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Production of Polymeric Nanofiber Membranes Containing Plant Extract and Investigation of Their Potential for Use on Wound Healing

Bitki Ekstraktı İçeren Polimerik Nanofiber Membranların Üretimi ve Yara İyileşmesi İçin Kullanım Potansiyellerinin Araştırılması

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ABSTRACT

In cases of skin tissue injury, such as severe burns, skin tissue layers can be extensively damaged. Various dressings have been developed to address different types of wounds, with functional polymeric dressings being the most popular. These advanced dressings are designed to accelerate wound healing. Incorporating plant-derived extracts and biological molecules into wound dressing materials is common. This study aimed to develop an electrospun nanofiber wound dressing by incorporating active ingredients extracted from the plant *Echium italicum* (Italian viper's bugloss), known for its efficacy in burn wound healing, into poly (lactic-co-glycolic acid) (PLGA). The nanofiber membrane wound dressings produced by electrospinning were subjected to various analyses. Morphological and structural characterization revealed that under physiological conditions, the membranes exhibit significant morphological decomposition and weight loss after 90 days of vitro degradation. *In vitro* cytotoxicity testing using the MEM extraction method demonstrated that the membranes were not cytotoxic. Based on the comprehensive analysis, it was concluded that the developed nanofiber membranes hold promise as potential wound dressings for the treatment of severe burn wounds.

Key Words

Polymeric wound dressing, *Echium italicum*, poly (lactic-co-glycolic acid), electrospinning.

ÖZ

Cilt dokusunda ciddi yanıklar gibi yaralanmalar olduğunda, deri katmanları büyük ölçüde hasar görebilir. Farklı yara tiplerini ele almak için çeşitli yara örtüleri geliştirilmiştir ve bunların arasında en popüler olanı fonksiyonel polimerik yara örtüleridir. Bitkisel özlerin ve biyolojik moleküllerin yara örtü malzemelerine dahil edilmesi yaygın bir uygulamadır. Bu çalışma, yanık yarası iyileşmesinde etkinliği bilinen *Echium italicum* bitkisinden elde edilen aktif bileşenler ile poli (laktik-ko-glikolik asit) (PLGA) polimeri blend edilerek elektroeğirme yoluyla üretilen nanofiber yara örtü malzemesi geliştirmeyi amaçlamaktadır. Elektroeğirme yoluyla üretilen nanofiber membranlı yara örtü malzemeleri çeşitli analizlere tabi tutulmuştur. Morfolojik ve yapısal karakterizasyon, membranların fizyolojik koşullar altında 90 günlük *in vitro* bozunma sonrasında önemli morfolojik ayrışma ve ağırlık kaybı sergilediğini ortaya koymuştur. MEM ekstraksiyon yöntemi kullanılarak yürütülen *in vitro* sitotoksisite testi, membranların sitotoksik olmadığını göstermiştir. Kapsamlı analize dayanarak, geliştirilen nanofiber membranların ciddi yanık yaralarının tedavisi için potansiyel yara örtü malzemeleri olarak umut vadettiği sonucuna varılmıştır.

Anahtar Kelimeler

Polimerik yara örtüsü, *Echium italicum*, poli (laktik-ko-glikolik asit), elektroeğirme.

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INTRODUCTION

The skin is the body's largest organ, and it mainly comprises the epidermis and dermis layers covering the body and in direct contact with the external environment [1]. With the disruption of skin integrity, scars are formed in the skin tissue. A wound is a disruption of tissue integrity, can occur on visible parts of the body as well as internal organs and tissues, and can be temporary or permanent. The breakdown of skin tissue integrity can result from injury or burns [2]. The most common burn wound types are first-, second-, third- and fourth-degree burns, depending on the duration of the heat exposure [3]. First-degree burns are commonly referred to as superficial burns, in which only the epidermis layer is damaged. Symptoms of burning include itching and dryness of the skin. Second-degree burns cause damage to both the epidermis and the dermis layers. Swelling (edema) can be observed in these burns. Third-degree burns destroy the epidermis and dermis layers. These burns are the most difficult to heal due to damage to nerve endings. Fourth-degree burns extend through the skin layers into the underlying tissues, possibly reaching the bone, and require surgical intervention.

Wound healing is the process by which tissue functionality is restored using various cells to repair damaged tissues. Wound healing involves the stages of hemostasis, inflammation, proliferation, and remodeling. During this healing process, various cell types, growth factors, chemokines, and proteins play roles in functional and efficient tissue regeneration [4].

Two types of dressings have been developed for the treatment of wounds during wound healing: traditional and modern. The most well-known and widely used traditional dressing is gauze. However, gauze and similar wound dressings slow down the wound healing process due to reasons such as not being able to provide a moist environment and not being protective against infection. As a solution to this, modern wound dressing materials have been developed [5]. Examples of modern dressings are hydrogel, foam, hydrocolloid, and nanofiber dressings. Nanofiber wound dressings are obtained by the electrospinning method, and since the fibrous structure formed resembles the structure of the natural extracellular matrix, it provides a favorable environment for the adhesion, development, and proliferation of cells during the wound healing phases [4].

Nanofiber dressings can be produced using natural or synthetic polymers by electrospinning method. Wound dressings made of nanofibers obtained by electrospinning have advantages over conventional wound dressings (i.e., accelerating the bleeding stopping (hemostasis), high absorption capacity and gas permeability) [6]. For tissue damage repair, the most suitable material should support the conditions necessary for cells to repair and proliferate. Fiber structures are chosen as the most appropriate materials due to their structural similarity to the extracellular matrix (ECM), high porosity, and interconnected pores. The electrospinning method is widely used to produce these fibrous structures. PLGA (poly (lactic-co-glycolic acid)), a synthetic polymer used to produce wound dressing materials via the electrospinning method, is a thermoplastic copolymer consisting of lactic acid (LA) and glycolic acid (GA) monomers [4,7]. PLGA exhibits an amorphous structure with a glass transition temperature ranging from 45°C to 55°C. It is soluble in solvents such as dimethylformamide, dichloromethane, and chloroform. PLGA finds broad applications in the biomedical field, particularly in drug delivery systems, surgical implants, and tissue scaffolds. It is biocompatible, biodegradable, mechanically robust, and easily electrospinnable. The extensive use of PLGA has been supported by FDA for clinical studies [8]. *Echium italicum* is a plant species in the Boraginaceae (Borage) family [9]. Owing to metabolites such as shikonin and alkannin, which are abundant in their roots and impart a red color, this plant has been used for wound healing from ancient times to the present day. The extraction of its active components by treating the roots with beeswax and certain oils has been ongoing for many years. *E. italicum* exhibits antioxidant, anti-inflammatory, antiproliferative, antidepressant, antiviral, antibacterial, and sebum-regulating properties. When used in wound healing, it promotes cell growth, proliferation, migration, and tissue granulation, and enhances epithelialization. It increases the mechanical elongation of wounds and imparts flexibility [10].

