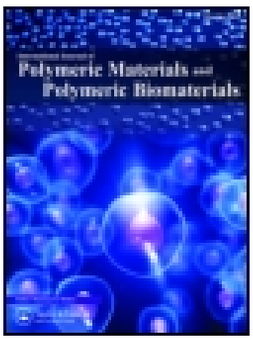

International Journal of Polymeric Materials and Polymeric Biomaterials

ISSN: 0091-4037 (Print) 1563-535X (Online) Journal homepage: https://www.tandfonline.com/loi/gpom20

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To cite this article: Nazende Nur Akşit, Seda Gürdap, Sevil Dinçer İşoğlu & İsmail Alper İşoğlu (2020): Preparation of antibacterial electrospun poly(D, L-lactide-co-glycolide)/gelatin blend membranes containing *Hypericum capitatum var. capitatum*, International Journal of Polymeric Materials and Polymeric Biomaterials, DOI: 10.1080/00914037.2020.1765354

To link to this article: https://doi.org/10.1080/00914037.2020.1765354

Published online: 18 May 2020.

INTERNATIONAL JOURNAL OF POLYMERIC MATERIALS AND POLYMERIC BIOMATERIALS
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Preparation of antibacterial electrospun poly(D, L-lactide-co-glycolide)/gelatin blend membranes containing *Hypericum capitatum var. capitatum*

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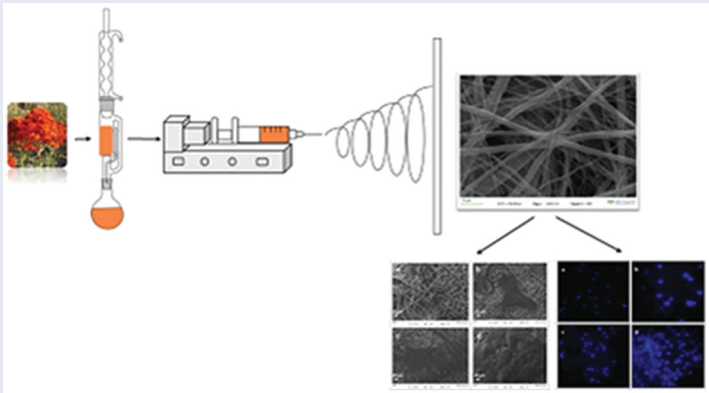
ABSTRACT

In this study, we fabricated poly(D, L-lactide-co-glycolide)/gelatin (PLGA/gelatin) membranes containing different amounts of *Hypericum capitatum var. capitatum* (HCC) extract (1, 5, 7.5, 10 wt%) by electrospinning technique. We investigated chemical, morphological, physical, and mechanical properties as well as in vitro degradation behavior of the electrospun membranes. We also evaluated the antibacterial activity of the electrospun membranes against *Escherichia coli* and *Staphylococcus aureus*. Viability, adhesion, and attachment of human fibroblast cells on the electrospun membranes on pre-set days were evaluated by the colorimetric CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS assay), scanning electron microscopy (SEM), and 4′,6-Diamidino-2-Phenylindole (DAPI) staining.

GRAPHICAL ABSTRACT



ARTICLE HISTORY
Received 23 December 2019
Accepted 27 April 2020

KEYWORDS
Electrospinning; *Hypericum capitatum var. capitatum*; wound dressing

1. Introduction

Since conventional approaches such as autograft- and allograft-based materials do not meet the clinical demands for skin regeneration, in recent years, the modern wound dressings such as films, gels, nano/microfibers, and membranes have been widely investigated as dermal substitutes. Among the available dressings, fibrous membranes have attracted significant attention as they possess suitable mechanical propertises comparable with skin, higher gas permission ability, high porosity, and the large surface-area-to-volume ratio[1].

Electrospinning is one of the versatile techniques to produce porous fibers structure with an average diameter from nano- to micrometer scale, which can mimic the native extracellular matrix (ECM)[2, 3]. Fiber diameter, morphology, and porosity of the electrospun membranes, which can be adjusted by variable parameters such as solution viscosity, solvent type, applied voltage, the flow rate of the electrospinning process, are closely related in terms of improving cell attachment and proliferation and become essential parameters to obtain adequate wound dressing material[4–6].

Synthetic and natural polymers can be utilized for preparing electrospun membranes as potential wound healing materials[7]. However, due to hydrophobicity and lack of surface ligand problems of synthetic polymers and the weakness of the processibility and mechanical properties of the natural polymers, the electrospun membranes prepared from their blends have been drawn considerable attention in the wound healing applications, recently[8]. Poly(D, L-lactide-co-glycolide) (PLGA), an FDA approved synthetic copolymer, has been widely used for absorbable sutures, absorbable surgical clips, and controlled release implants due to its biocompatible and biodegradable properties[9, 10]. Gelatin is a

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biopolymer derived from the hydrolysis of natural collagen existing in skin, tendon, cartilage, and bone tissues in the body. Since it has biological origin, and is non-immunogenic, biodegradability, biocompatibility, and commercially available at relatively low cost, gelatin has been used for various biomedical applications[11, 12].

Besides, the preparation of wound dressing materials containing herbal medicines becomes attractive to accelerate the wound healing process due to their antibacterial and antioxidant properties. These dressings also increase the epithelization through induction fibroblast cells[13–16]. *Hypericum* (Hypericaceae) is a well-known genus, which has been used for wound healing properties in Turkish folk medicine for a long time[17]. One of the species belonging to Hypericum genius, *Hypericum capitatum var. capitatum* (HCC), has significant properties such as antibacterial and antioxidant properties, which are crucial for the wound healing mechanism[18].

Similarly to our study, electrospun nanofibrous scaffolds made of synthetic/natural polymeric blends containing different growth factors, drugs, and also herbal extracts have been studied for wound dressing applications. Meng et al.[19] fabricated Fenbufen loaded electrospun PLGA and PLGA/gelatin nanofibers as a potential drug delivery system and showed that adding gelatin to membrane increase of hydrophilic drug release. In another study, Hu et al.[20] prepared Cefradine and 5-fluorouracil loaded PLGA/gelatin nanofibrous mats by emulsion electrospinning method to evaluate the potential as a scaffold for tissue engineering or drug delivery applications. Norouzi et al.[21] investigated PLGA/gelatin hybrid nanofibrous scaffolds encapsulating EGF for skin regeneration and demonstrated that the addition of gelatin to the PLGA membrane contributes to increased cell proliferation and attachment since gelatin has an RGD (Arg-Gly-Asp) sequence that enables the recognition of the integrin protein on the cell surface. In another study, Jia et al.[22] and Chuang et al.[23] reported that the poly(vinyl alcohol)/chitosan electrospun blend membrane enhances the adhesion and proliferation of fibroblasts. Also, Meng et al.[24] also reported that adding gelatin to PLGA electrospun membrane improves cell adhesion and proliferation. Liu et al.[10] produced an electrospun membrane consisting of 50/50 percent PLGA/collagen, and they showed that the presence of collagen in the membrane promotes fibroblast cell proliferation. Zhang et al.[11, 25] used coaxial electrospinning technique and post coating to prepare collagen- and gelatin-coated PCL nanofibers. Compared to post coating with gelatin, nanofibers coated with collagen exhibited more similarity to ECM structure, which improves the ability of the cell attachment onto the membranes. Furthermore, in some studies, the oil and extract of the genus *Hypericum*, such as *Hypericum perforatum*, loaded electrospun membranes have been investigated for wound dressing applications. Egri et al.[26] encapsulated *H. perforatum* oil in PEG capsules, which were held by the electrospun PEG membrane and evaluated as a potential wound dressing material. In another study, Maria Letizia Iabichella[27] studied the *in vitro* bacterial effect of electrospun PLLA scaffold with a mixture of *H. perforatum* and *Azadirachta indica* oil extracts for diabetic foot infections. Pourhojat et al.[28, 29] investigated the antibacterial *H. perforatum* extract loaded electrospun PCL nanofibers as a wound dressing and electrospun PLGA polymer nanofibers containing alcoholic extract of *H. perforatum* method to show the practical efficiency of the mats as an alternative for skin grafts.

