engineering, Scaffold
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Introduction

Nowadays, many people suffer from defective tissues and defective performances of their organs. Tissue engineering is consisted of cells or cell-precursors separation from tissue samples. Through passaging these cells in the culture medium, an increased number of cells are acquired. These cells are cultivated and incubated on a three-dimensional scaffold and finally, the cell-vector construct is grafted inside the patient's body.[1]

Natural materials and artificial polymers in the forms of fiber structures, porous sponges, woven nets, and hydrogels are used in the manufacture of various tissue scaffolds.[2] Natural materials such as collagen types 1 and 2, hyaluronic acid, other synthetic, biological materials, and their compounds have been clinically used in scaffolds manufacturing due to their flexibility, accessibility, or their ability to provide a macro-mechanical environment relative to the natural tissues.[3,4] Like collagen, fibrin scaffolds contain sites for cell adhesion,[5] and also cells can be directly linked to fibrin via their integrins.[1]

Through using PLGA in the form of microstructure, it would be possible to facilitate binding, growth, proliferation of chondrocytes, and also improve the production of extracellular matrix.[1] In addition, by composing these polymers, various characteristics of the polymers can be combined due to the control of the destruction, cell attachment, proliferation, and differentiation. As far as the activated polymers are widely used to overcome the problems associated with synthetic and natural polymers, various active groups provided by cell signals through activation can be added to the polymer.[2] For instance, to maintain cell phenotype and control scaffold degradation features, PLGA mesh has been combined with chondrocyte

fibrin glue or researchers could enhance the production of collagen type 2 using PLGA and cell-containing alginate. In an "*in vivo*" study, researchers have used the PLGA scaffold containing collagen type 1 for cell culture and as a result, uniform distribution of stable cells in terms of morphology with the formation of collagen type 2 has been observed.[6] Other similar examples in cartilage repair are poly glycolic acid (PGA)/alginate, poly lactid (PLA)/alginate, PLGA/ hyaluronic acid (HA), and PLGA/fibrin.[6] Chitosan (CS) nanoparticles were also composed of PLGA to fabricate scaffolds with improved hydrophilicity and mechanical characteristics. Increasing CS content and aligned nanofibers finally have led to the improvement of cell adhesion and proliferation.[7] In an attempt to induce the factors of chondrogenesis, researchers cultured human Adipose-Derived Stem Cells (hADSCs) on PLGA/fibrin hybrid scaffold containing avocado and soybean and found this compound suitable for the purpose of their study.[8]

Another usage of fibrin/PLGA composite combination has been shown in tissue engineering applications. Scientists have achieved a successful differentiation of Schwann Cell Like (SCLs), using fibrin-poly (lactic-co-glycolic acid) in the form of scaffolds due to the cultivation of rat Adipose-derived Stem Cells (rASCs). They observed that aligned electrospun fibrin/PLGA fibers improved the formation of Büngner-like structures in SCL and also, rASCs have been able to differentiate into activated proliferative SCLs. Therefore, these cells can change in response to minimum stimulus changes towards the promyelinating phenotype.[9] In addition, the presence of fibrin fibers together with PLGA fibers, which were spun separately from two syringe pumps toward the same collector target, led to a synergistic effect. This provides a micro-environment for the differentiation of the Umbilical Cord Blood Mesenchymal Stem Cells (UCBMSCs) towards the cardiac phenotype.[10]

In this paper, we focused on the production of composite materials made from natural materials (fibrin) and synthetic material (PLGA) in the form of aligned and unaligned electrospun fibers. By considering the technical issues of production, particularly the optimization of electro spinning parameters, some scaffolds have been produced to provide the possibility of binding, growth, and proliferation of mesenchymal cells derived from adipose tissue.

Materials and Methods

Materials

Poly (lactic-co-glycolic acid) (PLGA; 0.1 g/ml, Purasorb® PLG 8523; Purac, The Netherlands) with a copolymer ratio of 85/15 and fresh frozen fibrinogen and thrombin (frozen in vials, from Department of Anatomy, Isfahan University of Medical Sciences) has been used in the present study. Also, for the solvent phase 1,1,1,3,3,3-hexafluro-2-propanol (HFIP) (Merck & Co., Inc., Germany) was used.

Preparation of Polymer Solutions

In this study, we prepared and used polymer solutions with different ratios of PLGA and PLGA/Fibrin (Table 1). First, to prepare fibrin, we removed fibrinogen and thrombin from the freezer and kept them at room temperature, leading them to change into liquid state. They were then combined them with a 1:1 ratio. To produce solid fibrin, the mixture was placed at room temperature for several minutes. Then, the specified ratios of PLGA/Fibrin were solved in solvent 1,1,1,3,3,3-hexafluoro-2–propanol (HFIP), and the solutions with a concentration of 10% g/ml (w/v) were prepared. Finally, using a magnetic stirrer, the polymer solution was stirred for 5 hours so that a completely homogeneous solution was prepared.

Fabrication of Electrospun Scaffolds

To optimize the electrospinning parameters' values, we performed initial testing of potential values and defined optimum values for the purpose of this study as follows. The polymer flow rate was 0.8 mL/h, the distance between the needle tip and collection mandrel was 20 cm, the voltage of the device was set to 22 kV, and also the circumferential speed of the machine and speed of rotation of the collection mandrel cylinder were adjusted at 10 cm and 30 rpm, respectively. The resulting matrix scaffold was then placed in a vacuum oven and was vacuum-dried at 30 °C for 24 hours to completely remove solvents.

