



Porous electrospun poly(ϵ -caprolactone)/gelatin nanofibrous mat containing cinnamon for wound healing application: in vitro and in vivo study

Majid Salehi^{1,2} · Maryam Niyakan³ · Arian Ehterami⁴ · Saeed Haghi-Daredeh³ · Simin Nazarnezhad¹ · Ghasem Abbaszadeh-Goudarzi^{2,5} · Ahmad Vaez⁶ · Seyedeh Fatemeh Hashemi³ · Nariman Rezaei¹ · Seyed Reza Mousavi¹

Received: 11 July 2019 / Revised: 30 October 2019 / Accepted: 6 November 2019 / Published online: 18 November 2019
© Korean Society of Medical and Biological Engineering 2019

Abstract

In this study, cinnamon (cin) was loaded into poly(ϵ -caprolactone)/gelatin (PCL/Gel) nanofibrous matrices in order to fabricate an appropriate mat to improve wound healing. Mats were fabricated from PCL/COLL [1:1 (w/w)] solution with 1, 5 and 25% (w/v) of cinnamon. Prepared mats were characterized with regard to their microstructure, mechanical properties, porosity, surface wettability, water-uptake capacity, water vapor permeability, blood compatibility, microbial penetration and cellular response. The fabricated mats with and without cinnamon were used to treat the full-thickness excisional wounds in Wistar rats. The results indicated that the amount of cinnamon had a direct effect on porosity, mechanical properties, water uptake capacity, water contact angle, water vapor transmission rate and cell proliferation. In addition, the results of in vivo study indicated that after 14 days, the wounds which were treated with PCL/Gel 5%cin had better wound closure (98%) among other groups. Our results suggest that the cinnamon can be used as a suitable material for wound healing.

Keywords Cinnamon · Electrospinning · Gelatin · Poly(ϵ -caprolactone) · Mat

✉ Majid Salehi
Msalehi.te1392@gmail.com

Maryam Niyakan
qom.astane@yahoo.com

Arian Ehterami
arian.ehterami@srbiau.ac.ir

Saeed Haghi-Daredeh
haghi.s@shmu.ac.ir

Simin Nazarnezhad
smn.nazarnezhad@yahoo.com

Ghasem Abbaszadeh-Goudarzi
abbaszadehghasem@gmail.com

Ahmad Vaez
ahmadvaez@yahoo.com

Seyedeh Fatemeh Hashemi
fatemeh.hashemi71@gmail.com

Nariman Rezaei
nariman.rezaei67@gmail.com

Seyed Reza Mousavi
reza_mousavi91@yahoo.com

¹ Department of Tissue Engineering, School of Medicine, Shahrood University of Medical Sciences, Shahrood, Iran

² Tissue Engineering and Stem Cells Research Center, Shahrood University of Medical Sciences, Shahrood, Iran

³ Department of Medical Nanotechnology, School of Medicine, Shahrood University of Medical Sciences, Shahrood, Iran

⁴ Department of Mechanical and Aerospace Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran

⁵ Department of Medical Biotechnology, School of Medicine, Shahrood University of Medical Sciences, Shahrood, Iran

⁶ Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran

1 Introduction

It is as clear as crystal that skin is the largest organ in mammals and serves as a protective obstacle between the human body and the surrounding environment. It protects the underlying organs and guards the body as contrary to pathogens and microorganisms [1]. Wounds disrupt the skin function that may lead to water loss and fatal infections. Even though, the inherent healing ability of skin can regenerate a large area of this tissue. However in the vast injuries case, without appropriate treatment, satisfactory wound closure cannot be achieved [2]. A suitable wound dressing should provide appropriate properties such as maintain a moist environment in wound site, allow gas exchange between environment and wounded tissue and enhance epidermal migration [3].

Various types of wound dressings from decellularized membranes to hydrogels, foams, and sponges are used to treat wounds in clinic [4, 5]. Recently, nanofibrous structures because of their beneficial effects on the regeneration of different tissues have widely used [6, 7]. The nanofibers have high similarity to native extracellular matrix structure. Among different biomaterials were used for fabrication nanofibers, Polycaprolactone (PCL), because of its properties like non-toxic nature, high tensile strength, low melting point, and biodegradability are widely used for biomedical applications [8]. However, using PCL in tissue engineering is limited because of high hydrophobicity, lack of functional groups and neutral charge [9]. So, blending with other polymers with desired characteristics can decrease disadvantages of PCL. Gelatin is a protein that contains high contents of proline, glycine, and hydroxyproline [10]. Gelatin has been widely used as a wound dressing and as an absorbent pad during surgery [11]. The PCL/Gel scaffold has appropriate mechanical strength because of PCL, and good cell attachment, proliferation, and biodegradation because of gelatin [12, 13].

To improve wound healing process, researchers incorporation different kinds of bioactive agents in wound dressing. Cinnamon (Cin) has been used for a long time as a spice [14] and it was derived from a Greek word that means sweet wood, achieves from the inner bark of tropical evergreen cinnamon trees [15]. Cinnamon shows different biological functions such as anti-oxidant, anti-diabetic, anti-inflammatory, antibacterial, and anti-tumor effects. In addition, cinnamon is rich in essential oils and tannins which decrease microbial growth [16, 17].

All in all, previous studies indicated that due to favorable properties of PCL/gelatin mentioned above, it is widely used as a wound dressing [13, 18]. But to the best of our knowledge, we couldn't find electrospun nanofibers loaded with cinnamon for treating full thickness wound.

Therefore, PCL/Gel nanofibers loaded with cinnamon might be unique for wound healing application. In this study, the PCL/Gel nanofibrous mat containing different concentrations of cinnamon were developed by the electrospinning method and its properties were evaluated with different *in vivo* tests and the effect of them on the healing of skin injury in a rat model was evaluated.

2 Materials and methods

2.1 Chemicals

The materials and solvents were bought from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, USA) respectively unless otherwise noted.

2.2 Fabrication of PCL/gelatin fibrous mats containing cinnamon via electrospinning

Poly(ϵ -caprolactone) [PCL; $M_w = 48\text{--}90$ kDa] and gelatin (bovine skin, type B) with the same concentrations of 10% (w/v) were dissolved in acetic acid. The gelatin solution and the PCL solution were combined at the weight ratio of 1:1 and stirred for 24 h. The cinnamon (Sigma-Aldrich, W229210) was added into the PCL/Gel solution at the concentrations of 1, 5 and 25% weight of polymers and stirred at room temperature for 6 h. The solution was shifted to a 10 mL disposable syringe ending to an 18-gauge stainless steel needle. The syringe was placed into a syringe pump (Fanavaran Nano-Meghyas, Tehran, Iran) and the solution was fed at the rate of 0.4 mL/h. Electrospinning was started by applying a positive high voltage (20 kV) through a high voltage source (Fanavaran Nano-Meghyas, Tehran, Iran) between the mandrel and the needle. Modified 15 cm gap between collector and the needle tip was set at. For cross-linking fabricated mats, vapor of 10% (w/v) glutaraldehyde (Sigma-Aldrich, St. Louis, USA) was used for 16 h and after that completely washed with distilled water and dried at room temperature.

2.3 Characterization of fabricated mats

2.3.1 Scanning electron microscopy

The microstructures of the prepared mats were observed under a scanning electron microscope (SEM). For the beginning, a sputter coater (KYKY Technology Development, Beijing, China) was used to coat mats with gold for 250 s and then the coated mats were observed with SEM (KYKY Technology Development, Beijing, China) at an accelerating voltage of 30 kV.

2.3.2 Mechanical test

The tensile strength of the mats was investigated via a uni-axial tensile testing device (Santam, Karaj, Iran) with a 10 N load cell and extension rate of 1 mm/min. For this test, the samples were cut into 1 cm in width and 4 cm in length.

2.3.3 Porosity assessment

To calculate the percentage of the porosity of the fabricated mats, the liquid displacement technique and Eq. 1 was used [19];

$$\text{Porosity (\%)} = \frac{v1 - v3}{v2 - v3} \times 100 \quad (1)$$

In this formula, V1 and V2 are the initial volume of ethanol (96%) and the volume after immersing the mat respectively. In addition, V3 is the volume of the ethanol after the mat removed (after 10 min).

