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Morphology, drug release, antibacterial, cell proliferation, and histology studies of chamomile-loaded wound dressing mats based on electrospun nanofibrous poly(ε-caprolactone)/polystyrene blends

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Abstract: For the first time, it has been tried to achieve optimum conditions for electrospun poly(ε-caprolactone)/polystyrene (PCL/PS) nanofibrous samples as active wound dressings containing chamomile via D-optimal design approach. In this work, systematic *in vitro* and *in vivo* studies were carried out by drug release rate, antibacterial and antifungal evaluations, cell culture, and rat wound model along with histology observation. The optimized samples were prepared under the following electrospinning conditions: PCL/PS ratio (65/35), PCL concentration 9%(w/v), PS concentration 14%(w/v), distance between the syringe needle tip and the collector 15.5 cm, applied voltage 18 kV, and solution flow rate 0.46 mL h^{-1}. The FE-SEM micrographs showed electrospun PCL/PS (65/35) nanofibrous sample containing 15% chamomile had a minimum average diameter (~175 nm) compared to the neat samples (~268 nm). The drug released resulted in a gradual and high amount of chamomile from the optimized PCL/PS nanofibrous sample (~70%) in respect to PCL and PS nanofibers after 48 h. This claim was also confirmed by antibacterial and antifungal evaluations in which an inhibitory zone with a diameter of about 7.6 mm was formed. The rat wound model results also indicated that the samples loaded with 15% chamomile extract were remarkably capable to heal the wounds up to 99 ± 0.5% after 14 days post-treatment periods. The adhesion of mesenchymal stem cells and their viability on the optimized samples were confirmed by MTT analysis. Also, the electrospun nanofibrous mats based on PCL/PS (65/35) showed a high efficiency in the wound closure and healing process compared to the reference sample, PCL/PS nanofibers without chamomile. Finally, the histology analysis revealed that the formation of epithelial tissues, the lack of necrosis and collagen fibers accumulation in the dermis tissues for the above optimized samples. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 00B: 000–000, 2013.

Key Words: nanofibers, wound healing, histology, chamomile, drug release

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INTRODUCTION

The use of herbal extracts as biocompatible and nontoxic drugs without side effects in wound healing are highly favored compared to chemical and synthetic drugs. Since ancient times, a suitable material was used to cover a wound in order to prevent infections. Historically, honey pastes, plant fibers, and animal fats have been used as wound dressing materials.[1]

In recent years, electrospun nanofibrous mats have shown a great promise for the development of modern wound dressing materials.[2,3] This is due to their high specific surface area, excellent fluid drainage and a gradual drug release rate. There are limited research works on the use of electrospun biodegradable and biocompatible polymeric nanofibrous mats containing herbal extracts as wound dressing materials.[4–7] Karami et al.[8] investigated the effects of an herbal drug, thymol loaded poly(ϵ-caprolactone)/poly(lactic acid) (PCL/PLA) (50/50) as wound dressing samples. The results of this study showed that the nanofibrous mats containing 1.2% (v/v) thymol had a higher efficiency in the wound closure (~92.5%) compared to the commercial wound dressing, Comfeel®Plus, and gauze bandages (control) after 14 days post-treatment periods. In our previous work,[9] the use of a chemical drug, phenytoin sodium (PHT-Na) loaded poly(vinyl alcohol) (PVA) indicated that it was good candidate for cultivating mesenchymal stem cells and

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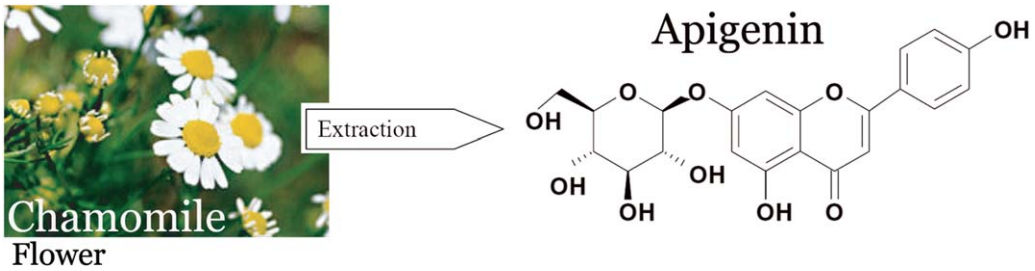

FIGURE 1. Chamomile flower and its flavonoid apigenin structure produced by extraction process. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

had remarkable effects on decreasing the wound area percentage (~3%) after 2 weeks. Also, the wound treated with PVA nanofibers containing PHT-Na, had a large number of myofibroblast and minimized necrosis in epidermis tissues.

The chamomile plant, *chamomilla recutita* (L.) Rauschert, is one of the Asteraceae family and a common medicinal plant. The therapeutic activity of chamomile belongs to different effective substances such as phenolics and flavonoids apigenin, quercetin, patuletin, luteolin, and their glucosides. Apigenin is quantitatively the most abundant flavonoid found in chamomile flower and shows a remarkable effect on the healing process of a wound[10] (Figure 1). A few research works has been reported on the effect of chamomile on the wound healing process and its unique properties for the promotion of the wound area closure. Jarrahi.[11] studied the effects of *matricaria chamomilla* extract on burned wound healing in albino rats. He showed that there was a significant difference between the animals of control groups, which received no treatment and those of treatment groups which treated with the extract dissolved in olive oil. The results indicated a complete wound healing after 61 days post-treatment periods. In another research work, Martins et al.[12] carried out a comparative analysis between *chamomilla recutita* and corticosteroid on wound healing. Their studies revealed that all the animal's wounds treated with chamomile extract exhibited a complete wound healing 9 days earlier before the triamcinolone acetonide and clobetasol propionate drugs. Subsequently, the histology observations showed that the wound treated with chamomile represented a total epithelium reparation and underneath connective tissues containing fibrosis and noninflammatory cells. The last evaluations on chamomile effects on rat wound healing were fully studied by Duarte et al.[13] They concluded that animal wounds treated with chamomile extract had the best results based on epithelialization and percentage of collagen fibers accumulation after 10 days post-treatment periods.

The use of chamomile extract in the above research works was mainly as an ointment or a pure extract directly deposited on the wound area. So far, a fully and systematic study of chamomile loaded electrospun polymeric nanofibrous mats as an active wound dressing material has not been ever reported in the literature.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing and optimizing processes.[14] This technique is an effective method for optimizing several variable parameters. Sukigara et al.[15] used RSM for modeling and optimizing electrospinning parameters in order to get nano-silk fibers and used regeneration method from domestic silkworm, *Bombyx mori*. Electrical field and silk concentration were used as variables for the control of fibers diameter in the distance between the syringe needle tip and the collector. Gu et al.[16] used polyacrylonitrile/*N*,*N*-dimethylformamide (PAN/DMF) solution as a precursor of carbon nanofibers and obtained electrospun nanofibers with diameters ranging from 200 to 1200 nm. They concluded that the concentration of polymeric solution played a key role in the average diameter of nanofibers. In our previous work,[17] developing a biomedical electrospun nanofibrous mats based on a PVA/PCL (80/20) hybrid with a defined drug release rate using tetracycline hydrochloride as a model drug was studied. The electrospinning conditions of these samples were optimized by D-optimal experimental design method. The drug loaded nanofibrous samples had a reduction in the average diameter (~200 nm) compared to the neat samples (~315 nm). Also, tetracycline hydrochloride release profiles in the nanofibrous and cast films samples revealed that Fickian diffusion was a dominant mechanism for electrospun PVA/PCL (80/20) hybrid samples.