Characterization of Scaffolds

For morphology characterization studies, SEM was done on thin gold-coated layer samples using the ZEISS SIGMAVP microscope (Carl Zeiss Inc, Oberkocken, Germany). To study chemical microanalysis, the FTIR spectroscopy test was performed by using the FTIR6300 MHZ (JASCO Corporation, Tokyo, Japan) test rig in the range of 400-4000 cm^{-1} and the scanning speed of 2 cm^{-1}.

To evaluate the fiber size distributions, SEM images were processed, and the mean values were reported.

The porosity of the scaffolds was studied by the image processing software developed in a previous study.[11] The results were reported in three thresholds. By calculating these thresholds, the grayscale images converted to binary. Changing the threshold resulted in visibility of various layers of nanofibers mat. Thresholds I and III show the scaffold porosity and the internal pore network connectivity, respectively.

We evaluated the mechanical properties of fabricated electrospun scaffolds according to the ASTM D 638 protocol. The tensile test was performed by the tensile test rig Zwick/Roell Z050 model (Zwick Roell AG and Zwick GmbH & Co. KG, Ulm, Germany) by placing test samples (dimensions of 1×3 cm and thickness of 0.2 mm) in the machine and subjecting them to a controlled load until

failure. The force was applied on samples along the direction of fibers deposition, and the average values of ultimate tensile strength, elasticity modulus, and elongation at the failure were calculated from the stress-strain curves.

The scaffold hydrophilicity was evaluated using a contact angle measurement system. We cut the nano-fiber mesh to a size of 12 mm^2 and placed it on a sample holder. Using a VCA Optima camera (AST Products, Inc. USA), the contact angles of 2 µl droplets were measured. To determine the absorption of fluids and scaffolds swelling, the dry specimens were first weighed (*W0*), and then after 24 hours' immersion in Phosphate Buffered Saline (PBS), their weight was again measured (*Wa*). The amount of PBS absorption was obtained by Eq. (1):

Eq. (1): PBS Absorption (%) = *([Wa – W0] / W0) * 100*

To calculate the biodegradability of scaffolds, samples were removed from PBS solution on days 1, 3, 7, 10, 15, 30, 45, 60, 75, 80, 85, and 90, and then they were dried off at 30 °C for 1 hour with a vacuum oven. The weight of the samples was then measured accurately (*Ws*). The Weight Loss Rate (WLR) of the scaffolds was calculated from Eq. (2):

Eq. (2): Weight loss rate = *([Ws – W0] / W0) * 100*

Cell Studies

Cell Separation and Cell Culture

In this study, human Adipose-derived mesenchymal Stem Cells (h-ADSCs) of the third passage was used. The cells were kindly gifted by Dr. Setayeshmehr (Department of Anatomy, Isfahan University of Medical Sciences). Frozen cells in the vial were removed from the Liquid Nitrogen Tank and were rapidly thawed in a 37 °C water bath until only a small ice pellet remains. The vial was also sprayed with 70% ethanol and placed into the hood. The cells were then gently re-suspended, counted, and slowly diluted into the required volume of pre-warmed culture medium. Cells were seeded at a density of 10.000/well (for the MTT tests) and 100,000/well (to reach the desired confluency for the morphological evaluations) to adhere and grow on the scaffold surface. The culture medium included DMEM low glucose, which was changed every three days. As the control sample, the cells were cultured in the bottom of the wells, containing a culture medium (without scaffolds). MTT tests were conducted 1, 3, and 7 days after primary culture.

Cell Survival Test

In each case, after drainage and washing with PBS, 200 µl of DMEM low glucose, and 20 µl of MTT solution (5 mg/ml) were added to each well of the 24-well plate. After incubation at 37 °C for 4 hours, the culture medium was removed. Then, 200 µl of dimethyl sulfoxide (DMSO) was added to each well. The plates were covered with aluminum foil and placed on a shaker for an hour. Then, 100 µl samples were poured into a 96-well plate in a dark room and were read by an optical absorption device (OD) over a wavelength of 549 nm. A similar process was performed on control samples to calculate the amount of cell survival (viability) as follows:

Eq. (3): Viability (%) = *([ODs – Odb] / [Odc – Odb]) * 100*

Where *ODds, ODb, and ODc* represent light absorption of a scaffold sample cultured, DMSO show light absorption in an empty plate, and the light absorption of control culture sample, respectively.

Morphological Evaluation of Cells on the Scaffolds

In this study, we used scaffolds containing the initial density of 100,000 cells. At first, 7-days culture samples were washed by PBS and then stabilized on the scaffold surface by glutaraldehyde solution 2% for 30 minutes. Samples were placed in a solution of 50, 70, 80, 90, and 100% ethanol (each for 30 minutes) and were kept in 100% ethanol for the duration of the imaging time. To evaluate the morphology of the cells on the scaffolds, the samples were first removed from the plate wells, and after evaporation of the residual alcohol, they were covered with a thin layer of gold and finally, SEM imaging was performed at a voltage of 14000 volts.

Statistical Studies

Data were analyzed by SPSS V20 software. The results were reported as mean ± SD and one-way ANOVA.

