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Antibacterial and Anti-biofilm Investigation of Electrospun PCL/gelatin/Lawsone Nano Fiber Scaffolds against Biofilm Producing Bacteria

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ABSTRACT

The aim of this study was to evaluate the antibacterial and anti-biofilm activity of polycaprolactone-gelatin (PCL/GEL) nanofibers containing lawsone against four pathogenic bacteria. Different concentrations of lawsone (2-hydroxy-1,4-naphthoquinone) (1, 3, 5 and 10%) were incorporated into PCL/GEL nanofibers via electrospinning technique. Presence of lawsone in the scaffold was confirmed by scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR). Antibacterial and anti-biofilm activities of lawsone loaded scaffolds against clinically isolated *Staphylococcus aureus*, Methicillin-resistant S*taphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Proteus mirabilis* were investigated by disc diffusion method. All scaffolds were able to inhibit the growth of *S. aureus* and MRSA. Additionally, PCL/GEL/lawsone 10% had bactericidal efficacy against *P. mirabilis*. Obtained results of the biofilm formation assay revealed that PCL/GEL/lawsone 10% inhibited the biofilm formation of *S. aureus*, MRSA and *P. mirabilis*. Due to good antibacterial and anti-biofilm activities of lawsone containing scaffolds, PCL/GEL 10% can be a good candidate for application in wound dressing patches.

Keywords: Antibacterial, Anti-biofilm activity, Lawsone, Polycaprolactone, Gelatin, Electrospinning

INTRODUCTION

Biofilm is regarded as a sessile community of bacterial cells encased in their extracellular matrix. This matrix is composed of different types of extracellular polymeric substances (EPS) such as exopolysaccharides, proteins, lipids, biosurfactants and extracellular DNA [1]. Biofilm producing bacteria are protected from antimicrobial agents, antibiotics and harsh environmental conditions [2]. The most commonly studied biofilm producing bacteria are *S. aureus*, Methicillin-resistant S. *aureus* (MRSA), *P. aeruginosa* and *P. mirabilis* [1,3,4]. They are opportunistic pathogens responsible for a wide range of nosocomial infections of burn wounds, respiratory and urinary tract [5,6]. Biofilm bacteria exhibit different gene expression mechanisms to enhance their tolerance to antimicrobial treatment [7,8]. It is estimated that biofilms account for up to 80% of chronic microbial infections in the body [9]. This biofilm-associated infections affect millions of people worldwide [8]. Previous reports point out the relation between biofilm, antibiotic resistance and application of anti-biofilm agents to prevent biofilm formation [10,11]. In recent years, investigation of natural and synthetic antimicrobial and anti-biofilm compounds has come to researcher's attention [7,12,13]. Medicinal plants are a potent resource of anticancer, antiviral, antimicrobial agents and have many pharmaceutical ingredients [14]. Among these natural compounds in literatures, naphthoquinones and their derivatives exhibite promising activities including antibacterial, antifungal, anti-inflammatory and anti-biofilm activities [15-21]. Lawsone (2-hydroxy-1,4-naphtho-quinone) is the active ingredient of *Lawsonia inermis* L. (commonly known as Henna). The ability of henna leaf extract for the inhibition or disruption of microbial-derived biofilm has been reported previously. For example, Lattab *et al*. investigated the antimicrobial and anti-biofilm activity of ethyl acetate and butanolic fractions of hydro-methanolic extract of *L. inermis* [3]. Likewise, antimicrobial activity of oily and alcoholic extracts of *L. inermis* was reported previously [22]. In another study, herbal extract was also

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capable of inhibiting the growth of microorganisms which cause burn wound infections [23].

In the past years, remarkable attention has been focused on the development of novel drug delivery systems for herbal drugs. Incorporation of herbal constituents in delivery systems have been aided to enhance their solubility, bioavailability, stability, pharmacological activity and protection from physical and chemical degradation [24]. Various novel herbal drug delivery systems such as liposomes, phytosomes, niosomes, microspheres, polymeric nanoparticles, nanocapsules and tissue engineered scaffolds have been developed recently [25,26]. Electrospinning is a versatile fiber production method using electrostatic force to produce nano to micron fibers. These fibers provide a high surface area porous nanostructure for drug delivery applications. Ahire et al. incorporated copper nanoparticles into poly L-lactic acid (PLLA) and poly(ethylene oxide) (PEO) polymers during electrospinning and this nanostructure, prevented the formation of biofilm produced by *P. aeruginosa and S. aureus* [13]. Fallah *et al*. [27] demonstrated a high throughput antibacterial activity of curcumin loaded PCL/GEL scaffold against MRSA and extended spectrum B lactamase (ESBL) producing bacteria. Basha *et al*. [21] reported the in vitro antimicrobial and in vivo wound healing activity of lawsone loaded chitosan microsphere. In a recent investigation, Hadisi *et al*. [28] explored the *in vivo* antibacterial and anti-inflammatory potential of electrospun henna/gelatin/starch nanofibers and proposed that henna leaf extract could be a promising agent to treat burn wound infections by preventing burn wound bacteria. Since lawsone is the main ingredient of henna, it seems that the addition of lawsone to electrospun nanofibers would have anti-biofilm potential. To the best of our knowledge, no previous study has investigated the anti-biofilm effect of the lawsone loaded electrospun scaffolds. In this study, we prepared electrospun PCL/GEL nanofibers containing different concentrations of lawsone and their effects on biofilm formation and bacterial growth were also investigated.