Amorphous polystyrene (PS) being a transparent and colorless material. It has very high electrical resistance and low dielectric loss. Also, PS is a hard, stiff, and very brittle polymer with remarkable water vapor permeability (WVP). On the other hand, PCL is a semicrystalline biodegradable polymer, belongs to the family of α-hydroxyl polyesters. This polymer has a suitable electrospinnability, very high mechanical properties, and biodegradability with low WVP.[2] As a result, blending PCL and PS with an optimum ratio and their electrospinning leads to a new product with interesting characteristics, which can be very valuable for using as active nanofibrous wound dressing materials.

The main purpose of this work was systematically to study and evaluate the PCL/PS blend nanofibrous mats containing chamomile extract for the wound healing acceleration. The electrospinning conditions for PCL/PS nanofibrous samples were determined by D-optimal design method. Moreover, the performance of the optimized samples in terms of drug release rate and wound healing process were analyzed using *in vitro* cell culture and *in vivo* experimental

TABLE I. D-Optimal Experimental Design to Study the Effects of PCL/PS ratio (X_1), PCL Concentration (X_2), PS Concentration (X_3), Flow Rate (X_4), Distance Between the Syringe Needle Tip and the Rotating Collector (X_5), and Applied Voltage (X_6) on the Nanofibers Diameter (Y) of Electrospun PCL/PS Blends

Run	X_1: Ratio (PCL/PS)	X_2: PCL %(w/v)	X_3: PS %(w/v)	X_4: Flow Rate (mL h^{-1})	X_5: Distance (cm)	X_6: Voltage (kV)	Y: Diameter (nm)
1	3	8	10	0.4	18	20	472.3
2	7	12	20	1	18	10	403.7
3	7	10	20	0.4	10	20	705.5
4	3	8	20	0.7	10	20	792.6
5	7	8	20	1	14	20	578.5
6	7	8	10	0.4	18	15	323.7
7	7	8	20	0.4	18	10	1203
8	7	12	10	0.7	10	20	627.9
9	5	10	15	0.7	14	10	939.4
10	7	8	10	1	18	10	724.6
11	3	12	10	1	10	10	1056.6
12	5	12	10	1	18	20	699.2
13	3	12	20	0.4	18	15	1909.1
14	5	8	20	1	18	20	453
15	7	8	10	1	18	10	730.4
16	3	12	20	0.4	10	10	2825.8
17	7	12	15	0.4	18	20	1053
18	3	8	10	0.4	10	10	1067.3
19	3	12	10	0.7	18	10	822.3
20	7	12	20	0.4	10	15	611.5
21	3	12	10	0.4	10	20	537.6
22	3	8	15	0.4	18	10	1856.8
23	3	8	20	1	14	10	384.4
24	3	8	20	1	14	10	380.8
25	7	8	10	0.4	10	20	410.7
26	7	10	10	1	18	20	399.3
27	3	8	10	1	10	20	1553.7
28	5	12	20	1	10	20	1217.6
29	7	12	10	0.4	14	10	1064.5
30	5	8	20	0.4	18	20	453.9
31	3	8	10	0.4	10	10	1168.3
32	3	12	10	0.7	18	10	830.4
33	5	10	15	0.7	14	20	501.9
34	7	12	10	0.4	14	10	1148.6
35	7	8	20	1	10	10	905.1
36	3	12	15	1	18	20	2070.4

rat wound model. Interesting results on the promoted rat wound healing process and skin regeneration were obtained for chamomile loaded electrospun PCL/PS (65/35) nanofibrous mats. These results are discussed in detail and useful suggestions have been proposed.

MATERIALS AND METHODS

PCL (biodegradable, weight average molecular weight of 90 kDa), was obtained from Sigma-Aldrich, Pillsburg, The Netherlands. PS (grade ST 316310, a synthetic polymer, weight average molecular weight of 70 kDa) was provided from Goodfellow Cambridge, Huntingdon, United Kingdom. Chamomile extract (purity about 99.9%, dark green to brown color) received from institute of medicinal plants (ACECR), Karaj, Iran. All the other chemicals were analytical reagent grades and used without further purification.

The electrospun PCL/PS nanofibrous samples were prepared with different ratios (30/70), (50/50), and (70/30) in a 7/3 solvent mixture of chloroform/DMF at different electrospinning conditions via D-optimal experimental design method. PCL and PS solution concentrations ranging 8–12 and 10–20% (w/v) were, respectively, made and 36 samples were electrospun via Design Expert V.8 (Stat-Easy, Minneapolis, MN) (Table I). On this basis, the effects of all the parameters, such as PCL/PS ratio (X_1), PCL concentration (X_2), PS concentration (X_3), solution flow rate (X_4), distance between the syringe needle tip and the collector (X_5), and applied voltage (X_6) on the average nanofibers diameters (Y), were investigated.

In order to prepare neat PCL/PS blend nanofibrous samples [Figure 2(a)], weighed PCL and PS granules were dissolved in a mixture of chloroform/DMF (7/3) as a solvent system and were stirred for about 2 h at room temperature. To prepare nanofibrous samples containing chamomile [Figure 2(b)], the weighed polymers and the drug were dissolved in 7 mL chloroform and then 3 mL DMF was added

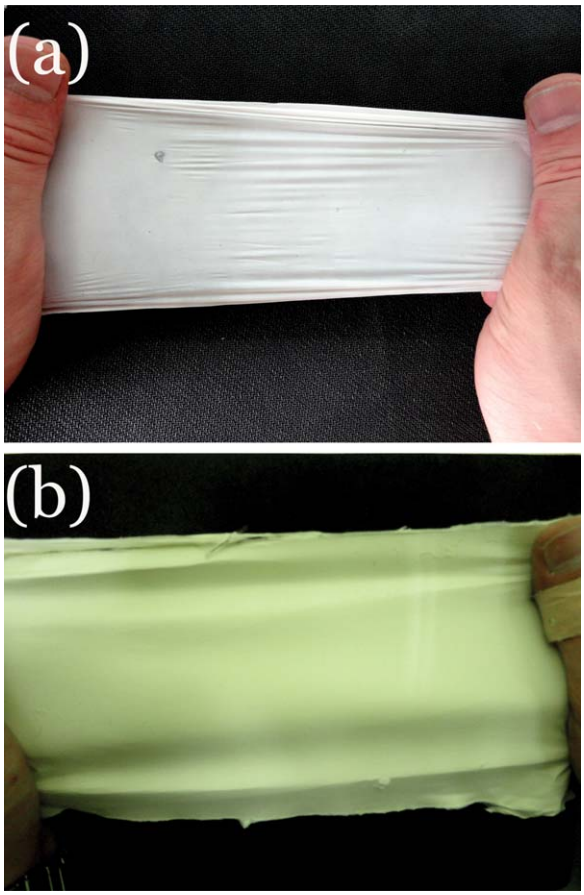

FIGURE 2. Schematics of electrospun neat and chamomile loaded optimized PCL/PS (65/35) blend nanofibrous wound dressings. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

later to the solution. The final concentration of the chamomile in the polymer solution was made to about 15%.[18,19]

The electrospinning device used for the preparation of the samples was a model eSpinner NF-CO EN/II from Asian Nanostructures Technology, (Tehran, Iran). The electrospinning conditions for PCL/PS nanofibrous samples were as follows: PCL/PS ratio = (65/35), PCL concentration = 9% (w/v), PS concentration = 14% (w/v), distance between the syringe needle tip and the collector = 15.5 cm, applied voltage=18 kV and solution flow rate=0.46 mL h^{-1}. In order to achieve fully dried electrospun nanofibrous samples and to insure the evaporation of the organic solvents from the spun fibers, the samples were dried at room temperature for about 12 h until a constant weight was attained.