2.3.4 Water uptake capacity

Equation 2 was used to investigate the water-uptake capacity of the mats [20].

$$\text{Water Uptake (\%)} = \frac{W1 - W0}{W0} \times 100 \quad (2)$$

In Eq. 2, W0 is the weight of dry samples and W1 is the weight of samples after immersion in distilled water at room temperature for 24 h. The average value of three samples for each dressing was reported.

2.3.5 Water vapour permeability (WVP)

To measure WVP of the fabricated mats, the flexible bottles permeation test (Systech, UK) was used. The bottles were capped with the dressings and incubated at 37 °C for 12 h and the mass of water lost through the dressing was measured. WVP was calculated at steady-state using Eq. 3;

$$\text{WVP} = \frac{W}{AT} \quad (3)$$

Where W indicates the mass of water lost, A stands for the area (1.18 cm²) and T is the exposure time [20].

2.3.6 Contact angle measurement

The contact angle test was used to evaluate the degree of hydrophilicity of fabricated mats. For this purpose, a static contact angle measuring device (KRUSS, Hamburg, Germany) was used and a water droplet was poured onto 3 parts

of each sample and the amount of angle formed between the water droplet and the surface of each dressing was averaged and reported.

2.3.7 Weight loss measurement

For weight loss measurement, the samples were cut into square shape with dimension of 2 × 2 × 5 cm and were immersed in 10 mL distilled water. The weight loss was calculated by using Eq. 4 [21].

$$\text{Weight loss} = \frac{W0 - W1}{W0} \times 100 \quad (4)$$

In the above formula, W0 and W1 are the initial weight of samples and the dry weight after removing from the media, respectively.

2.3.8 FTIR analysis

FTIR spectroscopy was used to characterize the presence of Cinnamon in the fabricated mats. For this purpose, FTIR spectrometer (ABB Bomem, Quebec, Canada) was used and IR spectra were recorded in the 400–4000 cm⁻¹ range with a resolution of 4 cm⁻¹.

2.3.9 Blood compatibility or hemolysis assay

Human blood was used to evaluate the compatibility of the mats. Whole human blood was anticoagulated and diluted with normal saline. 200 µl of blood samples were poured onto the mats and incubated at 37 °C for 60 min and after that centrifuged at 1500 rpm for 10 min. The supernatants were transferred to a 96-well plate and a BioTek Synergy 2 Multi-Mode Microplate Reader at 545 nm was used to read the absorbance values of each samples. Blood diluted in deionized water was considered as positive control while blood diluted in normal saline was considered as negative control. Hemolysis degree was calculated as Eq. 5 [22]:

$$\text{Hemolysis\%} = \frac{Dt - Dnc}{Dpc - Dnc} \times 100 \quad (5)$$

where Dt indicates the absorbance of the sample, Dnc is the absorbance of the negative control, Dpc is the absorbance of the positive control.

2.3.10 Microbial penetration

To evaluate capability of the mats to withstand against microbial penetration, each dressing was placed in 10 ml vials consisted 5 ml of Brain heart infusion (BHI) broth (Merck, Germany) (test area: 0.8 cm²). Bottles covered with the cotton ball consider as negative control and open

vials served as positive control. The tested vials were kept at ambient conditions for 3 and 7 days. The cloudiness of the mixture in any vial was indicative of microbial contamination. Spectrophotometric measurements were obtained at 600 nm in a microplate spectrophotometer ($n=3$).

2.3.11 Cell culture studies

The 3T3 mouse fibroblast cell line was cultured in the Dulbecco's modified Eagle's medium: nutrient mixture F-12 (DMEM/F12; Gibco, Grand Island, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco, Grand Island, USA), 100 unit/mL of penicillin and 100 $\mu\text{g}/\text{mL}$ of streptomycin in a humidified incubator at 37 °C with 5% CO_2 . The media was refreshed every 24 h. The mats were cut into appropriate sizes and then put into each well of a 96-well plate. The ultraviolet light (254 nm) irradiation was used for 30 min to sterilize both side of prepared mats in a laminar flow hood. The mats washed twice with PBS and once with DMEM/F12 and then 1×10^4 of 3T3 mouse fibroblast cell line seeded. To quantitatively assess the activity of cells cultured on the mats, the 3-(4,5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) according to a method as described previously was used [23]. The microplate-reader (Palm City, USA) at 570 nm was used to read the absorbance values of each samples ($n=3$).

2.3.12 In vivo wound healing study

A full-thickness excisional wound model was used to evaluate the healing capability of the fabricated mats. Animal experiments were approved by the ethics committee of Shahrood University of Medical Sciences and were carried out in accordance with the university's guidelines. Thirty healthy adult male Wistar rats (3 months old, weighing 250–270 g) were bought from Pasteur Institute, Tehran, Iran. For wound model creation, the animals were anesthetized by intraperitoneally injection of Ketamine 5%/Xylazine 2% (70 mg ketamine and 6 mg Xylazine/1 kg body weight). Their back was shaved and disinfected with ethanol and a full-thickness $1.5 \times 1.5 \text{ cm}^2$ was excised using a scalpel blade (Fig. 1). The animals were divided into five groups (6 rats per group) and the wounds were treated with the PCL/Gel, PCL/Gel/1%cin, PCL/Gel/5%cin and PCL/Gel/25%cin, and the sterile gauze as the negative control. The macroscopic changes in the wound site were recorded by a digital camera (Canon Inc., Tokyo, Japan) at 7 and 14 days post-wounding to evaluate the extent of wound closure. The pictures evaluated by using an image analyzing program (Digimizer, Ostend, Belgium). Wound closure was calculated by using Eq. 6 [24].



Fig. 1 A full-thickness $1.5 \times 1.5 \text{ cm}^2$ wound healing on the back of the Wistar rat

$$\text{Wound Closure (\%)} = \left(1 - \frac{\text{open wound area}}{\text{Initial wound area}} \right) \times 100 \quad (6)$$

14 days after post-wounding day, the animals were sacrificed by ketamine overdose injection and the wound tissue was harvested and fixed in 10% buffered formalin. For histopathological examinations, the specimens were processed and embedded in paraffin, sectioned, and stained with hematoxylin–eosin (H&E) and Masson's trichrome (MT). The prepared samples were examined under a light microscope (Carl Zeiss, Thornwood, USA) with a digital camera (Olympus, Tokyo, Japan) and photographed at $\times 40$, $\times 100$ and $\times 400$ magnifications.

2.4 Statistical analysis

The Origin Pro software (Version9, OriginLab, Northampton, MA) using a statistic on rows and two-sample t test on rows was used to statistically analyze the results and the data were expressed as a mean \pm standard deviation. In all of the evaluations, $p < .05$ was considered statistically significant.