MATERIAL AND METHODS

Chemicals

Poly (caprolactone) (PCL) (Mw 80 kDa), cell culture grade gelatin and 2-hydroxy-1,4-naphthoquinone (97%), acetic acid, dimethylformamide (DMF) were purchased from Sigma Aldrich Company (Germany). LB medium was purchased from Merck Chemicals (Germany).

Fabrication of Electrospun Nanofibers

PCL (15% w/v) and gelatin powder (15% w/v) separately dissolved in acetic acid (90% v/v) at 40 °C and stirred for 24 h at room temperature. Different concentrations of lawsone powder (1, 3, 5 and 10% w/v) were dissolved in DMF. PCL and gelatin were blended at a ratio of 2:1 to acquire a uniform solution. Then different lawsone concentrations in DMF, were added to the mixture and were stirred for another 30 min before electrospinning. The blend solution was electrospun with 15 kV applied voltage, 15 cm nozzle to collector distance and 0.5 ml h^{-1} flow rate. Nanofibers were allowed to dry under vacuum oven (Binder, Germany) at 37 °C.

Characterization of Nanofibers

Morphology of nanofibers was evaluated by scanning electron microscopy (SEM) at an accelerating voltage of 30 KV (SEM KYKY-EM3200, China). The related size distribution of nanofibers was graphed by image analysis software (ImageJ 1.518j, (NIH, Bethesda, MD, USA). Chemical structures of nanofibers and lawsone powder were characterized by Fourier transform infrared spectroscopy (FTIR, Bruker Tensor II, Germany). The spectra of the samples were recorded in 400-4000 cm^{-1} range with 4 cm^{-1} resolution. Mechanical properties of scaffolds were measured by a uniaxial tensile machine (SANTAM, STM20, Iran) with a load cell of 50 N. Four sheets (20 mm× 10 mm) of each PCL/GEL/lawsone (0, 1, 3, 5 and 10%) scaffolds were measured at a rate of 10 mm min^{-1}. In order to understand the effect of lawsone on the hydrophilicity of membranes, the contact angle of nanofibers was measured by Sessile drop method [29] and contact angle measuring system (Rame-Hart instrument, Model 100-0, USA). Three specimens were cut to 10 mm × 10 mm sheets for each test. To perform the degradation of nanofibers, scaffolds were immersed in one mL phosphate buffered saline (PBS) (pH = 7.4) and incubated at 37 °C for 14 days. At specific intervals, the samples were dried and weighted and the percent of weight loss (%) was calculated

by the following equation:

$$\text{Weight loss (\%)} = Mi - Md/Mi \times 100$$

Mi is the initial weight of the dry specimen before submersion in PBS and Md is the weight of the sample in its dry state after submersion in PBS for different time intervals.

In Vitro Antibacterial Study

Clinically isolated biofilm producing bacteria were a gift from Dr. Mojtaba Shakibaie (Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Iran). The antimicrobial activity of electrospun mats against both Gram-positive *S. aureus*, MRSA and Gram-negative *P. aeruginosa* and *P. mirabilis* was assessed using disk diffusion method according to Clinical and Laboratory Standards Institute [30]. Briefly, fresh bacterial cultures (10^5 colony forming units, $CFUml^{-1}$) were each spread onto LB agar (Himedia, India) and circular punched lawsone containing PCL/GEL scaffolds (15.6 mm diameter) were placed on the surface of the spread plates and then incubated at 37 °C for 24 h to determine the inhibition zone of bacterial growth. Each test was done in triplicate.

Biofilm Formation in the Presence of Scaffolds

The anti-biofilm activity of lawsone containing nanofibers was evaluated according to Ahire *et al*. [31] with some modifications. Briefly, *S. aureus*, MRSA, *P. aeruginosa* and *P. mirabilis* were cultured in tryptic soy broth (TSB) medium containing glucose (1% w/v) overnight. To reach 0.5 McFarland standard (1.5×10^8 $CFUml^{-1}$), they were allowed to grow at the next day until the turbidity of medium reach to 0.13 at OD 600 nm. The bacterial cultures were further diluted to reach 10^6 $CFUml^{-1}$ and 100 µl of each prepared inoculum was added to 900 µl of TSB medium in 24-well plate (SPL Life Sciences, Korea). The lawsone containing scaffolds (PCL/GEL/ lawsone 0, 1, 3, 5 and 10%) were inserted into each well. Bacterial suspension which was not exposed to nanofibers considered as positive control. After 24 h incubation of the plates at 37 °C, under static condition, non-adherent cells were removed and the plates were rinsed with PBS carefully. In order to fix the formed biofilm, plates were incubated at 60 °C for 1 h. Total biofilm was measured by staining of each well with crystal violet solution (0.01% w/v) for 20 min followed by washing with sterile deionized water and keeping at room temperature until drying. Afterward, 200 µl of acetic acid (33% v/v) was added to each well and the optical density was measured at 570 nm by Synergy2 microplate reader (BioTek, USA). All experiments were carried out in triplicate and mean of the obtained results was reported.

Effect of Lawsone Loaded Scaffolds on the Viability of Biofilm Producing Bacteria

The number of viable cells of biofilm bacteria was determined according to previous studies [32,33]. The UV-sterilized PCL/GEL/lawsone (0, 1, 3, 5 and 10%) nanofibers were each seeded into separated 24-well microplate. 10^5 $CFUml^{-1}$ of each bacterial culture in TSB medium, with 1% glucose, was added to the surface of mats and incubated at 37 °C for 24 h to allow microbial attachment and biofilm development. After incubation, the mats were removed and carefully washed with sterile PBS to remove any unattached cells slowly. The samples were then transferred to a new microtube with one ml PBS and vortexed vigorously to detached biofilm cells from the scaffolds. The solution was then serially diluted and 10 µL of each serial dilution was plated on TSA medium and $CFUml^{-1}$ were calculated after 24 h of incubation and reported as $CFUml^{-1}$.