To the best of our knowledge, for the first time in the literature, we fabricated electrospun PLGA/gelatin membranes containing *H. capitatum var. capitatum* (HCC) extract and investigated morphological, mechanical and physical properties such as hydrophilicity and degradation behavior of the membranes. We also evaluated the *in vitro* release profile of HCC, antibacterial activity, cytotoxicity, and *in vitro* cell attachment ability of electrospun membranes.

2. Materials methods

2.1. Materials

We purchased poly (D, L-lactide-co-glycolide) (PLGA, DL-Lactide: Glycolide copolymer, ratio M/M%: 75/25, M_W = 120,000) from Purasorb, Purac Biomaterials, gelatin (gel strength ~225 g Bloom, Type B) and 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI) from Sigma-Aldrich (USA), methanol, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), and agar from Merck, DMEM High Glucose from Biological Industries. Fetal Bovine Serum, Penicillin Streptomycin, and LB Broth were obtained from Gibco, USA. We collected the *H. capitatum var. capitatum* from Konya, Turkey. The Microbiology Department of the Erciyes University, Kayseri, Turkey kindly donated human fibroblast cells, *Escherichia coli* and *Staphylococcus aureus* strains to our laboratory. All other chemicals and solvents are used without further purification.

2.2. Methods

2.2.1. Extraction of HCC

Firstly, we extracted 100 g HCC in a Soxhlet with methanol at 60 °C for 24 h and removed the excess methanol from the extract by using a rotary evaporator (Buchi, Rotavapor R300). The final extract was dried under vacuum overnight[30]. The GC-MS analysis was performed for analysis of the chemical composition of HCC extract (Agilent 7890 B GC system equipped with a 5977 A series MSD). The capillary column was HP-5ms (30 m x 250 µm × 0.25 µm, Agillient 19091S-433). We kept the carrier gas helium at a constant flow rate of 3 mL/min. The temperature gradient, started at 50 °C, was held for 2 min and rose to 240 °C at a rate of 15 °C/min. Afterward, we injected one µl of HCC extract dissolved in methanol at 50 °C in the split-less mode. Compounds inside of HCC were detected using commercially available NIST libraries.

2.2.2. Fabrication of electrospun membranes

We fabricated PLGA/gelatin and PLGA/gelatin containing HCC membranes by electrospinning. Briefly, we first dissolved PLGA and gelatin pellets in HFIP (15% (w/v) with the ratio of 9:1 (v/v)) and added different amounts of HCC extract (1, 5, 7.5, and 10 wt%) into the blend solution. The mixture was stirred for 2 h to homogenize the HCC extract in the polymer solution. Polymer solutions were then electrospun from 10 mL syringe with 21 G needle (an inner diameter of 14.53 mm), flow rate of 1 m/h, and tip to collector distance of 15 cm. A high voltage (10 kV) was applied and a flat aluminum folio was used to collect random nanofibers.[28, 29]

2.2.3. Characterization of electrospun membranes

2.2.3.1. Transform infrared spectrometer (FT-IR). We characterized the chemical structure of the electrospun membranes by using Thermo Scientific Nicolet 6700 Fourier Transform Infrared Spectrometer (FT-IR) in the range of 400–4,000 cm^{-1}.

2.2.3.2. Morphology. Scanning Electron Microscopy (SEM) (Carl Zeiss EVO LS10, Germany) was used to investigate the fiber morphology of the electrospun membranes.

2.2.3.3. Swelling test. For the swelling test, we immersed the neat and electrospun PLGA/gelatin membranes containing HCC in distilled water for 5, 10, 15, 20, 25, and 30 min. Then, we weighed the samples after removing the surface water with a filter paper. The water content was calculated according to the following equations[10].

$$Wc(\%) = \frac{(W - Wo)}{Wo} \times 100 \quad (1)$$

where Wo and W are the weight of the samples before and after immersion in water for different times, respectively.

2.2.3.4. Mechanical analysis. The tensile strengths and elongation at the break of electrospun membranes were determined using Shimadzu Autograph AGS-X 10 kN device. We cut the samples into the strips with 20 mm in length, 20 mm in width possessing a thickness of 0.1 mm, and conducted tensile tests at the strain rates of 1×10^{-2} s^{-1} at room temperature.

2.2.3.5. In vitro drug release study. To investigate the *in vitro* drug release profile of membranes, we followed the Hypericin absorbance. Firstly, we calculated the hypericin contents of HCC and dissolved 50, 10, 1, 0.5, 0.1, 0.01 mg HCC extract in water. The calibration curve was drawn reading absorbance at 587 nm[31]. Hypericin percentage was calculated using the following equations,

$$Hyp\% = \frac{A}{780} \times \frac{100}{m} \quad (2)$$

where A is the measured absorbance, m the grams of extract, and 780 the specific absorbance of hypericin at 587 nm[28].

After determining the percentage of hypericin 10 mg membrane placed into 10 mL PBS solution, a specific time interval 1 mL sample was withdrawn from PBS solution, and a 1 mL fresh PBS solution was added to this medium. Withdrawn sample absorbance measured at 587 nm. The cumulative hypericin release calculated using the following equations.

$$\text{Cumulative percentage release } (\%) = \frac{\text{Volume of sample withdrawn (mL)} \times P(t-1)}{\text{Bath volume } (v)} + Pt \quad (3)$$

where Pt = Percentage release at time t; $P(t-1)$ = Percentage release previous to "t"[32].

2.2.3.6. Degradation study. To perform degradation study, we soaked the membranes with and without HCC in 20 mL PBS solution and kept at 70 rpm at 37 °C in a shaker incubator. One week later, we took the membranes from PBS solution, rinsed with distilled water, and dried under vacuum. After completely dried, membranes' weight and weight loss calculated using the following formula.

$$\text{weight} - \text{loss}(\%) = \frac{M_0 - M_t}{M_0} \times 100 \quad (4)$$

where M_0 and M_t are sample weights before and after incubation in PBS, respectively[33].

2.2.3.7. In vitro study of antibacterial activity. We evaluated the antibacterial activity of HCC containing membranes by using Kirby–Bauer disk diffusion susceptibility test against *E. coli* as a model gram-negative bacteria and *S. aureus* as a model gram-positive bacteria. Briefly, LB Broth culture was diluted to reach the Mc Farland turbidity standard. After dilution, we streaked the bacteria by using a sterile swab on the LB agar plate. Electrospun PLGA/gelatin membranes with and without HCC, and ampicillin disk were placed on the agar plate and incubated at 37 °C overnight, and zone diameter was measured[34] for showing the antibacterial activity of the membranes.