The electrospun fibers were coated with a thin layer of gold using a Bio-Rad E5200 auto sputter coater (Agar Scientific, Essex, United Kingdom). The morphology of the samples were observed by a field emission scanning electron microscopy (FE-SEM; Hitachi model SU8040, Krefeld, Germany), at 5000× magnification. The mean values of the nanofibers diameters from five different sections were measured and recorded.

An ultraviolet spectrometer (X-ma model 2000, Japan) was used for the determination of chamomile extract (a flavonoid apigenin) release rate. The maximum wavelength for apigenin in chloroform solvent was about 319 nm.[20] The calibration curve, with an r^2 value of 0.9981, was obtained from Beer–Lambert law according to the following linear equation:

$$A = \varepsilon \times 1 \times c = 2.98 \times c, \tag{1}$$

where, "A" is the absorption percentage, "c" is the concentration of the drug, "l" is the distance the light which travels through the material, and "ε" is the extinction coefficient of chamomile extract.

The degree of swelling, weight loss, water uptake, and WVP of PCL, PS, and PCL/PS nanofibrous samples were determined according to the following equations. These tests are fully explained in the literature.[2,8]

$$\text{Degree of swelling}\,(\%) = \frac{M - M_\text{d}}{M_\text{d}} \tag{2}$$

$$\text{Weight loss}\,(\%) = \frac{M_\text{i} - M_\text{d}}{M_\text{i}} \tag{3}$$

$$\text{Water up-take capacity}\,(\%) = \frac{M_\text{s} - M_\text{i}}{M_\text{i}} \tag{4}$$

$$\text{WVP}\ \left(\text{mg}\,.\text{cm}^{-2}.\text{h}^{-1}\right) = \frac{\Delta W}{A \times \Delta t} \tag{5}$$

where, "M" is the weight of swollen samples that were drip-dried with paper filter, "M_d" is the weight of the dried samples in an oven at 40°C until a constant weight was obtained, "M_i" is the initial weight of the samples, "M_s" is the weight of the sample after immersion in phosphate buffered saline (PBS), "ΔW" is the weight variations of the samples at every 1 h, "Δt" is the time interval and is equal to 1 h and "A" is the effective surface area of the nanofibrous samples in contact with water vapor and a humid environment.

The antibacterial and antifungal properties of the electrospun PCL/PS mat samples with and without chamomile were determined against a Gram-positive bacterium, *Staphylococcus aureus* (*S. aureus*) and a fungi, *Candida albicans* (*C. albicans*),[21] respectively. The antimicrobial and antifungal activities of the electrospun mat samples were studied by the disc diffusion method. This method performed in a Luria-Bertani medium solid agar Petri-dish.[22] The samples were cut into disc shapes 1 cm in diameter, sterilized under UV light for 2 h and placed on *S. aureus* and *C. albicans* cultured agar plates. Then, they were incubated for 24 h at 37°C, and the inhibitory zone was recorded.

The cell culture protocol in this work was based on our previous work.[9] Briefly, when accumulation of mesenchymal stem cells obtained from the human umbilical cord matrix (hUCM) reached to about 80% or in other words 1×10^6 hUCM cells mL^{-1} were cultured in each dish, they were seeded on the neat PCL/PS blend nanofibrous samples and the blend nanofibrous samples containing chamomile for evaluating *in vitro* cell adhesion and morphology studies after 6 days. In order to observe the cell morphologies on

the nanofibrous samples, FE-SEM investigation with 1000× magnification at 6-day intervals after culture times were studied. Cytocompatibility of the nanofibrous samples were characterized by using 3-4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) after 3 and 6 days. The optical density of the formazan solution was detected using an ELISA reader (Dana 3200, Iran) at 540 nm.

On the basis of ref. 9, a full-thickness square wound (2×2 cm^2) was cut from the back of each rat. The wounds were covered with a neat and chamomile loaded nanofibrous mats. The area of the wounds was measured every day up to 14 days. The percentage of the wound closure is defined by Eq. (6):

$$\text{Wound closure}\,(\%) = \frac{A_i}{A_0} \times 100, \tag{6}$$

where "A_i" and "A_0" are the wound area after the fixed time interval and initial wound area, respectively. For histology observations of re-epithelialization and granulation, the wound tissues samples were dissected at day 1, day 7, and day 14, fixed with 10% formalin and then stained with hematoxylin and eosin (H&E) reagents.

RESULTS AND DISCUSSION

Optimization of electrospun PCL/PS nanofibrous mats via D-optimal experimental design method

In order to achieve the minimum average nanofibers diameters for the electrospun PCL/PS samples, six different variables such as X_1: PCL/PS ratio (30/70, 50/50, and 70/30), X_2: PCL concentration (8–12% w/v), X_3: PS concentration (10–20% w/v), X_4: solution flow rate (0.4–1 mL h^{-1}), X_5: distance between the syringe needle tip and the collector (10–18 cm), X_6: applied voltage (10–20 kV), and (Y) as average nanofibers diameters were considered. According to Table I, a number of 36 runs were taken via D-optimal design approach. The analysis of variance (ANOVA) showed that the results were fitted with a quadratic polynomial equation as follows:

$$\begin{aligned} Y = {} & 17039.7 - 3081.1X_1 - 1820.2X_2 + 645.1X_3 - 981.7X_4 \\ & - 119.6X_5 - 1508X_6 - 706.1X_1X_2 - 160.8X_1X_3 \\ & - 2388.8X_1X_4 - 277.4X_1X_5 - 250.6X_1X_6 + 31.1X_2X_3 \\ & + 9.3X_2X_6 - 116.2X_3X_4 + 4.5X_3X_5 + 3.1X_3X_6 + 258X_4X_6 \\ & + 11.4X_5X_6 + 20814.8X_1^2 + 83.2X_2^2 - 28.7X_3^2 + 38.3X_6^2, \end{aligned} \tag{7}$$

After analyzing the Eq. (7), the minimum diameter for PCL/PS nanofibrous samples was about 268 nm. The optimized conditions for X_1, X_2, X_3, X_4, X_5, and X_6 were 65/35, 9% (w/v), 14% (w/v), 0.46 mL h^{-1}, 15.5 cm, and 18 kV, respectively. The regression coefficient (r^2) for the Eq. (7) was calculated about 0.91. Generally, every parameter that was eliminated in the above equation was because of their unimportance effects on the answer, had a $p > 0.05$.

Figure 3(a–l) shows 3D graphs of optimized effective parameters on the diameter of PCL/PS nanofibers. As it can be seen in Figure 3, the interactions of all the variables on the nanofibers diameters were nonlinear.

