

## Research Article

# Emulsion Electrospun Fiber Mats of PCL/PVA/Chitosan and Eugenol for Wound Dressing Applications

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Received 3 May 2019; Accepted 7 July 2019; Published 30 October 2019

Guest Editor: Bin Xu

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In recent years, the damaging effects of antimicrobial resistance relating to wound management and infections have driven the ongoing development of composite wound dressing mats containing natural compounds, such as plant extracts and their derivatives. The present research reports the fabrication of novel electrospun Polycaprolactone (PCL)/Polyvinyl Alcohol (PVA)/Chitosan (CS) fiber mats loaded with Eugenol (EUG), an essential oil, known for its therapeutic properties. The electrospun fiber mats were prepared via electrospinning from either water-in-oil (W/O) or oil-in-water (O/W) emulsions and characterized using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FT-IR), total porosity measurements, and water contact angle. The *in vitro* EUG release profile and antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* were also evaluated. The obtained results proved that the EUG was loaded efficiently into electrospun PCL/PVA/CS fiber mats and the two W/O and O/W emulsions prepared from the PCL/PVA/CS (7:3:1) and PCL/PVA/CS (3:7:1) revealed porosity within the ideal range of 60–90%, even when EUG was loaded. The measured contact angle values showed that the O/W emulsion exhibited a more hydrophilic character and the wettability noticeably decreased after adding EUG in both emulsion blends. Furthermore, the electrospun PCL/PVA/CS fiber mats demonstrated a rapid release of EUG during the first 8 hours, which enhanced gradually afterward (up to 120 hours). Moreover, an efficient antibacterial activity against *S. aureus* (inhibition ratios of 92.43% and 83.08%) and *P. aeruginosa* (inhibition ratios of 94.68% and 87.85%) was displayed and the *in vitro* cytotoxic assay demonstrated that the normal human dermal fibroblasts (NHDF) remained viable for at least 7 days, after direct contact with the produced electrospun fiber mats. Therefore, such findings support the biocompatibility and suitability of using these EUG-loaded electrospun PCL/PVA/CS fiber mats as a new innovative wound dressing material with potential for preventing and treating microbial wound infections.

## 1. Introduction

The integrity of the skin can be affected by several disorders. When the skin is injured, it becomes more susceptible to microbial infections, which can have negative consequences on the healing process [1, 2]. Therefore, the development of materials with suitable barrier properties using different antimicrobial agents have shown to prevent and suppress the microbial invasion and colonization by pathogenic bacteria [3]. However, it is still a challenge to achieve the release of these agents directly onto the damaged tissue to ensure the correct therapeutic dosage and prevent the wound from getting infections.

Recently, composite wound dressing mats produced by electrospinning have attracted research attention due to their unique properties like extremely high surface area, high porosity, and small pore size [4, 5]. In addition, these

materials are known in the wound management field for their capability to absorb excess exudate from the wound, while they create and maintain a moist environment. Moreover, electrospun fiber mats provide wound protection from mechanical trauma and bacterial colonization, exhibit high air, and oxygen permeability, as well as mimic the properties of natural extracellular matrix (ECM) ensuring additional support for cell growth and proliferation in order to promote the wound healing with the minimum scar formation [4–6]. Also, electrospinning has the ability to incorporate different types of bioactive or therapeutic agents, thus leading to the enhancing of the desirable wound healing properties [6]. Antibiotics, growth factors (GFs), vitamins, antimicrobial, analgesics, and anti-inflammatory compounds are among the most successful bioactive agents so far loaded into electrospun fiber mats [7]. Nevertheless,

medicinal plant extracts, and their derivatives, such as essential oils, have captured the attention of researchers due to their traditional therapeutic properties, cost-effectiveness, and availability [6].

Eugenol (EUG), a naturally occurring phenolic component extracted from cloves, is known for its analgesic, antimicrobial, antioxidant, anti-inflammatory, and anticarcinogenic properties and has demonstrated abilities to improve the healing process and tissue regeneration [8–10]. However, EUG presents poor water solubility, and its stability can be affected by chemical and enzymatic degradation, losses by volatilization or thermal decomposition [11, 12]. In order to overcome EUG disadvantages, emulsion electrospinning is of particular interest to successfully incorporate both hydrophilic and hydrophobic bioactive agents, while preventing the loss of their structural integrity and bioactivity [13].

Emulsion electrospinning is a novel and straightforward technique similar to the traditional electrospinning, where water-in-oil (W/O) or oil-in-water (O/W) emulsions are used instead of a conventional polymer solution [14, 15]. This modified electrospinning method does not require a special apparatus, neither a careful selection of the operating conditions to ensure desirable results. In addition, emulsion electrospinning has been used to improve the solubility of poorly soluble bioactive agents and, consequently, their therapeutic effectiveness. Furthermore, this approach also increases the affinity of the oil and water phases and plays a significant role in the stability of low molecular weight polymers and diluted polymer solutions [13].

In this context, the present work describes the innovative development of EUG-loaded into electrospun Polycaprolactone (PCL)/Polyvinyl Alcohol (PVA)/Chitosan (CS) fiber mats through W/O and O/W emulsions by nanospider technology, a needle-free electrospinning equipment, based on a rotating spinning electrode immersed into a liquid polymer bath. The modern nanospider technology differs from conventional electrospinning because it allows the formation of many Taylor cones (the source of nanofibers) simultaneously on the surface of the rotating spinning electrode, and hence this technology is highly productive and more effective to produce high-quality nanofibers [16, 17].

In this way, a nontoxic hydrophobic synthetic polymer, PCL, known for its many advantages over the main synthetic polymers was used to act as a protective barrier against external threats. However, the data available in the literature showed that cells are more prone to adhere, proliferate, and grow on a moderate hydrophilic surface than on a hydrophobic or super-hydrophilic surface [18, 19]. Therefore, CS, a naturally occurring polysaccharide, was selected for its abilities, namely by supporting cell adhesion and growth of several cell types and also by exhibiting hemostatic and antimicrobial properties [20]. Nevertheless, CS is difficult to electrospin into a fibrous structure and exhibits low mechanical properties, which restricts its use in medical applications [21, 22]. To overcome these limitations, CS has been blended with other biocompatible hydrophilic synthetic polymers, like PVA [23]. Herein, in this study, CS and PVA were blended in order to ensure efficient exudate management and provide a moist wound environment. Moreover,

the PVA/CS blend exhibits an inhibitory effect on microbial growth and promote cell adhesion and proliferation [24]. The biological abilities of EUG, mainly analgesic, anti-inflammatory, and antimicrobial properties have also been exploited to strengthen the healing process.