This study aimed to develop an easy-to-use and effective wound dressing for wound healing by extracting active compounds from *E. italicum* (viper's bugloss) into biodegradable poly (lactic-co-glycolic acid) (PLGA) copolymer. The characteristics of wound dressings based on electrospun nanofiber membrane structures and their mechanical properties were investigated. Furthermore, *in vitro* degradation and cytotoxicity tests of the membranes were conducted.

MATERIALS and METHODS

Materials

PLGA (PURASORB PLG 8523 L-lactide/Glycolide 85/15 molar ratio, 2.3 dl/g), was obtained from the Corbion N.V. company. *E. italicum* was collected in Yalova. For the determination of antioxidant compounds, gallic acid (Sigma Aldrich), Folin-Ciocalteu reagent (Merck), methanol (Tekkim), ethanol (Tekkim), $NaCO_3$ (Merck), $AlCl_3$ (Sigma Aldrich), Quercetin (Sigma Aldrich), NaOH (Sigma Aldrich) and $NaNO_2$ (Sigma Aldrich) were used. Chloroform and dimethylformamide (DMF) used as solvents were obtained from Sigma Aldrich (USA). For *in vitro* cytotoxicity studies, an MTS analysis kit was used (CellTiter 96® AQueous One Solution Cell Profiberation Assay, Promega). Dulbecco's Modified Eagle Medium (DMEM, high glucose), Fatal Bovine Serum (FBS), and Penicillin-Streptomycin antibiotic were purchased from Biological Industries (Israel). All other materials and solutions were used as received and stated unless otherwise stated. As shown in Table 1., the abbreviations of the produced nanofiber materials were used.

Extraction of Active Ingredients from *E. italicum* (EI) Plant

The root of the plant was collected in August – September; cut and dried with sunlight for 24 hours and ground into powder using a cylindrical shredder. The weighed powder plant root was transferred to a beaker and 55 gr of oil was added and left at room temperature for 1 hour to obtain the active ingredients. The resulting mixture was filtered with filter paper taken into a beaker and stored at 4°C.

Determination of Antioxidant Compound in *E. italicum* (EI) Plant

First, the plant powder was mixed with 50 ml of 96% ethanol and macerated for 48 hours. The filtered supernatant (by Whatman filter paper) was placed in a petri dish and dried in an oven at 37°C for 24 hours then stored at 4°C. To determine the total amount of phenolic compounds, the methodology described by Eruygur et al. was followed [11]. According to this method, total phenolic content was determined using the Folin-Ciocalteu reagent. 50 mg of the plant extract was mixed with 5 ml of methanol in a falcon tube and subjected to ultrasonic treatment for 10 minutes at 40°C. The sample was then centrifuged at 1000x g for 10 min then the supernatant was taken and stored at 4°C [12]. Gallic acid was used as a standard for constructing the calibration curve. The stock standard solution was prepared at a concentration of 10 mg/ml using methanol. From this stock solution, standard solutions of various concentrations (0.5 to 0.01 mg/ml) were prepared. Aliquots of 25 µl of both the extract and gallic acid solutions were

Table 1. The Nomenclature of the materials.

The Names of Materials	The Abbreviations of the Materials
PLGA nanofiber membrane (Control group)	PLGA
PLGA nanofiber membrane containing %10 (w/v) EIE	PLGA/EIE10
PLGA nanofiber membrane containing %12 (w/v) EIE	PLGA/EIE12
PLGA nanofiber membrane containing %15 (w/v) EIE	PLGA/EIE15
PLGA nanofiber membrane containing %20 (w/v) EIE	PLGA/EIE20

Table 2. Electrospinning parameters of PLGA solutions at different EIE concentrations.

	PLGA	PLGA/EIE10	PLGA/EIE12	PLGA/EIE15	PLGA/EIE20
Flow rate (ml/h)	0.8	0.75	0.75	0.75	0.75
Distance between injector tip and collector plate (cm)	15	17	17	17	18
Voltage (kV)	13	13	13.5	13	13

mixed with 100 µl of Folin reagent and allowed to react for 5 minutes. Subsequently, 80 µl of an 8% (w/v) $NaCO_3$ solution was added, and the mixture was incubated in the dark at room temperature for 90 minutes. After the incubation period, 100 µl of each sample was transferred to a 96-well microplate, and absorbance readings were recorded using a microplate reader (Biochrom EZ Read 400, UK) at 760 nm.

For the determination of total flavonoid content, the $AlCl_3$ colorimetric method was employed with quercetin as the reference standard. Standard solutions of quercetin, with concentrations ranging from 1 to 0.01 mg/ml, were prepared from a 5mg/ml stock solution of quercetin. For the plant extract, 25 mg was mixed with 5 ml of methanol in a Falcon tube and subjected to ultrasonic treatment at 40°C for 10 minutes. Following sonication, the sample was centrifuged at 1000 × g for 10 minutes, and the supernatant was transferred to a new tube. To establish the standard curve, 500 µl of distilled water was combined with 100 µl of the sample. Subsequently, 100 µl of 5% $NaNO_2$ solution was added to each tube and allowed to react for 6 minutes. After this period, 150 µl of a 10% $AlCl_3$ solution and 200 µl of 5 M NaOH solution were added, and the mixture was incubated at room temperature for 5 minutes. The absorbance values of the samples were measured at 492 nm using a microplate reader.