2.2.3.8. Cell viability assay. The viability of human fibroblast cells on the electrospun membranes was investigated by using MTS assay (Cell Titer 96® AQueous One solution; Promega, USA). Briefly, fibroblast cells were cultured in DMEM High Glucose medium supplemented with 10% FBS and 1% penicillin/streptomycin in cell culture plates in an incubator at 37 °C with 5% CO_2, and the media were replaced every 2 days. The electrospun membranes were sterilized with ethanol and kept under UV radiation for 2 h. Before cell seeding, electrospun membranes were kept in DMEM High Glucose media overnight. Following day, the electrospun membranes were placed into 96 well plates, and 10^4 cells were seeded on the membranes. After 24 and 48 h, 20 µL MTS reagent was added. After 2 h of incubation, membranes were removed from the plate, and absorbance was measured at 490 nm using Microplate Reader

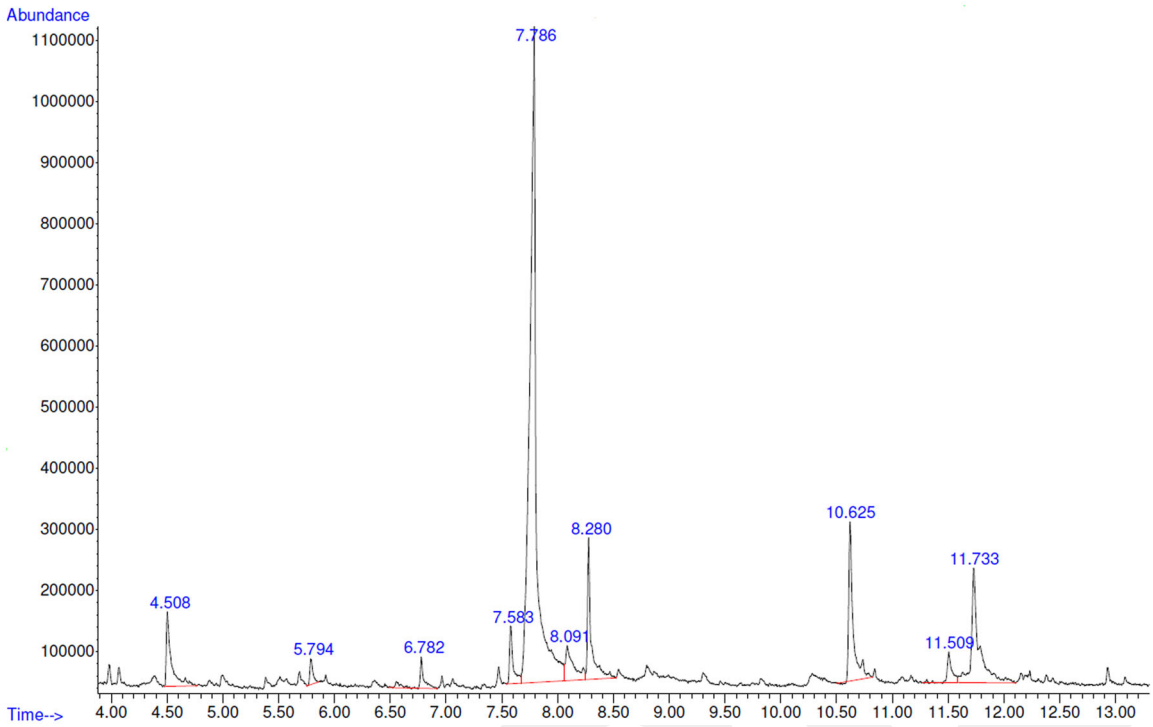

Figure 1. GC MS spectrum of HCC extract.

(Varioskan, Thermo Scientific), and cell viability (%) was calculated[35].

2.2.3.9. Cell attachment assay and DAPI staining. For cell attachment, electrospun membranes with and without HCC were placed into 96 well plates, and 10^4 cells/well were seeded on to the electrospun membrane. After 24 and 48 h, the membrane was removed from the plate and rinsed with PBS for removing the non-attached cells. Then the membranes were put in a glutaraldehyde solution (2.5%, v/v) to fix the cells for 2 h. After fixation, membranes were placed in gradient alcohol solution (30, 50, 70, 90%, v/v) and kept under vacuum to dry, and SEM images were taken.

For DAPI Staining, after fixation of the cells on to membranes with glutaraldehyde solution (2.5%, v/v), membranes were washed three times with PBS solution. 20 µL DAPI solution (300 Nm) was added to cover the surface of the membrane. After a 5-min incubation with protecting the light, PBS was used to remove excessive DAPI stain, and we took the cell images under the microscope.

3. Results and discussion

3.1. Gas chromatography-mass spectrometry (GC-MS) results

We analyzed the aerial part of HCC extract by using GC-MS. Figure 1 shows the retention time and the abundance of the active compounds in HCC extract. The name of the compound, retention time, molecular formula, molecular weight, peak area, and biological activity of phytochemicals are given in Table 1. Most abundant compounds inside of HCC extract were benzoic acid (RT: 7,786), Semicarbazone butyraldehyde (RT: 11,733), ^{1}H-Benzotriazole (RT: 10,625) respectively. Phytochemicals that give to HCC antibacterial, antifungal, antioxidant, and anti-inflammatory activity are listed in Table 1. Bagci and Yuce [44] showed that main constituents of HCC essential oils are α-pinene, caryophyllene oxide, hexadecanoic acid, β-caryophyllene and undecane and these constituents have antibacterial properties[45–47]. Boga et al.[18] evaluated phytochemical analysis and biological activity of HCC, and according to results, methanol extract of HCC showed antibacterial activity against *E. coli* and the DNA damage protective activity, which is responsible for antioxidant activity. The active compounds which are shown in Figure 1 have similar pharmacological activities of HCC in the literature[18, 30, 44]. In addition, the reason for the presence of different active substances in the HCC extract from the literature is that the plants are collected from different regions.

3.2. FT-IR spectra

Figure 2 shows the FT-IR spectra of the electrospun PLGA/gelatin membranes with and without HCC extract. All membranes showed characteristic peaks belong to PLGA and gelatin. The characteristic peaks of PLGA are C–O stretching at about 1,129 cm^{-1} and 1,752 cm^{-1}, C–O–C stretching at 1,182 cm^{-1}, and C–H stretching at about 1,453 cm^{-1}, respectively. Also, peaks of the amide I and amide II structure of gelatin were assigned to 1,646 cm^{-1} and 1,540 cm^{-1}. Furthermore, compared to neat membrane, HCC extract containing membranes have more broad absorption at 3,300 cm^{-1}, and this absorption increased depending on increasing amount of HCC extract in the membranes. Similar to our result, Dhayabaran et al.[14] and Maqbool

Table 1. Compounds in HCC extract.

No	RT	Name of compound	Molecular formula	MW	Peak area %	Activity
1	4.508	Dihydroxyacetone	$C_3H_6O_6$	90,078	4,44	Antifungal, vitiligo treatment[36]
2	5,794	3-Furanol, tetrahydro-	$C_4H_8O_2$	88,106	1,17	Intermediate to the AIDS drugs[37]
3	6,782	Maltol	$C_6H_6O_3$	126,11	6,781	Antioxidant[38]
4	7,583	2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	$C_6H_8O_4$	144,126	2,42	Antioxidant, antibacterial[39]
5	7,786	Benzoic acid	$C_7H_6O_2$	122,123	61	Antimicrobial[40]
6	8,091	Catechol	$C_6H_6O_2$	110,112	2,66	Antimicrobial[41]
7	8,280	Propanediamide	$C_3H_6N_2O_2$	102,093	6,01	
8	10,625	1H-Benzotriazole	$C_6H_5N_3$	119,127	7,81	Antimicrobial, antifungal[42]
9	11,509	4-Methyl-1H-benzotriazole	$C_7H_7N_3$	133,154	2,13	Antimicrobial, antifungal[42]
10	11,733	Butyraldehyde, semicarbazone	$C_5H_{11}N_3O$	129,163	10,90	Antibacterial, antifungal, antiviral and anti-inflammatory[43]

et al.[48] showed that adding HCC extract to the membrane exhibited a specific peak at around 3,400 cm^{-1} which was corresponded phenolic and flavonoid compound in HCC extract[49]. This specific peak occurs due to an increase in intermolecular hydrogen bonding which is attributed to N–H and OH–O stretching from the coming compound of HCC extract[24, 28, 29, 50].