Therefore, we present new findings claiming the development of new EUG-loaded electrospun PCL/PVA/CS fiber mats prepared from W/O and O/W emulsions, for wound dressing applications. The results obtained revealed that mixing PCL, PVA, and CS enhanced the final properties of the blend. CS formed miscible blends with PVA, which acted as a good emulsifying and dispersing agent and made CS/PVA blend compatible with the PCL. The antimicrobial and non-cytotoxic effect of EUG was also seen as a promising strategy to develop new innovative wound dressings, with the potential to prevent and treat microbial-resistant wound infections.

## 2. Materials and Methods

**2.1. Materials.** Polycaprolactone (PCL) (MW 80.000 g/mol) and Chitosan (CS) (MW 50.000–190.000 g/mol, degree of deacetylation 75–85%) and Eugenol (EUG) were purchased from Sigma-Aldrich. Polyvinyl Alcohol (PVA) (MW 115.000 g/mol) was purchased from VWR Chemicals. Chloroform (analytical grade), Dimethylformamide (DMF) (analytical grade), Glacial acetic acid, and Ethanol absolute were purchased from Fisher Chemical. Normal human dermal fibroblasts (NHDF) cells were acquired from ATCC—American Type Culture Collection. Brain Heart Infusion (BHI) Broth was provided from Panreac. Nutrient Agar (NA), Nutrient Broth (NB), and Agar for microbiology were purchased from Fluka. Sodium chloride (NaCl), Mueller-Hinton Broth (MHB), Dimethyl sulfoxide (DMSO) anhydrous  $\geq 99.9\%$ , Tween 80, Trysin, and 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma Aldrich. Phosphate-buffered saline (PBS), pH 7.4 was purchased from Alfa Aesar. All solvents were used as received without further purification.

**2.2. Determination of Minimum Inhibitory Concentration (MIC) of EUG.** Minimal Inhibitory Concentration (MIC) of EUG was applied against two bacterial strains: *Staphylococcus aureus* ATTC 6538 and *Pseudomonas aeruginosa* PA25 by the broth microdilution method according to NCLS M07-A6 guidelines. Briefly, EUG stock solution was prepared in DMSO (10% (v/v)) to yield a concentration of 20  $\mu\text{L}/\text{mL}$ . Serial dilutions of EUG were made in MHB with concentrations ranging from 10 to 1  $\mu\text{L}/\text{mL}$ . Then, overnight liquid bacterial cultures were adjusted to 0.5 McFarland turbidity standards with sterile water. Afterward, bacterial work suspensions were formed from 500  $\mu\text{L}$  of the 0.5 McFarland suspensions and 4500  $\mu\text{L}$  of MHB. A volume of 50  $\mu\text{L}$  of bacterial work suspensions and 50  $\mu\text{L}$  of the EUG dilutions were added into 96 multi-well polystyrene plates (Sigma-Aldrich). The multi-well plates were incubated for 24 hours at 37°C. Deposited bacteria (dot-shaped) in the bottom of each well were evaluated. The last well in the dilution series that showed deposit (bacterial killing) corresponded to MIC of EUG. All the determinations were performed in triplicate.

**2.3. Preparation of Electrospinning Emulsions.** PCL solution (8% w/v) was prepared by dissolving PCL in chloroform/DMF (volume ratio of 30:20) at 50°C under magnetic stirring until complete dissolution. Afterward, the solution was left to stir overnight to ensure proper dissolution of the PCL before being blended with the PVA/CS blend. CS solution (4% w/v) was prepared by dissolving CS in acetic acid (14%) at room temperature. Also, a PVA solution (10% w/v) in distilled water was prepared at 90°C. PVA and CS solutions were then mixed in two different ratios, 7:1 and 3:1 (v/v), to form the PVA/CS blend solution. The W/O emulsion blend 8% PCL/10% PVA/4% CS (7:3:1) was prepared by simultaneous adding of PVA/CS blend solution to PCL solution, followed by mixing with high-speed homogenizer (Techmatic S2), while the O/W emulsion blend 8% PCL/10% PVA/4% CS (3:7:1) was prepared by simultaneous adding of PCL solution to PVA/CS blend solution. The final mixtures were stirred at room temperature for 4 hours to ensure complete dissolution and to obtain uniform emulsions. PCL/PVA/CS blends were also loaded with EUG. The final concentration of the EUG was 5% (w/w) (based on the weight of the PCL powder), i.e., 5% over the weight of the fiber (owf) EUG. All emulsions were immediately used for electrospinning.

**2.4. Electrospinning.** The stable and homogenous emulsions were electrospun using Nanospider Technology (Nanospider laboratory machine NS LAB 500S from Elmarco s.r.o., Cezek Republic, <http://www.elmarco.com>). Electrospinning of the emulsions was carried out with a distance between the spinning electrode and collecting electrode of 13 cm at a driving voltage of 75.0 kV and a rotating spinning electrode of 9.6 rpm (60 HZ). The collection time was ~1.0 hour at 25°C and relative humidity up to 35%. The electrospun fiber mats were collected on polypropylene nonwoven fabric and were dried in the hood at room temperature till constant weight. The same electrospinning conditions were used to pure PCL and PVA/CS blend as a reference for electrospun PCL/PVA/CS fiber mats with and without loaded EUG.

## 2.5. Electrospun Fiber Mats Characterization

**2.5.1. Fourier Transform Infrared Spectroscopy (FT-IR).** The chemical composition of pure PCL, PVA/CS, and electrospun PCL/PVA/CS fiber mats with and without loaded EUG were analysed on FT-IR. Measurements were performed on Thermo-Nicolet is10 FT-IR spectrophotometer over the range 500–4000  $\text{cm}^{-1}$  with a spatial frequency resolution of 4  $\text{cm}^{-1}$  and each sample was scanned 64 times.

**2.5.2. Surface Morphology of Electrospun Fiber Mats.** The surface morphology of the electrospun PCL/PVA/CS fiber mats with and without loaded EUG was investigated with the help of scanning electron microscope (SEM) Hitachi S2700 at a high voltage of 20 kV. The fiber diameters were directly measured from SEM images using public domain software (Image J, National Institutes of Health, USA). After that, the average diameter and diameter distribution were determined by applying SPSS Statistics 21.0 software (SPSS Inc. Chicago, USA).

**2.5.3. Porosity Measurement.** The total porosity of the dry electrospun fiber mats was measured using a liquid displacement method, as described by Chitrattha et al. [25]. Ethanol was used as the displacement liquid because it readily penetrated into the pores of the matrices and did not induce shrinkage or swelling of these materials. Briefly, a preweighed porous membrane ( $W_s$ ) was immersed in a cylinder containing 20 mL of ethanol ( $W_1$ ) and placed in a water sonicator bath (Ultrasons-H, P-Selecta) for 40 min at 30°C to assist the penetration of ethanol into the porous structure. Subsequently, the volume in the sonicated cylinder containing porous membrane impregnated with ethanol was readjusted to 20 mL and weighed ( $W_2$ ). After that, the membrane saturated with ethanol was removed from the cylinder and the cylinder was reweighed ( $W_3$ ). The porosity ( $\epsilon$ ) of these porous materials was determined using the following Equation (1) [25]:

$$\epsilon(\%) = (W_2 - W_3 - W_s)/(W_1 - W_3) \times 100. \quad (1)$$

**2.5.4. Water Contact-Angle Determination.** The surface wettability of pure PCL, PVA/CS, and electrospun PCL/PVA/CS fiber mats with and without loaded EUG were determined through water contact angle (WCA) using a data physics contact angle system OCAH-200 apparatus. To accomplish that, deionized water droplets were placed on the surface of each sample at room temperature and the contact angle was calculated after 10 s of incubation time to avoid discrepancy in the contact angle values measured. The measurements were conducted on different sample locations and the average was reported as the contact angle of each sample.