3 Results

3.1 Morphology

Figure 2 shows the SEM micrographs of the fabricated mats. The nanofibers in all groups were dispersive manner,

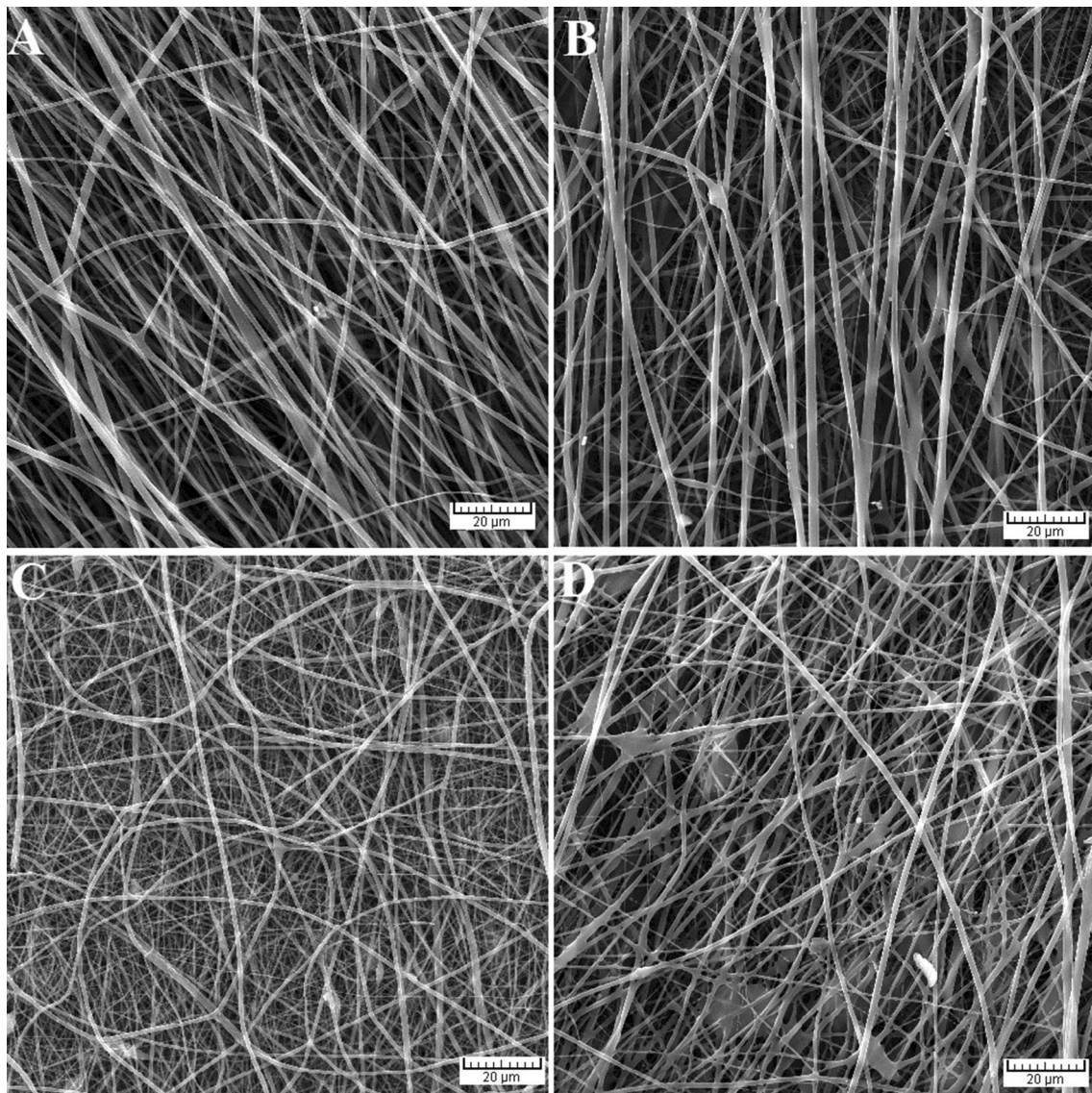


Fig. 2 Morphology of the wound dressings; **a** PCL/Gel, **b** PCL/Gel/1%cin, **c** PCL/Gel/5%cin and **d** PCL/Gel/25%cin

oriented randomly and forming a non-woven porous structure. The fibers diameter was evaluated by using Fiji/ImageJ (National Institutes of Health, Bethesda, USA) by analyzing 20 random points per image and the results were indicated in Table 1. Adding cinnamon significantly ($p < 0.005$) increased the average diameter of fibers from 891 ± 64 nm to 984 ± 97 nm.

3.2 Tensile strength

Enough tensile strength is necessary for wound mats to tolerate forces exerted during application [25]. Table 1 indicated that by increasing the concentration of cinnamon, the

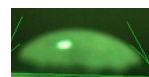
ultimate tensile strength decreased. The tensile strength of PCL/Gel was 2.83 ± 0.8 MPa while it was 2.23 ± 0.6 MPa for PCL/Gel/25%cin. The results indicated that the values of the ultimate tensile strength were not statistically significant.

3.3 Porosity

The porosity of the different groups was evaluated based on the liquid displacement method and the results were indicated in Table 1. The results showed that the porosity of all mats is high enough ($> 65\%$) to be used as a wound dressing [26]. Moreover, Table 1 revealed that by increasing the

Table 1 Characterization of fabricated mats

Samples	Fiber diameter (nm)	Tensile strength (MPa)	Porosity (%)	Water uptake capacity (%)	WVP (mg/cm ² h)	Contact angle (°)	Weight loss (after 2 weeks)
PCL/Gel	891 ± 64	2.83 ± 0.8	71.16 ± 1.04	12.86 ± 0.32%	9.23 ± 1.03%	53.19 ± 4.06	35.11 ± 2.36%
PCL/Gel/1in	914 ± 62	2.74 ± 0.1	73.2 ± 3.2	13.63 ± 0.15%	10.07 ± 3.01%	56.18 ± 2.03	36.2 ± 1.34%
PCL/Gel/5%cin	943 ± 116	2.67 ± 0.4	75.6 ± 5.4	13.91 ± 0.5%	11.2 ± 1.5%	57.33 ± 1.12	36.93 ± 1.09%
PCL/Gel/25%cin	984 ± 97	2.23 ± 0.6	76.8 ± 3.1	14.42 ± 0.6%	12.3 ± 3.8%	58.73 ± 1.16	38.89 ± 3.69%
Open container					29.76 ± 4.01%		



percentage of cinnamon, the porosity increased too. However, the differences between porosity values are not statistically significant.

3.3.1 Water uptake capacity and WVP

The water uptake capacity shows the capacity of the mat to absorb wound exudates [27]. The results of water-uptake capacity of the fabricated mats were demonstrated in Table 1. The PCL/Gel mat showed the water-uptake capacity of 12.86 ± 0.32% and by adding cinnamon to the mat, the water-uptake capacity was increased. For PCL/Gel/1%cin, PCL/Gel/5%cin and PCL/Gel/25%cin the water uptake capacity was 13.63 ± 0.15%, 13.91 ± 0.5%, 14.42 ± 0.6%.

Previous study indicated that an appropriate wound dressing should have ability to control the gas exchange to improve wound healing process. High WVP increasing the speed of dehydration in the wound site and consequently causes scar formation [28]. On the other hand, the low WVP makes the deposition of exudates and it makes delays to wound healing process and therefore, predisposes the wound for infection [29]. As can be seen from Table 1, by adding

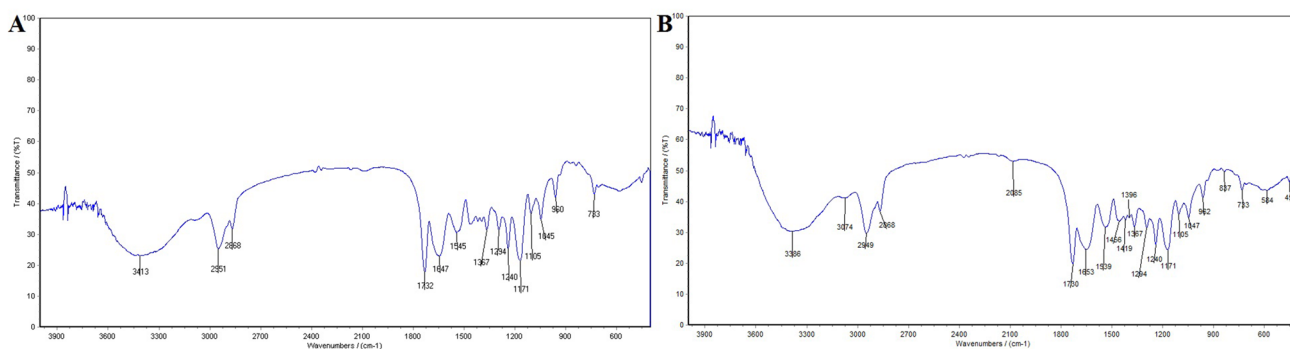
cinnamon to the mats, WVP increased. It is due to the percentage of porosity in the mat containing cinnamon. The differences between the values of Water uptake capacity and WVP of the mats were not statistically significant.