$CFUml^{-1}$ = colony number on plate × 100 × dilution factors

Furthermore, to confirm bacterial attachment, biofilm producing bacteria were cultured on lawsone loaded scaffolds for 24 h (as previously described) and nanofibers were then washed with PBS three times, fixed with paraformaldehyde 4% and photographed with SEM micrograph.

Statistical Analysis

All experiments were accomplished in triplicate and expressed as mean ± SD. The difference between groups was determined using Graph pad Prism software (version 6, San Diego, CA, USA) and one-way analysis of variance

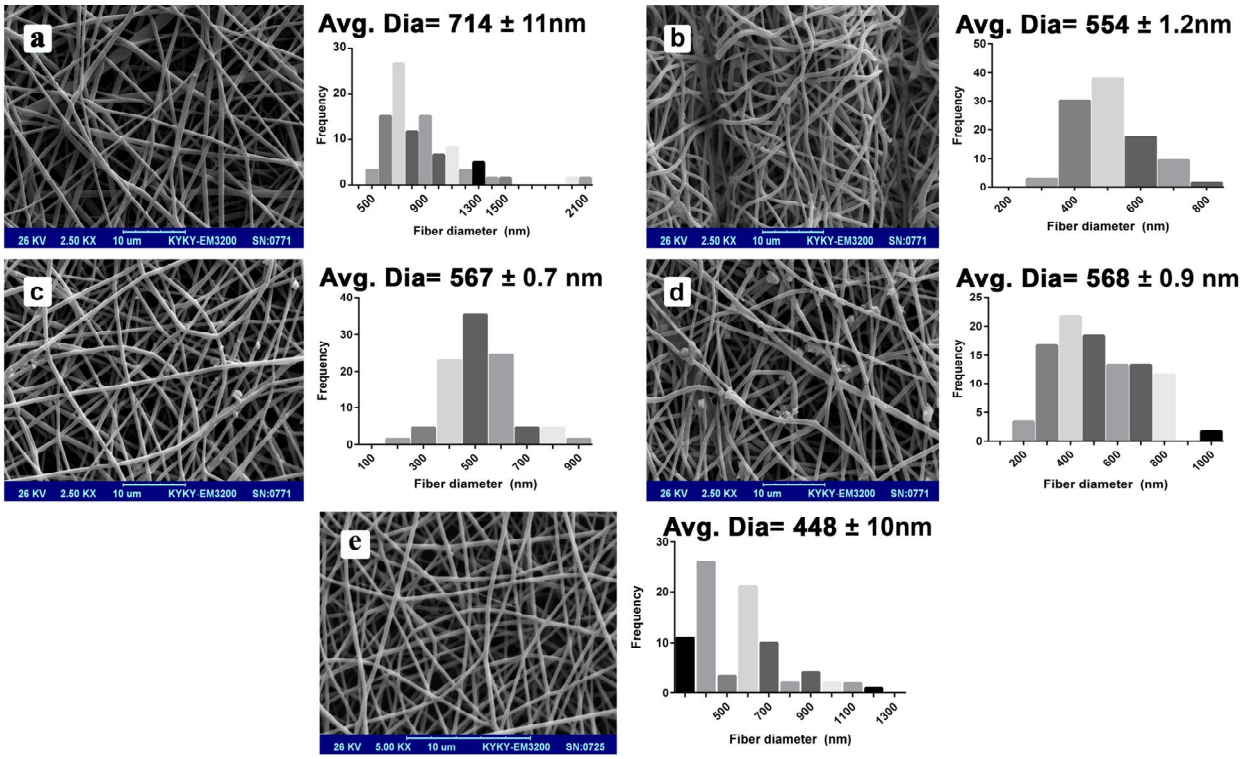

Fig. 1. The morphology and fiber diameter distribution of PCL/gelatin electrospun nanofibers from (a) PCL/GEL/lawsone 1%, (b) PCL/GEL/lawsone 3%, (c) PCL/GEL/lawsone 5%, (d) PCL/GEL/lawsone 10% and (e) PCL/GEL.

(ANOVA). *P*-values of less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Morphology Characterization of Electrospun Nanofibers

In this study, PCL and gelatin polymers were blended with different concentrations of lawsone (0, 1, 3, 5 and 10%) and electrospun under the controlled condition to produce similar density mats. All electrospinning parameters such as polymer concentration, solvents, flow rate, the distance between drum and jet were optimized. As shown in Fig. 1, uniform, smooth and bead free nanofibers were produced. SEM micrograph (Fig. 1) showed that mean fiber diameters were 448 ± 10, 714 ± 11, 554 ± 1.2, 567 ± 0.7 and 568 ± 0.9 nm for PCL/GEL/lawsone 0, 1, 3, 5 and 10%, respectively. In this study, lawsone addition increased the fiber diameter average from 448 nm in control scaffold to 714 nm in PCL/GEL/lawsone 1% significantly. Nevertheless, the increase in fiber diameter of other nanofibers (PCL/GEL/lawsone 3, 5, 10%) was not significant in comparison to PCL/GEL. The result of this study confirmed the findings of previous work which reported that the incorporation of natural compounds can increase fiber diameter [34].