**2.6. Release In Vitro of EUG from EUG-Loaded Electrospun PCL/PVA/CS Fiber Mats.** Release of EUG from EUG-loaded electrospun PCL/PVA/CS fiber mats was studied by UV-Vis spectrometry. To perform this assay, standard solutions of EUG with concentrations from 0.00  $\mu\text{L}/\text{mL}$  to 10.00  $\mu\text{L}/\text{mL}$  were prepared and a calibration curve was drawn at 282 nm (maximum wavelength of EUG). Samples (2 × 2 cm) of EUG-loaded electrospun PCL/PVA/CS fiber mats were immersed in 10 mL of phosphate-buffered saline (PBS, pH = 7.4) at 37°C with constant rotation at a speed of 100 rpm. Afterward, 3 mL of release medium was recovered at specified time intervals, ranging between 0 and 120 hours, and at the same time, an equal volume of the fresh PBS solution was replenished to maintain a constant volume. The concentration of EUG that remained in the release medium at each time point was quantified at 282 nm using UV-Vis spectrophotometer. All measurements were carried out in triplicate.

**2.7. Estimation of Antibacterial Activity of EUG-Loaded Electrospun PCL/PVA/CS Fiber Mats.** The antibacterial effect of EUG loaded into electrospun PCL/PVA/CS fiber mats was carried out according to E 2180-07 standard test method for determining the activity of incorporated antimicrobial agent(s) in polymeric or hydrophobic materials. *S. aureus* and *P. aeruginosa* were selected, once they are the most common bacteria present in wound infections [26].

To perform the assay, a bacterial suspension ( $1-5 \times 10^8$  CFU/mL) was added in an agar slurry previously prepared from 0.85 (w/v) NaCl and 0.3 (w/v) agar-agar in deionized water. Afterward, a thin layer of inoculated agar slurry was poured onto  $3 \times 3$  cm square samples of electrospun PCL/PVA/CS fiber mats with and without EUG loaded. The samples were evaluated immediately after adding the inoculated agar slurry ( $T_{0h}$ ) and after 18–24 hours in contact with the inoculated agar slurry at  $37^\circ\text{C}$  for 18–24 hours ( $T_{24h}$ ). For each sample, serial dilutions of the agar slurry were done with 0.85 (w/v) NaCl, plated in agar plates, and incubated for 18–24 hours at  $37^\circ\text{C}$ . The antimicrobial efficiency was quantitatively expressed in percentage of bacterial reduction (%R) using Equation (2) by comparing the CFU on the control samples (electrospun PCL/PVA/CS fiber mats),  $C$ , with the CFU on the samples loaded with 5% owf EUG (5% EUG-loaded electrospun PCL/PVA/CS fiber mats),  $A$  [27].

$$\text{Percentage Reduction (\%R)} = ((C - A)/C) \times 100. \quad (2)$$

According to Japanese Industrial Standard JIS L 1902:2002, the bacteriostatic or bactericidal effect of the electrospun PCL/PVA/CS fiber mats containing EUG was then calculated using Equations (3) and (4):

$$\text{Bacteriostatic activity value} = M_B - M_C, \quad (3)$$

$$\text{Bactericidal activity value} = M_A - M_C, \quad (4)$$

where  $M_A$  is the average of the common logarithm of the number of living bacteria in control samples immediately after adding the inoculated agar slurry ( $T_{0h}$ ),  $M_B$  is the average of the common logarithm of the number of living bacteria in control samples after 18–24 hours in contact with the inoculated agar slurry ( $T_{24h}$ ), and  $M_C$  is the average of the common logarithm of the number of living bacteria in samples containing 5% owf EUG after 18–24 hours in contact with the inoculated agar slurry ( $T_{24h}$ ).

**2.8. Characterization of the Cytotoxicity Profile of the EUG-Loaded Electrospun PCL/PVA/CS Fiber Mats.** The cytotoxic profiles of the produced electrospun PCL/PVA/CS fiber mats with and without EUG were performed *in vitro* following ISO 10993-5 (Biological evaluation of medical devices-Part 5: Tests for *in vitro* cytotoxicity). Briefly, the samples were placed in 24-well plates (Sigma-Aldrich) at the center of each well occupying  $<1/10$  of its area and then sterilized under UV irradiation ( $254 \text{ nm}$ ,  $\sim 7 \text{ mW cm}^{-2}$ ) for 1 hour. After that, normal human dermal fibroblast (NHDF) cells were used as a model to seed each well at a density of  $1 \times 10^4$  cells/well and incubated at  $37^\circ\text{C}$ , in an incubator under a humidified atmosphere containing 5%  $\text{CO}_2$ . After an incubation period of 1, 3, and 7 days, the mitochondrial redox activity of the viable cells was assessed through the reduction of the MTT into an insoluble blue formazan dye. In this way, the medium of each well was removed and it was replaced with a mixture of fresh culture medium and the MTT reagent solution. After 4 hours of incubation at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$  humidified atmosphere, the MTT solution was removed and DMSO was added to each well in order to dissolve the

formazan crystals. Finally, the absorbance of each sample was measured at  $570 \text{ nm}$  using a microplate reader (Biorad xMark microplate spectrophotometer). Wells containing cells cultured without the materials and wells containing cells cultured with EtOH (96%) were also included as a negative control ( $K^-$ ) and positive control ( $K^+$ ), respectively.

**2.9. Statistical Analysis.** Data were analyzed statistically by the one-way analysis of variance (ANOVA) and Tukey Post-Hoc test using SPSS 21.0 (SPSS Inc. Chicago, USA). Statistical calculations were based on a confidence level  $\geq 95\%$  (values of  $P < 0.05$  were considered statistically significant).

