Fabrication and Investigation of Electrospun Poly caprolactone /Gelatin/Egg white Nanofibers for Skin Tissue Engineering

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Abstract

Nowadays, the use of green materials has been expanded for biomedical and bioengineering. Egg white (EW) is a low-cost and abundant candidate for various biomedical applications. In this study, a nanofibrous scaffold based on EW blended with polycaprolactone/gelatin (PCL/Gel) was fabricated using electrospinning. The fabricated samples were characterized using Physicochemical analyses including SEM, FT-IR spectroscopy, tensile assay, and contact angle measurement. The optimized samples were used as scaffolds for cell culture. The average diameter of prepared nanofbers measured 215.9 nm to 434.1 nm. The FT-IR and DSC assays showed the physical blending of EW with PCL/Gel was appropriate and there wasn’t a new chemical reaction between them. The contact angle test indicated the hydrophilicity of the scaffolds was decreased from 26.25º to 116.5º by increasing the EW amount in the PCL/Gel (0%-15%). Furthermore, the electrospun PCL/Gel nanofibrous mat with 10% EW exhibited better bioactivity than other samples with different amounts of EW. Therefore, adding 10% of EW to PCL/Gel nanofibers can improve the efficiency of fibroblast culture.

This research introduced a nanofibrous scaffold for skin tissue engineering containing Gel and EW as low-cost and available materials that can be used for biological applications and also for productions like engineered leathers.

1. Introduction

Polymers are known as synthetic and natural and the natural types are used more than synthetic types for biological usages, due to their special features, including nontoxicity, bioactivity, and biodegradability [1, 2]. These materials obtain from natural sources such as plants and animals and they have been used in a different range of shapes including films, fibers, particles, and hydrogels[3]. There are a lot of biomedical products related to polymers, especially natural polymers. Although they are more important for some advanced usages such as tissue engineering and bioactive delivery systems [4]. But they are often costly or inaccessible, as they usually have difficult purification methods and limited sources [5]. Fortunately, nature itself can solve these problems. Egg white (EW) is an inexpensive and available natural material directly usable for different applications. EW has some proteins such as ovalbumin, ovotransferrin, and lysozyme [6], that can be used for biological activities, especially medical applications, due to their nice bioactivity, antibacterial activity, and biodegradability [7, 8]. Furthermore, they are appropriate for 3D cell culture systems [9]. They can shape into different structures such as fibers, hydrogels, or films[8].

Among the shapes, fibers are an important material with special properties. In particular, nanofibers as one-dimensional nanomaterials show a lot of important characteristics including large surface area, adjustable porosity, flexibility, and suitable mechanical performance [10, 11]. The mentioned features of nanofibers make fibers and nanofibers suitable for a variety of applications, especially healthcare, and biotechnologies, including drug delivery systems, tissue engineering, and wound healing [12, 13].
Nanofibers can be generated in various methods that the electrospinning is more important and known as a simple method to produce polymer nano fibers [14, 15]. The electrospun nanofibrous mats have unique characteristics that are suitable for a wide range of applications especially biotechnology and healthcare [16]. The diameters and morphology of the electrospun fibers can be optimized with the features of the polymer solution and the condition of the electrospinning process [17].

EW can be formed into nanofiber [18]. However, electrospinning of pure EW is difficult, due to its weak molecular structure. pure EW proteins have a spherical molecular structure that reduces their ability to build a structure of nanofiber [19, 20]. In addition, pure EW has limited use as a biomaterial and other advanced materials due to its poor mechanical strength. An effective approach to improve the mechanical properties of natural polymers is blending them with synthetic polymers. Mixing EW with different biopolymers has been proposed to enhance the mechanical properties and ability to form nanofibers [21, 22]. Mleko et al. obtained fiber from mixed cellulose/EW isolate. The results showed that the combination of EW with cellulose increases the mechanical strength and roughness of the fibers [18]. In another study, the effect of EW albumin on the electrospinning of polyethylene oxide solutions under different concentrations and pH conditions was assayed. The results indicated the morphology of the electrospun fibers was mainly influenced by the component of EW albumin [23].