Morphology of the neat and drug loaded electrospun PCL/PS mats

Figure 4(a,b) shows the FE-SEM micrograph images of neat and drug-loaded electrospun PCL/PS (65/35) nanofibrous samples. The uniformity and smooth surface of the nanofibrous samples was an indication of a good compatibility between chamomile and polymer-solvent system.[23] Similar to many other drugs, the results revealed that the addition of chamomile relatively reduced the average diameter of the nanofibers about from 268 to 175 nm. Similar results have been reported for tetracycline hydrochloride and PHT-Na loaded PCL/PLA (50/50) and PVA nanofibers, respectively.[2,9] This was attributed to viscosity reduction of polymer solution due to plasticizing role of chamomile in these polymeric chains.[24] Porosity and solid-state connectivity can also affect the cell adhesion and proliferation, the influence of which has been extensively reported in literature.[25,26]

The drug release rate of electrospun PCL, PS and optimized PCL/PS nanofibrous mats containing chamomile

As indicated in Figure 5, the release profiles of chamomile with a concentration of 15% loaded electrospun PCL, PS, and PCL/PS (65/35) nanofibrous mats were plotted. As a result, the electrospun PS sample containing the drug shows a lower release amount compared to of the electrospun PCL and blend samples. It was due to the chamomile incompatibility with PS molecular structure, which resulted in the minimum drug release rate in PBS. On the other hand, PCL is an amorphous polymer and has a good compatibility with chamomile similar to tetracycline hydrochloride loaded PCL which had a higher release rate than that of tetracycline hydrochloride loaded poly(lactic acid) (PLA) as a crystalline polymer.[2] Therefore, the electrospun PCL sample containing chamomile exhibited a burst and sharp release before 10 h. The release profile of chamomile loaded PCL nanofibrous sample tend to become plateau with a constant slope by immersing in PBS after 10 h. It means that there was no released drug for electrospun PCL nanofibrous sample containing chamomile from 10 to 48 h. The chamomile release profile for PCL/PS nanofibrous sample was gradual throughout the release time. It can be concluded that a sufficient amount of chamomile (~70%) always existed on the wound area, which can promote the wound healing process. However, the higher accumulative drug release cannot always confirm the efficiency of the nanofibrous samples in the wound dressing application because having a gradual drug release is a more significant parameter when the wound is covered during post-treatment period.

Degree of swelling, weight loss, water uptake, and water vapor permeability of the optimized samples

Degree of swelling and weight loss are two important parameters for controlling the drug release rate from electrospun nanofibers. Figure 6(a,b) shows the swelling and

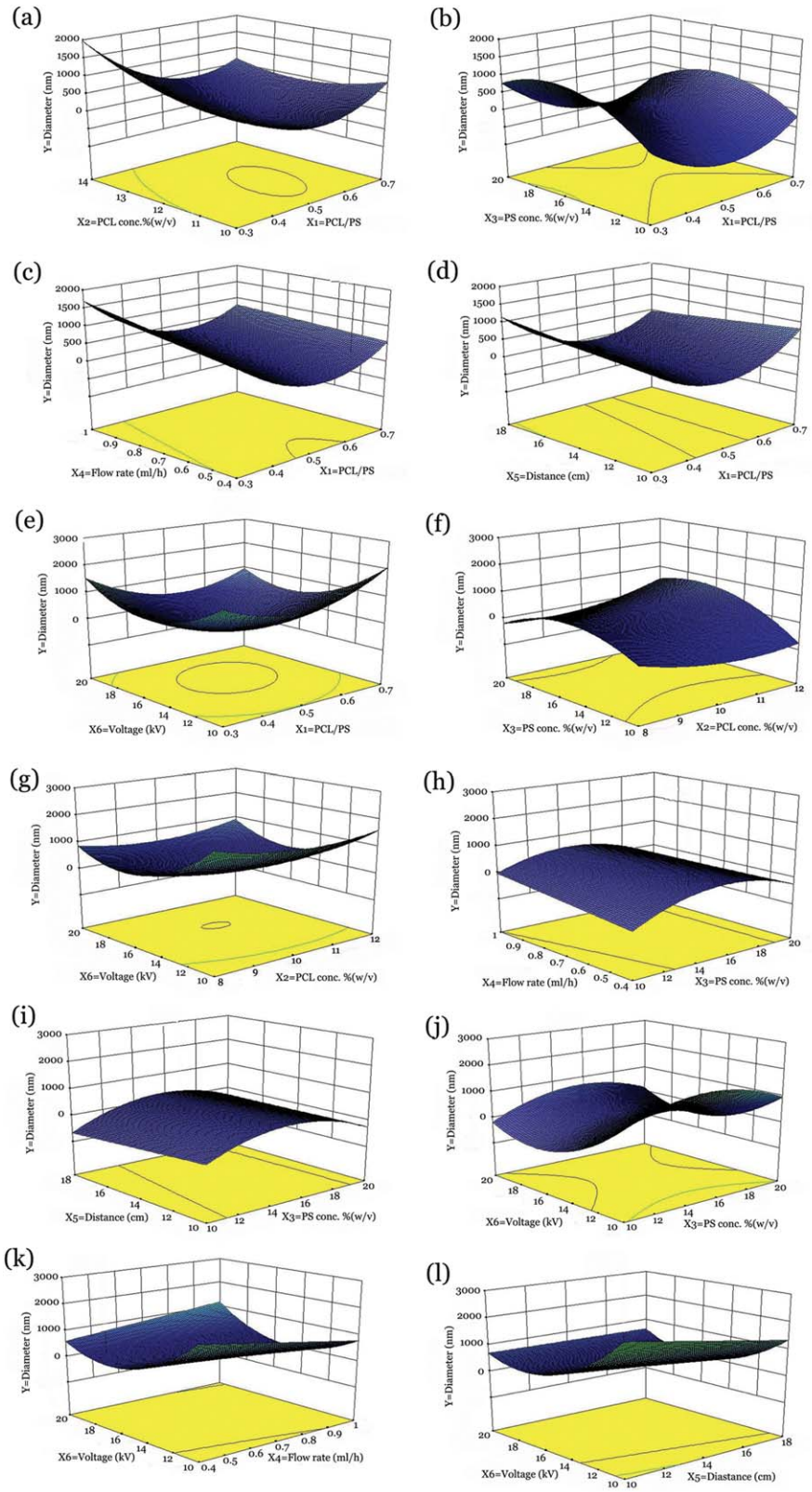

FIGURE 3. Effect of different electrospinning process variables on average nanofibers diameters for PCL/PS mats. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

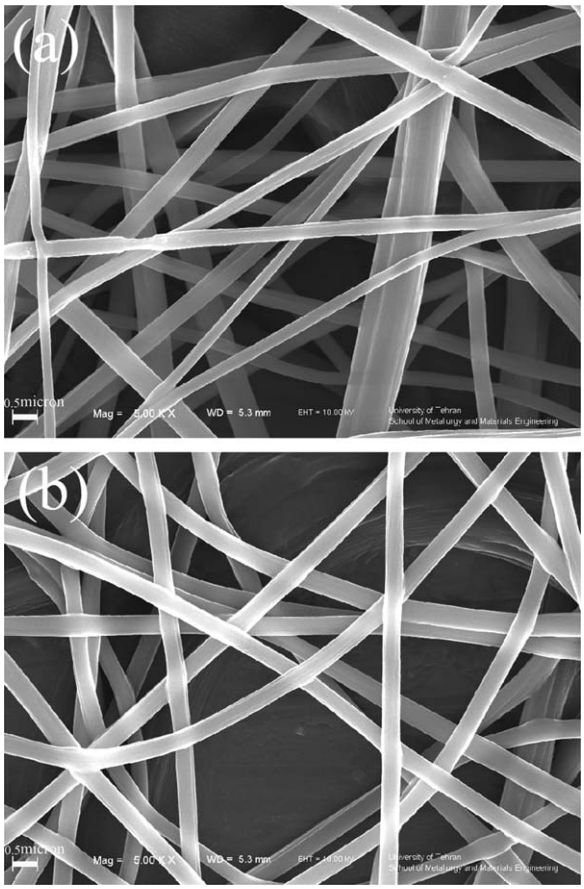

FIGURE 4. FE-SEM micrographs of electrospun polymeric solution optimized by D-optimal design method: (a) neat PCL/PS (65/35) nanofibers, (b) PCL/PS (65/35) nanofibers with 15% chamomile (PCL concentration 9% (w/v), PS concentration 14% (w/v), distance between the syringe needle tip to the collector 15.5 cm, flow rate 0.46 mL h^{-1} and applied voltage 18 kV) (the scale bars are 0.5 μm).