### 3. Results and Discussion

**3.1. Determination of Minimum Inhibitory Concentration (MIC) of EUG.** Minimal Inhibitory Concentration (MIC) of EUG against *S. aureus* and *P. aeruginosa* was found to be  $4.25 \mu\text{L/mL}$  and  $3.00 \mu\text{L/mL}$ , respectively. Several other studies have confirmed the significant antibacterial activity of EUG against *S. aureus* and *P. aeruginosa*. For example, Sanla-Ead et al. [28] described MIC of  $25.00 \mu\text{L/mL}$  against *S. aureus* and  $50.00 \mu\text{L/mL}$  against *P. aeruginosa*, which were higher than those found in this study. However, the MIC values for specific alkaloids obtained from plants, namely the bioactive properties of these natural compounds depend on various factors, including the environmental condition and seasonal variables, as well as the geographical location and method of extraction.

#### 3.2. Electrospun Fiber Mats Characterization

**3.2.1. Fourier Transform Infrared Spectroscopy (FT-IR).** The FT-IR spectra of pure PCL, PVA/CS and electrospun PCL/PVA/CS fiber mats with and without EUG were acquired and represented in Figure 1. The FT-IR spectrum of pure PCL exhibits its characteristic peaks, Figures 1(a)(i) and 1(b)(i). Peaks at  $2866.05$  and  $2945.76 \text{ cm}^{-1}$  are attributed to symmetric and asymmetric  $\text{CH}_2$  stretching vibrations. At  $1722.51 \text{ cm}^{-1}$  occurs an intense, sharp peak, which corresponds to carbonyl stretching vibration of the ester group, while the peaks at  $1293.80$ ,  $1239.67$ , and  $1164.76 \text{ cm}^{-1}$  are associated for C-O and C-C stretching, asymmetric and symmetric-C-O-C stretching, respectively [29]. On the other hand, the characteristic bands of both PVA and CS are displayed in the FT-IR spectrum of the PVA/CS blend. Figures 1(a)(ii) and 1(b)(ii) shows the characteristic peaks at  $1558.80 \text{ cm}^{-1}$  and  $1653.25 \text{ cm}^{-1}$ , corresponding to N-H bending vibrations in secondary amides and C=O stretching vibrations of the amide bond, respectively. Moreover, the spectrum of PVA/CS blend exhibits a broad peak at  $3118.56 \text{ cm}^{-1}$ , which is assigned to O-H and N-H stretching.

Additionally, the emulsion blends of PCL/PVA/CS (Figures 1(a)(iii) and 1(b)(iii)) display a sharp peak at around  $1720.00 \text{ cm}^{-1}$  which corresponds to the PCL and a broad peak in the region between  $3000$  and  $3500 \text{ cm}^{-1}$  that belongs to PVA/CS blend. Likewise, the FT-IR spectra of electrospun

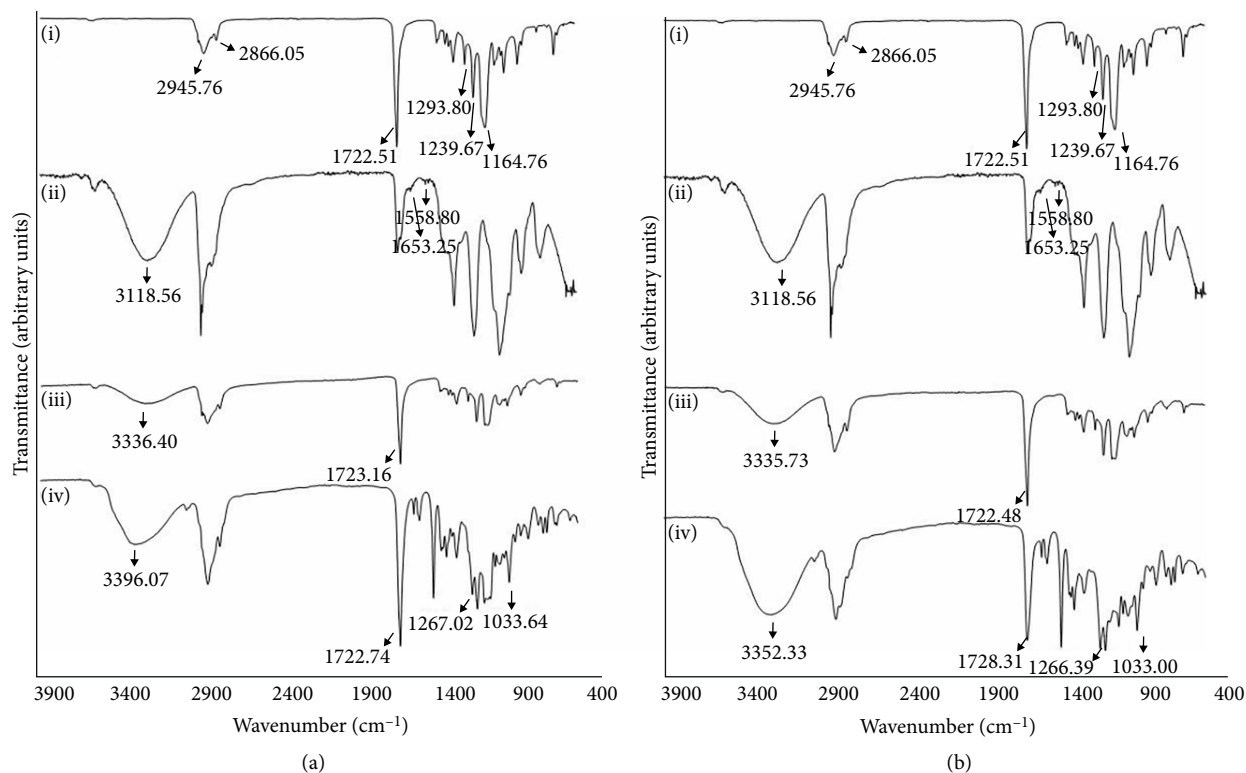


FIGURE 1: FT-IR spectrums of pure PCL (i), PVA/CS (ii) and electrospun PCL/PVA/CS fiber mats from W/O emulsion (a) and from O/W emulsion (b). Electrospun PCL/PVA/CS fiber mats without loaded EUG (iii) and with 5% owf EUG (iv).

PCL/PVA/CS fiber mats containing EUG (Figures 1(a)(iv) and 1(b)(iv)) exhibit those peaks and other characteristic peaks at around 1033.00 and 1267.00 cm<sup>-1</sup>, regarding the stretching vibration of symmetric and asymmetric C-O-C, which demonstrate the presence of the methoxy group of EUG. Moreover, the presence of all characteristic peaks of the components used to produce the electrospun fiber mats proves the successful blending of the EUG with the PCL/PVA/CS emulsions.

However, the FT-IR analysis suggests that there is no evidence of chemical bonding between each component of the blend because new absorption peaks are not seen in FT-IR spectra. Nevertheless, some chemical interactions could have occurred among carboxyl, amino, and hydroxyl groups of the PCL, PVA, CS, and bioactive agent. These interactions can be suggested by slight displacement of absorption peaks, as well as by different peak intensities and peak widths.