There are some reports of a combination of gelatin (Gel) and EW for medical applications. Babaei et al. produced Gel/EW hydrogels. Then, they removed the Gel component by washing with water. The hydrogels showed a high degree of swelling and water absorbance capacity [21].

Furthermore, Cardenas et al. reported a hydrogel made from EW ovomucin and Gel as an implantable biomaterial for tissue engineering. Results showed different concentrations of Gel and EW ovomucin affected the morphology and resistance of the hydrogels [24]. You et al. blended EW and silk fibroin at different ratios to obtain composite film for biomedical applications. They reported that increasing the amount of silk fibroin increased the breaking strength of the films while increasing the amount of EW increased the flexibility and elasticity of the composite samples. In addition, they reported that the presence of EW in the film enhanced cell viability [25].

In this research, PCL/Gel nanofibers were first optimized, and then the optimized sample was selected for combination with different amounts of EW to make PCL/Gel/EW nanofibrous scaffolds. The morphology and physicochemical properties of nanofibrous scaffolds were investigated. Then, the biological properties of the samples were assayed in order to evaluate the interaction of samples with cells and its effect on cell growth.

2. Materials And Methods

2.1 Materials

PCL (MW = 80000 g/mol) and Gel (type A) were supplied from Sigma Aldrich Co. Also, formic acid and acetic acid (Sigma Aldrich Co) was used as the solvent. Fresh eggs were prepared locally and EW was
separated carefully from the egg yolk to prepare EW powder. Phosphate buffer saline (PBS) solution was purchased from Cyto Matin Gene, Iran.

2.2 Preparation of the PCL/Gel/EW solutions

EW was separated from the egg yolk and homogenized at 3000 rpm. Then, it was poured into a silicone mold and dried at 40°C. The dried EW was powdered and stored in a dry environment at 4°C [25, 26]. At first, three concentrations of 20, 22 and 24% (w/v) of PCL and Gel (70:30, w/w) were prepared using the mixed solvents of acetic acid and formic acid (3:1, v/v) and stirred for 6 h.

To prepare the EW solution, different amounts of EW powder (0, 5, 10 and 15%, w/v) compared to the PCL/Gel were dissolved in the mixed solvent of acetic acid and formic acid (3:1, v/v) for 30 min at ambient condition. Then, PCL and Gel (70:30, w/w) were added into the EW solution at an optimum concentration (22%, w/v) and stirred for 6 h at ambient temperature.

2.3 Electrospinning of nanofibers

For the electrospinning process, each of the solutions was transferred separately into a 1-ml syringe and placed between the clamps of a syringe pump (Pars Nanoris, Iran). The syringe needle was connected to a high-voltage device (Pars Nanoris, Iran). Nanofibers were produced using two different voltages, distance between needle and collector and a feeding rate of 0.25 mL/h to obtain the best conditions for making nanofibrous mats. The conditions of samples and electrospinning have been mentioned in Table 1. The process has been done with the temperature of 25º C and the humidity of 65%.
2.4 Characteristics of the nanofibrous scaffolds

To determine a suitable range of polymer solution concentration and effective factors in the electrospinning process, an optical microscope was used to initially check the uniformity and quality of the electrospun fibers. For this purpose, a part of the fibers produced during electrospinning was collected on a slide and then observed under a light microscope under 400x magnification. After determining the range of polymer concentration and suitable electrospinning conditions, the surface morphology of the nanofibers was observed using a field emission scanning electron microscopy FE-SEM (Philips EM 208S, USA). All nanofibrous mats on the aluminum foil were coated with a 7 nm layer of gold before the scanning. At least 100 fibers of each FE-SEM image were considered to calculate the average diameter of fibers with Digimizer software. In addition, degraded samples were observed with FE-SEM to investigate their morphological changes after soaking in PBS solution.