Results

Chemical, Morphological and Mechanical Characterization of PLGA and PLGA/Fibrin Scaffolds

Investigations on the elements and chemical compounds of fibrin and PLGA were performed by EDX and FTIR. The presence of fibrin and PLGA-related chemical elements and compounds through EDX and FTIR have been investigated. According to Figure 1a and Figure 1b, in the PLGA/30% Fibrin hybrid scaffold, in addition to carbon and oxygen, nitrogen was present, which is an indicator of the presence of amide groups in the fibrin molecular structure.

The results of FTIR spectroscopy on two PLGA and PLGA/30% Fibrin scaffolds are shown in Figure 1c. The absorption peaks were observed in the presence of PLGA in the range of 1184 cm^{-1} and 1090 cm-1 for C-O tensile strengths and 1763 cm^{-1} for tensile strains C = O. The three absorbing peaks observed in 1654 cm^{-1}, 1546 cm^{-1} and 1267 cm^{-1}, are related to the first, second and third types of amide, respectively; which can be assigned to the fibrin structure of PLGA/30%Fibrin hybrid electrospun scaffold.

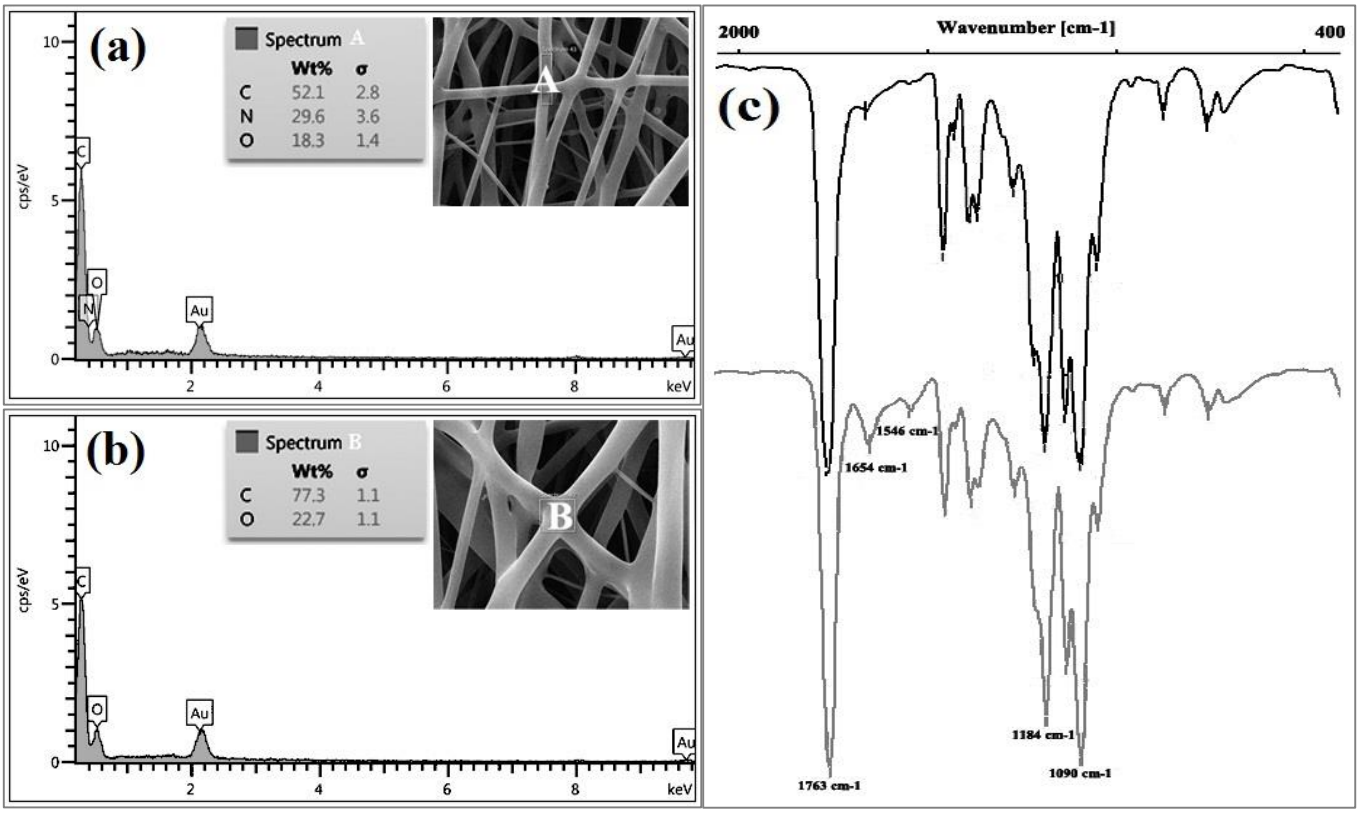

Figure 1. EDX Spectrum of PLGA/30%Fibrin **(b)** and Pure PLGA **(a)** Nano-fiber Scaffolds with the Percentage of Carbon, Nitrogen, and Oxygen in the Selective Points; The FTIR Spectrum of the PLGA/30%Fibrin and the Pure PLGA Nano-fibrous Scaffold (Gray and Black) **(c)**.