Similar results were presented by Ajallouei et al. [20]. The FT-IR spectrum of PLGA/CS/PVA nanofibers displayed the characteristic peaks of PLGA, CS, and PVA, and when they removed the PVA from the blend fewer hydroxyl groups were detected as shown by a narrower and shorter peak at 3400–3700 cm<sup>-1</sup>. Furthermore, Zargham et al. [19] demonstrated by FT-IR that olive oil was successfully embedded within the PCL/Olive oil composite nanofibers. Nevertheless, in the FT-IR spectrum of the PEO/CS/PCL/Olive oil composite nanofibers were not evident chemical bonding between the PEO/CS and PCL/Olive oil.

**3.2.2. Surface Morphology of Electrospun Fiber Mats.** SEM micrographs and fiber diameter distributions of EUG-loaded electrospun PCL/PVA/CS fiber mats, prepared via electrospinning from either W/O or O/W emulsions, are shown in Figure 2. The electrospun PCL/PVA/CS fiber mats produced from W/O emulsion displayed beads and fibers, known as the spindles or bead-on-a-string morphologies, with an average diameter of  $379.05 \pm 161.95$  nm, Figure 2(a). When 5% owf EUG was incorporated, nanofibers with similar morphology were produced with a mean diameter of  $387.07 \pm 179.51$  nm, Figure 2(b). In turn, the electrospun PCL/PVA/CS fiber mats produced from O/W emulsion, with and without EUG loaded, demonstrated thinner and more uniform fibers with fewer beads, with an average diameter of  $174.47 \pm 38.93$  nm and  $199.90 \pm 48.86$  nm, Figures 2(c) and 2(d), respectively. It showed that the PVA, which acts as an emulsifying agent, is the most critical component of the blend which controls the process of emulsion electrospinning [30]. Thus, PVA decreases the surface tension between immiscible phases and contributes towards the formation of more uniform nanofibers [30, 31]. Moreover, the O/W emulsions are especially useful for controlled or sustained release of the oil-soluble bioactive compounds, as the EUG.

Hajiali et al. [32] obtained similar results to Sodium Alginate (SA)-Polyethylene Oxide (PEO) nanofibers and SA-PEO containing 5% v/v of Lavender oil nanofibers. In both cases, 85% of fibers exhibited diameters in the range of 50–125 nm, with a most representative percentage between 75 and 100 nm. This result confirmed that the addition of

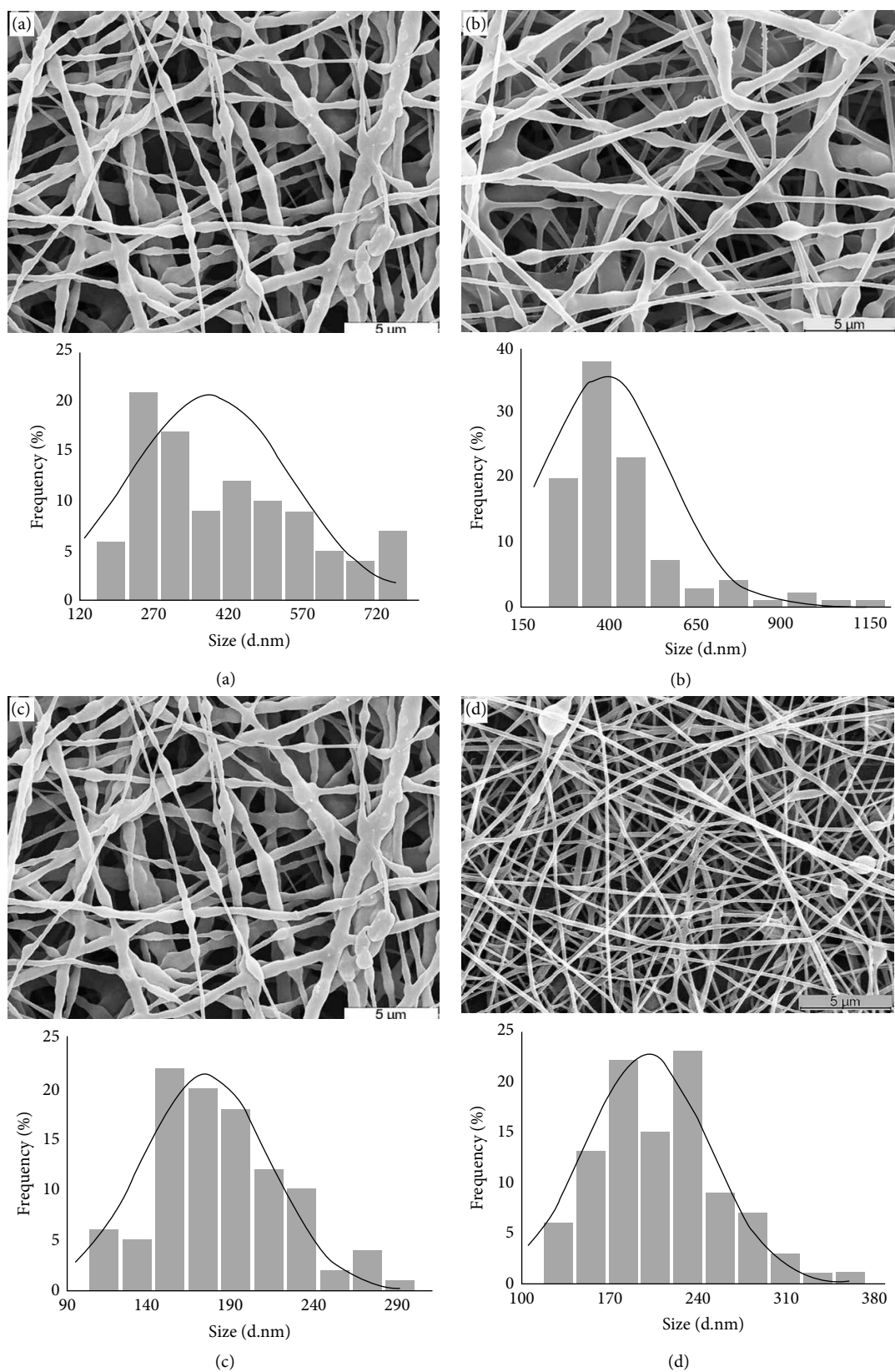


FIGURE 2: SEM images with fiber diameters distribution obtained from electrospun (a) PCL/PVA/CS fiber mats prepared via W/O emulsion without EUG loaded, (b) and with 5% owf EUG, (c) PCL/PVA/CS fiber mats prepared via O/W emulsion without EUG loaded, (d) and with 5% owf EUG.



essential oil to the polymer solution did not have a significant effect on fiber diameters.