The IR spectrum of prepared PCL/Gel/EW nanofibers was performed using FT-IR unit (Hartmann and Braun-MB100, Canada) to confirm the presence of EW and investigation of the chemical compositions between PCL, Gel and EW. For this purpose, a small piece of PCL/Gel/EW nanofiber and pure materials were powdered and mixed with KBr separately to make a pill via pressing. Then the spectra were determined over the range of 400–4000 cm\(^{-1}\) at 8 scans per minute. OMINIC software was used for baseline correcting, normalizing and analyzing FT-IR spectrum.
To measure the surface hydrophilicity of the prepared samples, the static water contact angle measurement was investigated using a contact angle measuring equipment DCAT 11 based on ASTM D5964. Initially, a small piece of each nanobrous mat was placed on a smooth surface. Then a 5 µl water droplet was carefully dropped by a micropipette on the surface of the samples at room temperature. The images of the contact angle were taken using a high-resolution digital camera (Canon, Japan) and the contact angle was measured using the Digitizer software [27].

Differential scanning calorimetry (BÀHR-Thermoanalyse GmbH, Germany) study of pure EW, PCL/Gel and PCL/Gel/EW nanobrous mat was performed in the temperature ranging from ambient temperature to 150ºC with rating 10ºC/min and in a nitrogen atmosphere. For this purpose, approximately 0.5 mg of each fully dried sample was poured into aluminum pans and the melting point and crystallization temperature of them were measured from the heating scans [28].

Mechanical properties of the PCL/Gel/EW nanobrous mats including tensile strength, Young’s modulus and elongation at break were determined by a tensile apparatus (Zwick 1446-60, Germany) at room temperature with a 20 N load cell under a cross-head rate of 5 mm/min according to ASTM D638. The thickness of the samples was measured using a micrometer. The size of the samples and the gauge distance were 5 x 30 mm2 and 20 mm, respectively. Each nanobrous mat was measured with three samples. The stress-strain curves were used to determine the mechanical properties of the nanobrous scaffolds [29].

2.5 Cell preparation and biological study

Cell tests were performed to investigate the biological effect of the presence of different amounts of EW (0, 5, 10 and 15% rather than polymer) in the PCL/Gel nanofibers. At first, the samples were cut with a diameter of 10 mm and immersed in ethanol (70% v/v) for 1 hour and then placed under UV light for 8 hours for sterilization. In this study, human epidermal fibroblast was cultured on the sterilized scaffolds in DMEM (Gibco) supplemented with 1% antibiotics (penicillin/streptomycin, Sigma-Aldrich) and 10% (v/v) fetal bovine serum (FBS, Gibco) in T75 cell culture flasks at 37° C in 5% CO₂. The medium of the cells was renewed every three days [30].

The MTT test is a method based on the regeneration and breaking of the yellow MTT tetrazolium crystals by one of the enzymes of the mitochondrial respiratory cycle and the formation of purple crystals insoluble in water. This test is one of the important methods to check the non-toxicity of cell culture scaffolds and at different culture times; one of the advantages of this method, which distinguishes it from other methods, is the elimination of the steps of washing and cell separation, which usually cause the loss of part of the cells.

The non-cytotoxicity test was performed according to the ISO/EN-10993-5 standard (1999). According to this method, 40 µl of 5 mg/ml 3- [4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT, Sigma-Aldrich) solution in PBS solution was added to the cell cultured nanofibrous scaffolds and the negative control sample (cell culture without the presence of nanofibrous scaffold) on the 3th and 7th
days. Then the samples were incubated in a CO$_2$ incubator at 37°C for 4 h. Then, 400 µl of DMSO (Sigma-Aldrich) was added to each well for 5 minutes, and the number of dissolved formazan crystals was read through absorbance measurement at 570 nm by ELISA reader device. The amount of color change in MTT solution is directly related to the amount of growth and proliferation of cells, because the living cells form formazan and it causes the color change in the MTT solution [31].

### 2.6 Cell morphology on the nanofibrous scaffolds

The morphology of the cells on the PCL/Gel/EW scaffolds was assayed using FE-SEM. After seeding the fibroblasts on the scaffolds for 3 days and 7 days, they were immersed in 2.5% glutaraldehyde in PBS as a fixation solution for 45 min and then incubated for 30 min. After that, the samples were dried using a dryer and then the FE-SEM images were taken to investigate the morphology and observe the cells on the scaffolds [32].

### 2.7 Statistical Analysis

All the data in this study were reported as mean ± SD based on at least triplicates for each experiment. One-way analysis of variance was performed between groups for significant differences. All statistical analyses were performed using SPSS statistic software (Release 26.0.0 for Windows) and differences were considered significant in $p < 0.05$.