3.3. Fiber morphology of electrospun membranes

It was shown in the literature that the electrospun membranes prepared from the PLGA/gelatin solution with a ratio of 9:1 (w/w) exhibited uniform fiber structure without any bead formation[24]. Thus, we started with this ratio and adjusted the electrospinning parameter as a 10 kV of the applied voltage, 1 mL/h of flow rate, and 15 cm of distance between tip and collector. Image J software was used to calculate the mean diameter by randomly selecting 25 the fiber samples from SEM images. Figure 3 shows the average fiber diameter of the electrospun membranes. The average fiber diameter of neat electrospun PLGA/gelatin membrane was calculated as 1.83 ± 1.02 µm, whereas the mean fiber diameter of electrospun PLGA/gelatin/1, 5, 7.5, and 10 wt% HCC membranes were calculated as 1.05 ± 0.56 µm, 0.7 ± 0.29 µm, 0.51 ± 0.11 µm, 0.49 ± 0.2 µm, respectively. Generally, adding gelatin to the PLGA solution decreased the viscosity of the solution, which leads to smaller fiber diameter and broad fiber distribution. Increasing of HCC amount within the membranes caused the fiber diameter to decrease as a result of the decrease in the viscosity of the polymer solution.

Figure 4 shows the SEM micrographs of electrospun PLGA/gelatin membranes with and without HCC extract. Nezarati et al.[51] showed that decreasing polymer solution viscosity reduced fiber diameter as a result of decreasing of viscoelastic forces, which resist to fiber deformation given electrical field. In contrast, higher viscosity provides higher viscoelastic forces, which results in larger fiber diameter. Also, the low viscosity leads to an enhance asymmetric instability (Rayleigh instability), which causes to decrease the mean fiber diameter of the membranes[52–54]. Additionally, In Figure 3, the membrane containing the HCC has round shape structures, which are assumed to come from the HCC extract.

3.4. Contact angle

One of the important parameters of wound dressing materials for cell interaction is the surface hydrophilicity of the material. To determine surface hydrophilicity, we performed the contact angle test for the PLGA/gelatin electrospun membranes with and without HCC extract. Figure 5 shows the contact angle values of the membranes. We measured 113.72° for the neat membrane, whereas 85.19°, 62.5°, 37.14°, 36.02° for electrospun PLGA/gelatin/1 wt%, 5 wt%, 7.5 wt%, 10 wt% HCC, respectively. Membrane without the HCC surface has already a hydrophilic character. Comparison of HCC containing membranes to the neat

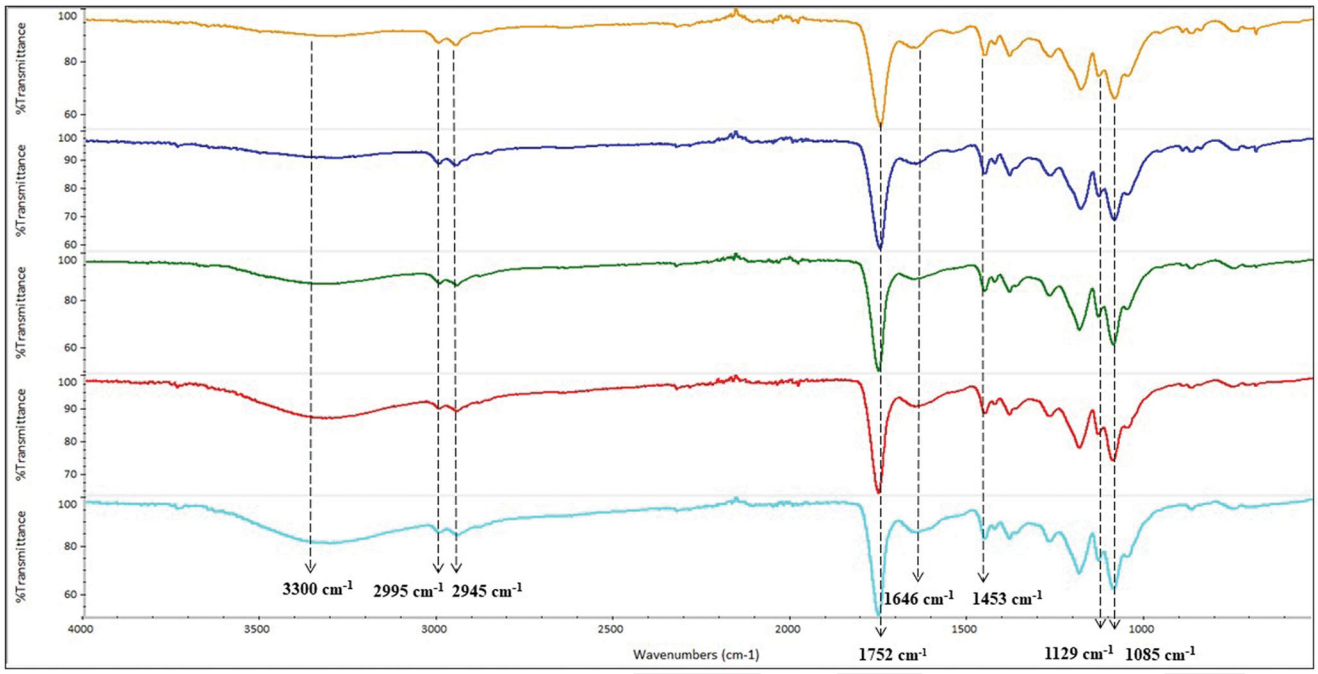

Figure 2. FT-IR spectrum of (a) PLGA/gelatin, (b) PLGA/gelatin/1 wt% HCC, (c) PLGA/gelatin/5 wt% HCC, (d) PLGA/gelatin/7.5 wt% HCC, and (e) PLGA/gelatin/10 wt% HCC.

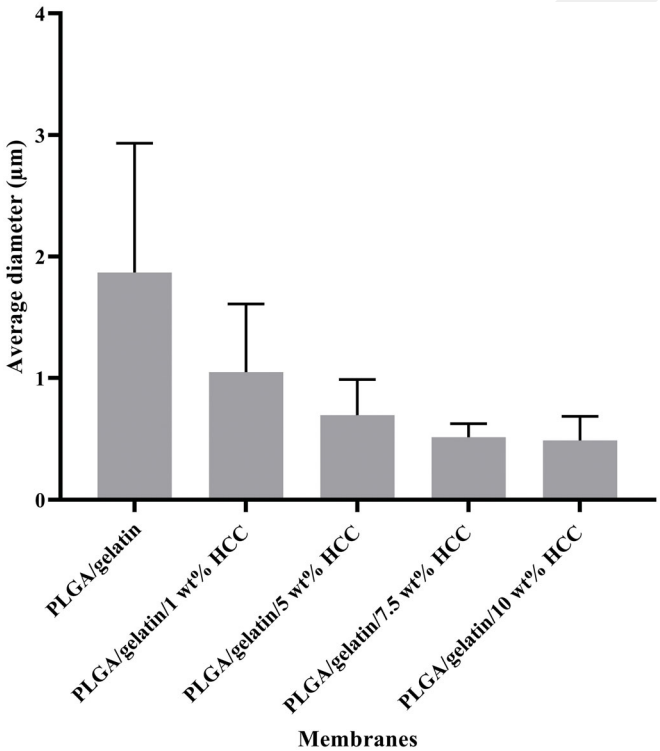

Figure 3. The average fiber diameters of electrospun PLGA/gelatin membranes with and without HCC. Error bars represent ± SD.

membrane, adding HCC decreased contact angle values depending on the increasing amount of HCC. The contact angle value is related to membrane surface hydrophilicity, and as the surface hydrophilic character increases, the contact angle value decreases. According to the FTIR result, the addition of the HCC extract to the membranes increased the N–H and O–H stretching, and these bonds contributed to the material hydrophilic properties, which leads a decrease in the contact angle values. Fu et al.[55] demonstrated that increased surface hydrophilicity, enhanced cell interaction with the membrane due to improved cell behavior such as initial binding, proliferation, and cell differentiation. Therefore, the addition of HCC to the membrane made to membrane more hydrophilic and biocompatible.