FTIR Spectroscopy

The IR spectra of lawsone and PCL/GEL/lawsone have been shown in Fig. 2. Referring to lawsone, the characteristic peaks at 775 cm^{-1} (C-H bending of aromatic rings), 1457 cm^{-1} (C=C stretching of aromatic compounds), 1585, 1641 and 1679 cm^{-1} (C=O stretching), and 3169 cm^{-1} (OH stretching) were observed. This is in accordance with the signature peaks of lawsone as reported in the literature [28,35]. The IR spectra in PCL/GEL consists of several distinct peaks at 3299 cm^{-1} (OH stretching), 2863 and 2936 cm^{-1} (C-H stretching), 1465 cm^{-1} (CH_2 bending),

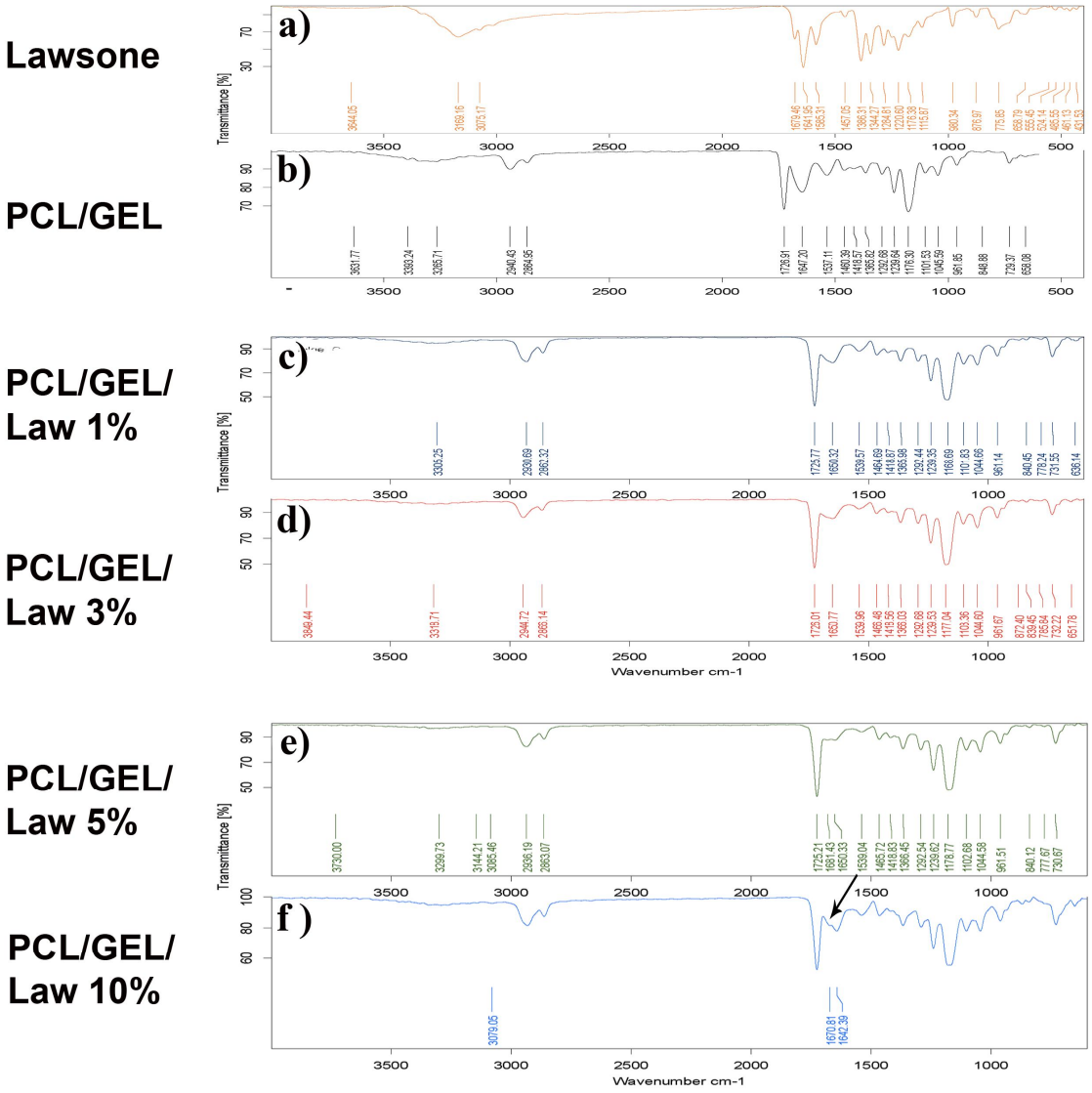

Fig. 2. The IR spectra of (a) pure lawsone, (b) PCL/GEL, (c) PCL/GEL 1%, (d) PCL/GEL 3%, (e) PCL/GEL 5% and (f) PCL/GEL 10% nanofibers. The black arrow shows the peak at 1679 cm^{-1}.

1366 cm^{-1} (CH_3 bending), and 1044-1102 cm^{-1} (C-O stretching). After the addition of lawsone to the PCL/GEL mats, the characteristic peaks of lawsone overlapped with the peaks of gelatin and PCL. However, a peak was appeared at around 1679 cm^{-1} and 3075 in the IR spectra of the mats containing 5 and 10% lawsone. These peaks are related to the C=O stretching and aromatic C-H stretching in lawsone structure respectively and confirms the successful incorporation of lawsone in the PCL/GEL nanofibers.

