

Fabrication and characterization of PCL nanofibrous networks containing ZnO and Ag for enhanced antibacterial properties

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Article Information	Abstract
<p>Article history:</p> <p>Received: 2025-01-07</p> <p>Accepted: 2025-07-14</p>	<p>This study investigates the manufacturing and characterization of nanofibrous networks consisting of poly(ϵ-caprolactone) (PCL) and its nanocomposites, incorporating ZnO and Ag nanoparticles, and their effectiveness in antibacterial properties. The focus is on the fabrication of PCL-ZnO (ZnO:1 wt.%) and PCL-ZnO-Ag (ZnO: 1 wt.% and Ag: 0.5 wt.%) structures using the electrospinning technique. Scanning electron microscopy (SEM) showed the successful formation of beadless and smooth nanofibers for all three samples, with average nanofiber diameters of approximately 409.17 ± 106.99 nm for PCL, 226.65 ± 76.34 nm for PCL-ZnO, and 167.97 ± 24.26 nm for PCL-ZnO-Ag. However, incorporating ZnO and Ag nanoparticles led to a reduction in nanofiber diameter. Energy Dispersive X-ray (EDX) analysis confirmed the presence of ZnO and Ag nanoparticles in PCL-ZnO-Ag nanofibrous webs. The water contact angle increased with ZnO incorporation, showing hydrophobic behavior, while Ag addition increased surface hydrophilicity in comparison with the PCL-ZnO sample. Antibacterial activity tests exhibited the capability of PCL-ZnO and PCL-ZnO-Ag structures against both Gram-negative (<i>E. coli</i>) and Gram-positive (<i>S. aureus</i>) bacteria. The combined effects of ZnO and Ag nanoparticles in PCL-ZnO-Ag resulted in broader antibacterial activity, promising significant potential for wound dressing applications.</p>
<p>Keywords:</p> <p>PCL, ZnO, Ag, Antibacterial properties, Electrospinning.</p>	

1 INTRODUCTION

Developing advanced materials with proper antibacterial properties has gained importance in various fields, including biomedical, healthcare, and environmental applications [1-2]. Electrospinning is regarded as a versatile and superior technique to producing uniform ultrafine nanofibers for wound regeneration applications [3-5]. Electrospun nanofibers, with their high surface area-to-volume ratio and tunable properties, have been presented as a promising structure for creating antibacterial materials [6-7]. The electrospinning method offers a multitude of advantages, making it an indispensable technique in the field of nanofiber fabrication. One of its prominent strengths lies in its versatility, as it serves a wide range of polymer solutions, including both natural and synthetic polymers, as well as non-biodegradable and biodegradable variants to craft intricate nanofibrous webs [8-11]. In recent years, the application of electrospun nanofibers loaded with antibacterial materials has gained high attention within the scientific studies. These nanofibers have appeared as a promising substitutes for developing highly efficient and biocompatible antibacterial materials. They are attractive because of their great features, including an increased surface area, better contact with bacteria, the controlled release of active agents, biodegradability, biocompatibility, and non-toxicity [12]. Furthermore, these nanofibers exhibit

multifunctional physicochemical properties that can be carefully modified to fit the requirements of antibacterial effectiveness.

Current research focuses on using the biodegradable polymer poly (ϵ -caprolactone) (PCL) blended with antibacterial nanomaterials to create an effective structure for wound healing applications. PCL, a semicrystalline, aliphatic, and thermoplastic polyester, has a range of remarkable properties, including potent miscibility and excellent mechanical properties [13]. These distinctive characteristics render PCL an attractive biomaterial, finding extensive applications in biomedical engineering [14]. To cater to specific end-uses, PCL can be skillfully blended with a diverse range of materials, creating electrospun nanofiber webs centered around PCL [15-16]. In the case of antibacterial materials, integrating nanoparticles with inherent antibacterial properties into the electrospun nanofiber is a common method. This integration is achieved by uniformly dispersing nanoparticles within the polymer solution before the electrospinning process [10, 17-18]. The widely employed approach, known as the direct method, disperses nanoparticles into the electrospinning polymer solution. By modulating the quantity of added nanoparticles, the properties of the resulting nanofibers can be precisely controlled. This method has successfully incorporated a variety of metal and metal oxide

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nanoparticles (e.g., copper, silver, TiO₂, ZnO) into various biodegradable polymeric matrices [19]. Among the nanoparticles with antibacterial properties, ZnO and Ag stand out as desirable options due to their distinctive characteristics. ZnO nanoparticle as a kind of metal oxide has the appropriate ability to kill different types of bacteria [20]. ZnO has been incorporated in some natural polymers such as gelatin [21], and synthetic polymers such as PLA. It has been approved that adding ZnO nanoparticles into chitosan-based fibers enhances the antibacterial effect significantly [22-23]. Virovska et al. [22] conducted a study on the antibacterial properties of PLA/ZnO nanofibers and found that these structures exhibited not only bactericidal effects but also excellent photocatalytic activity. Additionally, biomimetic nanofibrous scaffolds of PCL/hydroxyapatite nanoparticles were electrospun using various concentrations of ZnO to determine the optimal range that strikes a balance between osteoregeneration and biocompatibility [24].