3.5. Swelling test

Since the swelling capacity of wound dressing materials is an essential feature for the absorption of wound exudate from the wound site, we investigated the swelling capacity of electrospun PLGA/gelatin membranes with and without HCC extract (Figure 6). All membranes were swollen very rapidly in 5 min and reached equilibrium after 5 min. The swelling ratio of the neat PLGA/gelatin membrane was reached 190% while the swelling ratios of PLGA/gelatin/1 wt%, 5 wt%, 7.5 wt%, 10 wt% HCC membranes were reached 338, 332, 327, 322%, respectively. According to the FT-IR spectra and contact angle results, the addition of HCC into PLGA/gelatin membranes caused significant increasing the hydrophilicity of the membranes due to the increase of the hydrogen bonding, which is the reason of the water absorption capacity. Also, Pourhojat et al.[28, 29] showed that adding to *H. perforatum* extract to the membrane contributed to increasing the hydrophilic properties and absorption capacity of the membranes.

3.6. Mechanical properties

Figure 7 shows the tensile stress-strain curves of PLGA/gelatin membranes with and without HCC extract. Table 2 tabulates ultimate tensile strength, Young's modulus, and

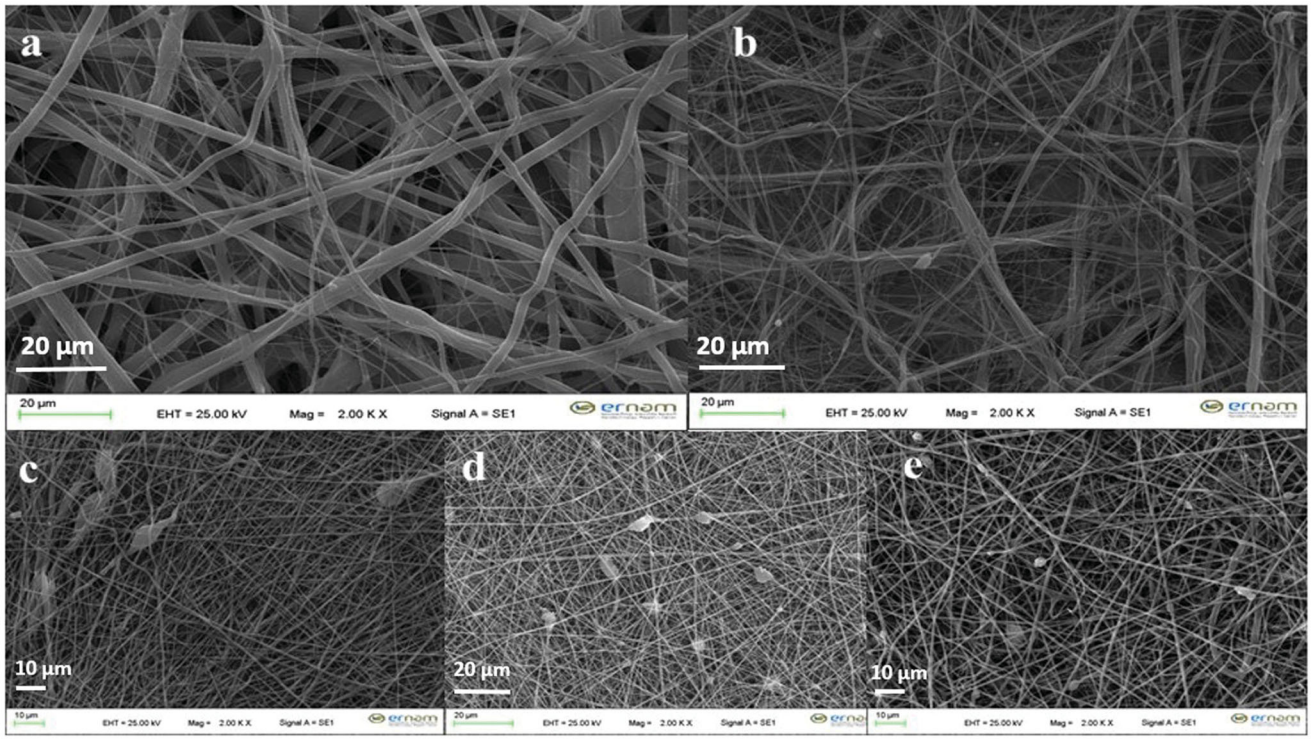

Figure 4. SEM images of (a) PLGA/gelatin, (b) PLGA/gelatin/1 wt% HCC, (c) PLGA/gelatin/5 wt% HCC, (d) PLGA/gelatin/7.5 wt% HCC, and (e) PLGA/gelatin/10 wt% HCC.

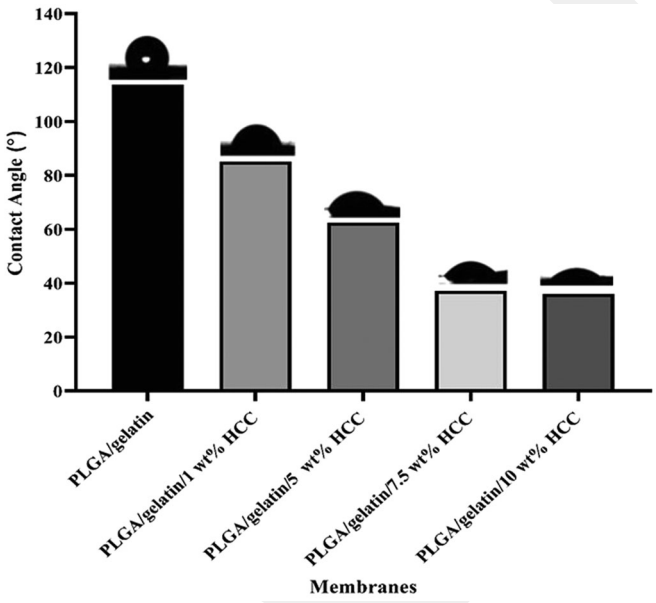

Figure 5. Contact angle results for electrospun PLGA/gelatin membranes with and without HCC.

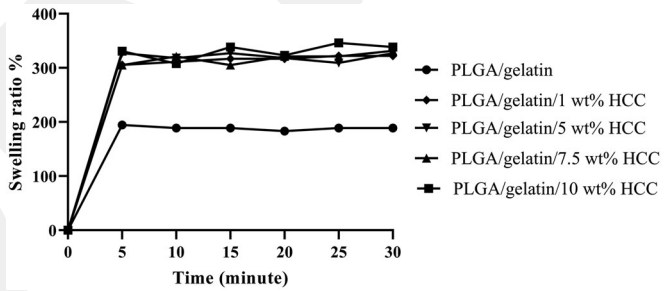

Figure 6. The swelling ratio (%) of PLGA/gelatin membranes with and without HCC extract.