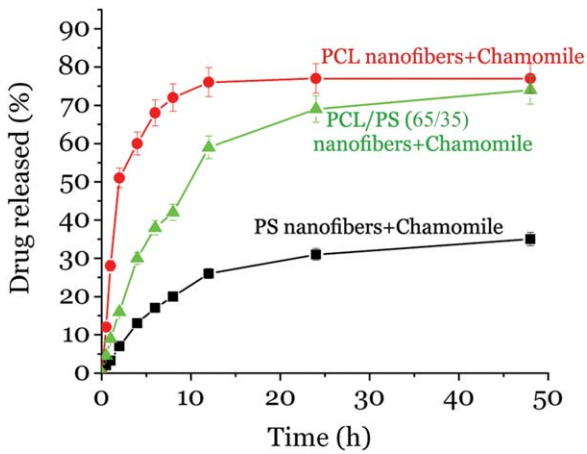

FIGURE 5. Release profiles of chamomile (15%) from (●) PCL nanofibers, (▲) PCL/PS (65/35) nanofibers, and (■) PS nanofibers during 48 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

weight loss for neat PCL, PS, and 65/35 PCL/PS nanofibrous samples in the release environment (PBS) at 37°C for 1, 6, 24, and 48 h. As it can be seen in Figure 6(a), the order of swelling percentages for these samples is as follows: PCL nanofibers > PCL/PS nanofibers > PS nanofibers. It means that PCL nanofibrous samples due to their amorphous structure have the highest swelling than that of the blend and PS nanofibrous samples. Figure 6(b) shows that PCL nanofibrous samples have the highest weight loss about of 0.6% after 48 h. However, although these polymeric nanofibrous samples have a little weight loss but it is not significant due to their hydrophobic molecular structures.

One of the effective methods for increasing WVP of PCL is its blending with PS. In this work, the WVP of electrospun PCL/PS nanofibers was increased up to 7.9 mg cm^{-2} h^{-1} (Table II). In our previous work, it was reported that the value of WVP for an effective polymer wound dressing must be in the range of 8.3–10.4 mg cm^{-2} h^{-1}.[3] On the other hand, a high value of water uptake for an electrospun wound dressing material due to fluid absorption of a wound

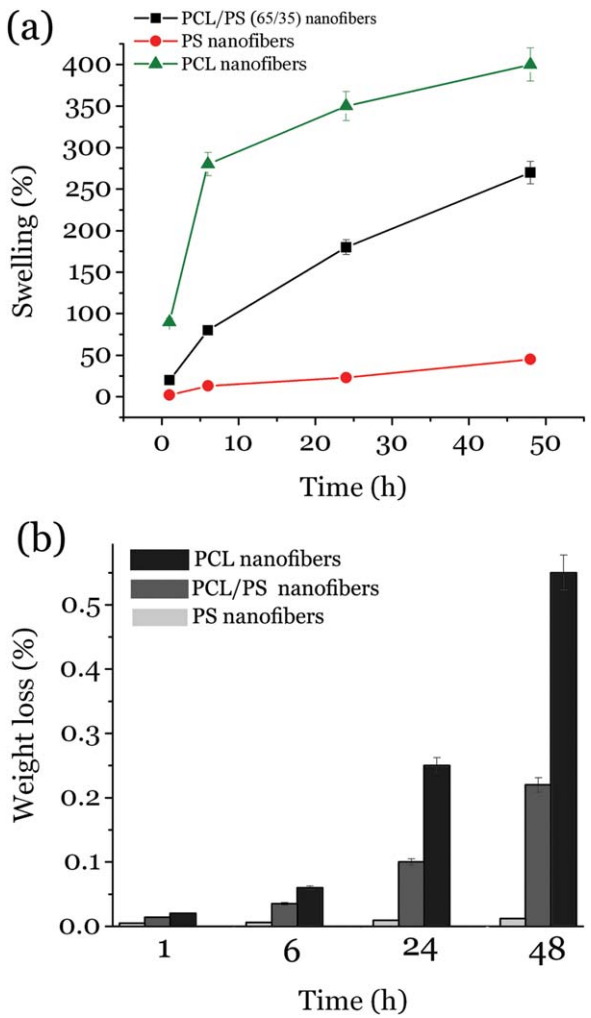

FIGURE 6. (a) The degree of swelling (%) and (b) the weight loss (%) of PCL, PS, and 65/35 PCL/PS for nanofibrous mat samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE II. WVP and Water Uptake of the PCL, PS, and PCL/PS (65/35) Electrospun Nanofibrous Samples

Property	Sample		
	PCL Nanofibrous Mat	PS Nanofibrous Mat	PCL/PS (65/35) Nanofibrous Mat
Water vapor permeability (WVP) (mg cm^{-2} h^{-1})	3.6 ± 0.2	8.5 ± 0.7	7.9 ± 0.4
Water uptake (%) after 48 h	500 ± 50	50 ± 5	220 ± 25

surface is essential. The results after 48 h showed that PCL as an amorphous polymer and PCL/PS nanofibrous samples have water uptake percentages of about 500 and 220%, respectively.

Antibacterial and antifungal activities of PCL/PS nanofibrous samples with and without chamomile

The antibacterial and antifungal properties of chamomile loaded PCL/PS nanofibrous mats against two different microorganisms, *S. aureuas* as a bacteria and *C. albicans* as a fungi were evaluated. As it can be seen in Figure 7(a,b), chamomile has a good effect on inhibition of *S. aureus* [Figure 7(a)] and *C. albicans* [Figure 7(b)] growth. The results also revealed that this herbal drug has the same efficiency on the extent of inhibitory zones for both *S. aureus* and *C. albicans* (~7.6 mm). The reason for this inhibitory behavior refers to the flavonoid, apigenin, which displays a key role for antifungal properties against *C. albicans*.[27]