Production of Polymeric Nanofiber Membranes Containing *E. italicum* Extract (EIE)

E. italicum extract (EIE) and PLGA polymer solutions were prepared at concentrations of 10%, 12%, 15%, and 20% (w/v). Additionally, a 10% (w/v) PLGA solution was prepared as a control. A chloroform/DMF (4:1) mixture was used as the solvent. The solution was stirred overnight at room temperature at 120 rpm using a magnetic stirrer. Electrospinning was conducted using an assembly comprising an OptoSense (Orlando, Florida) high-voltage source, a syringe pump (New Era Pump Systems, Inc., New York), and an aluminum collector plate. All electrospinning experiments were performed at room temperature, with variations in the parameters of voltage (kV), solution flow rate (ml/h), and the distance between the injector tip and the collector plate (cm). The optimal values for these parameters were determined, and nanofiber membranes were produced. As detailed in Table 2, the parameters varied for each concentration group.

Characterization of Membranes

Scanning Electron Microscopy (SEM, FEI Inc., Inspect S50, EDAX Inc., Octane Prime) was used to determine the surface properties, fiber morphology, and diameter of the nanofiber membranes. After the samples were covered with a thin gold layer, they were examined using an electron beam accelerated to 15 kV.

To determine the *in vitro* degradation rate of the nanofiber membranes, the samples were cut into 2x2 cm and weighed. Then, to prevent contamination in sterile falcon tubes, they were immersed in phosphate-buffered solution (PBS, pH=7.4) containing 2% sodium azide (NaN_3) and incubated in an oven at 37°C. Samples were removed from the solution at regular time intervals (7, 14, 30, 60, 90 days), washed with ultrapure water and dried at 37°C for 24 hours and weighed again. The percentage weight loss was calculated based on the initial and final weight of each sample using the following formula:

(Wi-Wf)/(Wi) x100
(Wi: Initial weight, Wf: Final weight)

To detect the presence of EIE in the membrane, 1x1 cm sized samples were analyzed with FTIR-ATR (Perkin Elmer, Spectrum 100, USA) in the wavelength range of 4000-650cm^{-1}.

The tensile strength and % elongation values of the membranes were measured using a universal tester (Zwick/Roell, Germany). Test samples were prepared by cutting 1x6 cm. The test was carried out at a constant tensile speed of 200 mm/min using a 10kN force under atmospheric conditions with 50% relative humidity at 23°C. Five different measurements (n=5) were performed for each sample type and their mean values; standard deviations were reported.

Thermogravimetric differential thermal analysis (Thermal Gravimetric Analysis/Dynamic Thermal Analysis (TG/DTA) System (Seiko, TG/DTA 6300)) was performed to determine the thermal properties of the PLGA/EIE membrane and to determine the amount of EIE and PLGA contained in the membrane. Analysis was carried out in a nitrogen environment with a temperature range of 29-500°C and a heating rate of 10°C/min.

Table 3. Cytotoxic response index.

Cytotoxic Response	Reactivity	Pass/Fail
0-1	None	Pass
1-3	Slight	Pass
3-5	Mild	Retest
5-7	Moderate	Fail
7-8	Severe	Fail

The water contact angle was measured to analyze wettability, hydrophilic, and hydrophobic properties of nanofiber membranes. Measurements were carried out at room temperature using deionized water. Membranes were cut in the form of 1x1 cm and placed on a moving layer. Deionized water was dropped on them using a microsyringe. The contact angle of the water droplet was recorded using a microcamera.

The cytotoxicity index of the membranes produced at this stage of the study was determined using the MEM-extraction test according to ISO 10993/EN 30993. Latex rubber and polystyrene (PS) were used as positive and negative controls, respectively. For the test, each membrane was prepared at an extraction rate of 6 cm^2/ml and sterilized under UV light for 20 minutes. Sterilized samples were dipped in DMEM containing 10% FBS and 1% penicillin/streptomycin solution. Next, the cells were incubated in a 37°C water bath with constant shaking speed for 24 h. At the end of the period, the extraction fluids were sterilized with the aid of a 0.20 µm pore diameter filter.

L929 mouse fibroblast cells (ATCC®, CCL-1™) were incubated at 37°C, 5% CO_2, and 90% humidity to create a half-full layer. After 24 hours of incubation, the culture medium was aspirated, and sample extracts were added to each well. Cells were cultured with extraction solution for 24, 48, and 72 h. After 24 and 48 h, the percentage of confluency, percentage of floating cells, and change in cellular morphology were evaluated under an optical microscope and scores were recorded.

The reduction in growth rates after 72 hours of incubation was determined with the MTS analysis kit. FBS-free DMEM and MTS reagent (5:1 ratio) were added to each well and incubated at 37°C for 3 h in a 5% CO_2 incubator. The absorbances of the samples at 490 nm were then recorded using a microplate reader (Biochrom EZ Read 400, UK). Scores from the microscopic evaluation were corrected for the negative control and mean. Percent growth inhibition was determined by MTS analysis and scored after correcting for the value of the negative control. The final cytotoxic response was calculated by adding these two scores and the samples were evaluated according to Table 3.

RESULTS and DISCUSSION

Determination of Antioxidant Compound in *Echium italicum* (EI) Plant

To determine the total amount of phenolic compounds, a calibration curve was drawn using the absorbance values of solutions containing different concentrations of gallic acid, which was selected as the standard. The equation of the calibration curve was determined as $y = 1.9775x + 0.0040$ and $R^2 = 0.9957$ (Figure 1). The total phenolic compound amount of the sample was measured at the same wavelength and calculated as mg gallic acid equivalent (GAE) / g extract ± standard error using this calibration equation.

The total amount of flavonoid compounds was determined by the $AlCl_3$ colorimetric method. The quercetin calibration curve was drawn using the absorbance and concentration values obtained from the microplate reader. The equation of the calibration curve was determined as $y= 0.2644x + 0.0315$, $R^2= 0.9704$ (Figure 1). The total amount of flavonoid compounds was calculated as mg quercetin equivalent/g extract ± standard error using this calibration equation. The total amounts of flavonoid and phenolic compounds obtained using the equations are presented in Table 4. It has been shown that the values we obtained are compatible with the values in the literature [11].