Mechanical Characterization of Nanofibers

Mechanical properties of nanofibers are important parameters for biomedical applications such as drug carriers and tissue engineering so they should be strong and flexible enough to withstand external forces [36]. To understand the effect of lawsone content on the mechanical properties of the scaffold, the tensile tests were conducted and the strength, strain at break and Young's modulus values of PCL/GEL and PCL/GEL containing (1, 3, 5 and 10%)

lawsone electrospun nanofibers have been shown in Table 1. The tensile strength of PCL/GEL, PCL/GEL/lawsone 1%, PCL/GEL/lawsone 3%, PCL/GEL/lawsone 5% and PCL/GEL/lawsone 10% was 1.79 ± 0.1, 0.955 ± 3.6, 0.87 ± 0.22, 0.611 ± 0.054 and 0.38 ± 0.19, respectively. As depicted in Table 1, the tensile strength and young modulus were decreased with the increase in the lawsone content. The result indicated that the presence of lawsone in nanofibers mats exerted a lowering effect in mechanical properties. This result was expected because lawsone was incorporated into nanofibers by poor hydrogen bonds and weakened intermolecular forces between PCL and gelatin polymers in electrospun nanofibers. Yousefi *et al.* [37] observed that inserted henna extract into electrospun nanofibers reduced mechanical properties of chitosan-polyethylene oxide (PEO) mats. Similar results were also reported that incorporation of herbal extracts reduced tensile strength and Young's modulus of electrospun nanofibers. This reduction in tensile strength may be due to plasticizing effect of lawsone in the nanofibrous structure [28,37].

Hydrophilicity and Biodegradability of Nanofibers

PCL is a hydrophobic polymer. It has been approved by Food and Drug Administrator (FDA) to be used as drug delivery device and implant [38]. In this study, we blended PCL with gelatin to improve its surface chemistry. To determine the lawsone effect on the hydrophilicity of scaffolds, water contact angle of four different lawsone containing scaffolds was measured. As shown in Fig. 3, following the addition of lawsone, a significant decrease in water contact angle observed. By the addition of lawsone, the contact angle decreased from 76.26 in PCL/Gel to 30.55 in PCL/GEL/lawsone 10%. Surprisingly, lawsone is an approximately insoluble compound in water, but its incorporation increased the hydrophilicity of membranes. A possible explanation for this result may be the hydroxyl group on lawsone, which can interact with water.

In the current study, the biodegradation of electrospun PCL/GEL nanofibers with different concentrations of lawsone was determined with regard to the weight loss of nanofibers in aqueous medium over 10 days. As it has been shown in Fig. 4, it can be observed that, in all nanofibrous mats, the weight loss increased over the course of days. The weight loss was not significant in the first day of experiment and five days following immersion of nanofibers in PBS, the weight loss was statistically significant (34 ± 6.2) in PCL/GEL/lawsone 10%. On tenth day, PCL/GEL/lawsone 5% and 10% had the highest weight loss versus PCL/GEL/lawsone1, 3% and PCL/GEL mats. Although gelatin is a hydrophilic polymer, lawsone incorporation in PCL/GEL scaffold affected weight loss more significantly. As PCL/GEL/lawsone 5% and 10% had the highest degradation after 10 days. This finding can be related to lawsone effect in lowering the intermolecular forces between PCL and gelatin and increase biodegradation of nanofibers. Similar studies confirmed that presence of herbal extracts increases the biodegradation of electrospun nanofibers [28,39].

In Vitro Antimicrobial Activity

In the next step of our study, the antimicrobial potential of lawsone containing nanofibers were examined against biofilm producing bacteria. PCL/GEL (as negative control), had no antimicrobial activity on both Gram-negative and Gram-positive bacteria. The inhibition growth zone diameter of PCL/GEL with different concentrations of lawsone has been shown in Table 2. These results demonstrate that antibacterial activity of mats increased by increasing concentration of lawsone. PCL/GEL/lawsone 10% inhibited the growth of *S. aureus,* MRSA and *P. mirabilis*, while PCL/GEL/lawsone 1%, 3% and 5% had an inhibitory effect on *S. aureus* and MRSA (Gram-positive ones). Resistance of *P. aeruginosa* and *P. mirabilis* is ascribed to the outer membrane of Gram-negative bacteria, which act as a barrier to protect the cell from bacteriostatic or bactericidal agents. The present finding is consistence with earlier studies which reported that lawsone has antimicrobial effect against Gram-positive bacteria such as *Bacillus subtilis* and MRSA [40-42]. Notwithstanding Sanchez-Calvo [43] reported no antibacterial and antifungal activity for synthetic lawsone (2-hydroxy-1,4-naphthoquinone) using the broth microdilution method. In another study, butanolic fraction of *L. inermis* had antimicrobial and antibiofilm property against *P. aeruginosa* [3].