Moreover, Gholipour-Kanani et al. [33] produced PCL/PVA/CS nanofibrous mats by adding PVA to PCL/CS in 2:1:1.33 (PCL/PVA/CS) blend ratio and obtained uniform fibers with diameters in the range of  $136 \pm 37.00$  nm. This study revealed a mean diameter lower than the average diameter of fibers found for PCL/PVA/CS (7:3:1) ( $379.05 \pm 161.95$  nm) and PCL/PVA/CS (3:7:1) ( $174.47 \pm 38.93$  nm), confirming that both the composition and ratio of each component in the blend influence the fiber diameters.

**3.2.3. Porosity Measurement.** The wound dressings' porosity contributes to a correct gas, nutrient, and fluid exchange, as well as drug release. The porosity of a material is also essential to support cell adhesion and proliferation in the wound site [25].

Herein, the electrospun PCL/PVA/CS fiber mats produced from W/O and O/W emulsions presented a total porosity of  $88.74 \pm 2.27\%$  and  $90.46 \pm 2.15\%$ , respectively, as demonstrated in Table 1. The porosity values slightly decreased when the ratio of PCL in the emulsion blend increased, namely to W/O emulsion, and when EUG was loaded. Moreover, the electrospun PCL/PVA/CS fiber mats prepared from W/O emulsion loaded with EUG exhibited a total porosity of  $84.44 \pm 3.10\%$ , while a value of  $88.52 \pm 4.09\%$  was observed from the O/W emulsion loaded with EUG.

The total porosity values of the electrospun PCL/PVA/CS fiber mats, with and without EUG, are within the preferred range of 60–90% for an effective wound healing process [34]. Indeed, the high porosity exhibited by the produced electrospun fiber mats is more proper to provide a suitable pore structure for cell migration, nutrient exchange, and development of a new ECM, although it depends on the wound characteristics and the aim of wound management [25].

**3.2.4. Water Contact-Angle Determination.** Surface wettability of electrospun fiber mats is one of the most desirable material properties to the wound dressing applications, which may influence the initial adhesion and migration of cells, as well as their proliferation to the wound site [29, 35]. Therefore, the contact angle between water droplets and the electrospun fiber mats (WCA) was determined to assess the wettability of the pure PCL, PVA/CS, and electrospun PCL/PVA/CS fiber mats with and without EUG (Figure 3).

In this study, a hydrophobic polymer, PCL,  $90.2 \pm 7.30^\circ$ , was blended with a hydrophilic polymer PVA/CS blend,  $28.8 \pm 5.50^\circ$ , to improve the hydrophilic features of electrospinning emulsions [36]. WCA values of  $61.2 \pm 4.24^\circ$  and  $42.85 \pm 3.95^\circ$  were obtained for electrospun PCL/PVA/CS fiber mats prepared from W/O and O/W emulsions, respectively. The O/W emulsion presented a more hydrophilic character than W/O emulsion, due to inherent hydrophilic nature of both PVA and CS. However, the incorporation of EUG through the electrospun fiber mats was more hydrophobic. WCA values of  $70.70 \pm 4.62^\circ$  and  $59.37 \pm 5.11^\circ$  were displayed for electrospun PCL/PVA/CS fiber mats loaded with 5% owf EUG from W/O and O/W emulsions, respectively. Hence, the addition of EUG improves the most hydrophilic character of the O/W emulsion.

TABLE 1: Porosity measurement of electrospun PCL/PVA/CS fiber mats with and without loaded EUG.

Samples	Porosity (%)
W/O emulsion blend PCL/PVA/CS	$88.74 \pm 2.27$
W/O emulsion blend PCL/PVA/CS + 5% owf EUG	$84.44 \pm 3.10$
O/W emulsion blend PCL/PVA/CS	$90.46 \pm 2.15$
O/W emulsion blend PCL/PVA/CS + 5% owf EUG	$88.52 \pm 4.09$

Similar results found by Cui et al. [35] showed that increasing the PVA-SbQ amount in comparison with the Zein amount resulted in a lower WCA. The reason for this was because Zein, a predominantly corn-based protein, exhibits a more hydrophobic character due to a higher amount of hydrophobic (non-polar) amino acids than hydrophilic (polar) ones.

**3.3. Release In Vitro of EUG from EUG-Loaded Electrospun PCL/PVA/CS Fiber Mats.** Figure 4 shows the cumulative release of 5% owf EUG loaded into electrospun PCL/PVA/CS fiber mats produced from W/O and O/W emulsions. The release profiles revealed a burst effect of  $65.05 \pm 3.58\%$  and  $54.61 \pm 3.19\%$  during the initial 8 hours, followed by a continuous slow and sustained release over the next days. The observed initial burst could be due to the EUG adsorbed on or near the surface of the electrospun nanofibers, while the sustained release might be due to the diffusion of EUG from electrospun nanofibers. During the period of incubation,  $80.37 \pm 2.19\%$  and  $70.15 \pm 1.63\%$  of total EUG were released from W/O and O/W emulsions, respectively. This result can be explained by the higher affinity of EUG for PCL phase, which results in a faster EUG release from W/O emulsions. In addition, the EUG loaded into electrospun PCL/PVA/CS fiber mats prepared from O/W emulsion need to pass through the shell PVA/CS barrier before being released. In turn, the PVA/CS blend exhibits better wettability than the PCL and, consequently, the PVA/CS matrix is easily penetrated by the release medium, releasing the EUG quickly. Therefore, the initial EUG burst release observed is a predictable result, due to the chosen polymer blend to produce the electrospun fiber mats and selected method. The burst release can improve the therapeutic effect and accelerate the healing process, once EUG is suitable to reach some relief at the beginning of wound treatment and to stimulate an adequate initial inflammatory response, essential to proper tissue repair.

These results are in agreement with Motealleh et al. [37] in that electrospun PS fibers exhibited a lower chamomile release than electrospun PCL fibers. In this study, PCL revealed greater compatibility with the chamomile extract and its flavonoid apigenin. A similar trend was displayed by EUG loaded into W/O emulsion blend.

Controlling the rapid initial release of the biologically active compounds can be needed to treat specific types of wounds at different stages of healing. A variety of approaches have been employed to reduce and avoid the occurrence of an early burst release. Among them, the capacity of diffusion of a bioactive agent from electrospun nanofibers and the wetting properties of the electrospun nanofibers have been explored to achieve a desirable release profile. In addition, environmental factors,