As Figure 2 illustrates, the SEM of the aligned and unaligned electrospun scaffolds provide homogenous fibrous mats without any bead and water droplet and with fibers in the thickness of 0.1 to 3.5 µm. By adding fibrin to the polymeric solution used in electro-spinning, the thickness of fiber is reduced. Also, there are fibers with proper orientation and several curved fibers in the aligned electrospun scaffolds. Mostly, the thickness of fiber is less in comparison to the random samples.

We calculated the porosity percentage and internal correlation of cavities in samples by SEM image processing a software developed in a previous study[11] (see Figures 2c and b). The figures show that all of the scaffolds have a porosity ranging from 79.28 to 82.32%. The internal correlation of cavities was calculated in the range of 20.26 to 25.4%. As Figure 2 depicts, by adding fibrin to polymeric solutions, slight changes in porosity variations and internal correlation of scaffold cavities were observed. On the other hand, there is no significant difference in the porosity and internal relation of the cavities between the aligned and unaligned electrospun scaffolds (p>0.05).

The mechanical properties of the specimens are shown in Table 1. The results reveal that the highest and lowest tensile strengths are associated with the aligned electrospun PLGA scaffold (130.0 ± 10.70 MPa) and the unaligned electrospun PLGA/20% Fibrin scaffold (11 ± 1.41 MPa), respectively. The tensile strength of the aligned elelctrospun samples is greater than the unaligned one. Also, by adding fibrin to PLGA, the tensile strength and modulus of elasticity of scaffolds decreased. Additionally, the results illustrate 20-40% sample elongation at the failure in aligned electrospun scaffolds and 10-25% for unaligned electrospun scaffolds.

The contact angle of the aligned and unaligned electrospun PLGA scaffolds is in the range of 120 to 137 degrees, which indicates the hydrophobicity of the specimens. By increasing the percentage of fibrin in the polymer solution used in the electrospinning, we can obtain more hydrophilic aligned electrospun scaffolds.

PBS Absorption Capacity and Degradation Ratio of Scaffolds

The aligned and unaligned electrospun PLGA/30%Fibrin scaffold had the highest amount of absorption capacity after 24 hours of immersion in PBS solution (95.9 ± 1.9% and 90.59 ± 1.8%, respectively) (Figure 3a). The PBS absorption capacity of the scaffolds has been enhanced by increasing the fibrin content. There is no significant difference between the PSB absorption capacity of the aligned and unaligned electrospun scaffolds (p>0.05).

Figure 3b shows the weight loss process of scaffolds during a 90-day incubation period in a PBS solution. After four weeks, the destruction of aligned and unaligned electrospun scaffolds were between 20% and 35%. From