3.4 Wettability

The water contact angles for the PCL/Gel and PCL/Gel/25%cin mats were 53.19 ± 4.06° and 58.73 ± 2.16° respectively (Table 1). As Cinnamon is a hydrophobicity material, by adding it to the fabricated mats, the contact angle increased [30]. The differences between the contact angle results were not statistically significant.

3.5 Degradation studies

The weight loss results over the period of 2 weeks experiment are reported in Table 1. As cinnamon is a degradable material [31], by increasing the amount of cinnamon in the prepared mats, the degradation rates increased but differences were not statistically significant.

**Fig. 3** FTIR spectra of **a** PCL/Gel and **b** PCL/Gel/25%cin

3.6 Microstructure characterization of prepared mat

Figure 3 shows the FTIR spectra of fabricated mat. The band observed at 2951 cm^{-1} (asymmetric CH₂ stretching), 2868 cm^{-1} (symmetric CH₂ stretching), 1732 cm^{-1} (carbonyl stretching), 1294 cm^{-1} (C–O and C–C stretching) and 1240 cm^{-1} (asymmetric COC stretching) [32]. Common bands of protein appeared at approximately 1647 cm^{-1} (amide I) and 1545 cm^{-1} (amide II), corresponding to the stretching vibrations of C=O bond, and coupling of bending of N–H bond and stretching of C–N bonds, respectively. The amide I band at 1647 cm^{-1} was attributable to both a random coil and α -helix conformation of gelatin [33]. The peaks at 1171 cm^{-1} and 1105 cm^{-1} are attributed to the stretching vibrations of C–O and the C–OH deformation vibration. The peak at 960 cm^{-1} is assigned to the C–H bending vibration absorption, and the peak at 733 cm^{-1} is assigned to benzene rings CH vibration absorption. The peak at 1456 cm^{-1} is very characteristic for an alcohol C–OH within the bending vibration absorption. The peak at 1456.1419 and 584 cm^{-1} corresponds to the vibration absorption of alkenes.

3.7 Blood compatibility or hemolysis assay

Hemolysis directly related to the blood compatibility of materials and it shows the release of hemoglobin into plasma due to damage of erythrocytes. Hemolysis rate of all mats were less than positive control, and this difference is significant (Fig. 4a). The hemolysis rate was increased by adding cinnamon to the fabricated mat but the differences were not significant.

3.8 Microbial penetration through the wound dressings

The antimicrobial nature of the wound dressing prevents wound site from wound infection. The results of microbial penetration through the mats are shown in Fig. 4b. In the test tubes containing BHI broth that was capped with the mats containing cinnamon, the optical density of the microbial contamination was higher but not significant

than PCL/Gel mat and lower than negative control (BHI broth in a glass test tube sealed with an airtight cap) after 3 and 7 days of starting experiment. The optical densities of the BHI broth in the positive control was significantly higher than all mats and negative control ($p < 0.05$).

3.9 In vitro and in vivo study

The MTT assay was performed to assess the cytocompatibility of mats towards the 3T3 cells and the results are shown in Fig. 4c. PCL/Gel/5%cin had higher absorbance values among other groups after 24 h and 72 h. While PCL/Gel/25%cin, because of the hydrophobicity and cytotoxicity of the cinnamon, the absorbance decreased significantly ($p < 0.05$).

Figure 5a demonstrates the macroscopic appearance of the wounds covered with the sterile gauze (negative control), PCL/Gel, PCL/Gel/1%cin, PCL/Gel/5%cin and PCL/Gel/25%cin mats 7 and 14 days after surgery. The photographic evaluation indicated that there was a sign of inflammation in the negative group while, there was no sign of inflammation or infection in other groups. Moreover, after 2 weeks, the wound site in the control group were still hemorrhagic whereas the groups covered with mats specially PCL/Gel/5%cin became better at the same time. In addition, to quantify the wound-healing process, the wound closure was determined (Fig. 5b). The negative control had the wound closure of $30.1 \pm 2.47\%$ and 53.4 ± 2.29 at 7 and 14 days after wounding, respectively. By covering wounds with mats, the wound closure increase. These values for PCL/Gel, PCL/Gel/1%cin, PCL/Gel/5%cin and PCL/Gel/25%cin after 7 days were 41.06 ± 4.32 , 45.5 ± 1.66 , 70.12 ± 6.21 and 65.54 ± 2.78 and after 14 days were 57.2 ± 0.47 , 63.16 ± 3.14 , 98.13 ± 2.35 and 79.38 ± 4.11 respectively. The wound size reduction in PCL/Gel/5%cin group was significantly higher than other mats and the control group in each time interval ($p < 0.05$).

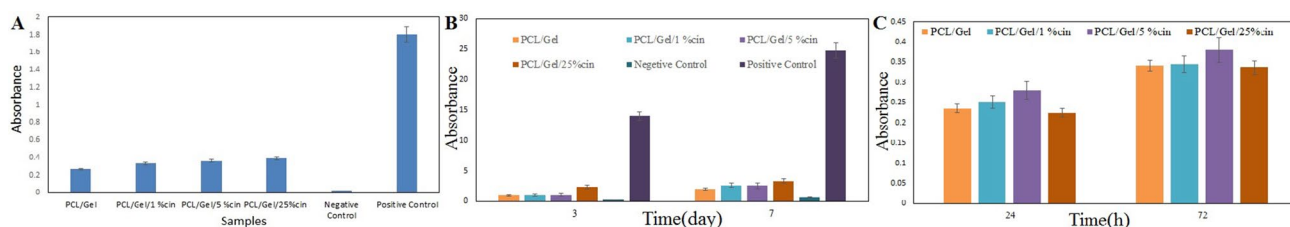


Fig. 4 a Blood compatibility histogram of the mats, b the extent of microbial penetration through the mats and c MTT assay histogram after 24 and 72 h of cell seeding

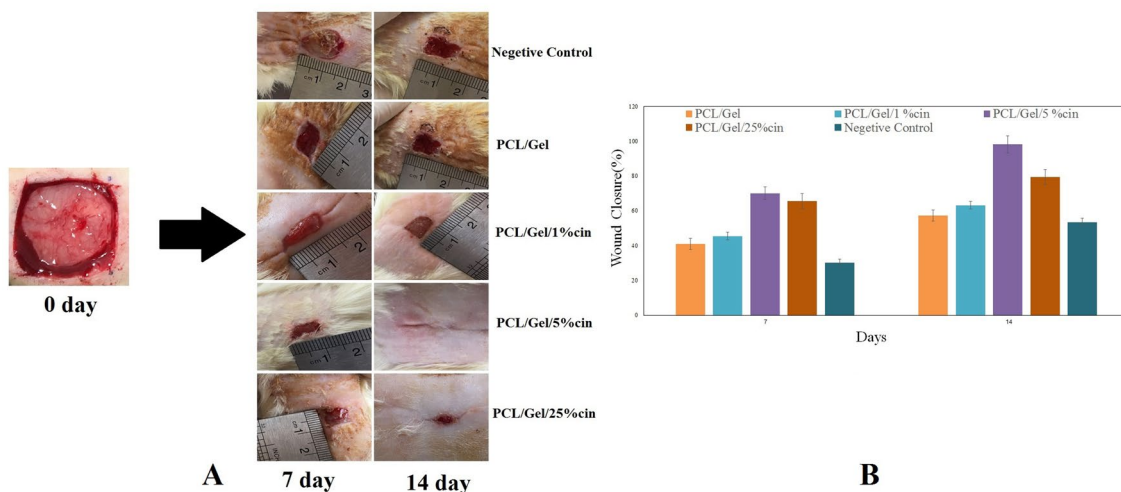


Fig. 5 In vivo wound-healing results: **a** macroscopic appearances of the wounds treated with the sterile gauze, PCL/Gel, PCL/Gel/1%cin, PCL/Gel/5%cin, PCL/Gel/25%cin mat 7 and 14 post-wounding and **b**

histogram comparing the wound closure percentages of experimental groups at the end of 7th and 14th days post-wounding

3.10 Histopathological results

Histopathological findings of positive and negative control groups are illustrated in Fig. 6. In the PCL/gelatin treated group, a thick layer of crusty scab covered the wound (Fig. 6, asteroid), and several PMN cells infiltrated to the wound area (Fig. 6, thin arrows). PCL/Gel/1%cin treated group showed the initiation of epidermal proliferation (Fig. 7, thick arrow). The regeneration of wounds in PCL/Gel/25%cin treated animals was better than those of PCL/gelatin and PCL/Gel/1%cin treated groups, the epidermal layer was completely formed and remodeled (Fig. 6, thick arrow). Among all groups, PCL/Gel/5%cin group showed more resemblance to normal skin tissue (positive control) with a rejuvenation of hair follicles, sebaceous glands and typical epidermis (Fig. 6, thick arrow).