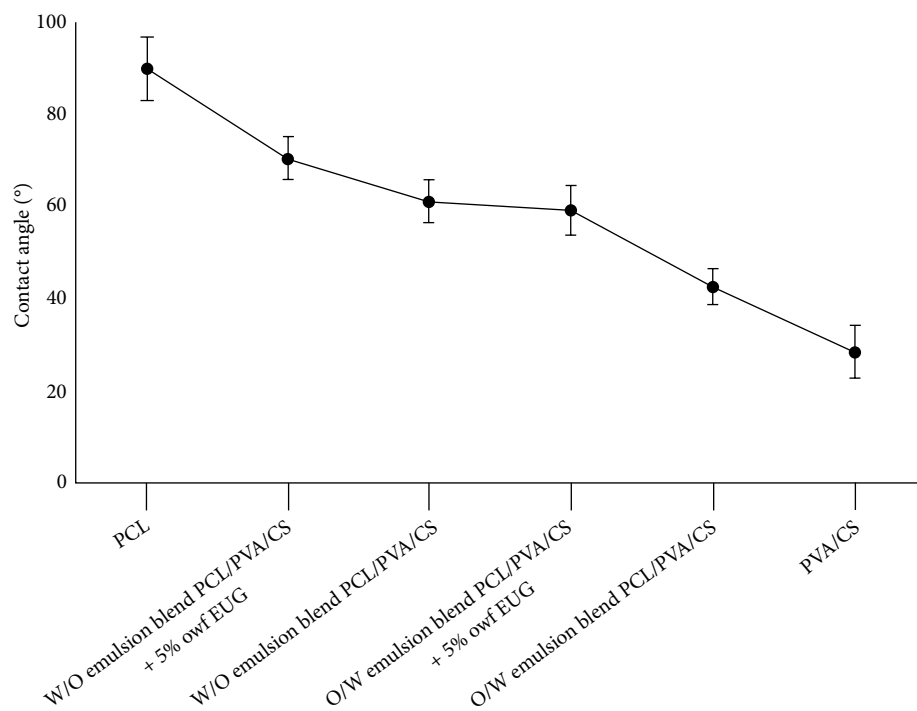
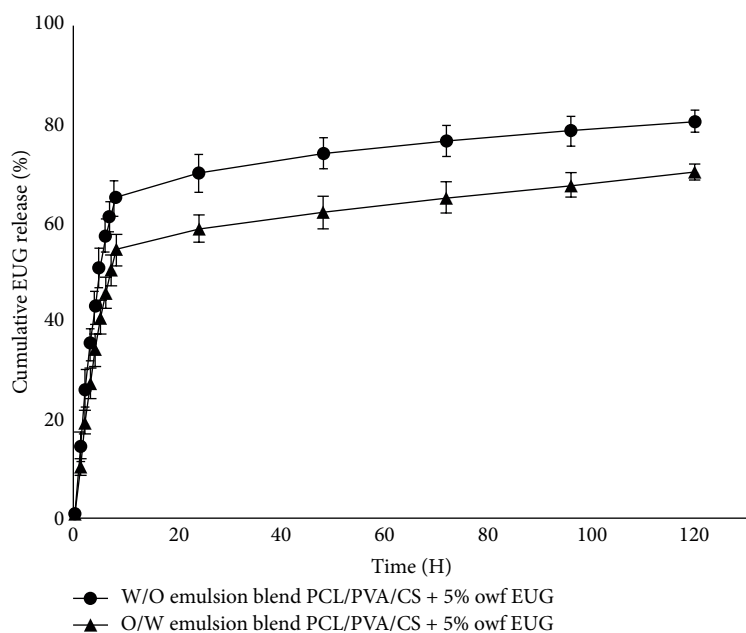


FIGURE 3: Variation of contact angle on pure PCL, PVA/CS and electrospun PCL/PVA/CS fiber mats prepared from W/O and O/W emulsions, with and without EUG.



Time (h)	W/O emulsion	O/W emulsion
0	0.68 ± 0.02	0.60 ± 0.03
1	14.44 ± 3.02	10.38 ± 1.59
2	26.05 ± 4.20	19.47 ± 2.50
3	35.29 ± 3.29	27.34 ± 3.08
4	42.84 ± 3.40	34.18 ± 3.32
5	50.73 ± 4.07	40.63 ± 3.20
6	57.37 ± 3.51	45.56 ± 3.16
7	61.31 ± 3.16	50.43 ± 3.09
8	65.05 ± 3.58	54.61 ± 3.19
24	69.84 ± 3.69	58.63 ± 2.79
48	73.85 ± 3.10	61.92 ± 3.28
72	76.42 ± 3.03	64.84 ± 3.16
96	78.47 ± 2.96	67.51 ± 2.56
120	80.37 ± 2.19	70.15 ± 1.63

FIGURE 4: *In vitro* release study of EUG loaded into electrospun PCL/PVA/CS fiber mats at a physiological pH of 7.4 in a PBS buffer solution for 120 hours.

such as temperature, pH, or light response, have been considered as a stimulus for bioactive agent release.

3.4. Estimation of Antibacterial Activity of EUG-Loaded Electrospun PCL/PVA/CS Fiber Mats. The antibacterial

efficiency of EUG loaded into electrospun PCL/PVA/CS fiber mats was quantitatively expressed as a percentage of bacterial reduction (%R) and evaluated after 24 hours against *S. aureus* and *P. aeruginosa*, two bacteria commonly present in wound infections [26].



TABLE 2: Antibacterial efficiency of EUG loaded into electrospun PCL/PVA/CS fiber mats against *S. aureus* and *P. aeruginosa*, expressed in percentage of bacterial reduction (%R).

Samples	<i>S. aureus</i>			<i>P. aeruginosa</i>		
	CFU/mL	Growth reduction (%)		CFU/mL	Growth reduction (%)	
W/O emulsion blend	0h	8.86E+04	—	0h	1.77E+06	—
PCL/PVA/CS	24h	3.04E+06	—	24h	6.02E+07	—
W/O emulsion blend		2.30E+05	92.43%		3.20E+06	94.68%
PCL/PVA/CS+5% owf EUG						
O/W emulsion blend	0h	9.78E+04	—	0h	2.21E+06	—
PCL/PVA/CS	24h	2.51E+06	—	24h	1.90E+08	—
O/W emulsion blend		4.25E+05	83.08%		2.31E+07	87.85%
PCL/PVA/CS+5% owf EUG						

TABLE 3: Antibacterial activity values (bacteriostatic and bactericidal activity values).

Samples	<i>S. aureus</i>		<i>P. aeruginosa</i>	
	$M_B - M_C$	$M_A - M_C$	$M_B - M_C$	$M_B - M_C$
W/O emulsion blend PCL/PVA/CS + 5% owf EUG	1.12	-0.23	1.27	-0.26
O/W emulsion blend PCL/PVA/CS + 5% owf EUG	0.77	-0.64	0.92	-1.02