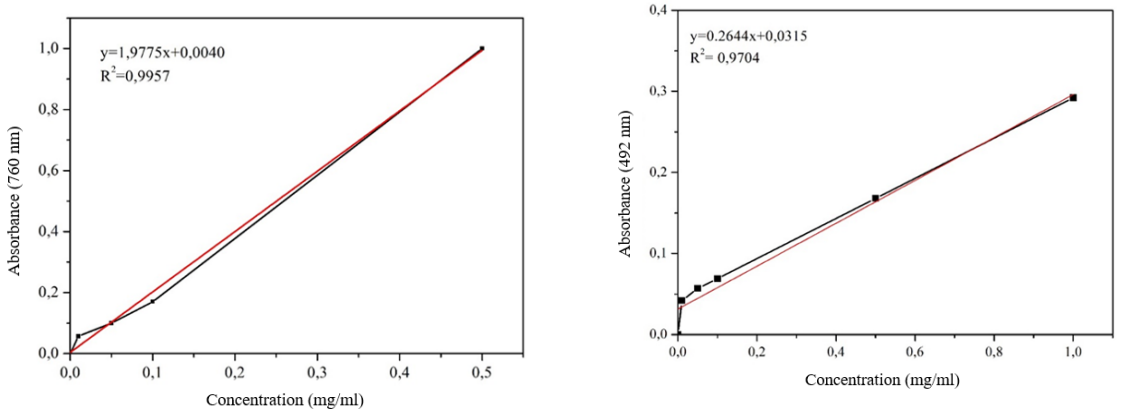

Figure 1. Standard calibration curves of gallic acid (left) and quercetin (right).

Table 4. The total amounts of flavonoid and phenolic compounds in *E. italicum* extract.

Amount of Total Phenolic Compound	Amount of Total Flavonoid
15.9 ± 0.15 mg GAE/gr extract	60.8 ± 0.043 mg quercetin /gr extract

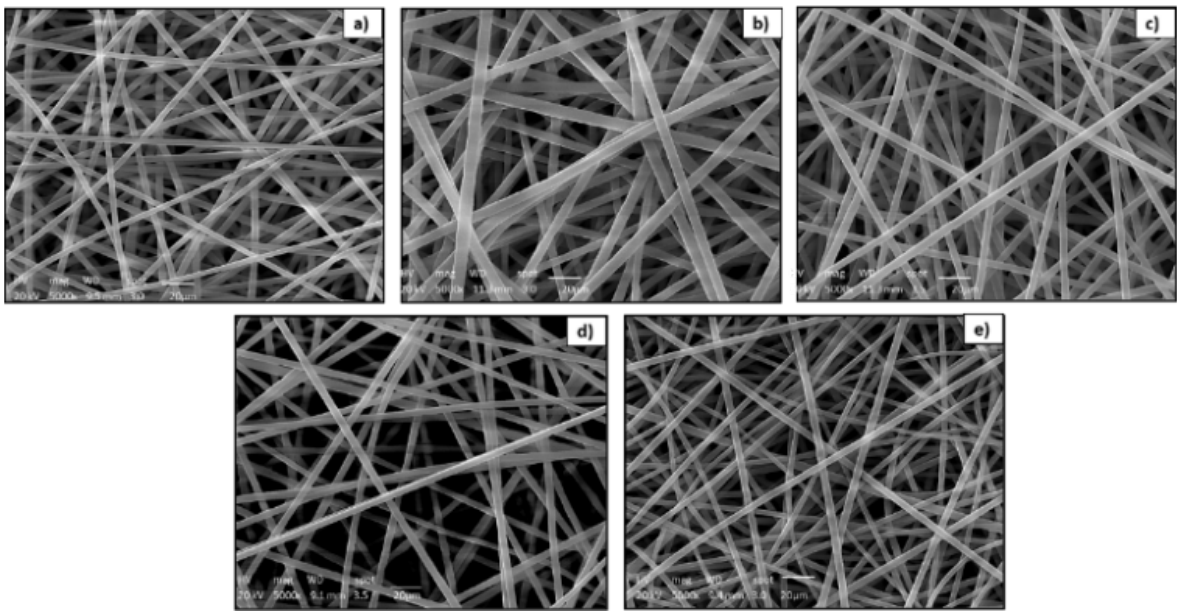

Figure 2. SEM images of a) PLGA, b) PLGA/EIE10, c) PLGA/EIE12, d) PLGA/EIE15, e) PLGA/EIE20 membrane (5000x).

Morphological Evaluation

According to SEM analysis, the morphological properties of membranes were similar for each concentration (Figure 2). All observed membranes were continuous, beadless, and homogeneous. Thus, it can be concluded that the tuned parameters are well optimized for each concentration.

The average diameters of nanofibers are listed in Table 5. According to the obtained data, the highest diameter was observed for a PLGA/EIE20 membrane ratio of 1878 nm, whereas the diameter of the membrane produced using PLGA was measured as 910 nm. The diameters of the nanofibers increased with increasing solution concentration. In this case, it was determined that there was a direct proportionality between the amount of EIE in the membranes and the diameter of the nanofibers. If the solution concentration increases, the viscosity and the diameter of the fibers increases [13]. In addition, the diameter of the fibers increased with the amount of extraction of EIE. For this reason, the PLGA/EIE10 membrane was selected as the most suitable sample.

in vitro Biodegradation Studies

The degradation rate of the PLGA polymer varies according to the Poly (lactic acid)/ Poly (glycolic acid) (PLA/PGA) ratio in its content. *in vitro* biodegradation studies show that the ester bonds in the structure of PLGA are hydrolyzed with water over time and the bonds break and the Mn and Mw values of the polymer begin to decrease. PLGA polymer with a 50:50 PLA/PGA ratio degrades in 1-2 months, 75/25 PLGA in 4-5 months, and 85/15 PLGA in 5-6 months [14].

In this experiment, the prepared membranes were immersed in PBS, and washed with distilled water after 7, 14, 30, 60, and 90 days, they were dried in a drying oven at 37 ℃ for 24 hours, and they were weighed again to determine whether there was any weight loss, and the percentage of weight loss was calculated.