Biofilm Formation and Bacterial Growth in the Presence of Nanofibers

The effect of lawsone on biofilm formation by *S.*

Table 1. Mechanical Properties of the PCL/GEL/lawsone (1, 3, 5 and 10%) Scaffolds and PCL/GEL as Control. The Data have Beencollected as Mean ± Standard Deviation (SD) Obtained from Three Independent Experiments

Nanofiber scaffolds	Stress at breaking (Mpa)	Strain (%)	Modulus (Mpa)
PCL/GEL	1.79 ± 0.1	42 ± 3.1	4.261 ± 2.43
PCL/GEL/lawsone 1%	0.95 ± 3.6	56.6 ± 2.63	1.68 ± 2.7
PCL/GEL/lawsone 3%	0.87 ± 0.22	62.9 ± 0.7	1.38 ± 1.16
PCL/GEL/lawsone 5%	0.611 ± 0.054	47.98 ± 0.92	1.27 ± 0.98
PCL/GEL/lawsone 10%	0.38 ± 0.19	92.97 ± 4.7	0.41 ± 3.71

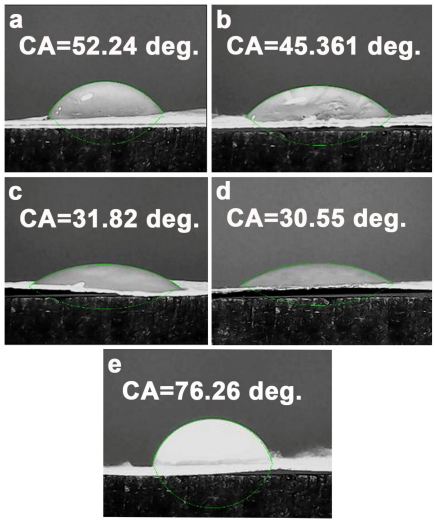

Fig. 3. Water contact angle of the nanofiber's surface. (a), (b), (c), (d) and (e) show the contact angle of PCL/GEL 1%, PCL/GEL 3%, PCL/GEL 5%, PCL/GEL 10% and PCL/GEL, respectively.

aureus, MRSA, *P. mirabilis* and *P. aeruginosa* has been shown in Fig. 5. The biofilm formation was decreased by increasing lawsone in nanofibers significantly ($P < 0.0001$). PCL/GEL (as negative control) had no anti-biofilm activity against biofilm producing strains. Biofilm formation of *S. aureus* was decreased from 80.4% ± 0.5 in PCL/GEL/lawsone 1% to 11 ± 5.56% in PCL/GEL/lawsone 10%. The amount of MRSA biofilm was also decreased from 67 ± 7.6 in PCL/GEL/lawsone 1% to 5.3 ± 3 in PCL/GEL/lawsone 10%. For *P. mirabilis*, biofilm content was decreased by increasing lawsone and reached from 97.7 ± 1.3 in PCL/GEL/lawsone 1% to 22.9 ± 3.24 in PCL/GEL/lawsone 10%. No remarkable difference in biofilm formation by *P. aeruginosa* was observed between PCL/GEL and PCL/GEL/lawsone 1%, 3% and 5%. In contrast, biofilm produced by *P. aeruginosa* was significantly reduced in PCL/GEL/lawsone 10% (83.3 ± 5.6).

SEM illustration of produced biofilm by *S. aureus, P. mirabilis,* MRSA and *P. aeruginosa* on PCL/GEL/lawsone 5% and PCL/GEL has been shown in Fig. 6. SEM micrograph of biofilm formation on the surface of PCL/GEL/lawsone 1, 3 and 10% was similar to that of PCL/GEL/lawsone 5% (data not shown). As presented in

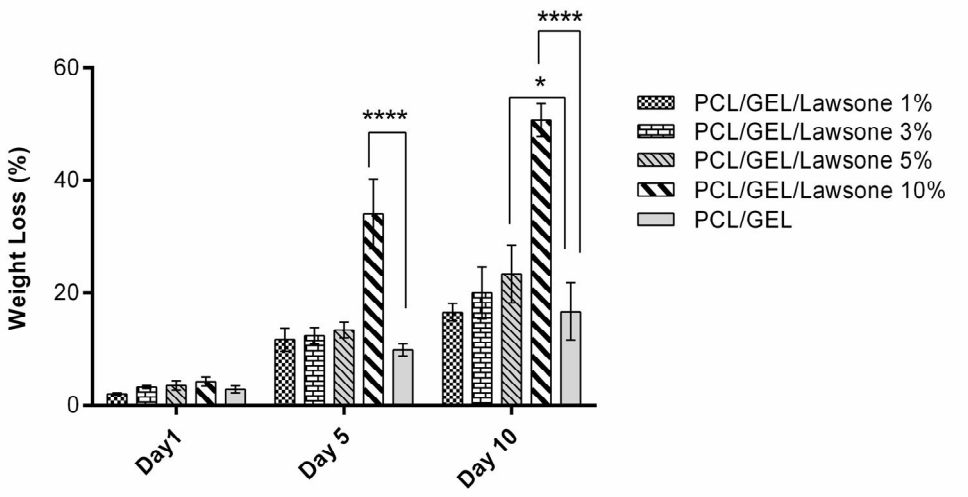

Fig. 4. The weight loss of different PCL/GEL mats containing lawsone and PCL/GEL, as control, after 10 days of incubation in PBS at 37 °C.

Table 2. Inhibition Growth Zone Diameter (mm) Data of Scaffolds with Different Lawsone Content against Selected Bacteria

Nanofiber scaffolds	*S. aureus*	*P. mirabilis*	MRSA	*P. aeruginosa*
PCL/GEL	-	-	-	-
PCL/GEL/lawsone 1%	27.4 ± 0.01	-	18.1 ± 0.1	-
PCL/GEL/lawsone 3%	27.9 ± 0.02	-	18.5 ± 0.06	-
PCL/GEL/lawsone 5%	33.4 ± 0.08	-	18.52 ± 0.2	-
PCL/GEL/lawsone 10%	37.6 ± 0.41	18.6 ± 0.2	19.5 ± 0.05	-

Figs. 6a and c, compared to PCL/GEL nanofibers (Figs. 6e and g), the amount of biofilm in *S. aureus* and MRSA was reduced considerably.