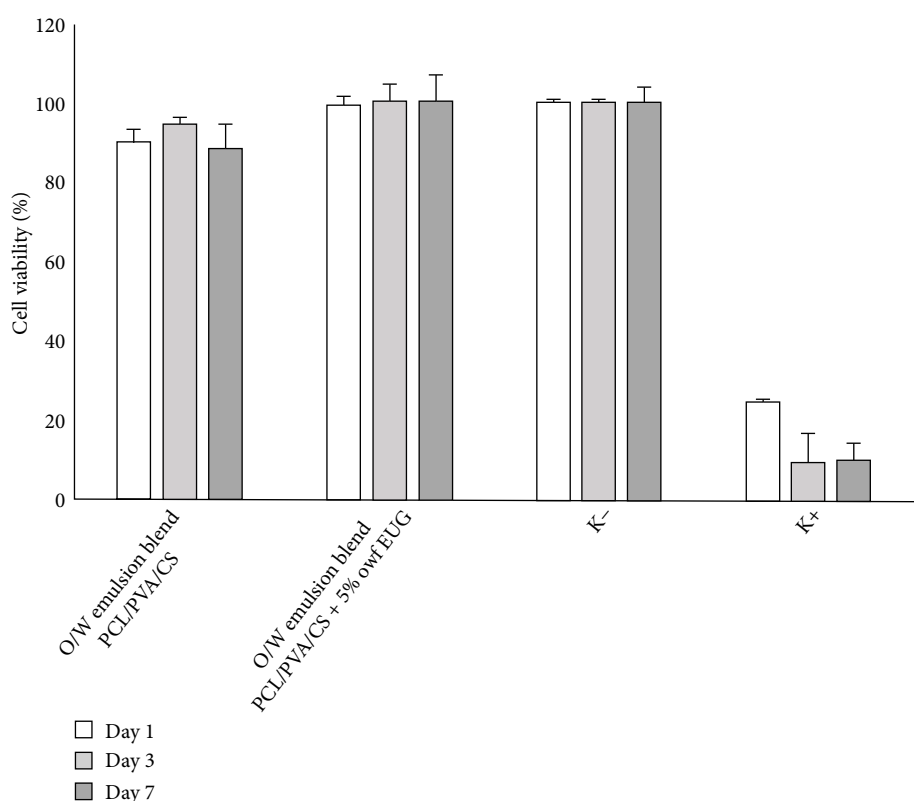


FIGURE 5: NHDF cell viability percentage after 1, 3, and 7 days in direct contact with the electrospun PCL/PVA/CS fiber mats prepared from O/W emulsion, with and without loaded EUG.

The results showed an inhibitory effect against selected bacteria (Table 2). Interestingly, electrospun PCL/PVA/CS fiber mats obtained from W/O emulsion revealed a higher bacterial reduction against *S. aureus* (92.43%), comparatively to O/W emulsion (83.08%). The same pattern was observed

for *P. aeruginosa*. The W/O emulsion showed an increased inhibitory effect in bacterial growth (94.68%) when compared to O/W emulsion (87.85%).

These results can be explained by the difference in EUG's affinity for the polymeric blend. Such values are in agreement

with those obtained for EUG release at 24 hours ( $58.63 \pm 2.79\%$  from O/W emulsion and  $69.84 \pm 3.69\%$  from W/O emulsion (Figure 4)), where a higher EUG release from W/O resulted in a stronger inhibition of bacterial growth.

Moreover, the electrospun PCL/PVA/CS fiber mats loaded with 5% owf EUG proved to have a bacteriostatic effect (Table 3). The bacteriostatic activity values were 1.12 and 0.77 for *S. aureus*, while for *P. aeruginosa* were 1.27 and 0.92 to W/O and O/W emulsions, respectively.

Therefore, the antibacterial efficiency of the electrospun PCL/PVA/CS fiber mats, conferred by natural properties of CS, can be strengthened with the use of EUG, as a natural antibacterial agent.

The hydrophobic nature of EUG was responsible by its mechanism of action against common pathogens present in wounds. Thus, when the EUG is partitioned into the lipid bilayer of the bacterial membrane, it changes its permeability and, consequently, rupture and release of cellular contents occur [5].

Bai et al. [38] reported the production of PCL/Chitosan nanofibers that were incorporated with tea tree oil (TTO) to improve their antibacterial ability.

**3.5. Characterization of the Cytotoxicity Profile of the EUG-Loaded Electrospun PCL/PVA/CS Fiber Mats.** The cytotoxic profile of the electrospun PCL/PVA/CS fiber mats with and without loaded EUG was evaluated from O/W emulsions, once these samples displayed a better capability to be applied as wound dressing materials. The results showed that the produced fiber mats did not induce any cytotoxic effect on the NHDF cells for at least 7 days, as shown in Figure 5. Moreover, the incorporation of 5% owf EUG into the emulsion blend did not compromise the cell viability. Therefore, these data reinforce the suitability of the EUG-loaded electrospun PCL/PVA/CS fiber mats for wound healing applications.

## 4. Conclusion

In this study, EUG, an essential oil extracted from cloves, was successfully loaded into electrospun PCL/PVA/CS fiber mats in an amount of 5% (w/w), based on the weight of PCL powder, via electrospinning from either W/O or O/W emulsion.

The results attained showed that the produced electrospun PCL/PVA/CS fiber mats with and without loaded EUG presented a high porous structure similar to the fibrous structure of native ECM. Moreover, these electrospun nanofibers exhibited surfaces with moderate wettability, which are able to provide suitable moisture at the wound site and better fibroblast attachment and proliferation. In addition, the antibacterial ability of EUG loaded into electrospun PCL/PVA/CS fiber mats was examined against *S. aureus* and *P. aeruginosa* and displayed the potential to inhibit bacterial growth.

The *in vitro* EUG release study showed a burst release of EUG in the first 8 hours, followed by a continuous slow and sustained release over the next days. The O/W emulsion revealed a better release behaviour compared to W/O emulsion, once the cumulative EUG release from W/O emulsion resulted in a faster release rate.

Therefore, the incorporation of plant essential oils, such as EUG, which exhibit unique therapeutic properties, into electrospun fiber mats from O/W emulsion revealed better results and proved to be a noncytotoxic and highly promising approach for improving the wound healing process.

## Data Availability

The data used to support the findings of this study are included in the article.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

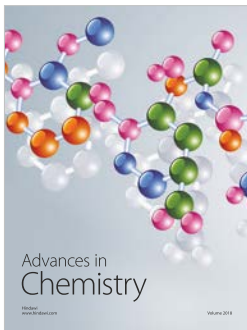
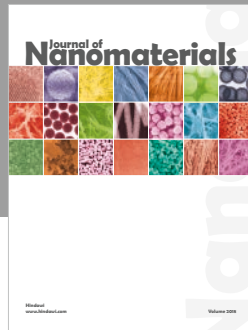
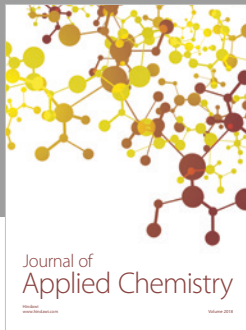
## Acknowledgments

The authors acknowledge the Portuguese Foundation for Science and Technology (FCT) for funding the PhD grant PD/BD/113550/2015. The manuscript was presented as a poster in the AMiCI WG2 workshop “Antimicrobial Coatings Applied in Healthcare Settings—Efficacy Testing”, Berlin, Germany.

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