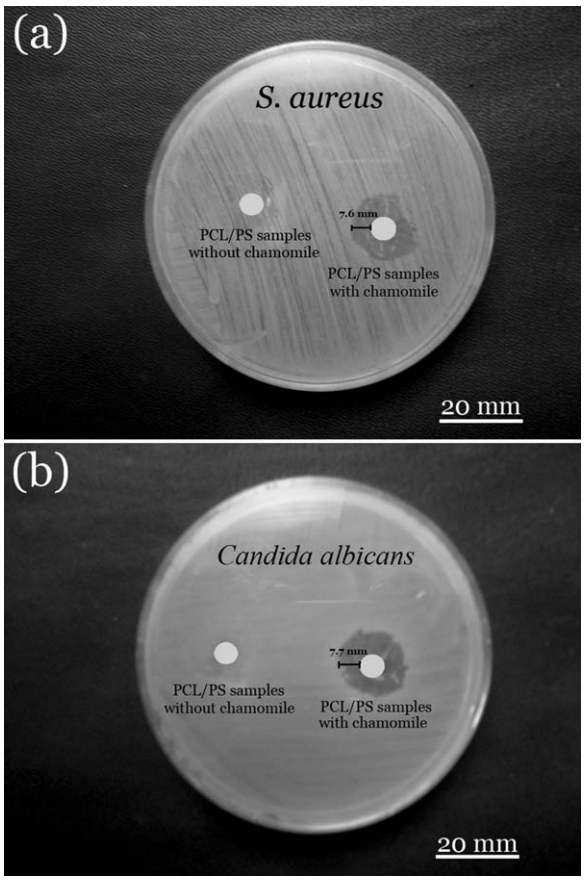

FIGURE 7. Antibacterial and antifungal activities of the electrospun PCL/PS (65/35) nanofibrous mats containing 15% chamomile against (a) *S.aureus* and (b) *Candida albicans* (the scale bars are 20 mm).

In vitro cell adhesion and proliferation

In vitro cytotoxicity test is usually carried out on a wound dressing to evaluate its nontoxicity. Figure 8 shows a UV absorbance at 540 nm illustrating the viability of mesenchymal stem cell on the neat and 15% chamomile-loaded PCL/PS (65/35) blend electrospun nanofibrous samples. After 3 days, the UV absorbance (quantity) of the cell seeding on the neat and drug-loaded nanofibrous samples as well as control (cells in the culture environment DMEM-F12) were 0.13 (0.05×10^6), 0.5 (0.22×10^6), and 2.25 (1×10^6), respectively. The viability and proliferation of stem cells produced similar results for the above samples after 6 days as follows: 0.2 (0.09×10^6), 1.95 (0.9×10^6), and 2.5 (1.1×10^6), respectively. It can be concluded that the cell viability capacity of the nanofibrous sample containing chamomile reached to 90% that of control.

The morphology of seeded stem cells on the neat and drug loaded samples after day 6 incubation time was observed by FE-SEM and the results are shown in Figure 9(a,b). Figure 9(a) shows the cells' proliferation on the neat PCL/PS nanofibrous sample. Figure 9(b) demonstrates the cell growth on the PCL/PS nanofibrous sample containing 15% chamomile. Comparing these figures resulted in the accumulation of stem cells at day 6 on the sample with the drug is about 10 times higher than that of the neat sample. Finally, it can be concluded that chamomile was a more effective cell proliferation drug compared to PHT-Na.[9]

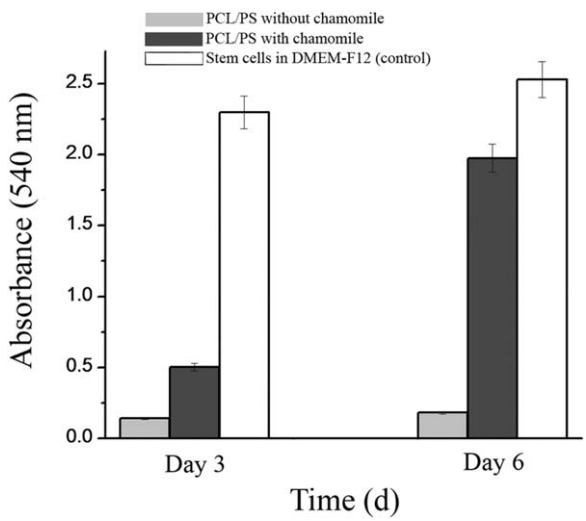

FIGURE 8. The effect of chamomile (15%) on PCL/PS nanofibers viability after 3 and 6 days.

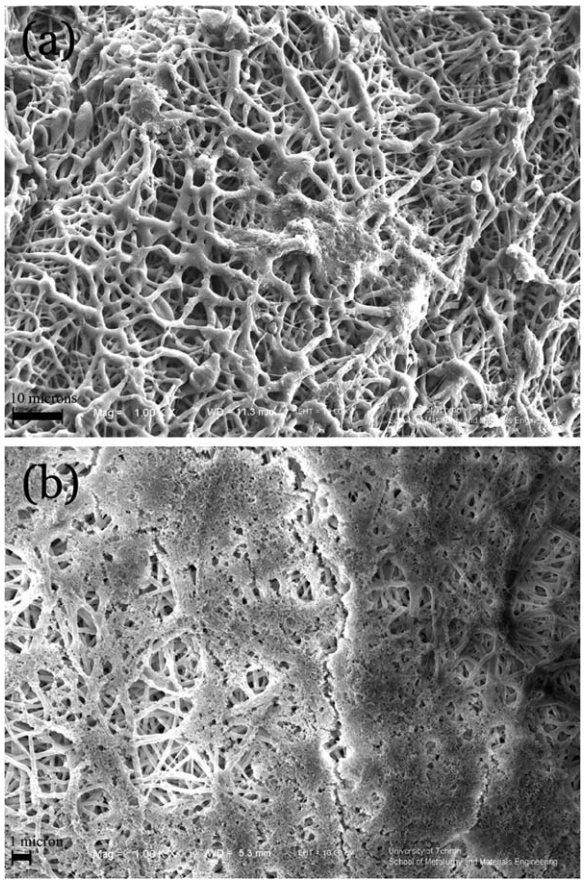

FIGURE 9. FE-SEM micrographs of mesenchymal stem cells after six days seeded on (a) neat PCL/PS nanofibers, (b) PCL/PS nanofibers containing 15% chamomile (the scale bars are 1 and 10 μm).

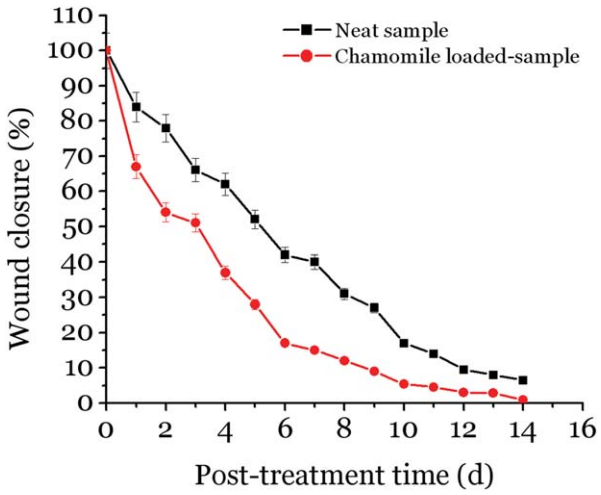

FIGURE 11. The surface area of wounds treated with neat PCL/PS nanofibers and PCL/PS nanofibers containing chamomile during 14 days post-treatment time. (Faster wound healing and its surface reduction is clearly observed for PCL/PS nanofibers containing chamomile in different days). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In vivo rat wound model and histology investigation