Another well-established therapeutic agent for infection prevention is silver (Ag), which is extensively utilized in wound dressings due to its potent antibacterial properties. Among the various antibacterial nanostructures, silver nanoparticles (AgNPs) are the best-known, showcasing a broad spectrum of antibacterial activity [25]. Lopez-Esparza *et al.* [26] studied the antibacterial silver nanoparticles incorporated into the PCL nanofiber web to strong resistance against Gram-positive and Gram-negative bacteria. Bhullar *et al.* [27] investigated the incorporation of antibacterial silver nanoparticles into a PCL nanofiber web to enhance its resistance against both Gram-positive and Gram-negative bacteria. Also, Augustine *et al.* reported the successful fabrication and characterization of electrospun PCL membranes incorporated with biosynthesized silver nanoparticles, showcasing their potential for wound dressing applications [28].

The optimized concentration of nanoparticles in electrospun webs like PCL is essential and depends on the specific application and the desired properties of composite materials. However, studies typically report that the ZnO nanoparticle concentration in PCL electrospun webs is generally up to 10 wt.% [29-30]. Low concentrations of ZnO nanoparticles can contribute to antimicrobial activity without significantly affecting the electrospinning process. Also, high nanoparticle concentration can lead to agglomeration, where nanoparticles cluster together rather than dispersing uniformly[31]. Augustine *et al.* [29] designed novel tissue-engineering scaffolding materials with antibacterial properties, and enhanced tissue proliferation abilities made from electrospun mats of PCL fibers filled with ZnO nanoparticles in different concentrations (0.1 wt.% to 6 wt.%). They observed that at a concentration of 1 % ZnO nanoparticles, the proliferation of fibroblast cells was significantly improved and showed higher cell attachment and proliferation in comparison to other concentrations. Ag nanomaterials have been incorporated into PCL webs for anti-infection activity. However, the potential toxicity of Ag nanoparticles in the skin, kidney, respiratory system, hepatobiliary system, immune system, and reproductive system cannot be ignored [32], which limits the wide clinical applications of Ag-containing PCL scaffolds in high concentrations. Another research focused on the fabrication of PCL electrospun membrane incorporated by various concentrations of silver nanoparticles (0.05 wt.% to 1

wt.%) and found better mechanical properties of PCL-Ag nanocomposite membranes in 0.5 wt.% of silver nanoparticle content and high antibacterial activity against both *S. aureus* and *E. coli* in 1 wt.% of Ag nanoparticles [28].

This study presents the first instance in the literature where a tri-component combination of poly(ϵ -caprolactone) (PCL), zinc oxide (ZnO), and silver (Ag) nanoparticles has been successfully fabricated into nanofibers using the electrospinning technique. The synergistic incorporation of both ZnO and Ag nanoparticles within the PCL matrix offers a novel approach that significantly alters the physicochemical properties of the resulting nanofibers. This dual-functional nanocomposite structure, with improved antibacterial property, presents a promising and innovative approach for advanced wound dressing applications.

2 MATERIALS AND METHODS

2-1 Materials

PCL polymer (Avg. Mol. wt. 80,000 g mol⁻¹), Ag nanoparticles (~ 100 nm diameter), and ZnO nanoparticles (~60 nm diameter) were supplied by Sigma Aldrich, St. Louis, USA. Glacial acetic acid was purchased from Merck Company, Germany. All components were used as received without further purification.

2-2 Preparation of PCL-ZnO-Ag nanofibers

To prepare the PCL solution, a 15 wt.% concentration was achieved by dissolving 1.5 g of PCL pellets in 10 mL of 90% v/v acetic acid at room temperature. Subsequently, the solution was magnetically stirred for 30 minutes to ensure a homogeneous mixture[28].

Subsequently, 1 wt.% of ZnO and 0.5 wt.% of Ag (calculated based on the dry weight of PCL used for the polymeric solution) were added to 90% v/v acetic acid. The concentration of nanoparticles selected according to the previous investigation is explained in the introduction section[28-29]. The total concentration of nanoparticles, including ZnO and Ag, was 1.5 wt.%.