The effect of lawsone scaffolds on bacterial growth and viability is illustrated in Fig. 7. Although cell viability of biofilm producing bacteria was decreased in all lawsone treated nanofibers, the viability reduction observed in PCL/GEL/lawsone 5% and 10% was statistically significant ($P < 0.05$). PCL/GEL/lawsone 5% and 10% inhibited *S. aureus* growth completely. In PCL/GEL/lawsone 10% group, bacterial viability of MRSA and *P. mirabilis* was decreased (99.9 ± 0.03% for MRSA and 37.3 ± 1.9% for *P. mirabilis*). PCL/GEL/lawsone 5%, inhibited growth of MRSA to 47.7 ± 0.8%. PCL/GEL had no effect on biofilm producing bacteria. None of lawsone loaded scaffolds had significant effect on reduction of *P. aeruginosa* viability.

Based on these findings, lawsone incorporated in PCL/GEL electrospun nanofibers inhibited cell growth and biofilm formation of the tested bacteria. Biofilm formation is a serious thread in medicine, and established biofilms can tolerate antimicrobial and phagocytosing agents, making biofilms difficult to eradicate [44]. Nowadays many studies are being concentrated on the utilization of antimicrobial and anti-biofilm compounds for inhibition of bacterial growth, even in planktonic or sessile forms. Several investigations reported the extensive antimicrobial activity of *L. inermis* (henna) upon nosocomial pathogens like *staphylococcus aureus,* Methicillin-resistant *Staphylococcus aureus*, *Enterococcus faecium*, *Bacillus subtilis*, *Escherichia coli*, *proteus mirabilis*, *Klebsiella pneumoniae*,

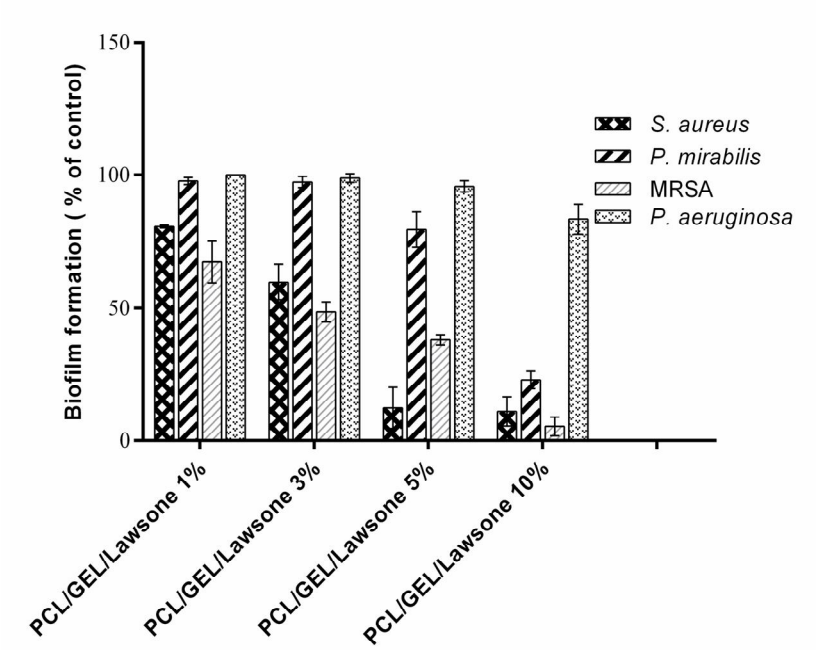

Fig. 5. Biofilm formation of *S. aureus*, *P. mirabilis*, MRSA and *P. aeruginosa* on PCL/GEL/lawsone 1%, 3%, 5% and 10% after incubation for 24 h. PCL/GEL containing biofilm forming bacteria in the absence of lawsone were designed as control.

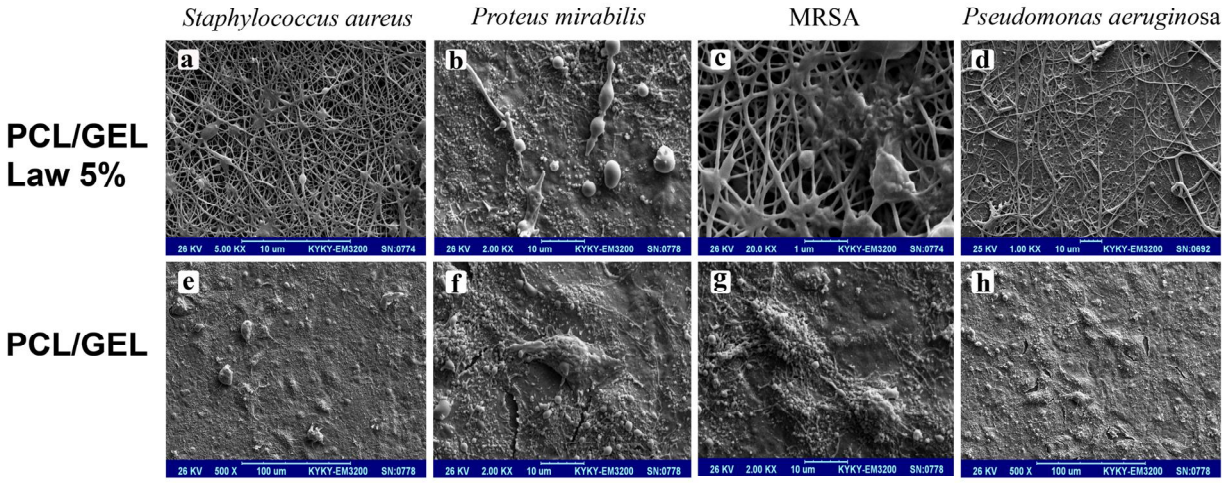

Fig. 6. Scanning electron microscopy (SEM) images of produced biofilm by *S. aureus*, *P. mirabilis*, MRSA and *P. aeruginosa* on PCL/GEL/lawsone 5% nanofibers after 24 h of incubation.