The histological findings of Masson's trichrome (MT) stained sections indicated that PCL/Gel/1%cin group had the greatest collagen fiber synthesis, maturation, and arrangement among other groups (Fig. 7). On the other hand, the minimum rate of collagen fiber synthesis and deposition was in PCL/gelatin and negative control groups.

3.11 Histomorphometric analysis

Histomorphometric assessment for experimental groups has been presented in Fig. 8. PCL/Gel/5%cin group had the best re-epithelialization ($p < 0.01$). Blood vessels in PCL/Gel/1%cin group treated animals were significantly higher than the negative control, PCL/gelatin, and PCL/Gel/25%cin groups ($p < 0.05$). Moreover, the best results for regeneration was observed in the PCL/gelatin/5%cin.

4 Discussion

In the current study, PCL/Gel mats were fabricated by electrospinning method with different amounts of cinnamon for wound healing. Wound healing is a biological process which contains four sequential stages after the wound occurred: hemostasis, inflammation, proliferation and maturation [34]. Various studies evaluated different wound dressings to improve this process. The selection of wound dressings depends on the type of wound and on the patient's need for recovery; hence, different types of wound dressings have emerged. As an ideal wound dressing should have a structure that resembles the natural skin's structure, and nanofibers have ability to mimic a natural nanometer dimension of the tissue, due to their physical features, are able to mimic the healthy extracellular matrix network found in the skin [35]. In general, using nanofiber as wound dressing has several advantages; As nanofibers has appropriate biological features, some physical features such as small interstices and a large surface area to volume ratio, allow for enhanced hemostasis at the injury site, and therefore, this mat may substitute for the addition of hemostatic agents which would have adverse effects in the body [36]. Moreover, nanofiber mats can not only absorption of exudates from the wound but also keep the wound site moisty [37]. In addition, different drugs can be loaded in nanofibers to improve wound healing. Previous study indicated that the PCL/Gel have capability to overcome the shortage of natural and synthetic polymers. It is an appropriate biomaterial with good biocompatibility, physical and chemical properties [38].

Porosity and mechanical properties are two important factors that should consider in designing and fabricating a suitable wound dressing [39]. Porosity is critical for oxygen

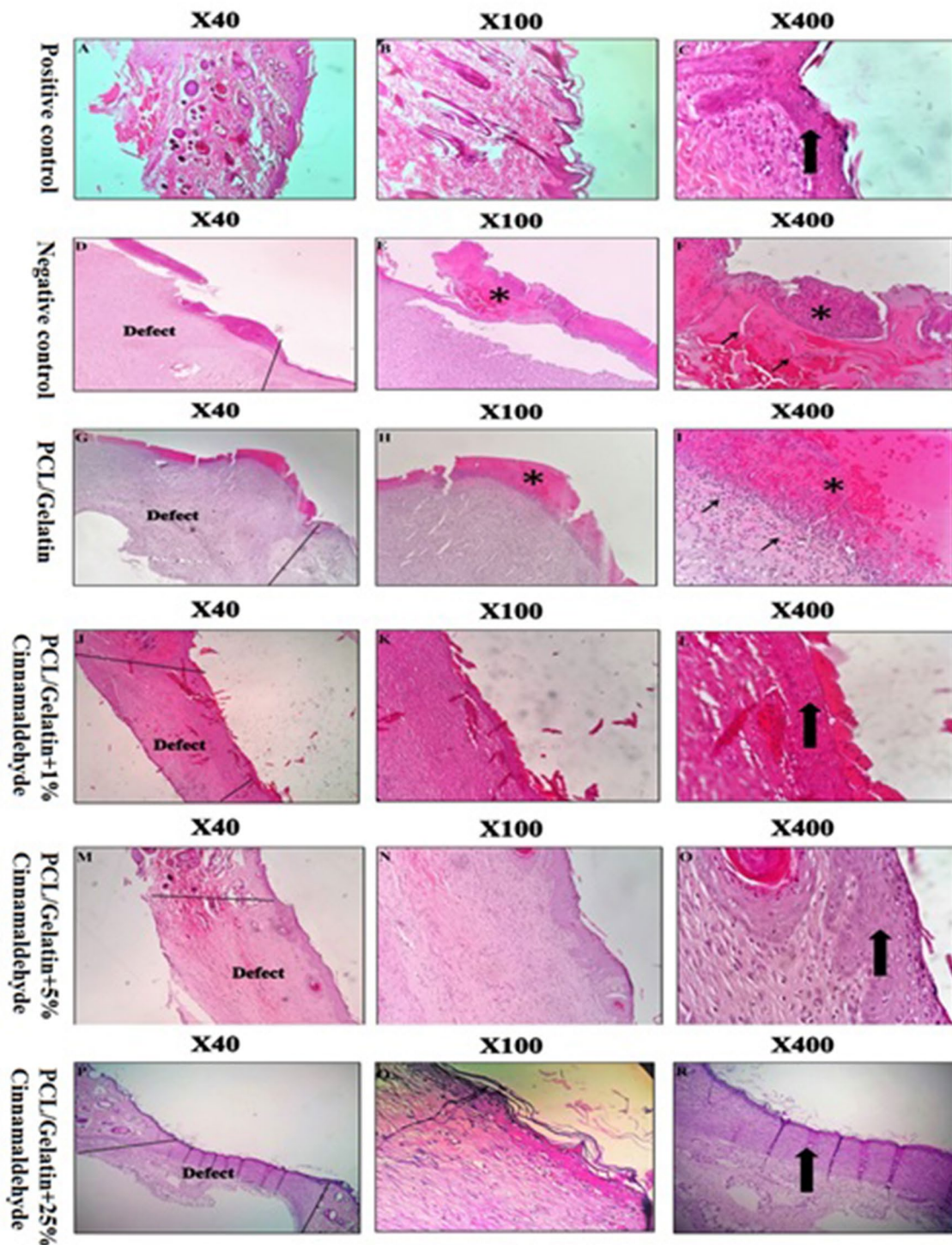


Fig. 6 H&E stained microscopic sections of healed incisions in rats at 14 days, *D* defect

and nutrient transport into the wound site, and the mechanical properties are necessary for the mats to tolerate the forces during surgical operations, physiological activities etc. [40, 41]. The best range for the porosity of wound dressing is 60–90% [42]. However, the porosity of fabricated PCL/

Gel nanofibrous mats in this study is around 70–80%. The mechanical strength of mat are completely related to the porosity and the formation of fibers [43]. So, the high porosity and random fibers made very low mechanical properties [44]. By increasing cinnamon content from 1 to 25%,