The solution was subjected to 30 minutes of sonication. Following this, PCL was introduced into the ZnO and ZnO-Ag solutions, and the resulting mixtures were stirred for 12 h to create PCL-ZnO and PCL-ZnO-Ag blends. Notably, the blend solutions exhibited a homogeneous, colorless, and transparent appearance, with no signs of phase separation or precipitation observed. Table 1 provides a summary of the composition of the samples.

Table 1 Composition of samples

Sample No.	Nanofiber Sample	PCL (wt.%)	ZnO (wt.%)	Ag (wt.%)
1	PCL	15	0	0
2	PCL-ZnO	15	1	0
3	PCL-ZnO-Ag	15	1	0.5

An approximate volume of 10 mL of the blend solution was carefully dispensed into a plastic syringe equipped with a needle and then subjected to the electrospinning process to produce nanofiber webs. During the initial stage, meticulous optimization of the electrospinning parameters was carried out

to ensure the formation of uniform and bead-free nanofibers. The applied voltage, distance between the nozzle and collector, and feed rate were adjusted to 18 kV, 13 cm, and 1 mL h⁻¹, respectively. Each type of nanofiber, including PCL, PCL-ZnO, and PCL-ZnO-Ag, was collected individually on a rotating collector. After the fabrication process, the nanofibers were dried and carefully peeled off the aluminum foils for further studies. Inside the electrospinning chamber, the temperature and relative humidity were maintained at 28 °C and 55%, respectively. Figure 1 illustrates the process of nanofiber production and provides a visual representation of the final characteristics of the nanofibers.

2-3 Characterization and physicochemical specification of the prepared nanofiber webs

The morphology of the nanofibers was characterized using Scanning Electron Microscopy (SEM) with a Hitachi SEM, S-4160 model. The nanofiber diameter was determined by measuring the diameter of 30 fibers from different parts of the samples and calculating their average using Image J software. To investigate the distribution of ZnO and Ag nanoparticles in the PCL structure, Energy Dispersive X-ray (EDX) analysis was performed using a Tescan Mira II model detector attached to the SEM instrument. EDX maps of the webs were obtained under a nitrogen atmosphere without any previous coating. The functional groups present in PCL, PCL-ZnO, and PCL-ZnO-Ag nanofibers were identified using Fourier Transform Infrared Spectroscopy (FTIR) within the range of 400 – 4000 cm⁻¹.

The surface wettability of the fabricated samples was measured by determining the static water contact angle (WCA) at room temperature using a contact angle goniometer (JIKAN, CAG10 9610IL58300 model) equipped with a digital camera. An 8 µl drop of deionized water was placed onto the nanofibrous substrate, and measurements were taken at three different locations on the surface of the nanofibers. The average contact angle with standard deviation was reported.

2-4 Antibacterial study

The antibacterial activity of PCL, PCL-ZnO, and PCL-ZnO-Ag samples was evaluated using the Kirby-Bauer agar

diffusion test. Two bacterial strains, *Staphylococcus aureus* (*S. aureus*-G+) as Gram-positive bacteria and *Escherichia coli* (*E. coli*-G-) as Gram-negative bacteria, were used in this study. Discs with a diameter of 5 mm were cut from each of the three different nanofiber compositions (PCL, PCL-ZnO, and PCL-ZnO-Ag) and placed on the surface of Mueller-Hinton Agar (MHA) plates, which were previously inoculated with the respective bacterial strains. A standard antibiotic disc (Ampicillin, 25 µg disc-1) was the positive control. The culture plates were then incubated at 37 °C for 24 hours in an incubator. After incubation, the diameter of the inhibition zone around each disc was measured using a ruler in millimeters (mm). The experiment was repeated three times to obtain average values, and the results were reported as mean ± standard deviation (SD).

3 RESULTS AND DISCUSSIONS

3-1 Morphological evaluation

Using the electrospinning method, nanofibrous webs comprising PCL, PCL-ZnO, and PCL-ZnO-Ag were successfully fabricated. SEM images in Figure 2 showcase the beadless and smooth structures of the three nanofibrous samples. Through Image J software, we measured the average nanofiber diameters to be approximately 409.17 ± 106.99 nm for PCL, 226.65 ± 76.34 nm for PCL-ZnO, and 167.97 ± 24.26 nm for PCL-ZnO-Ag. Our results demonstrate that incorporating ZnO and Ag nanoparticles led to a reduction in nanofiber diameter, which can be attributed to their presence in the polymeric solution. Particularly noteworthy, the PCL-ZnO-Ag sample exhibited the most minor nanofiber diameter, significantly lower than the other groups ($P < 0.05$). Lopez-Esparza *et al.* [26] demonstrated that an increased concentration of Ag nanoparticles resulted in nanofibers with smaller diameters. In contrast, a lower concentration of Ag nanoparticles led to thicker PCL nanofibers. Incorporating metallic nanoparticles enhances the conductivity and electrical charge of the solution, leading to a reduction in nanofiber diameters. These conductive nanoparticles modify the behavior of the polymer solution in the electric field during the electrospinning process, thereby facilitating the formation of thinner nanofibers [33]