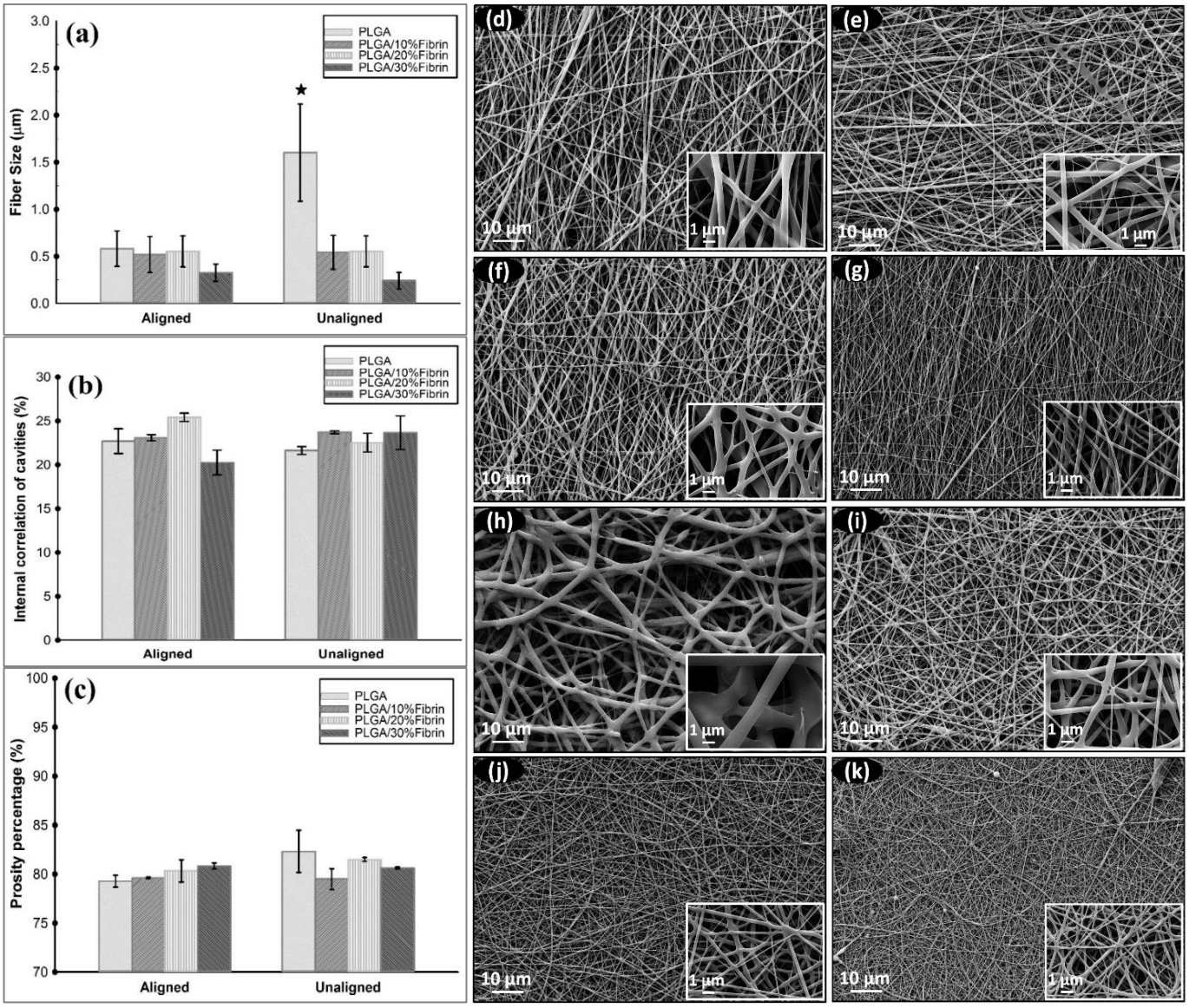

Figure 2. Gradual Reduction of Fibers Thickness by Adding Fibrin to the Polymeric Solution Used in Electro Spinning **(a)**; Porosity Percentage **(c)** and Internal Correlation of Cavities **(b)**; Mean ± SD. One-way ANOVA. *p<0.05; Scanning Electron Microscope Micrographs of the Aligned and Unaligned Electrospun Scaffolds; **(d)**: PLGA-al; **(e)**: PLGA/10%Fibrin-al; **(f)**: PLGA/20%Fibrin-al; **(g)**: PLGA/30%Fibrin-al; **(h):** PLGA-unal; **(i):** PLGA/10%Fibrin-unal; **(j):** PLGA/20%Fibrin-unal; **(k):** PLGA/30%Fibrin-unal.

Table 1. Characteristics of Nano-fiber Scaffolds

Nomencladure	(W/V) %	PLGA/Fibrin	Mechanical Properties			Contact Angle
			Elongation at the Failure (%)	Young's Modulus (Mpa)	Ultimete Tensile Strength (Mpa)	
PLGA-al	10	10:0	37.46 ± 1.82	24.18 ± 6.57	130 ± 10.70	137 ± 2
PLGA/10%Fibrin-al	10	9:1	39.24 ± 10.21	6.36 ± 0.23	46 ± 10.96	130 ± 1
PLGA/20%Fibrin-al	10	8:2	31.80 ± 6.16	10.00 ± 1.22	76 ± 18.35	125 ± 2
PLGA/30%Fibrin-al	10	7:3	19.10 ± 3	6.27 ± 3.56	46 ± 34.11	126 ± 1
PLGA-unal	10	10:0	20.44 ± 11.09	5.09 ± 2.47	20 ± 3.37	125 ± 0
PLGA/10%Fibrin-unal	10	9:1	10.35 ± 3.10	5.32 ± 3.74	23 ± 15.41	120 ± 3
PLGA/20%Fibrin-unal	10	8:2	12.78 ± 4.26	2.11 ± 1.02	11 ± 1.41	123 ± 3
PLGA/30%Fibrin-unal	10	7:3	24.23 ± 6.5	3.03 ± 0.66	17 ± 3.90	127 ± 2

the 60th day, the scaffold degradation made from PLGA-Fibrin polymer solutions was significantly higher than the PLGA-made scaffolds (pure PLGA). Increasing the percentage of fibrin led to more degradable scaffolds.

Attachment, Survival and Cell Proliferation on PLGA and PLGA/Fibrin Scaffolds

Using the MTT test, h-ADSCs were evaluated on the first, third, and seventh days after culture. As the control group,

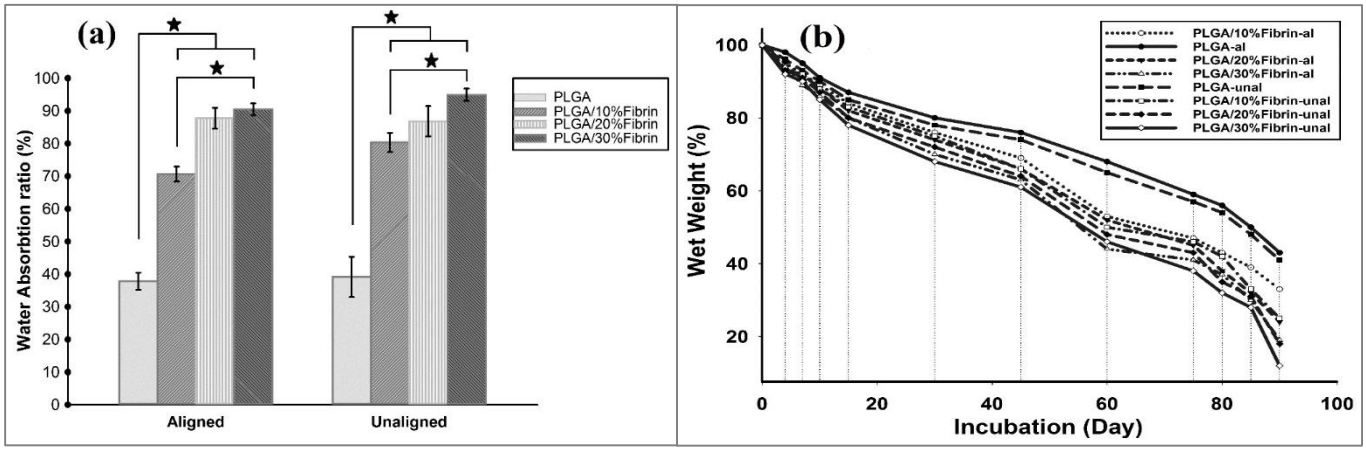

Figure 3. Water Absorption Ratios of the Aligned and Unaligned Electrospun PLGA/Fibrin Nano-fiber Scaffold with Different Ratio of PLGA:Fibrin **(a)**; Dry Weight Percentages of Aligned and Unaligned Electrospun Scaffolds in a 90-day Incubation **(b)**. Mean ± SD. One-way ANOVA. *p<0.05.