### 3. Results And Discussions

The properties and characteristics of nanofibrous structures used in various applications are of particular importance. Since part of this research is related to the field of tissue engineering and cell culture, the nanofibrous structures prepared for this purpose must have special conditions and properties; because it will play a significant role in the interaction between cells and the nanofibrous structure as a cell culture substrate. One of the characteristics of nanofibers, which is very important in the discussion of cell culture, is the morphology of the fibers. The morphology of nanofibers and nanofibrous structures consisting of the electrospinning process is influenced by several factors such as factors related to the polymer solution, factors related to the electrospinning process, and also environmental conditions. Since some of these factors, according to the conducted studies, have a greater effect on the properties and morphology of nanofibers, and also because it is not possible to investigate the effect of all the factors involved in the production of nanofibers, only a number of factors with the highest coefficient of influence are investigated and among them the optimal sample is determined.

### 3.1. Morphology of PCL/Gel/EW nanofibers

In this research, a solution of PCL, Gel, and EW was used to make the nanofibrous scaffold for skin tissue engineering. Initially, the optimal PCL/Gel nanofibers were determined and then different amounts of EW were added to it. As previously mentioned, electrospinning of nanofiber can be affected from a variety of factors, including type of raw materials, concentration of solution, solution flow rate, applied voltage, and
distance between nozzle to collector [33]. According to the results of the preliminary tests and determination of the appropriate range of the concentration using optical microscope images, three concentrations of 20, 22 and 24% w/v were selected for the production of PCL/Gel nanofibers.

Other factors can also affect the electrospinning process are applied high voltage and the distance between the nozzle and the collector. Increasing the applied voltage can make the lower diameter of the fibers and vice versa. However, it should be noted that increasing the voltage difference until higher than the permissible limit increases the possibility of producing beaded or not uniform fibers because it causes excessive throwing of the jet. In order to determine the optimal electrical conditions, the applied voltage was considered to be 16 and 18 kV. Furthermore, the distance between the nozzle and the collecting has an effect on the fiber diameter and morphology. This distance should be optimal for the electrospinning process. The distance higher than a certain limit, can make beaded fibers and the distance less than a certain limit, increases the probability of making strip fibers or a film layer on the collector; in this research, distances of 15 and 18 cm were chosen.

Figure 1 indicates the FE-SEM images and diameter distribution of the PCL/Gel nanofibers. It can be seen; the average diameter of nanofibers has increased by increasing the concentration of the solution from 20 to 24% w/v. The concentration of polymer solution is one of the important factors that effect on the diameter and morphology of fibers. This factor affects the viscosity and surface tension of the solution. If the concentration of the electrospinning solution is less than the permissible limit, the electrospun jet separates before reaching the collector plate and form a droplet or bead. On the other hand, if the concentration of the solution is higher than the permissible limit, the fiber is not sufficiently stretched and make thick fibers. For this reason, it is necessary to perform electrospinning of polymer solutions in an optimal concentration range to form fibers with uniformity and suitable diameter [13]. However, there is no significant difference between the average diameter of 20% and 22% PCL/Gel nanofibers; but beaded fibers are observed in 20% PCL/Gel nanofibrous mat and also, the distance of 15 cm is not enough to remove the solvent of the jet and make fused fiber in the intersection points of the fibers. Also, the nanofibers made of the 20% PCL/Gel solution are not enough uniform and it can be seen that 2% increasing in concentration has improved the morphology and uniformity of the fibers. Therefore, the concentration of 22% was chosen as the optimal sample due to the uniformity and an appropriate average diameter. Also, among the electrospinning conditions, a distance of 18 cm and an applied voltage of 16 kV were determined due to less variance of the fiber's diameter.