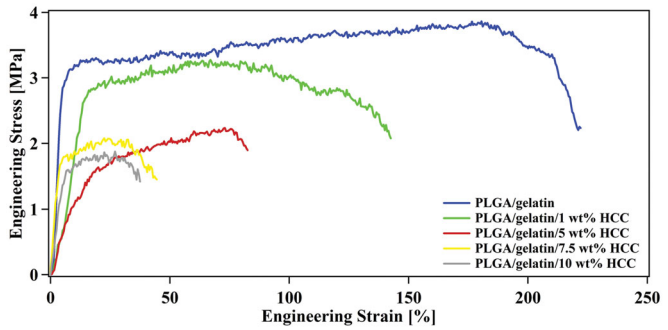

Figure 7. Stress–strain curves of electrospun PLGA/gelatin membranes with and without HCC extract.

fracture strain. The ultimate tensile strength of PLGA/gelatin was 3.86 MPa, with a decrease in the maximum tensile strength value while the HCC extract percentage increased from 1 to 10. On the other hand, the ductility of neat PLGA/gelatin was around 237%, and it was the highest ductility value among all membranes. Similar to the ultimate tensile strength, the ductility percentage reduced when the amount of HCC extract increased. Young's modulus of PLGA/gelatin was 58.63 MPa, and it was the highest value when compared to membranes containing HCC. The addition of HCC extract to the membrane, which reduces the viscosity of the solution, causes to decrease average of the fiber diameter and can result in reduced the mechanical properties of the membrane. Meng et al.[24] and Liu et al.[55] showed that when gelatin concentration increased in the PLGA membrane result in reducing mechanical properties.

Table 2. Mechanical properties of electrospun PLGA/gelatin membranes with and without HCC extract.

Sample name	Ultimate tensile strength (MPa)	Elongation (%)	Young's modulus (MPa)
PLGA/gelatin	3.8597	237.036	58.63095
PLGA/gelatin/1 wt% HCC	3.2743	155.035	42.89453
PLGA/gelatin/5 wt% HCC	2.2358	82.535	13.78142
PLGA/gelatin/7.5 wt% HCC	2.0782	59.499	42.22584
PLGA/gelatin/10 wt% HCC	1.8756	41.001	29.85896
Native skin		35-115	4.6-20

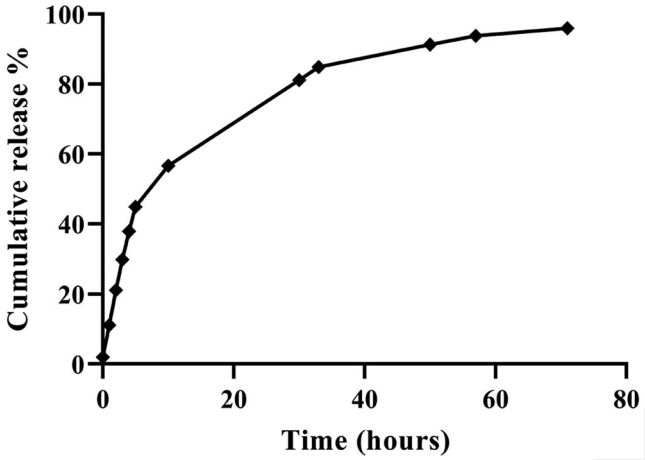

Figure 8. Release behavior of electrospun PLGA/gelatin membranes with and without HCC extract.

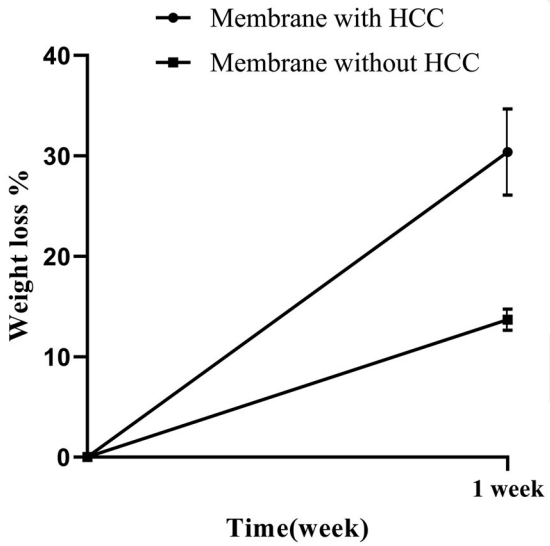

Figure 9. Weight loss of membranes with and without HCC extract.

Nevertheless, compared with membranes used for wound healing material in the literature, the membrane with HCC mechanical property value was sufficient to be used as wound healing material and mechanical property values of membrane with and without HCC were similar to mechanical properties of skin tissue[8, 56].

3.7. Release study

The release behavior of electrospun PLGA/gelatin membranes containing HCC was determined by following hypericin content using a UV spectrophotometer at 590 nm. Figure 8 shows that the first 24 h, 80% of hypericin released from the membrane and after 80 h, the release value reached 100%. This rapid release from the membrane can be explained by the absorption of HCC content on the membrane surface and causes a burst effect due to its rapid dissolution in PBS. Similar to our result Pourhojat et al.[29] showed the burst release of *H. perforatum* from the PLGA membrane is due to the weak physical and hydrophobic interaction of the extract with the membrane, which is similar to our results. The *Hypericeaece* family has an inducing effect on fibroblast cells, and such release behavior may increase fibroblast proliferation, which leads to an improvement in the healing process[57]. Pourhojat et al.[29] also suggested that wound healing would enhance because rapid HCC release provided rapid antimicrobial environmental conditions in the opened wound area. All results have shown that this rapid release may be beneficial for the wound healing process.

3.8. Degradation study

In one week of degradation study, the weight loss of the electrospun membranes with and without HCC reached 13.7%, 30.4%, respectively (Figure 9). Similar to our results, Liu et al.[10] showed that the weight of the PLGA membrane decreased by 30% in 4 weeks and by about 10% in 1 week. It can be seen from Figure 9 that the degradation ratio of the membrane containing HCC extract was higher than the neat membrane. HCC release from the membrane resulted in the loss of membrane weight as well as PLGA and gelatin degradation, leading to an increase in the degradation rate of the membranes.

3.9. Antibacterial activity

The antibacterial activity of membranes containing HCC extract was investigated against *E. coli* and *S. aureus* by Kirby Bauer Disk Diffusion Method and compared with neat membrane and ampicillin disk. Figure 10 shows the images of the antibacterial study of the membranes. The PLGA/gelatin/1 wt% HCC had no antibacterial effect. Membrane showed an antibacterial effect when HCC percentage increased to 5%, and there was zone inhibition of the PLGA/gelatin/7.5 and 10 wt% HCC membranes, and zone inhibition enhanced due to the increase of HCC percentage. We indicate the zone inhibition diameters in Table 3. Sokmen et al.[30] evaluated the antibacterial activity of extracts of aerial part of HCC in chloroform, water, and acetone extract. Their result demonstrated that chloroform and acetone extract showed zone inhibition against *Bacillus*