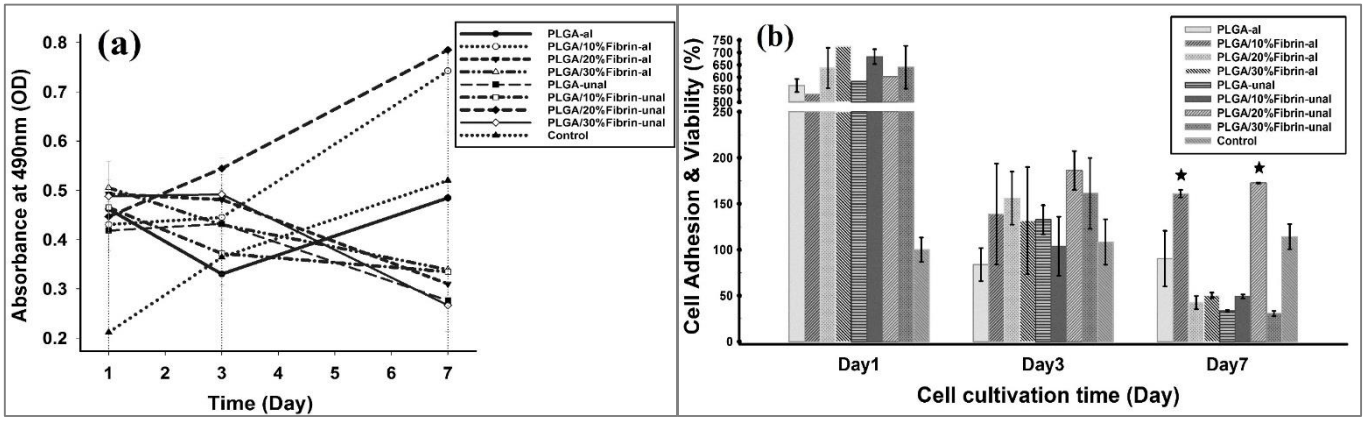

Figure 4. MTT Test Results (Light Absorption at 490 nm). Connectivity and Cell Survival Results **(a)**; Cell Adhesion and Viability on the Aligned and Unaligned Eletrospun PLGA/Fibrin Scaffolds and Control Samples after 1, 3 and 7 Days after Cultivation Time **(b)**. Mean ± SD. One-way ANOVA. *p<0.05.

24 cells were planted on the plate surface. The light absorption at 549 nm wavelength in all specimens except aligned electrospun PLGA/10% Fibrin and unaligned electrospun PLGA/20% Fibrin samples decreased gradually up to the seventh day (see Figure 4a). On the seventh day, the adsorption in these two scaffolds was significantly higher than the others (p<0.05). The results of cell binding and cell proliferation (Figure 4b) indicate that in the first 24 hours after culture, higher cell proliferation on scaffolds has occurred in comparison with control samples. Also, higher cell death has been found in control samples. The number of cells on the scaffolds gradually reduced (the number of cells attached to scaffolds decreased to about 1/3 from the initial state on the third day). This trend continues until the seventh day after the aligned electrospun PLGA/10%Fibrin and the unaligned electrospun PLGA/20% Fibrin scaffold carry a higher cell count than the control samples.

Cell-Scaffold Interaction Behavior

Figure 5 depicts the SEM images from scaffolds carrying cells on the seventh day in which the presence of h-ADSCs is observed in all the images. The growth orientation of the cells on aligned electrospun scaffolds is well aligned with the direction of the fibers. However, the cells on unaligned electrospun scaffolds grow and distribute accidentally and inconsistently. The cells' morphology on the aligned and unaligned electrospun scaffolds represents a spindle-like shape. The aligned electrospun PLGA scaffolds, aligned electrospun PLGA/10% Fibrin scaffolds, and unaligned electrospun PLGA/20% Fibrin scaffold carries more human adipose-derived Mesenchymal Stem Cells than the others.