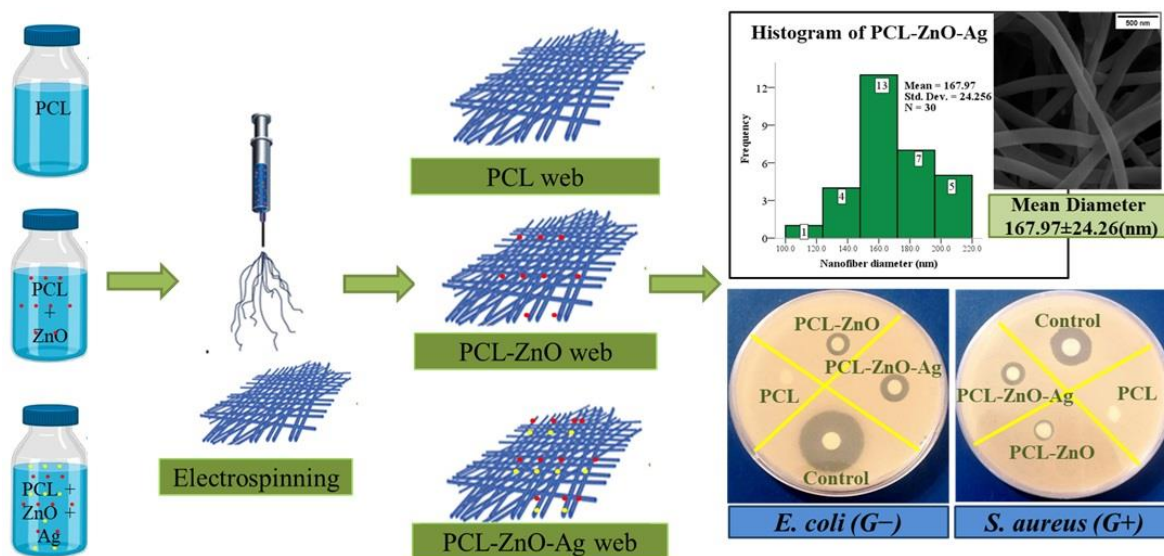


Fig. 1 Schematic illustration of the electrospinning process to fabricate PCL, PCL-ZnO, and PCL-ZnO-Ag samples and further studies