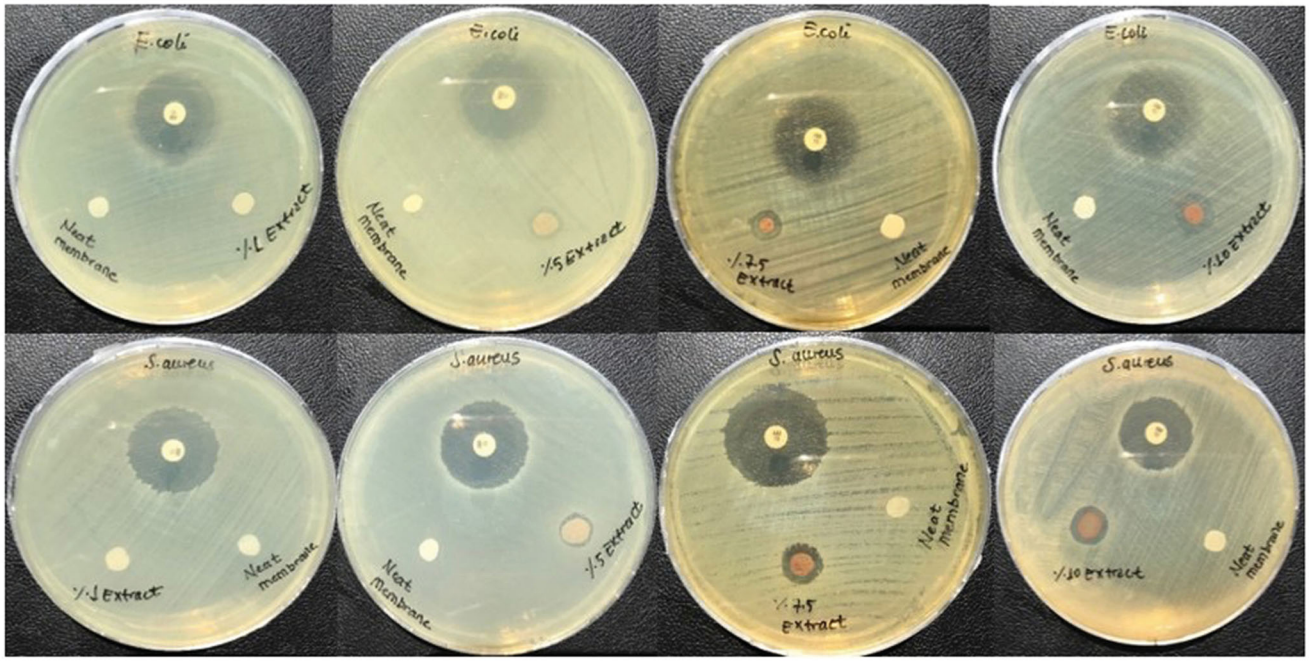

Figure 10. Antibacterial effect of (a) PLGA/gelatin and PLGA/gelatin/1, 5, 7.5, and 10 wt% HCC against *E. coli*, (b) PLGA/gelatin and PLGA/gelatin/1, 5, 7.5, and 10 wt% HCC against *S. aureus*.

Table 3. Zone inhibition diameter of PLGA/gelatin membranes with and without HCC.

	Escherichia coli	*Staphylococcus aureus*
PLGA/gelatin	–	–
PLGA/gelatin/1 wt% HCC	–	–
PLGA/gelatin/5 wt% HCC	10 mm	10 mm
PLGA/gelatin/7.5 wt% HCC	10 mm	11 mm
PLGA/gelatin/10 wt% HCC	12 mm	12 mm
Ampicillin (10 mcg) disk	25 mm	25 mm

cereus, *S. aureus*, and *Clostridium perfringens*. However, water extract only showed antibacterial activity against *S. aureus*. In addition to the literature information on the antibacterial properties of HCC, according to our GC MS result, the compounds in the HCC extract, such as 2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one, benzoic acid, catechol, 1H-Benzotriazole, 4-Methyl-1H-benzotriazole, Butyraldehyde semicarbazone, enhance the antibacterial properties to extract[58–60]. This result shows that the addition of HCC to the membranes provides antibacterial properties against *E. coli* and *S. aureus*.

3.10. Cell viability assay

Human fibroblast cells were seeded onto electrospun PLGA/gelatin membranes with and without HCC, and cell viability was evaluated using the MTS assay. Briefly, 10^4 cells were seeded onto membranes in 96 well plates and wells without membrane were selected as a control group. According to the results of the MTS for 24 h and 48 h, membranes with and without HCC had no toxic effect, and cell viability was observed above 90%, which approves the biocompatibility of the membranes (Figure 11). In the

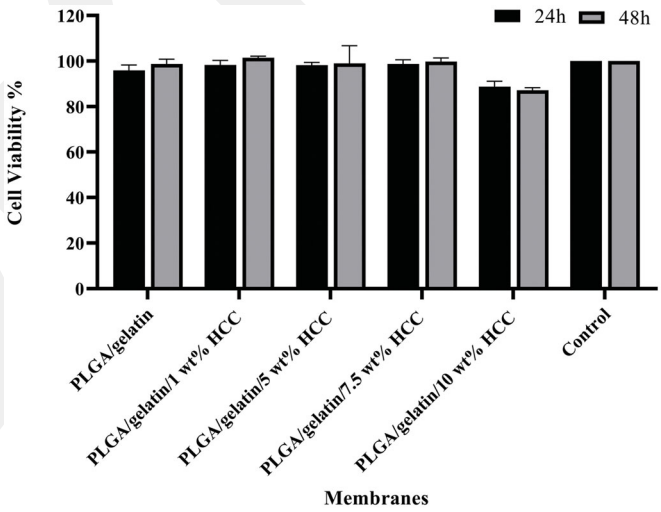

Figure 11. Cell viability (%) of PLGA/gelatin membranes with and without HCC.

literature, it was shown that PLGA/gelatin membranes do not cause toxicity on fibroblast cells[11, 21, 24].

3.11. Cell attachment assay

Attachment of human fibroblasts onto electrospun PLGA/gelatin membranes with and without HCC was shown using DAPI staining and SEM analysis in Figures 12 and 13, respectively. The nuclear was stained with DAPI and visualized by Fluorescence microscopy. According to Figure 12, it was shown that cell nuclei present on the membrane. Additionally, HCC adding to the membrane increased the number of cells attached onto the membrane in pre-determined time period indicating that the cells adhered to the membrane and continued to grow. FT-IR and contact angle