Discussion

The results of the FTIR pictogram (Figure 1c) indicate the presence of absorption peaks of the $C-O$ tensile bond and the $C=O$ tensile strains in the same range as previous studies.[12,13] The results show the presence of PLGA in the structure of pure PLGA scaffold as well as the presence of amide of type I, II, and III (related to the fibrin molecular structure) in electrospun PLGA/30% Fibrin hybrid scaffold

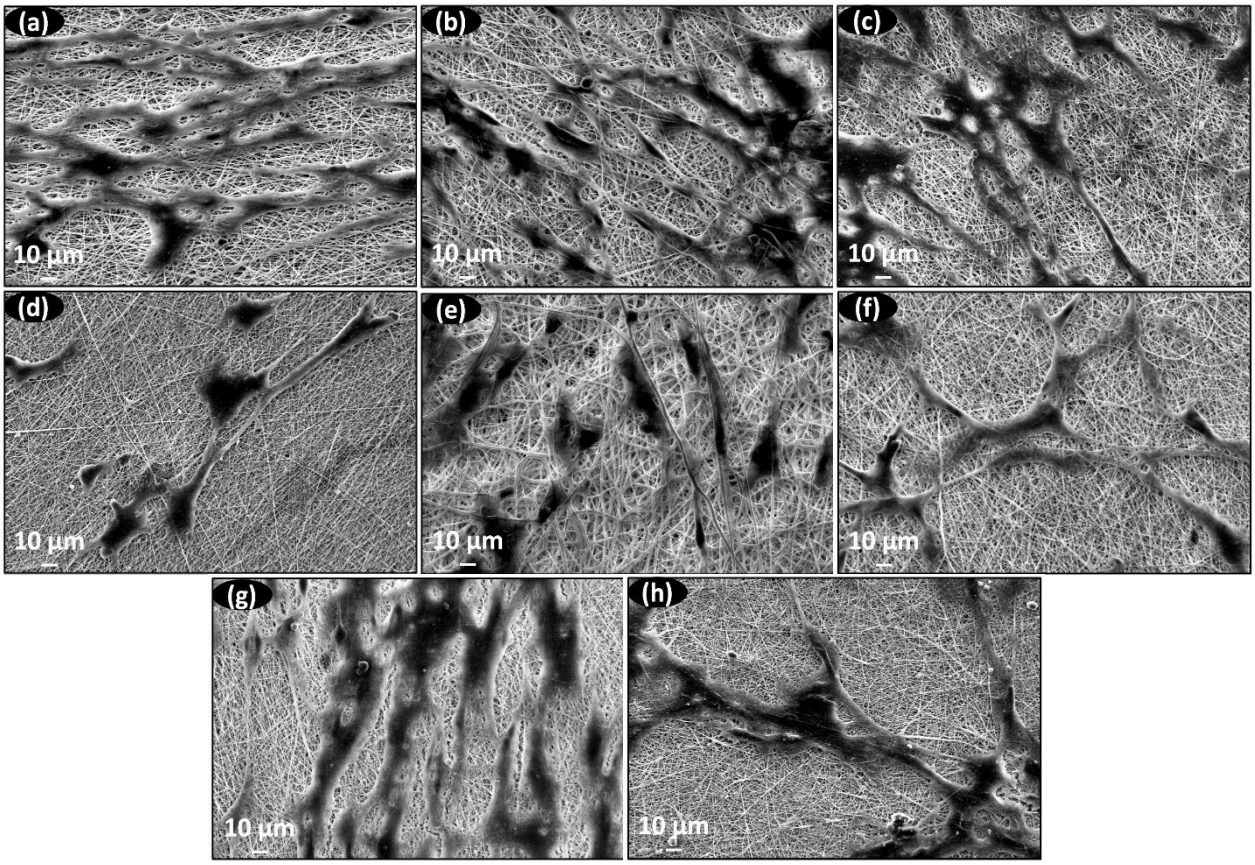

Figure 5. The SEM Micrograph of h-ADSCs Cultivated on Scaffolds with Different PLGA:Fibrin Ratios. **(a):** PLGA-al; **(b):** PLGA/10%Fibrin-al; **(c):** PLGA/20%Fibrin-al; **(d):** PLGA/30%Fibrin-al; **(e):** PLGA-unal; **(f):** PLGA/10%Fibrin-unal; **(g):** PLGA/20%Fibrin-unal; **(h):** PLGA/30%Fibrin-unal

and the absorption peaks are close to previous studies.[13-15] In addition, EDX chemical microanalysis confirmed the presence of nitrogen along with the carbon and oxygen elements in the PLGA/Fibrin hybrid scaffold (Figure 1). The morphology investigation of the fibers in aligned and unaligned electrospun scaffolds (Figure 2) showed that homogeneous fibrous mattresses without any bead and water droplet which is in agreement with the results of a previous study[16] in terms of the thickness of the fibers. By adding fibrin to the polymer solution used in electro spinning, the thickness of the fiber was reduced due to the viscosity reduction.[17] However, through spinning separate PLGA and fibrin fibers from two syringe pumps toward the same collector target, researchers produced PLGA-Fibrin composite scaffolds with higher fiber thickness.[10] Aligned electrospun scaffolds depicted favorable nanofibers and several curved fibers. In these samples, the thickness of the fibers was less due to the mandrel rotation and its tension effect on the fibers.[7]

A Scaffold with a minimum porosity of 80% is appropriate for most tissue engineering applications. Such a scaffold can provide a suitable base for cell adhesion, growth, and nutrition.[18,19] The results of porosity measurements of scaffolds suggested that the scaffolds have proper porosity and the internal correlation of cavities is also suitable for the purpose of tissue engineering applications.[11] These results are less than those that have been measured using the methanol inhalation method on the PLGA-Fibrin sponge scaffolds[20] and greater than those that have been measured by Asti et al. on PLGA and PLGA/HA.[21]