According to biodegradation studies, the % reduction in membrane weight after 7, 14, 30, 60, and 90 days were recorded. No weight loss was observed in the samples at the end of the 7th and 14th days. At the end of the 30th day, although decomposition started in the PLGA and PLGA/EIE10 samples, no weight loss was observed in the other three samples. At the end of 60 days, weight loss of PLGA, PLGA/EIE10, PLGA/EIE12, PLGA/EIE15, and PLGA/EIE20 were 4.04, 3.88, 1.2, 1.3 and 1.38, respectively.

Based on these data, the EIE content first increased and then slowed down the degradation of the PLGA/EIE10 sample. It was thought that the animal fat content in the EIE, and therefore the ester bonds and long hydrocarbon chains, might influence the degradation slow down. When the amount of ester bonds increased, water molecules broke these bonds, and the degradation time increased. At the end of the 90th day, weight loss was 4.056 % in the PLGA sample, 5.49% in the PLGA/EIE10 sample, 4.08% in the PLGA/EIE12 sample, 4.34% in the PLGA/EIE15 sample, and 1.91% in the PLGA/EIE20 sample. With increasing EIE content, the degradation rate increased, whereas a decrease was observed in the PLGA/EIE20 sample. According to weight loss, the optimum EIE ratio in the membranes was 10% (Figure 3).

Figure 4 and 5 show SEM images of the fabricated membranes after 60 and 90 days of *in vitro* biodegradation testing. Decomposition occurs as water molecules in the PBS solution hydrolyze ester bonds within the membrane structure. Structural changes were observed in all fibers. The fibers reached a breaking point at which fragmentation and breakage occurred at various locations, and an increase in surface roughness was noted. Concurrently, the fibers exhibited thinning, and some areas became flattened. Membranes containing only PLGA exhibited the most significant degradation by day 60. In samples analyzed after 90 days, the membrane containing PLGA/EIE10 demonstrated the highest degree of degradation.

FTIR Analysis

The results of the FTIR-ATR analysis performed to determine the chemical structure of nanofiber surfaces are shown in Figure 6. According to the FTIR spectrum, characteristic bands of PLGA were observed as the C=O bond stretch band at approximately 1752 cm^{-1} and the C–O–C stretch band at 1090 cm^{-1} as strong characteristic absorption bands [15]. The peak seen at 1088 cm^{-1} represented the C-O stretch band. The presence of stretching of the C-H bond was observed in the 2944 cm^{-1} band [16].

Since EIE has fatty acids such as alpha-linolenic acid (ALA), stearidonic acid (SDA), linoleic acid (LA), gamma-linolenic acid (GLA), bands observed at the extract spectrum between 2800 and 3000 cm^{-1} can be attributed to fatty acids [15]. Therefore, it is seen that the intensity of these bands increases with increasing EIE content. In addition, EIE contains shikonin and alkanine as secondary metabolites, which have characteristic bands at the same wavelengths as PLGA. Therefore, the above-mentioned C=O and C–O–C tensile bands were also seen in the FTIR spectrum of EIE-containing materials. The addition of EIE to the PLGA polymer did not make a significant difference except at wavelengths of 2850-3000 cm^{-1}.

Table 5. Average nanofiber diameters of membranes produced at different concentrations.

Samples	PLGA	PLGA/EIE10	PLGA/EIE12	PLGA/EIE15	PLGA/EIE20
Average diameter of membranes (nm)	910	1023	1178	1240	1878

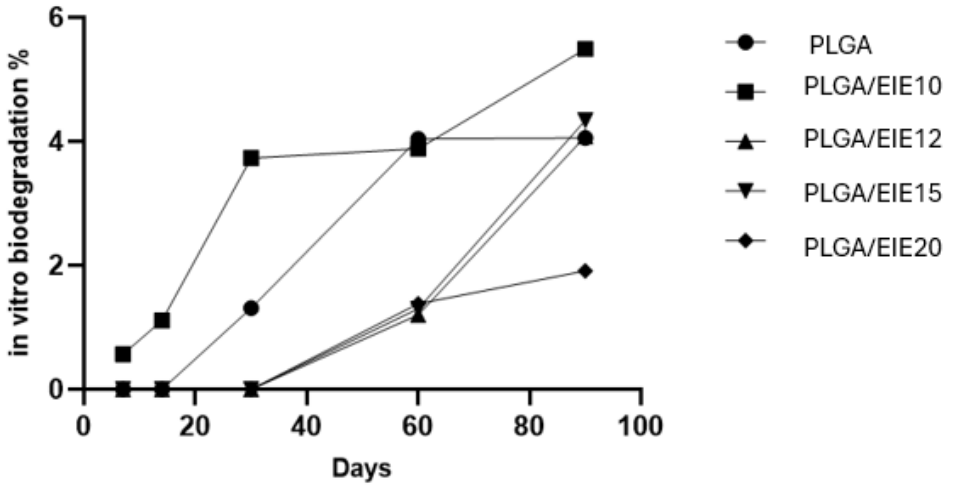

Figure 3. The graph of in vitro biodegradation.