After that, different amounts of EW were added to the PCL/Gel solution of optimal sample and the PCL/Gel/EW nanofibers were produced using electrospinning. Figure 2 shows the Fe-SEM images, diameter distribution and nanofiber diameter diagram of the PCL/Gel/EW nanofibers. The addition of EW into the PCL/Gel has increased the average diameter of the fibers compared to the sample without EW from 215.9 nm to 434.1 nm. And with the increase of EW concentration, the average diameter of the fibers became $231.7 \pm 50.5$, $276.2 \pm 67.9$ and $434.110 \pm 8.4$, respectively [20, 34]. In a similar study, Kuppan et al. [35], reported that the pretense and amount of Gel in PCL/Gel solution has direct effect on average diameter of the electrospun nanofibers and increasing the amount of Gel has increased the fiber
diameters. Since EW and Gel are similar in terms of chemical structure and both of them have amino acids, they have a similar behavior in the electrospinning process and effecting in diameter changes. So, increase of the EW concentration increases the average diameter of nanofibers.

3.2 Physico-chemical properties of the PCL/Gel/EW nanofibers

The presence of EW within the nanofibrous scaffolds was investigated by FT-IR (Fig. 3 (A)). It shows the PCL and Gel are partially similar to each other, due to their similar chemical bonds. According to the IR spectra and the studies of other similar works, the typical peaks were at 2945 and 2867 cm\(^{-1}\) related to the asymmetric and symmetric stretching of CH\(_2\) bonds. Further peaks were at 1730 and 1295 cm\(^{-1}\) related to carbonyl stretching and both of C-O and C-C stretching, respectively [36]. As well as, peaks were at 1240 and 1170 cm\(^{-1}\) related to symmetric stretching are common in both of the polymers.

The absorption spectrum in the peaks of 1200 to 1266 cm\(^{-1}\), which indicates the bending of C-H, O-H and CH\(_2\) bonds, confirms the presence of PCL and Gel [37, 38]. It is also possible to see the presence of NH\(_2\) amide groups in the IR spectrum related to Gel. Peaks at 3443, 1650, and 1540 cm\(^{-1}\) correspond to NH amide bond, amide I and amide II (N-H), respectively, confirm the presence of Gel in the nanofiber structure [27, 39]. The peaks of 1651, 1542 and 1240 cm\(^{-1}\) were observed at the IR spectra of, which corresponds to the stretching vibration of C = O, amid II and amid III, respectively [40]. According to the IR spectra of the PCL/Gel/EW nanofibers, it can be seen that no new chemical reactions were created after mixing the three substances.

The hydrophilicity of the nanofibrous mats was investigated by the contact angle method and the results are shown in Fig. 3 (B-E). This cost-effective method is useful to assay the effect of additives on wettability properties. Hydrophilicity of nanofibrous scaffolds is one of the important properties of nanofibers that use as biomaterial. The addition of EW to the PCL/Gel nanofibers resulted an increasing contact angle compared to the PCL/Gel nanofibers without EW. The contact angle values were 26.25 ± 7º, 83.5 ± 2º, 100.5 ± 6º, and 116.5 ± 4º for the PCL/Gel/EW nanofibers containing 0, 5, 10 and 15% EW, respectively. In fact, the hydrophilicity of the nanofibers has been decreased with increasing the amount of EW. EW proteins contain both hydrophilic and hydrophobic amino acids. In general, the proteins that make up the EW are aggregated and have a spherical appearance. Hydrophobic amino acids are mainly located in the center of the Spherical structure, and hydrophilic amino acids accumulate on the surface of the protein and are surrounded by water molecules. However, the bonds between the chains are relatively weak and break easily [41]. PCL/Gel nanofiber has a high hydrophilicity due to the presence of Gel in its structure. By adding EW to the PCL/Gel nanofibers, it seems that the hydrophilic amino acids of the EW join to the hydrophilic groups of Gel and the hydrophobic amino acids have a greater effect on the nanofiber surface. Therefore, the presence of EW in the PCL/Gel nanofibers and increasing the amount of that, the water contact angle of the blended nanofibers is increased, which means a decrease in the surface hydrophilicity of the samples.
There are some studies with similar results. Lu et al. [40], made the electrospun polyvinyl alcohol/EW nanofibers with different amount of EW for wound dressings. Their results of the water contact angle indicated that the water contact angle increased with the increasing the amount of EW into the nanofibers, and it's related to the binding of hydrophilic amino acids of EW to the hydrophilic functional groups of polyvinyl alcohol.