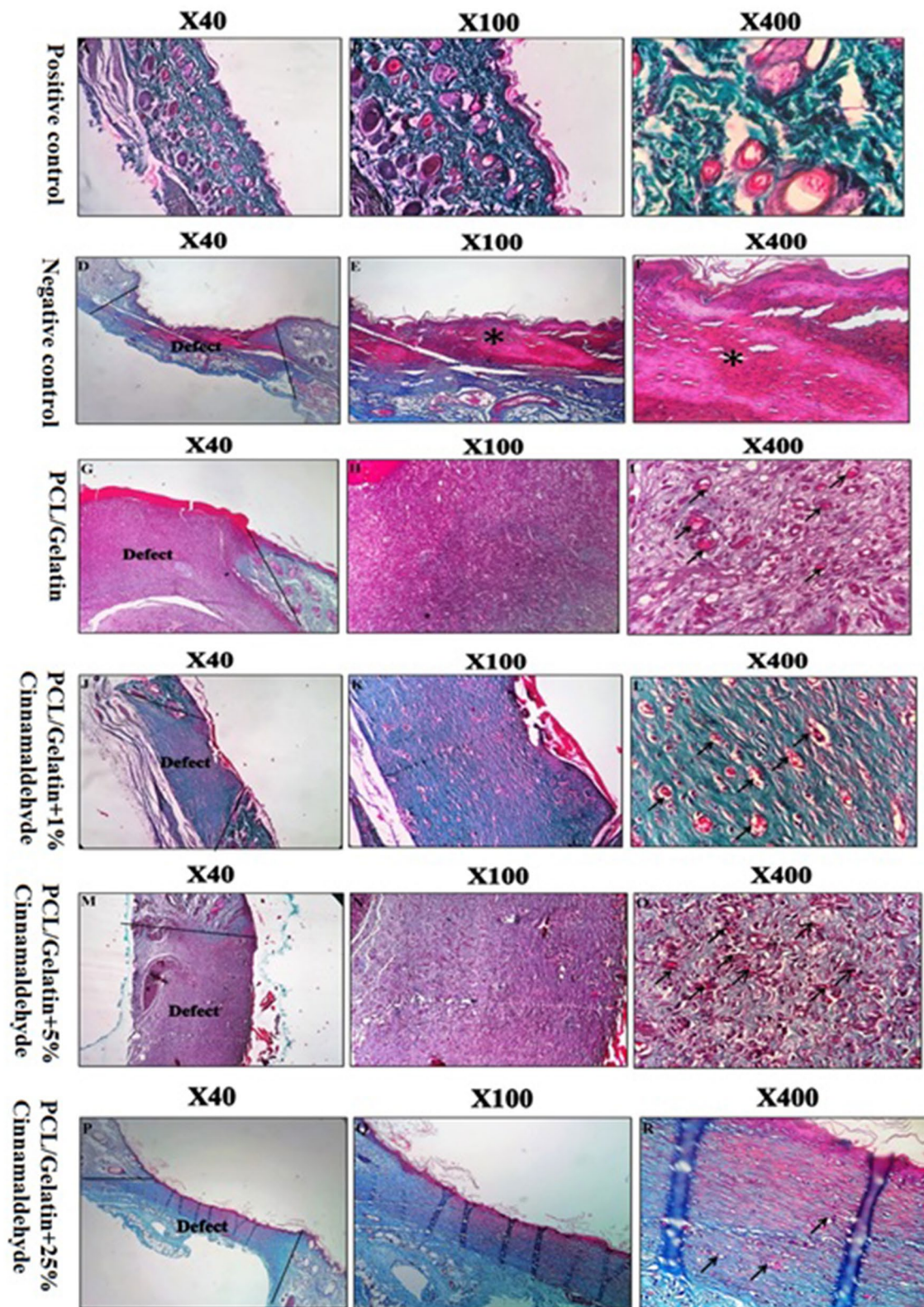


Fig. 7 MT stained microscopic sections of healed incisions in rats at 14 days, *D* defect

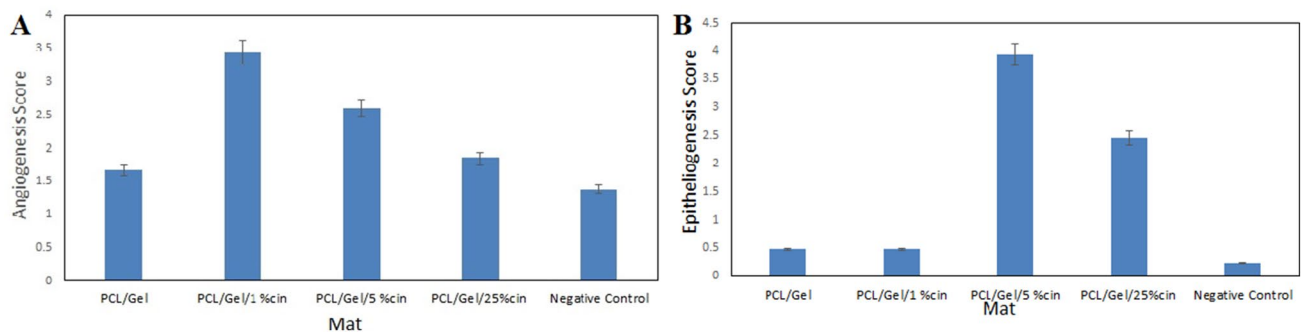


Fig. 8 **a** Histomorphometric study: angiogenesis and **b** histomorphometric study: epithelialization

the mechanical properties declined as a result of improved porosity and the fibers became random instead of align.

Wettability of wound dressing indicated the tendency of dressing to attach to the wound bed and its ability to absorb wound exudates [45]. The hydrophobicity nature of cinnamon should decrease the wettability, and consequently, the water uptake capacity of the mats. But, based on the previous study, by increasing percentage of porosity, the amount of water uptake capacity and WVP increased too [46]. In this study, by increasing concentration of cinnamon, the porosity of scaffolds was increased and therefore, water uptake capacity and WVP increased too. Regarding contact angle results, the values for all fabricated mats in this study and values obtained from commercial non-adhesive wound dressings produced by Coloplast (Biatain) and Smith & Nephew (ALLEVYN) were almost the same [47].

Base on the blood compatibility results, the mats containing cinnamon are better than other groups. It due to cinnamon properties that can decrease platelet aggregation [48]. The results of microbial penetration showed the ability of PCL/Gel mat containing cinnamon to stop bacterial invasion through electrostatic interactions between PCL/Gel/Cin and surface negative charge of bacteria. It can be attribute to the antibacterial properties of cinnamon [49].

The MTT assay results indicated that all fabricated mats were non-toxic for the wound-healing applications. Previous study indicated the antiapoptotic and proliferative effect of cinnamon on the fibroblasts cells [50]. It is confirmed by our study specially PCL/Gel/5%cin mat showed the highest cell viability in the MTT assay among other groups. By increasing amount of cinnamon, as it is a hydrophobic material, the cell cannot attach to the mats very well and consequently, the cell proliferation decreases. Based on it, the MTT results for PCL/Gel/25%cin is lower than other mats.

The in vivo evaluation showed that the efficacy of cinnamon on wound healing. The PCL/Gel/5%cin mat had higher wound closure among other mats and it is significantly better than the gauze-treated wound. Based on the previous studies, cinnamon is an anti-oxidant material which is widely

used for the treatment of many diseases such as diabetes, hypertension, and cardiovascular diseases [14, 51]. Antioxidants materials have ability to reduce the ROS-induced damages and consequently improve the wound healing by [52]. 80% of Cinnamon composition includes eugenol, cinnamaldehyde, and linalool [53]. Eugenol effects on inflammation through the reducing prostaglandin biosynthesis. Cinnamaldehyde has anti-inflammatory properties [54]. Indeed, Cinnamaldehyde has antibacterial activity against Gram-positive and Gram-negative bacteria [49]. Linalool's decreases nitric oxide and activating analgesic paths of cholinergic and glutamatergic compounds and therefore it has analgesic and anti-inflammatory effects [55, 56]. All in all, antioxidant, anti-inflammation, and antimicrobial of the cinnamon accelerate wound healing. On the other hand, PCL/Gel/25%cin group showed the negative effect of cinnamon in in vivo and in vitro study. It is due to the toxicity of high doses of cinnamon which is concluded by Adawiyah Ahmad etc. [57].

Various studies used nanofiber mats as wound dressing to improve wound healing process. For instance, Samadian etc. used electrospun cellulose acetate/gelatin/hydroxyapatite mats to improve wound healing. Their results indicated that after 14 days, about 90% of wound was healed [58]. Other studies which was used PCL/collagen mat containing insulin-chitosan nanoparticles reported that about 95% of crated wound healed after 14 days in rat model [28]. Frazamfar etc. reported that Taurine-loaded poly(ϵ -caprolactone)/gelatin electrospun mat could improve wound healing process and about 92% of the wound in rat model was healed after 14 days [59]. The results of the current study indicated that about 98% of wound was healed.