The biologic sustainability of neat and 15% chamomile loaded electrospun PCL/PS nanofibrous samples were assessed using a wound-healing wistar rats. Figure 10 exhibits the photograph pictures of wound healing procedure, which were treated with neat and drug loaded PCL/PS nanofibrous mats for 1, 7, and 14 days post-treatment periods. All the wound surfaces were 100% at the first day and reduced in about 40 and 15% when the wound treated with neat and chamomile loaded PCL/PS blend nanofibrous mats at day 7, respectively. This trend seemed interesting when examined after 14 days showing satisfactory results in Figure 11. The wound closure percentage for the above samples was about 10 and 0.5%, respectively. Additionally, the wound closure efficiency of 65/35 (PCL/PS) nanofibrous mats containing chamomile compared with a commercial wound dressing, Com®feel (without silver particles) and/or gauze bandage (control) was evaluated. According to reference,[9] it can be concluded that the

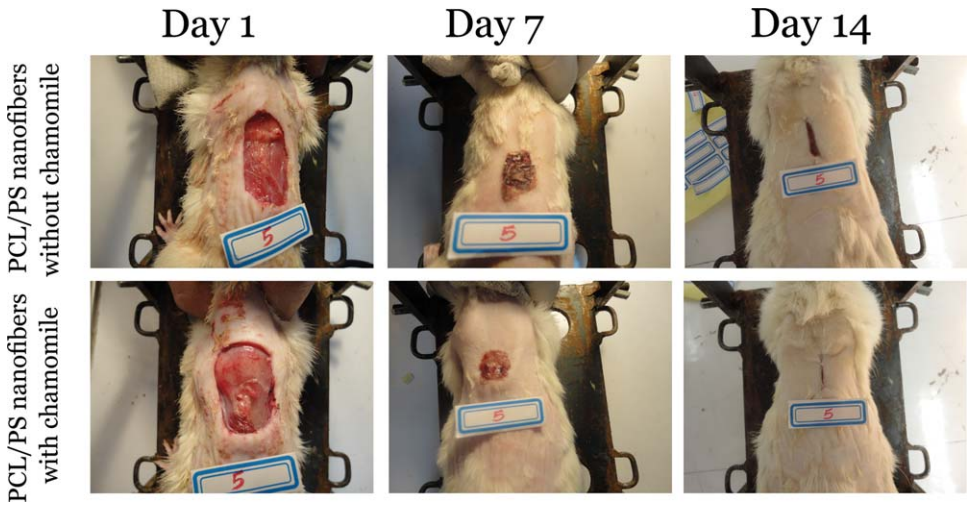

FIGURE 10. Photographic images from skin wounds treated with neat PCL/PS nanofibers and PCL/PS nanofibers containing chamomile at day 1, day 7, and day 14 post-treatment periods. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

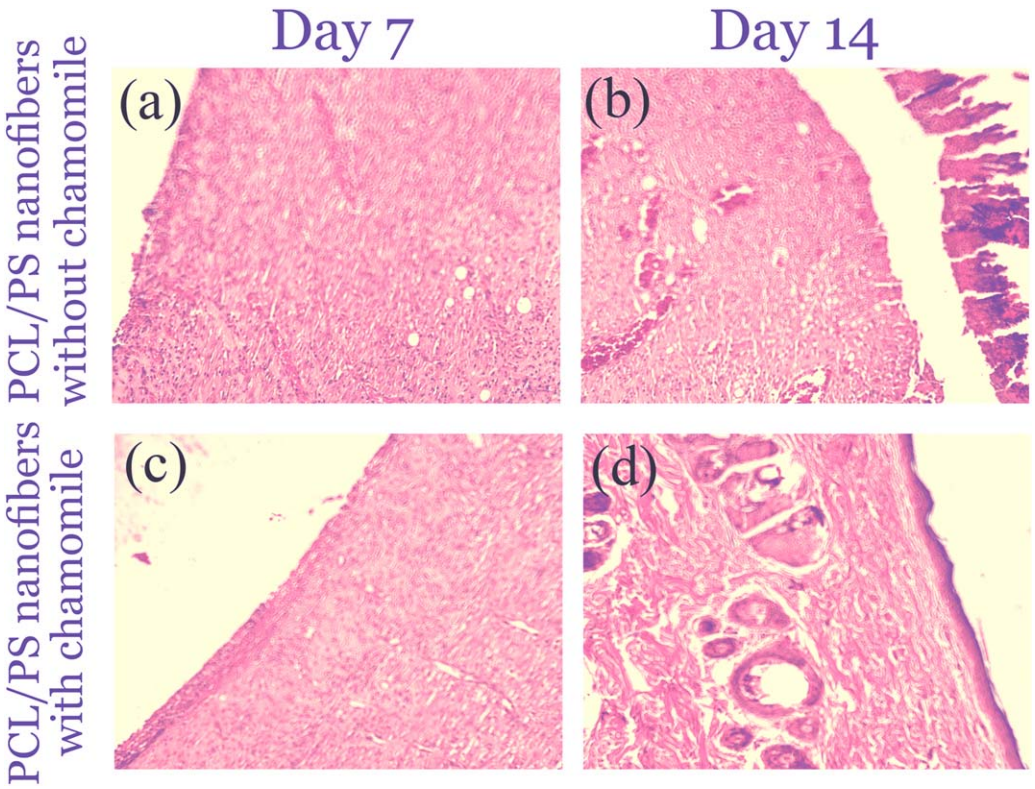

FIGURE 12. Representative histology (H&E staining, 100×) after day 7 (a, c) and day 14 (b, d), after day 7 post-treatment, the wounds treated by PCL/PS nanofibers containing chamomile have new epidermis and necrosis. Also, granulating tissue is observed (c); after day 14 post-treatment, the wound healing trend is very suitable for the dressing (b, d) and formation of new epidermis for wounds treated by PCL/PS nanofibers containing chamomile is remarkable. Some strong dermis also shows the skin has no damage in the dissected tissues samples from the back of the rats. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

chamomile loaded PCL/PS sample had a higher percentage than that of Com®feel and the control on wound healing which were calculated about 11 and 19%, respectively, after 14 days post-treatment time.

In order to study the effect of neat and chamomile loaded PCL/PS nanofibrous samples, (H&E) staining was carried out. Figure 12(a–d) shows a histology study for the treated wounds. Also, Figure 12(a,c) demonstrates the wounds treated with neat and the drug loaded samples at day 7. These results indicated that the inflammatory phase with no epithelial tissues were existed [Figure 12(a)], and this trend changed to the presence of collagen fibers accumulation in dermis tissues with a weak formation of re-epithelialization when the wound treated with electrospun PCL/PS nanofibrous sample containing chamomile [Figure 12(c)]. The wound healing effects after 14 days for nanofibrous sample containing chamomile showed remarkable results in re-epithelialization process along with formation of granulating tissues in the dermis layer [Figure 12(d)]. It means that healing process of the wound treated with PCL/PS nanofibrous sample containing chamomile with no necrosis was accomplished and covered by fibroblast cells. Moreover, the wound histology treated with PCL/PS (65/35) nanofibers containing chamomile exhibited a complete epithelial tissue as far as it was enabled to connect the two edges of the opened wound and contracted the granulation tissue. Consequently, the wound area was smaller and mitosis cells were formed upon the basal layer.