Furthermore, the contact angle results of Unalan et al. [30], showed that the surface wettability of PCL/Gel nanofibers was decreased with the increase in the amount of an additive such as clove essential oil, which might be due to the loss of free functional groups on the Gel. But in another study, Renkler et al. [42], prepared PCL nanofibers containing EW and the results of hydrophilicity analysis showed that the water contact angle of the PCL nanofiber decreased from 90º to 70º with addition of EW, which means an increasing of hydrophilicity of PCL/EW nanofibers. The result is related to the absence of hydrophilic chemical groups in PCL, and the hydrophilic amino acids of EW have more access to water molecules; Therefore, the surface hydrophilicity of the PCL/EW nanofiber is higher than pure PCL nanofiber.

It should be mentioned that the reduction of surface hydrophilicity of the nanofibers using for cell culture samples should not reach a level that causes disruption of cell adhesion and as a result decrease of their growth and proliferation. Therefore, it seems that the amount of adding EW into the PCL/Gel nanofiber structure has a limit and an optimal point.

DSC analysis was performed to prove the presence of EW in the structure of PCL/Gel nanofibers and to investigate possible new chemical reactions. Figure 3 (F) shows the DSC diagrams of EW, PCL/Gel nanofibers, and PCL/Gel/EW. As it is clear in the figure, the DSC diagrams of EW and PCL/Gel nanofibers have endothermic peaks around 41 and 67°C, respectively. it can be seen that both peaks are present in the DSC diagram of PCL/Gel/EW. It is also observed that the intensity of the peaks in both diagrams of EW and PCL/Gel nanofiber is higher than the peak intensity in nanofibers made off all three materials. In fact, the crystallinity of the nanofibers decreased with the addition of EW to the PCL/Gel nanofiber.

The nano fibrous scaffold should be biodegradable to allow for successful skin regeneration. The FE-SEM images of the nanofibers with different amounts of EW at 3, 7 and 21 days (Fig. 4) after degradation showed that the surface of nanofibers became rough. The structure of the nanofibers without EW has been preserved in 7 days after destruction, but in 21 days, some parts have changed in the fibrous structure. The intensity of the nanofibrous degradation shows a significant increase with increasing the amount of EW into the PCL/Gel. It reveals that fibrous structure of the PCL/Gel containing 15% EW has completely disappeared on the 21st day. Also, the degradation and deformation of the nanofibers with a larger diameter has been slower than the finer nanofibers. Therefore, it is expected that nanofibers with a wider diameter distribution create more porosity in their structure during the degradation time, and this parameter can help to the process of cell culture.

According to the degradation images, it is clear that the sample containing 10% EW on the 7th and 21th days still has thick fibers, due to the larger diameter distribution than the other samples. For this reason, it
is expected that the PCL/Gel nanofibers containing 10% EW is more effective than other samples in terms of the presence of more porosity due to degradation.

Different factors including of substance, morphology, porosity and structure of fibers can affect on the mechanical properties of fibrous scaffolds. Figure 5 (A-C) shows the stress-strain curve, elongation at break, tensile strength and Young’s Modulus of the PCL/Gel/EW nanofibrous scaffolds, respectively. All the curves represented an initial linear elastic behavior and it can be used to calculate the Young’s Modulus of each sample. The Young’s Modulus, elongation at break and tensile strength for PCL/Gel nanofibrous mat without EW are 0.57 ± 0.06 MPa, 18.9 ± 1.6% and 3.8 ± 0.65 MPa, respectively. Furthermore, it can be seen that the Young’s Modulus, elongation at break and tensile strength of the nanofibrous scaffolds decreased gradually with the increase of EW content. The tensile strength of the PCL/Gel nanofibers with 5, 10 and 15% of EW is 2.44.57 ± 0.59, 1.98 ± 0.69 and 1 ± 0.28 MPa, and the elongation at break of the samples with 5, 10 and 15% of EW is 18.8 ± 4.2, 13.4 ± 2.41 and 10.7 ± 3.67%, respectively. These results showed the flexibility of nanofibers with higher amounts of EW could be attributed to the presence of globular protein of EW in the structure. Crystallinity is another parameter that has an important effect on mechanical properties. As the DSC test shows the crystallinity of the PCL/Gel nanofibers has been decreased with increasing the EW content, it can be considered another reason for decreasing of mechanical strength of the samples with increasing the EW contents [25, 32].