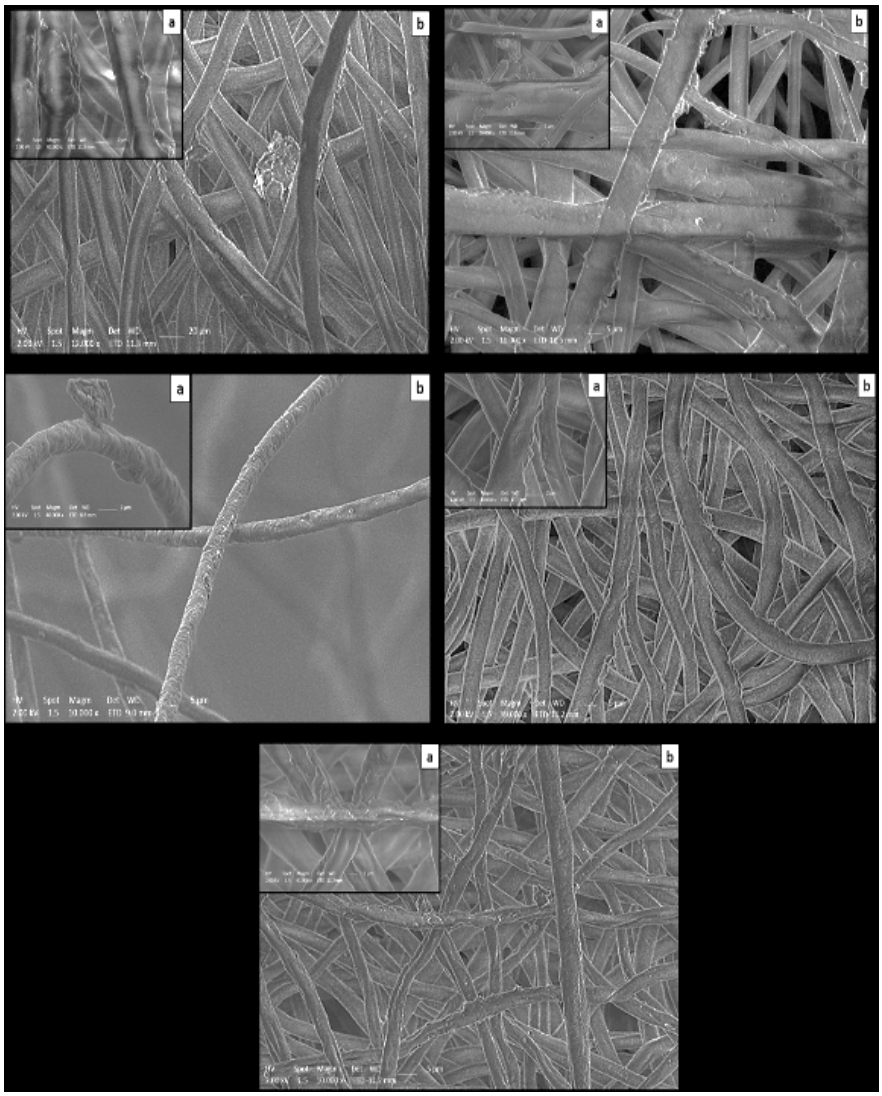

Figure 4. SEM images of membranes after 60 days * PLGA (a) 40.000x, b) 12.000x), PLGA/EIE10 (a) 20.000x, b) 10.000x), PLGA/EIE12 (a) 40.000x, b) 10.000x), PLGA/EIE15 (a) 40.000x, b) 10.000x), PLGA/EIE20 (a) 40.000x, b) 10.000x), respectively.

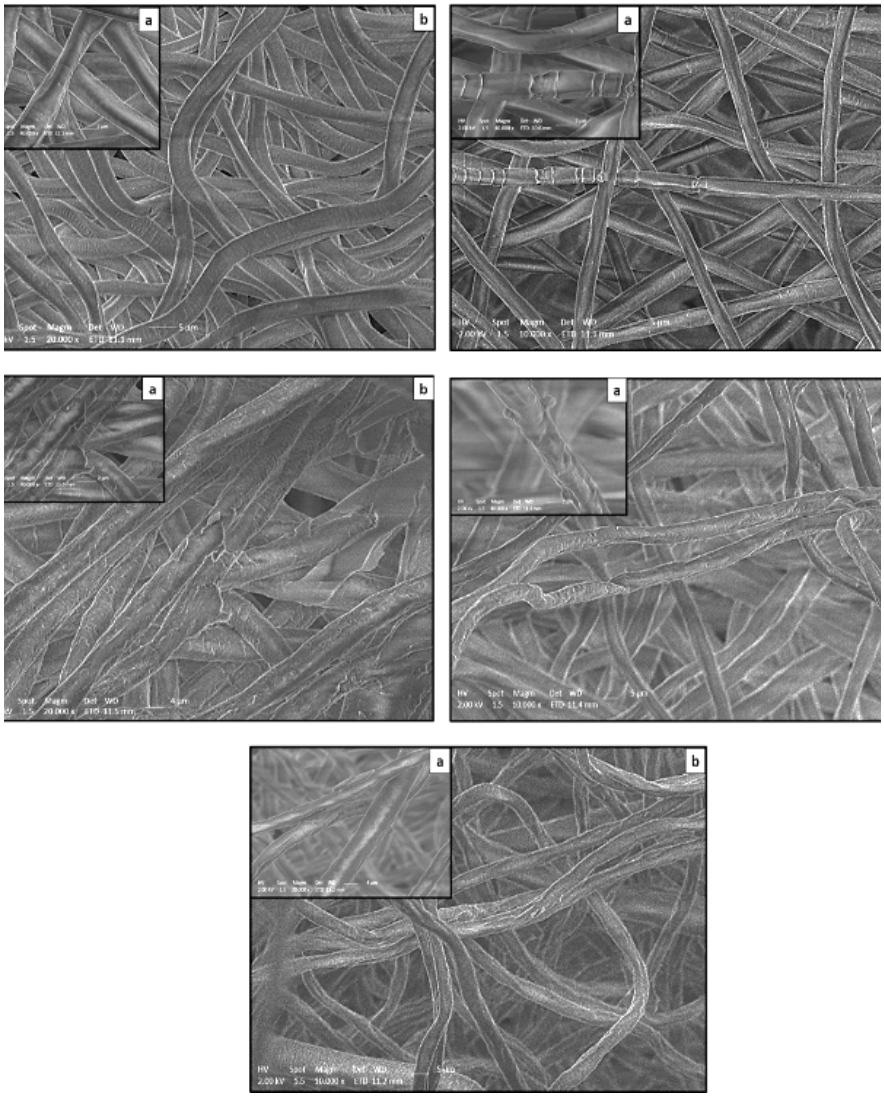

Figure 5. SEM images of membranes after 90 days *PLGA (a) 40.000x, b) 20.000x), PLGA/EIE10 (a) 40.000x, b) 20.000x), PLGA/EIE12 (a) 40.000x, b) 20.000x,), PLGA/EIE15 (a) 40.000x, b) 10.000x), PLGA/EIE20 (a) 20.000x, b) 10.000x), respectively.

Mechanical Test

In order to use as a wound dressing, the material needs to have some properties such as high elasticity, high tensile strength, and high elongation. PLGA is a synthetic and biodegradable polymer that has all these properties. A tensile test was performed to evaluate the effect of the addition of EIE on the mechanical properties of the membranes and results were presented in Table 6. According to these tests, the material that exhibited the highest tensile strength was the sample containing PLGA. Nevertheless, EIE addition to the nanofiber materials resulted in decreases in both the tensile strength and elongation values, but they were still sufficient for the application.