CONCLUSIONS

PCL/PS (65/35) nanofibrous mat samples containing 15% chamomile extract were prepared as an active wound dressing by an electrospinning process under optimum conditions. In order to evaluate and characterize the efficiency of these samples, a series of systematic tests including *in vitro* release rate and cell culture as well as *in vivo* animal model along with histology observation were carried out. FE-SEM morphology studies revealed that chamomile has a good compatibility with the polymer-solvent system. The obtained average nanofibers diameter after addition of the drug was decreased from 268 ± 3 to 175 ± 2 nm. UV–vis spectroscopy results showed that PCL/PS nanofibrous mats containing an herbal drug, chamomile extract had the best release behavior compared to PCL and PS nanofibrous samples. This trend was due to a gradual and continuous release rate of chamomile from the electrospun PCL/PS nanofibers into PBS after 24 h. Mesenchymal stem cells culture on PCL/PS nanofibrous mats showed that chamomile drug had exceptional properties as a cell growth agent, increased cell accumulation and a compatible environment for their viability and proliferation. *In vivo* studies on rat wound for PCL/PS

nanofibrous wound dressing samples with and without drug loading exhibited that simultaneous use of chamomile drug and PS as a tissue cultured polystyrene (TCPS) with a nanofibrous structure can produce an increased rate of re-epithelialization and collagen fibers accumulation in dermis and finally an increased rate of granulation tissue formation after 14 days post-treatment periods.

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REFERENCES

1. Majno G. The Healing Hand: Man and Wound in the Ancient World. Cambridge: Harvard University Press; 1975.
2. Zahedi P, Karami Z, Rezaeian I, Jafari SH, Mahdaviani P, Abdolghaffari AH, Abdollahi M. Preparation and performance evaluation of tetracycline hydrochloride loaded wound dressing mats based on electrospun nanofibrous poly (lactic acid)/poly (ϵ-caprolactone) blends. J Appl Polym Sci 2012;124:4174–4183.
3. Zahedi P, Rezaeian I, Ranaei-Siadat SO, Jafari SH, Supaphol P. A review on wound dressing with an emphasis on electrospun nanofibrous polymeric bandages. Polym Adv Technol 2010;21:77–95.
4. Sikareepaisan P, Suksamrarn A, Supaphol P. Electrospun gelatin fiber mats containing an herbal centella asiatica extract and release characteristic of asiaticoside. Nanotechnology 2008;19:1–10.
5. Uyar T, Nur Y, Hacaloglu J, Besenbacher F. Electrospinning of functional poly (methyl methacrylate) nanofibers containing cyclodextrin-menthol inclusion complexes. Nanotechnology 2009;20:1–10.
6. Opanasopit P, Ruktanonchai U, Suwantong O, Panomsuk S, Ngavhirunpat T, Sittisombut C, Suksamran T, Supaphol P. Electrospun poly (vinyl alcohol) fiber mats as carriers for extracts from the fruit hull of mangosteen. J Cosmet Sci 2008;59:233–242.
7. Suwantong O, Opanasopit P, Ruktanonchai U, Supaphol P. Electrospun cellulose acetate fiber mats containing curcumin and release characteristic of the herbal substance. Polymer 2007;48: 7546–7557.
8. Karami Z, Rezaeian I, Zahedi P, Abdollahi M. Preparation and performance evaluations of electrospun poly (ϵ-caprolactone), poly (lactic acid) and their hybrid (50/50) nanofibrous mats containing thymol as an herbal drug for effective wound healing. J Appl Polym Sci 2013;129:756–766.
9. Zahedi P, Rezaeian I, Jafari SH. *In vitro* and *in vivo* evaluations of phenytoin sodium-loaded electrospun PVA, PCL and their hybrid nanofibrous mats for use as active wound dressings. J Mater Sci 2013;48:3147–3159.
10. Achterrath-Tuckermann U, Kunde R, Flaskamp E, Issac O, Thiemer K, Pharmakologische von kamillen-inhaltsstoffen. V. untersuchungen uber die spasmolytische wirkung von kamillen-inhaltsstoffen und von kamillosan an isolierten meerschweinchen-ileum. Planta Med 1980;39:38–50.
11. Jarrahi M. An experimental study of the effects of matricaria chamomilla extract on cutanous burn wound healing in albino rats. Nat Prod Res 2008;22:422–427.
12. Martins MD, Marques MM, Bussadori SK, Martins MAT, Pavesi VCS, Mesquita-Ferrari RA, Fernandes PSF. Comparative analysis between chamomilla recutita and corticosteroids on wound healing: An *in vitro* and *in vivo* study. Phytoter Res 2009;23: 274–278.
13. Duarte C, Quirino M, Patrocinio M, Anbinder A. Effects of chamomilla recutita (L.) on oral wound healing in rats. Med Oral Patol Oral Cir Bucal 2011;16:716–721.
14. Raymond HM, Montgomery DC. Response Surface Methodology: Process and Product Optimization Using Designed Experiments. New York: Wiley; 2002.
15. Sukigara S, Ganghi M, Ayutsede J, Micklus M, Ko F. Regeneration of *bombyx mori* silk by electrospinning, Part 2: Process optimization and empirical modeling using response surface methodology. Polymer 2004;45:3701–3708.
16. Gu SY, Ren J, Vansco GJ. Process optimization and empirical modeling of electrospun polyacrylonitrile (PAN) nanofiber precursor of carbon nanofibers. Eur Polym J 2005;41:2559–2568.
17. Zahedi P, Rezaeian I, Jafari SH, Karami Z. Preparation and release properties of electrospun poly (vinyl alcohol)/poly (ϵ-caprolactone) hybrid nanofibers: Optimization of process parameters via D-optimal design method. Macromol Res 2013;21:649–659.
18. Abdolghaffari AH, Mahdaviani P, Fallah-Bonekohal S, Ghasemi-Niri SF, Hagiaghaei R, Banan-Khojaste SM, Baeeri M, Abdollahi M. Wound healing effect of rosemary and chamomile combination in rat. Pharmacologyonline 2010;3:139–145.
19. Kerr J. The use of essential oils in healing wounds. Int J Aromather 2002;12:202–206.
20. Svehlikova V, Bennett RN, Mellon FA, Needs FW, Piacente S, Kroon PA, Bao Y. Isolation, identification and stability of acylated derivatives of apigenin 7-o-glucoside from chamomile (Chamomilla recutita [L.] rauschert), Phytochemistry 2004;65:2323–2332.
21. Ahmed FH, El Badri AA, Ibrahim MMK, El Shahed AS, El Khalafawy HMM. Comparative studies of antifungal potentialities for some natural plant oils against different fungi isolated from poultry. Fats Oils 1994;45:260–264.
22. Maneerung T, Tokura S, Rujiravanit R. Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. Carbohydr Polym 2008;72:43–51.
23. Zeng J, Yang L, Liang Q, Zhang X, Guan H, Xu X, Chen X, Jing X. Influence of the drug compatibility with polymer solution on the release kinetics of electrospun fiber formulation. J Control Release 2005;105:43–51.
24. Zamani M, Morshed M, Varshosaz J, Jannesari M. Controlled release of metronidazole benzoate from poly (ϵ-caprolactone) electrospun nanofibers for periodontal diseases. Eur J Pharm Biopharm 2010;75:179–185.
25. Zhou Y, Yang D, Chen X, Xu Q, Lu F, Nie J. Electrospun water-soluble carboxyethyl chitosan/poly (vinyl alcohol) nanofibrous membrane as potential wound dressing for skin regeneration. Biomacromolecules 2008;9:349–354.
26. Xie J, Li X, Xia Y. Putting electrospun nanofibers to work for biomedical research. Macromol Rapid Commun 2008;29:1775–1792.
27. Kedzia B. Antimicroorganisms activity of oil, chamomillae and its components. Herba Polonica 1991;37:29–38.