pseudomonas aeruginosa [17,45-47]. Triveni *et al.* [48], showed that leaf extract of henna extract had promising antibiofilm activity against MRSA. Moreover, Lattab *et al.* [3] introduced hydromethanolic extract of henna leaves as a potent anti-biofilm and antibacterial compound against *P. aeruginosa*. In recent years, utilization of antibacterial and

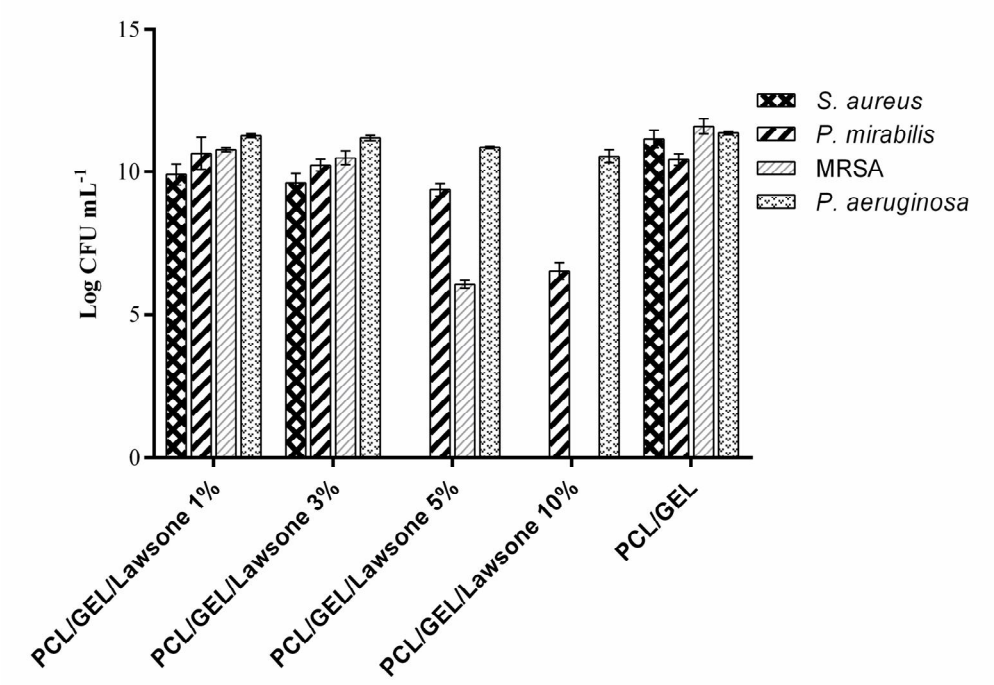

Fig. 7. Effect of PCL/GEL/lawsone mats on growth of four biofilm producing bacterial strains. Each well received 10^5 CFUml^{-1} of each bacterial species after 24 h incubation at 37 °C.

anti-biofilm compounds in regeneration medicine and wound dressing mats has come to attention. For example, there are several reports about employment of herbal extracts, with antimicrobial capability, incorporated in different bioactive electrospun nanofibers to accelerate wound healing process and skin repair [27,28,37]. Hadisi *et al.* constructed electrospun gelatin-oxidized starch nanofibers containing henna extract for treating second-degree burn wounds [28]. This study confirmed that antimicrobial activity of nanomatrix is due to the presence of various phenolic compounds such as 2-hydroxy-1,4-naphthoquinone in the henna extract that could interact with bacterial cell wall and inactivate their function. Other studies also represented excellent anti-biofilm activity of chemical compounds [13,31]. A literature review revealed no report on the anti-biofilm activity of neither lawsone nor lawsone loaded electrospun PCL/gelatin nanofibers. Results of the present work demonstrated that higher concentrations of lawsone containing nanofibers such as PCL/GEL/ lawsone 5% and PCL/GEL/lawsone 10% had substantial effect of biofilm inhibition and bacterial eradication effect.

CONCLUSIONS

Collectively our results indicate that all lawsone containing scaffolds had antibacterial activity against *S. aureus* and MRSA and PCL/GEL/lawsone 10% prevented the growth of *P. mirabilis* either. A significant anti-biofilm activity in inhibition of biofilm observed in all biofilm strains, while PCL/GEL/lawsone 3%, 5% and 10% had an inhibitory effect in detachment studies. This study has demonstrated for the first time that lawsone can be incorporated in PCL/gelatin nanofibers via electrospinning and showed potential antibacterial and anti-biofilm activities against biofilm producing bacteria. This study corroborates our approach of using lawsone scaffold in situations where common antibiotic therapies are ineffective. This research will serve as a base for further studies in the production of wound dressing bandages to treat burn wounds.

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