3.3. Investigation of biocompatibility

To study the biological activities of the prepared PCL/Gel/EW nanofibers, fibroblast cells were cultured on the nanofibers for 3 and 7 days. Figure 6 shows the absorbance at 570 nm for cell cultured nanofibers compared to the control sample (cell culture without the nanofibrous scaffolds). It is observed that the amount of absorption on the 3th and 7th days for all the nanofibrous scaffolds is higher than the control samples. It means that nanofibrous scaffolds are non-toxic for cells and also have positive effects on cell growth.

Cell growing of the PCL/Gel containing 5% EW is slightly reduced compared to the PCL/Gel without EW, but this difference is not significant at the 5% significance level. While the results of cell growing on the PCL/Gel contains 10% EW, are significantly better than the scaffold contains 5% EW and this change is more noticeable after 7 days culturing. On the other hand, the results show that increasing the EW from 10 to 15% in PCL/Gel scaffold reduced the cell growth even less than PCL/Gel without EW [42, 43].

3.4 Cell morphology on PCL/Gel/EW nanofibrous mats

Cell adhesion and morphology of cells grown on the scaffolds were investigated using FE-SEM images. Figure 7 shows the FE-SEM images of the fibroblast cultured on the PCL/Gel nanofibers containing different amounts of EW, after 3 and 7 days. It can be seen that three days after cultivation, cells are well attached to the surface of nanofibers. Also, after seven days, the cells have grown well on the nanofibers and penetrated into the structure of the fibers, especially into the pores of the mats. On the other hand, it
can be seen that the adhesion and penetration of cells inside the PCL/Gel/EW nanofibers is better than the sample without EW. Accordingly, the in vitro cell culture and morphology results indicated that PCL/Gel nanofiber with different amounts of EW are biocompatible.

4. Conclusion

In summary, the bio-based scaffold was made via incorporation of PCL, Gel and EW to make nano fibrous mat to develop skin tissues. The prepared nanofibers contain different amount of EW had a largest diameter (434.1 nm) rather than the nanofibers without EW (215.9 nm). The presence of PCL, Gel and EW was proved using FT-IR and DCS. The hydrophilicity of scaffolds was decreased with increasing the amount of EW. The PCL/Gel/15% EW scaffolds indicated enhanced mechanical strength compared with PCL/Gel scaffolds without EW. Furthermore, the results of cell growing on the PCL/Gel contains 10% EW, are significantly better than the PCL/Gel scaffold with other amounts of EW. Hence, the PCL/Gel/10% EW nanofibrous scaffold showed great potential for skin tissues engineering.

Declarations

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability Statement

Not applicable.

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References


Figures
Figure 1

FE-SEM images display the morphology and fiber diameter of PCL/Gel 7:3 nanofibres with concentration of 20% (N1-N4), 22% (N5-N8) and 24% (N9-N12) and electrospinning conditions according to the table 1.
Figure 2

FE-SEM images of PCL/Gel nanofibres with (A) 5%, (B) 10%, (C) 15% EW and (D) diagram of average diameter of PCL/Gel/EW nanofibres.
Figure 3

(A) FT-IR spectra of EW, Gel, PCL and PCL/Gel/EW nanofibers. (B-E) Water contact angle of PCL/Gel nanofibers with 0, 5, 10 and 15% EW, respectively. (F) DSC thermogram of EW, PCL/Gel and PCL/Gel/EW.
Figure 4

FES-EM images of PCL/Gel nanofibers with different amounts of EW at 3, 7 and 21 days after degradation.
Figure 5

Stress-strain curve, (B) Elongation at break and (C) Tensile strength and Young's Modulus of the PCL/Gel/EW scaffolds.
Figure 6

The absorbance percentage at 570 nm in the scaffolds in day 3, and 7 after seeding.
Figure 7

FE-SEM micrographs of the scaffolds in days 3 and 7 after seeding fibroblast.