Thermal Gravimetric Analysis

TGA analysis was performed between 25-500 ℃ to determine the thermal properties of nanofiber materials and EIE active compounds (Figure 7). Thermal degradation and evaporation with thermal increase were identified as causes of weight loss. When the weight loss percentage of nanofibers against temperature increase is examined, no weight loss occurred in any nanofiber up to 250°C. The reason is that nanofibers show thermal stability up to 300°C [17]. In the literature, Zhu et al. [18] reported that degradation of PLGA began after 225 °C and the TGA result in this study was consistent with the literature. The weight losses of PLGA, PLGA/EIE10, PLGA/EIE12, PLGA/EIE15, PLGA/EIE20, and extract-only samples (EIE) depending on the temperature were 98.7%, 99.5%, 99%, 99%, 98.5%, and 99.6%,

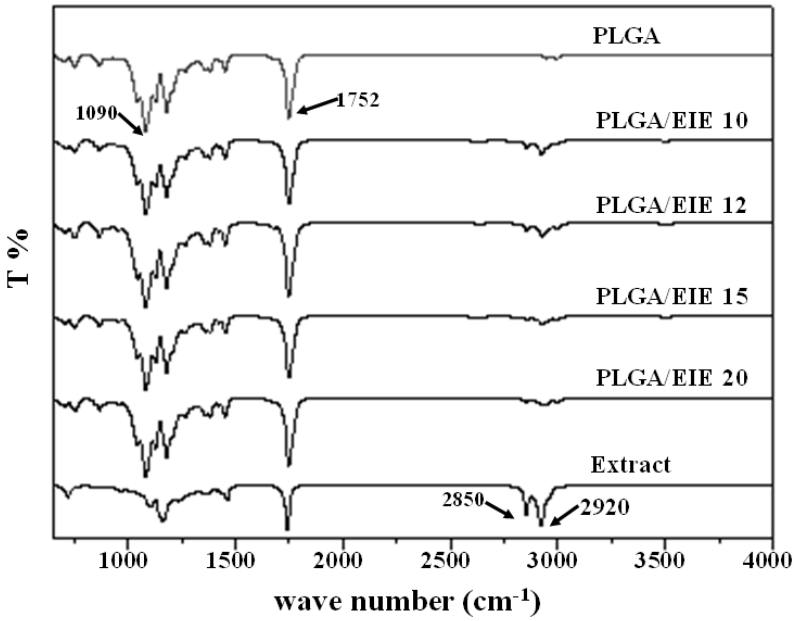

Figure 6. FTIR spectra of the membranes.

Table 6. Mechanical properties of the produced membranes.

Sample	Tensile strength (MPa)	Strain %
PLGA	0.23	207
PLGA/EIE10	0.11	142
PLGA/EIE12	0.07	88
PLGA/EIE15	0.10	163
PLGA/EIE20	0.08	112

respectively. After thermal degradation was completed, all nanofibers left some residue. When the TGA graph of the EIE sample was analyzed, it was observed that decomposition occurred over a very wide temperature range, such as 127-467°C.

Therefore, when plant extract containing active ingredients was added to PLGA, it was seen that the thermal resistance decreased. The oil additive in EIE reduced the polymer-polymer interaction in the materials, thereby reducing the thermal stability. Based on the TGA results, PLGA-containing nanofiber dressing materials containing different plant extract concentrations can beat body temperature (37°C) without degradation.

Water Contact Angle

A water contact angle test was performed to determine the hydrophobicity and hydrophilicity of the nanofibrous materials produced at different concentrations. As shown below, water droplets were dropped on the different concentrations of PLGA/EIE and control PLGA nanofiber membranes, and contact angles were measured. Then, these contact angles were averaged and reported as shown in Table 6. Figure 8 shows images taken with a microscopic camera after water was applied to the samples. It was observed that the contact angle of the materials initially decreased but then increased with increasing EIE content. Since the PLGA polymer is hydrophobic, the membrane containing only PLGA exhibited a high contact angle. The decreased water contact angle of the PLGA/EIE10 sample can be attributed to the increased hydrophilicity and wettability of the membranes upon the addition of EIE. It is hypothesized that the –OH groups in EIE contribute to this increased hydrophilicity. However, an increase in the contact angle was noted for samples containing higher EIE concentrations than for the PLGA/EIE10 sample. Therefore, the optimum concentration for these samples was determined to be 10%.

Indirect Cytotoxicity Test

The indirect cytotoxicity was determined using the MEM-extraction method. The values were obtained after 24 and 48 h. The cell viability values determined

by MTS analysis are presented in Figure 9. According to the results of the analysis, cell viability was found to be higher in 10% and 15% of samples compared to other membranes. The membrane with the lowest cell viability was 20%. After combining the results obtained from both microscopic evaluation and cell viability testing, it can be clearly stated that membrane-leachable components can be considered non-cytotoxic with a cytotoxic index between 1.70 and 2.77.

CONCLUSION

The skin is the largest organ in the human body and serves as the primary barrier against external factors and the initial point of contact with these factors. A wound represents the body's response to an external influence such as heat, chemicals, or sharp objects that encounter the skin. The healing process of a wound involves a series of phases: hemostasis, inflammation, proliferation, and remodeling (maturation). The duration of wound healing can vary depending on the severity of the wound. Contemporary wound care has shifted from traditional dressings, such as bandages, to advanced wound dressings that maintain a moist environment conducive to healing. Modern wound dressings include hydrocolloids, polyurethane foams, hydrogels, and nanofiber dressings. Nanofiber wound dressing materials are typically electrospun from natural or synthetic polymers. Owing to their nanoscale dimensions, these materials enhance moisture and oxygen permeability and facilitate the formation of new skin tissue through the active role of the polymer. Recent studies have also incorporated plant extracts known for their wound-healing properties into these materials.