5 Conclusion

In this study, PCL/Gel mat with different concentration of cinnamon was fabricated and examined for healing of full-thickness excisional wound. The results indicated that the

PCL/Gel/5%cin mat had the highest cell proliferation among other groups. The in vivo study results showed the favorable wound-healing efficacy of the prepared mats especially PCL/Gel/5%cin. Therefore, our results provide evidence supporting the applicability of using cinnamon for wound healing.

Acknowledgements The present study was supported by Shahroud University of Medical Sciences, Shahroud, Iran (Grant No. 1398.086).

Authors' contributions All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethics approval Animal experiments were approved by the ethics committee of the Shahroud university of medical sciences (ethical code: IR.SHMU.REC.1398.086) and were carried out in accordance with the university's guidelines.

References

- Naseri-Nosar M, Salehi M, Farzamfar S, Azami M. The single and synergistic effects of montmorillonite and curcumin-loaded chitosan microparticles incorporated onto poly (lactic acid) electrospun film on wound-healing. *J Bioact Compat Polym.* 2018;33(3):239–53.
- Gantwerker EA, Hom DB. Skin: histology and physiology of wound healing. *Facial Plast Surg Clin N Am.* 2011;19(3):441–53.
- Kamoun EA, Kenawy E-RS, Chen X. A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings. *J Adv Res.* 2017;8(3):217–33.
- Chen S, Liu B, Carlson MA, Gombart AF, Reilly DA, Xie J. Recent advances in electrospun nanofibers for wound healing. *Nanomedicine.* 2017;12:1335–52.
- Farzamfar S, Salehi M, Ehterami A, Naseri-Nosar M, Vaez A, Zarnani AH, Sahrapeyma H, Shokri M-R, Aleahmad M. Promotion of excisional wound repair by a menstrual blood-derived stem cell-seeded decellularized human amniotic membrane. *Biomed Eng Lett.* 2018;8(4):393–8.
- Farzamfar S, Naseri-Nosar M, Samadian H, Mahakizadeh S, Tajerian R, Rahmati M, Vaez A, Salehi M. Taurine-loaded poly(ϵ -caprolactone)/gelatin electrospun mat as a potential wound dressing material: in vitro and in vivo evaluation. *J Bioact Compat Polym.* 2017. <https://doi.org/10.1177/0883911517737103>.
- Liu M, Duan X-P, Li Y-M, Yang D-P, Long Y-Z. Electrospun nanofibers for wound healing. *Mater Sci Eng C.* 2017;76:1413–23.
- Mohamed RM, Yusoh K (2016) A review on the recent research of polycaprolactone (PCL). In: *Advanced Materials Research.* vol 1134. Trans Tech Publ; 2016. p. 249–255.
- Coombes A, Rizzi S, Williamson M, Barralet J, Downes S, Wallace W. Precipitation casting of polycaprolactone for applications in tissue engineering and drug delivery. *Biomaterials.* 2004;25(2):315–25.
- Bagher Z, Atoufi Z, Alizadeh R, Farhadi M, Zarrintaj P, Moroni L, Setayeshmehr M, Komeili A, Kamrava SK. Conductive hydrogel based on chitosan-aniline pentamer/gelatin/agarose significantly promoted motor neuron-like cells differentiation of human olfactory ecto-mesenchymal stem cells. *Mater Sci Eng C.* 2019;101:243–53.
- Kweon H, Yoo MK, Park IK, Kim TH, Lee HC, Lee H-S, Oh J-S, Akaike T, Cho C-S. A novel degradable polycaprolactone networks for tissue engineering. *Biomaterials.* 2003;24(5):801–8.
- Gautam S, Dinda AK, Mishra NC. Fabrication and characterization of PCL/gelatin composite nanofibrous scaffold for tissue engineering applications by electrospinning method. *Mater Sci Eng C.* 2013;33(3):1228–35.
- Chong EJ, Phan TT, Lim IJ, Zhang Y, Bay BH, Ramakrishna S, Lim CT. Evaluation of electrospun PCL/gelatin nanofibrous scaffold for wound healing and layered dermal reconstitution. *Acta Biomater.* 2007;3(3):321–30.
- Ranasinghe P, Piger S, Premakumara GS, Galappaththya P, Constantine GR, Katulanda P. Medicinal properties of 'true' cinnamon (*Cinnamomum zeylanicum*): a systematic review. *BMC Complement Altern Med.* 2013;13(1):275.
- Ballai M. In vitro and in vivo activity of *Cinnamomum verum*. *J Med Sci.* 2008;8(4):425–8.
- Jayaprakasha GK, Rao LJ, Sakariah KK. Chemical composition of volatile oil from *Cinnamomum zeylanicum* buds. *Z Naturforschung C.* 2002;57(11–12):990–3.
- Farahpour M, Habibi M. Evaluation of the wound healing activity of an ethanolic extract of Ceylon cinnamon in mice. *Vet Med.* 2012;57(1):53–7.
- Naseri-Nosar M, Farzamfar S, Sahrapeyma H, Ghorbani S, Bastami F, Vaez A, Salehi M. Cerium oxide nanoparticle-containing poly(ϵ -caprolactone)/gelatin electrospun film as a potential wound dressing material: in vitro and in vivo evaluation. *Mater Sci Eng C.* 2017;81:366–72.
- Salehi M, Naseri-Nosar M, Ebrahimi-Barough S, Nourani M, Khojasteh A, Farzamfar S, Mansouri K, Ai J. Polyurethane/gelatin nanofibrils neural guidance conduit containing platelet-rich plasma and melatonin for transplantation of Schwann cells. *Cell Mol Neurobiol.* 2018;38(3):703–13.
- Salehi M, Farzamfar S, Bastami F, Tajerian R. Fabrication and characterization of electrospun PLLA/collagen nanofibrous scaffold coated with chitosan to sustain release of aloe vera gel for skin tissue engineering. *Biomed Eng Appl Basis Commun.* 2016;28(05):1650035.
- Salehi M, Bagher Z, Kamrava SK, Ehterami A, Alizadeh R, Farhadi M, Falah M, Komeili A. Alginate/chitosan hydrogel containing olfactory ectomesenchymal stem cells for sciatic nerve tissue engineering. *J Cell Physiol.* 2019. <https://doi.org/10.1002/jcp.28183>.
- Ai A, Behforouz A, Ehterami A, Sadeghvaziri N, Jalali S, Farzamfar S, Yousefbeigi A, Ai A, Goodarzi A, Salehi M, Ai J. Sciatic nerve regeneration with collagen type I hydrogel containing chitosan nanoparticle loaded by insulin. *Int J Polym Mater Polym Biomater.* 2019;68(18):1133–41.
- Naseri-Nosar M, Salehi M, Ghorbani S, Beiranvand SP, Goodarzi A, Azami M. Characterization of wet-electrospun cellulose acetate based 3-dimensional scaffolds for skin tissue engineering applications: influence of cellulose acetate concentration. *Cellulose.* 2016;23(5):3239–48.
- Gong C, Wu Q, Wang Y, Zhang D, Luo F, Zhao X, Wei Y, Qian Z. A biodegradable hydrogel system containing curcumin encapsulated in micelles for cutaneous wound healing. *Biomaterials.* 2013;34(27):6377–87.
- Unnithan AR, Gnanasekaran G, Sathishkumar Y, Lee YS, Kim CS. Electrospun antibacterial polyurethane–cellulose acetate–zein composite mats for wound dressing. *Carbohydr Polym.* 2014;102:884–92.
- Samadian H, Salehi M, Farzamfar S, Vaez A, Ehterami A, Sahrapeyma H, Goodarzi A, Ghorbani S. In vitro and in vivo evaluation