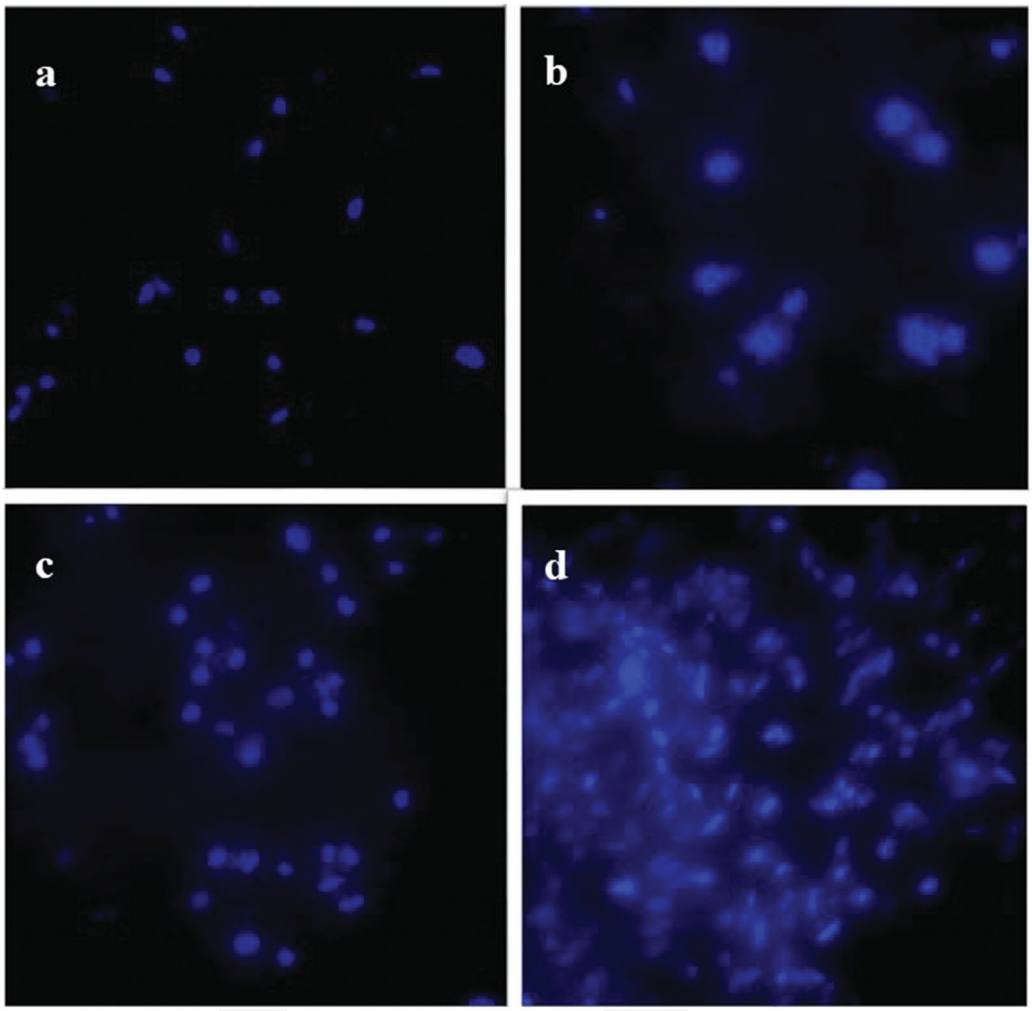

Figure 12. DAPI staining of (a) PLGA/gelatin at 24 h, (b) PLGA/gelatin at 48 h, (c) PLGA/gelatin/7.5 wt% HCC at 24 h, and (d) PLGA/gelatin/7.5 wt% HCC at 48 h.

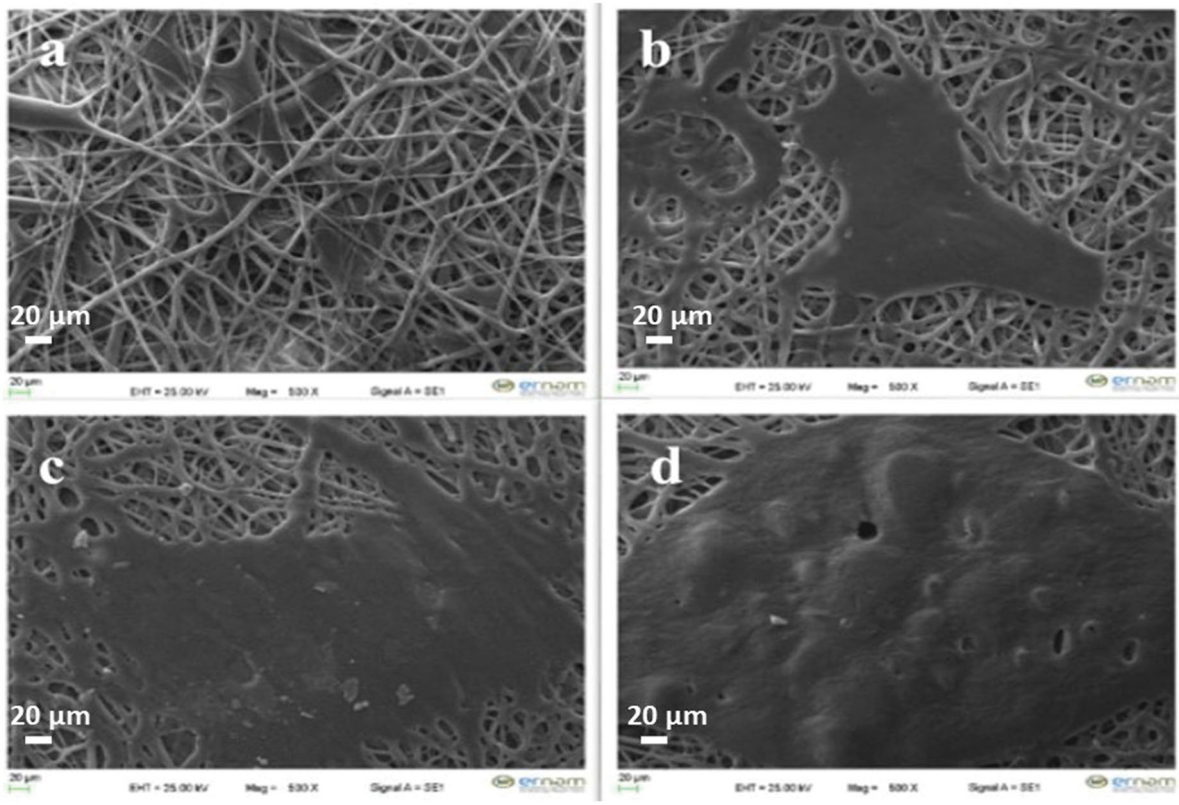

Figure 13. SEM images of (a) PLGA/gelatin at 24 h, (b) PLGA/gelatin at 48 h, (c) PLGA/gelatin/7.5 wt% HCC at 24 h, and (d) PLGA/gelatin/7.5 wt% HCC at 48 h.

results provide the addition of HCC to the membrane increased the hydrophilicity of the membrane. Kim et al.[61] showed that increasing membrane hydrophilicity improves cell attachment on the membrane. Yadollah-Damavandi et al.[57] used *H. perforatum* gel consisting of 5 wt% and 10 wt% extract and showed that the numerical fibroblast density and collagen volume density increased depending on the percentage of *H. perforatum*, which demonstrate that *H. perforatum* has an inducing effect on fibroblast proliferation. Also, Öztürk et al.[62] showed that *Hypericum* species wound healing mechanism might be related to fibroblast migration and simulation of collagen synthesis. Additionally, Rao et al.[16] showed that pro healing activity of *Hypericum* spp increasing wound contraction rate and granulation tissue breaking, which results in enhanced epithelialization. The results approve that the addition of HCC to the membrane increases cell binding and biocompatibility.

4. Conclusions

In this study, we prepared antibacterial PLGA/gelatin membranes containing HCC, a traditional medicinal herbal, by the electrospinning technique. We first extracted HCC and analyzed the chemical composition with GC-MS. After the extraction phase, we prepared PLGA/gelatin blend solutions (9:1, v/v) containing different amounts of HCC extract (1, 5, 7.5, 10 wt%) and electrospun the solutions to obtain membranes with a bead-free and uniform fiber structure. According to SEM images, membranes without HCC had bead free and uniform fiber structure. The average fiber diameters of neat membrane and HCC containing membranes were measured as 1.83 ± 1.02 µm (neat), 1.05 ± 0.56 µm (1 wt%), 0.7 ± 0.29 µm (5 wt%), 0.51 ± 0.11 µm (7.5 wt%), 0.49 ± 0.2 µm (10 wt%), respectively, which shows that an increase in HCC concentration resulted in a reduction in fiber diameter. Membrane without HCC was the highest contact angle value with 113.72° and adding HCC to the membrane reduced contact angle value, resulting in increased membrane hydrophilicity. The release profile of the extract in the electrospun membranes was followed by detecting hypericin content, and 80% HCC was released in the first 24 h. The degradation ratio of HCC containing membrane was higher than the neat membrane because the release of HCC from the membrane caused a decrease of membrane weight and the degradation rates of membranes with and without HCC were 13.7% and 30.4%, respectively. The antibacterial activity of electrospun membranes against *S. aureus* and *E. coli* was tested by using Kirby-Bauer disk diffusion susceptibility test and membranes with a ratio of 5 wt% and above showed antibacterial properties. The biocompatibility of electrospun PLGA/gelatin and HCC extract containing electrospun PLGA/gelatin membranes were evaluated by *in vitro* cell attachment and proliferation studies and membrane with HCC and without HCC did not exhibit a toxic effect on human fibroblast cell and addition of HCC to the membrane increased cell adhesion. According to all results, the membrane containing HCC is promising material as a wound dressing material.

Acknowledgments

The authors would like to thank Hacettepe University Advanced Technologies Research and Application Center (HUNİTEK), Ankara, for their help in the gas chromatographic and mass spectrometric measurements.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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