In this study, various concentrations of solutions derived from. *E. italicum* extract and PLGA polymer, which is commonly used in skin treatments, were prepared and electrospun under optimized conditions to produce nanofiber wound dressing materials. Based on the results of mechanical testing, TGA analysis, *in vitro* biodegradability and cytotoxicity assessment, the optimal concentration for nanofiber membrane production was determined to be PLGA/EIE10. One of the significant advantages of this nano-sized fiber wound dressing material is its ability to maintain the oxygen and moisture balance

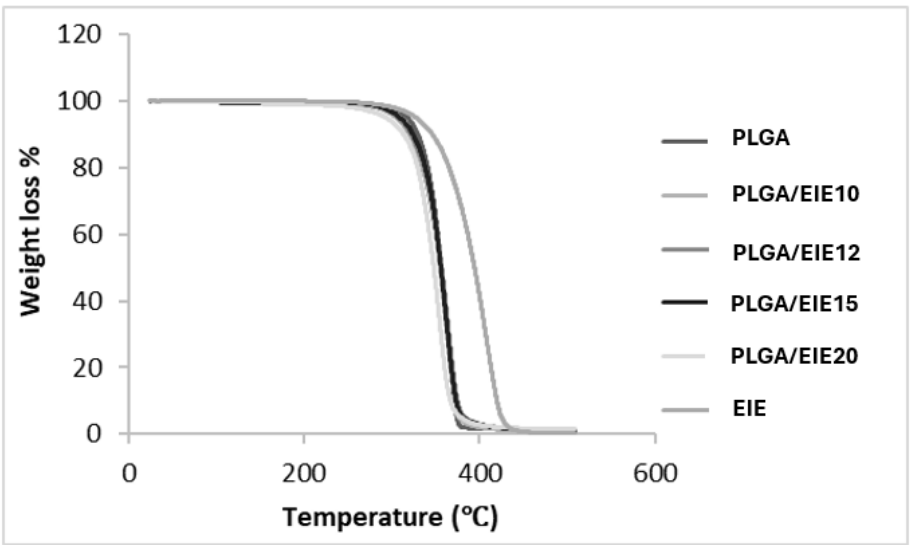

Figure 7. TGA graphs at different concentration.

Table 6. Water contact angles of membranes.

Samples	Contact Angle (°)
PLGA	125,79
PLGA/EIE10	121,70
PLGA/EIE12	126,86
PLGA/EIE15	129,66
PLGA/EIE20	122,78

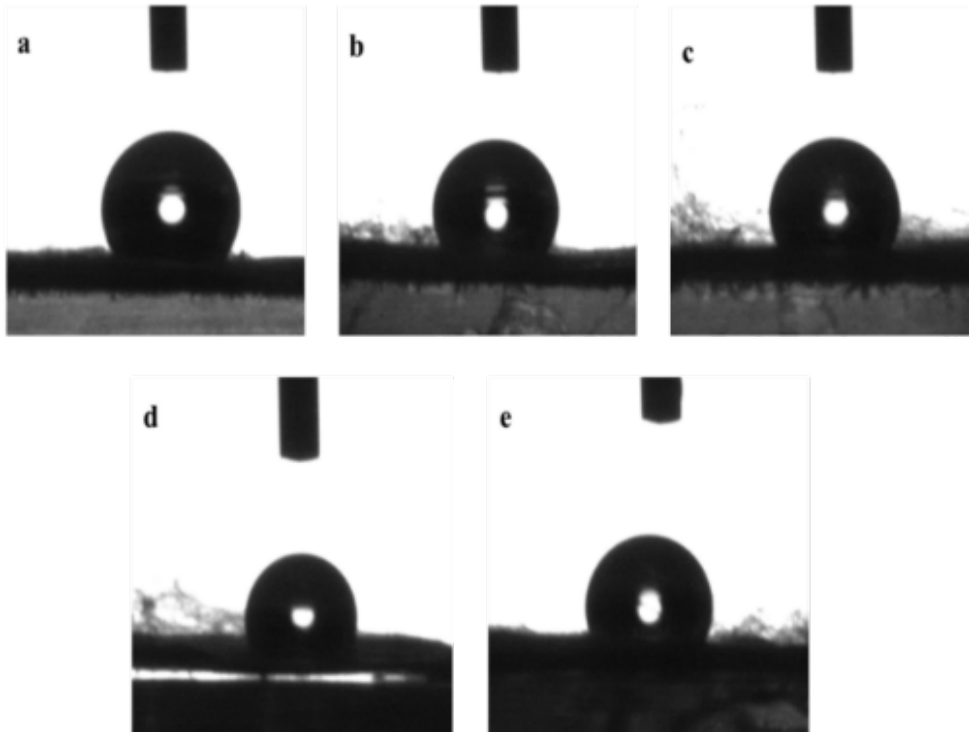

Figure 8. The images of the contact angle of the samples; a) PLGA, b) PLGA/EIE10, c) PLGA/EIE12, d) PLGA/EIE15, e) PLGA/EIE20.

Table 7. Cytotoxic index values of membranes after 24 and 48 hours.

	PLGA	PLGA/EIE10	PLGA/EIE12	PLGA/EE15	PLGA/EIE20
24 h	1.75	2.04	2.05	2.19	1.92
48 h	1.75	2.76	2.77	2.70	2.64
Results	1-3 Pass	1-3 Pass	1-3 Pass	1-3 Pass	1-3 Pass

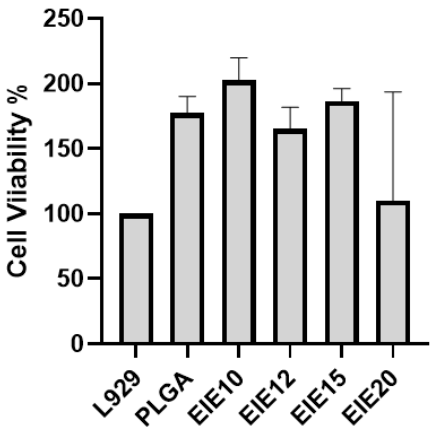

Figure 9. Cell viability of L929 cells after incubation with control and sample extracts for a period of 72 h (n=3).

at the wound site. Additionally, it is biocompatible, biodegradable, flexible, and possesses robust mechanical properties. It does not inhibit cell growth, thereby promoting the formation of new skin tissue and minimizing wound traces. The findings of this study suggest that the enhanced fiber mats are well-suited as wound dressing materials for the treatment of severe burn wounds.

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