- of electrospun cellulose acetate/gelatin/hydroxyapatite nanocomposite mats for wound dressing applications. *Artif Cells Nanomed Biotechnol.* 2018;46:1–11.
27. Archana D, Dutta J, Dutta P. Evaluation of chitosan nano dressing for wound healing: Characterization, in vitro and in vivo studies. *Int J Biol Macromol.* 2013;57:193–203.
 28. Ehterami A, Salehi M, Farzamfar S, Vaez A, Samadian H, Sahrpayma H, Mirzaei M, Ghorbani S, Goodarzi A. In vitro and in vivo study of PCL/collagen wound dressing loaded with insulin-chitosan nanoparticles on cutaneous wound healing in Rats model. *Int J Biol Macromol.* 2018;117:601–9.
 29. Nangia A, Hung C. Design of a new hydrocolloid dressing. *Burns.* 1989;15(6):385–8.
 30. Chen H, Hu X, Chen E, Wu S, McClements DJ, Liu S, Li B, Li Y. Preparation, characterization, and properties of chitosan films with cinnamaldehyde nanoemulsions. *Food Hydrocoll.* 2016;61:662–71.
 31. Michiels J, Missotten J, Dierick N, Fremaut D, Maene P, De Smet S. In vitro degradation and in vivo passage kinetics of carvacrol, thymol, eugenol and trans-cinnamaldehyde along the gastrointestinal tract of piglets. *J Sci Food Agric.* 2008;88(13):2371–81.
 32. Ghasemi-Mobarakeh L, Prabhakaran MP, Morshed M, Nasr-Esfahani M-H, Ramakrishna S. Electrospun poly(ϵ -caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering. *Biomaterials.* 2008;29(34):4532–9.
 33. Ki CS, Baek DH, Gang KD, Lee KH, Um IC, Park YH. Characterization of gelatin nanofiber prepared from gelatin–formic acid solution. *Polymer.* 2005;46(14):5094–102.
 34. Brisbois EJ, Handa H, Meyerhoff ME. Recent advances in hemocompatible polymers for biomedical applications. In: Puoci F, editor. *Advanced polymers in medicine.* New York: Springer; 2015. p. 481–511.
 35. Bhattarai N, Edmondson D, Veisheh O, Matsen FA, Zhang M. Electrospun chitosan-based nanofibers and their cellular compatibility. *Biomaterials.* 2005;26(31):6176–84.
 36. Hassiba AJ, El Zowalaty ME, Nasrallah GK, Webster TJ, Luyt AS, Abdullah AM, Elzatahry AA. Review of recent research on biomedical applications of electrospun polymer nanofibers for improved wound healing. *Nanomedicine.* 2016;11(6):715–37.
 37. Zhang Y, Lim CT, Ramakrishna S, Huang Z-M. Recent development of polymer nanofibers for biomedical and biotechnological applications. *J Mater Sci Mater Med.* 2005;16(10):933–46.
 38. Zhang Y, Ouyang H, Lim CT, Ramakrishna S, Huang ZM. Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds. *J Biomed Mater Res B Appl Biomater.* 2005;72(1):156–65.
 39. MacEwan MR, MacEwan S, Kovacs TR, Batts J. What makes the optimal wound healing material? A review of current science and introduction of a synthetic nanofabricated wound care scaffold. *Cureus.* 2017;9(10):e1736.
 40. Cipitria A, Skelton A, Dargaville T, Dalton P, Huttmacher D. Design, fabrication and characterization of PCL electrospun scaffolds—a review. *J Mater Chem.* 2011;21(26):9419–53.
 41. Ho J, Walsh C, Yue D, Dardik A, Cheema U. Current advancements and strategies in tissue engineering for wound healing: a comprehensive review. *Adv Wound Care.* 2017;6(6):191–209.
 42. Zhu X, Cui W, Li X, Jin Y. Electrospun fibrous mats with high porosity as potential scaffolds for skin tissue engineering. *Biomacromolecules.* 2008;9(7):1795–801.
 43. Moroni L, De Wijn J, Van Blitterswijk C. 3D fiber-deposited scaffolds for tissue engineering: influence of pores geometry and architecture on dynamic mechanical properties. *Biomaterials.* 2006;27(7):974–85.
 44. Ma PX, Elisseeff J. *Scaffolding in tissue engineering.* Boca Raton: CRC Press; 2005.
 45. Kim SE, Heo DN, Lee JB, Kim JR, Park SH, Jeon SH, Kwon IK. Electrospun gelatin/polyurethane blended nanofibers for wound healing. *Biomed Mater.* 2009;4(4):044106.
 46. Hsieh W-C, Chang C-P, Lin S-M. Morphology and characterization of 3D micro-porous structured chitosan scaffolds for tissue engineering. *Colloids Surf B Biointerfaces.* 2007;57(2):250–5.
 47. Yang Y, Hu H. Spacer fabric-based exuding wound dressing—Part II: comparison with commercial wound dressings. *Text Res J.* 2017;87(12):1481–93.
 48. Fuli A, Zhong Z, Congkun X, Lanfang K. Component analysis of essential oils from cinnamon and their inhibition action against platelet aggregation. *China Pharm.* 2009;22:019.
 49. Lee H-S, Ahn Y-J. Growth-inhibiting effects of Cinnamomum cassia bark-derived materials on human intestinal bacteria. *J Agric Food Chem.* 1998;46(1):8–12.
 50. Tung Y-T, Chua M-T, Wang S-Y, Chang S-T. Anti-inflammation activities of essential oil and its constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) twigs. *Bioresour Technol.* 2008;99(9):3908–13.
 51. Lee J-S, Jeon S-M, Park E-M, Huh T-L, Kwon O-S, Lee M-K, Choi M-S. Cinnamate supplementation enhances hepatic lipid metabolism and antioxidant defense systems in high cholesterol-fed rats. *J Med Food.* 2003;6(3):183–91.
 52. Baumann LS, Md JS. The effects of topical vitamin E on the cosmetic appearance of scars. *Dermatol Surg.* 1999;25(4):311–5.
 53. Chericoni S, Prieto JM, Iacopini P, Cioni P, Morelli I. In vitro activity of the essential oil of *Cinnamomum zeylanicum* and eugenol in peroxynitrite-induced oxidative processes. *J Agric Food Chem.* 2005;53(12):4762–5.
 54. Mohammadi A, Mohammad-Alizadeh-Charandabi S, Mirghafourvand M, Javadzadeh Y, Fardiazar Z, Effati-Daryani F. Effects of cinnamon on perineal pain and healing of episiotomy: a randomized placebo-controlled trial. *J Integr Med.* 2014;12(4):359–66.
 55. Peana AT, Marzocco S, Popolo A, Pinto A. (–)-Linalool inhibits in vitro NO formation: probable involvement in the antinociceptive activity of this monoterpene compound. *Life Sci.* 2006;78(7):719–23.
 56. Peana AT, Paolo SD, Chessa ML, Moretti MD, Serra G, Pippia P. (–)-Linalool produces antinociception in two experimental models of pain. *Eur J Pharmacol.* 2003;460(1):37–41.
 57. Ahmad RA, Serati-Nouri H, Majid FAA, Sarmidi MR, Aziz RA. Assessment of potential toxicological effects of cinnamon bark aqueous extract in rats. *Int J Biosci Biochem Bioinform.* 2015;5(1):36–44.
 58. Samadian H, Salehi M, Farzamfar S, Vaez A, Ehterami A, Sahrpayma H, Goodarzi A, Ghorbani S. In vitro and in vivo evaluation of electrospun cellulose acetate/gelatin/hydroxyapatite nanocomposite mats for wound dressing applications. *Artif Cells Nanomed Biotechnol.* 2018;46(sup1):964–74.
 59. Farzamfar S, Naseri-Nosar M, Samadian H, Mahakizadeh S, Tajerian R, Rahmati M, Vaez A, Salehi M. Taurine-loaded poly(ϵ -caprolactone)/gelatin electrospun mat as a potential wound dressing material: In vitro and in vivo evaluation. *J Bioact Compat Polym.* 2018;33(3):282–94.