Adding fibrin has no significant effect on the cavity and internal bonding of the cavities. However, it was predicted that by adding the fibrin protein structure, the fibers are placed on each other and their interaction increases and as a result the porosity of the scaffolds decreases.[22]

Compared to the natural polymers of the body and tissues, the tensile strength and modulus of elasticity of the scaffolds are suitable for cartilage tissue engineering.[7,23] The tensile strength of aligned electrospun samples is greater than unaligned electrospun scaffolds which confirms the results of previous studies.[10,19,24] By adding the fibrin to PLGA, the viscosity of the solution and then the diameter of the fibers decreased, therefore the tensile strength and modulus of elasticity have been decreased.[22]

All scaffolds are hydrophobic (Table 1). As the fibrin increases, the contact angle of the droplet on the scaffolds is slightly reduced and so the scaffolds have become a bit more hydrophilic. By spinning the PLGA and fibrin fibers from two different syringes at the same time, researchers produced PLGA-Fibrin composite scaffolds which is much more

hydrophilic than PLGA (the contact angles of the drop of water were 84.3 ± 0.73 and 138.58 ± 4.91 degree, respectively). By adding gelatin or chitosan to PLGA, more hydrophilic composite scaffolds were obtained (PLGA-gelatin: 31 ± 4.8 and PLGA/30% Chitosan: 79 degree).[7,20]

According to the results of Figure 3a, aligned and unaligned electrospun PLGA/30%Fibrin scaffolds had the maximum, and the pure aligned and unaligned electrospun PLGA scaffolds had the minimum capacity of fluids after 24 hours' immersion in a PBS solution. These differences are due to the porosity of the scaffolds (the higher the porosity, the higher the absorption rate).[25] In addition, with increasing fibrin percentage, the absorption rate of scaffolds increases due to the hydrophilicity of fibrin protein.[20]

To study scaffolds degradation, we measured their weight loss over a 90-day period. Our results suggested that using composite materials, we can control the rate of scaffold degradation and produce composite scaffolds with high and rapid degradation (Figure 3b).[6] After the 30th day, a higher degradation rate of fibrin-scaffolds than pure scaffolds was observed. Also, the results show that the improvement was more noticeable after 60 days. In addition, the higher percentage of fibrin led to a more degradation rate due to the presence of OH groups in the amide-fibrin structures as reported in previous studies.[10,26]

The results of MTT (Figure 4a) showed that the survival of h-ADSCs in all samples gradually dropped from the first to the third and the seventh day after the cultivation. On the seventh day after culturing, only on aligned electrospun PLGA/ 10% Fibrin and unaligned electrospun PLGA/20% Fibrin scaffolds, the number of cells was greater than control samples which is in line with the findings of the study by Wei et al.[20] In fact, the presence of fibrin along with PLGA has a positive effect on the enhancement of cell binding and proliferation.[27]

The presence of a spindle-like h-ADSCs is observed in all SEM images on the seventh day after the initial culture (Figure 5). These cells have spread wells on the surface of the scaffolds, and the results show a growth aligned orientation with the direction of fiber spinning in directional senses in the aligned electrospun mats. However, random and non-oriented cell distribution happen on the random scaffolds. The spinning-like morphology of the cells on aligned electrospun scaffolds and relatively round morphology on unaligned electrospun scaffolds were observed. Also, in confirmation of the MTT test results, based on SEM images, the aligned electrospun PLGA/10% Fibrin scaffold and especially the unaligned electrospun PLGA/20% Fibrin scaffold carry more cells which is due to the important role of the scaffold in cell attachment and survival.[10]

Conclusion

In this investigation, composite scaffolds with different proportions of PLGA and fibrin were produced by the electro spinning method. Scaffolds were cultured by the mesenchymal cells of adipose tissue in a laboratory environment, in order to use in cartilage tissue engineering. The presence of fibrin and PLGA in composite scaffolds was confirmed by FTIR and EDX results. The results of the morphology of the fibers indicated the presence of homogeneous fibrous mats, without any beads and water droplets in all samples. The fibers orientation in the aligned oriented samples and the thickness of the fibers was less than random samples due to the rotation of the collector and the elasticity of the fibers. All of the scaffolds used in this investigation had acceptable mechanical properties in comparison with body tissues and natural polymers for the purpose of tissue engineering. The results also revealed that the capacity of scaffolds increased, which facilitates the penetration of food into structures and the release of waste materials. MTT results indicated the binding and survival of h-ADSCs on all scaffolds, in particular, the absorption of 490 nm wavelength on the seventh day of culture on the aligned electrospun PLGA/10% Fibrin and unaligned electrospun PLGA/20% Fibrin scaffold was more significant than the control sample and was also significantly higher than the other samples as well. In addition, SEM images of the scaffolds illustrated the presence of the spindle-like cells along with the fibers in the aligned electrospun mats. Meanwhile, these images showed that the aligned electrospun PLGA/10% Fibrin scaffold and especially the unaligned electrospun PLGA/20% Fibrin scaffold carry more numbers of cells, which is verified by MTT results. Therefore, the two mentioned scaffolds are considered as optimal options for future investigations.

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Authors' Contributions

Conceptualization: MR, EP, MN. Data curation: MN. Investigation: MN, MR, MS. Formal analysis: MN, MR, EP. Methodology: MN. MR. Writing–original draft preparation: MN. Writing–review and editing: MN, MR, MS.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

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