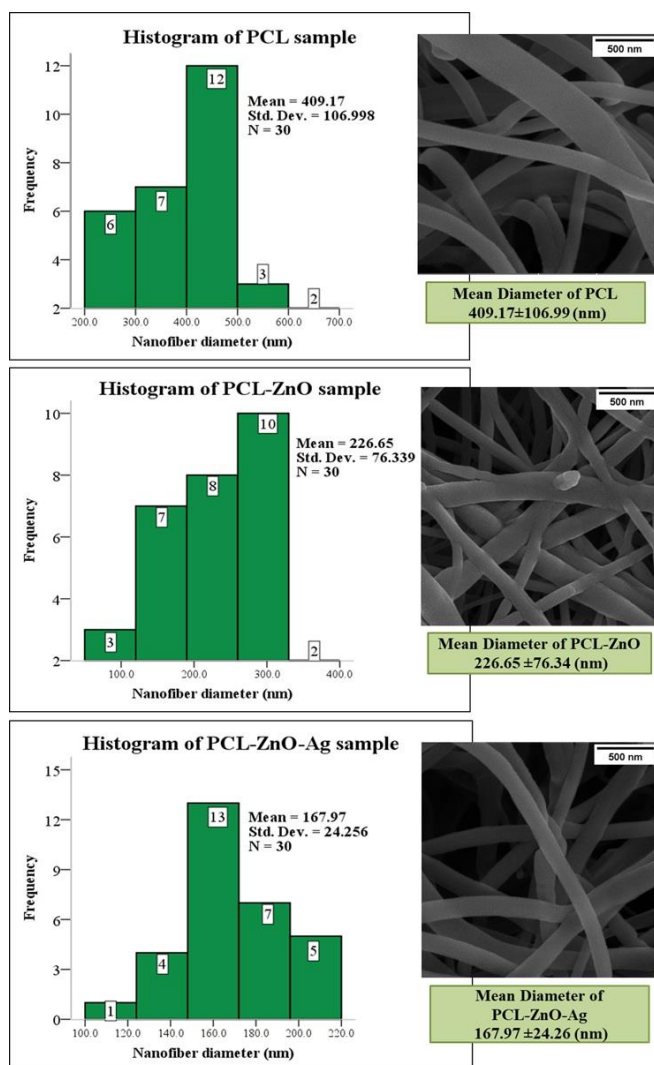


Fig. 2 SEM pictures of PCL, PCL-ZnO, and PCL-ZnO-Ag samples, including the corresponding diameter distribution diagram

EDX analysis of PCL and PCL-ZnO-Ag was performed to determine the elemental composition of the samples. EDX analysis detected the presence of ZnO and Ag nanoparticles in the PCL samples, confirming their successful incorporation during the electrospinning process. Table 2 and Figure 3 further validated the presence of ZnO and Ag nanoparticles in the PCL and PCL-ZnO electrospun nanofibers. As expected, carbon (C) and oxygen (O) – the specific elements of PCL – were present in all samples, constituting approximately 80.13% and 19.87% of the elemental composition, respectively. In the PCL-ZnO nanofibrous webs, an additional element related to Zn was observed, indicating the successful addition of ZnO nanoparticles. Moreover, an increase in the elemental composition of O could be attributed to the presence of this element in the structure of ZnO nanoparticles. Similarly, the presence of Ag in the PCL-ZnO sample was confirmed by EDX analysis, affirming the successful blending of PCL polymer solution with ZnO and Ag nanoparticles. EDX map images in Figure 3 also demonstrated the distribution of Zn and Ag on the PCL nanofibrous webs, providing further visual evidence of their incorporation.

Table 2 Elemental composition of PCL, PCL-ZnO, and PCL-ZnO-Ag samples by EDX

Samples	Elemental composition (%)			
	C	O	Zn	Ag
PCL	80.13	19.87	0	0
PCL-ZnO	71.78	24.54	3.68	0
PCL-ZnO-Ag	72.86	22.78	2.61	1.75

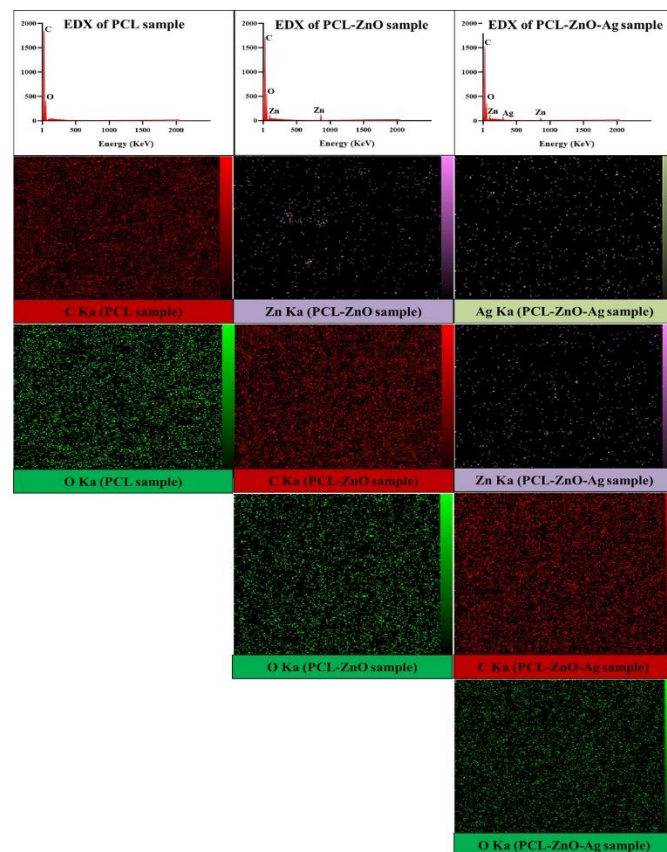


Fig. 3 EDX graphs including maps of PCL-ZnO and PCL-ZnO-Ag nanofibrous webs show Zn and Ag elements

3-2 FTIR analysis

FTIR is a widely used analytical technique for studying the chemical structure and molecular interactions of polymeric materials, including polycaprolactone (PCL) and its composites with ZnO and Ag, such as PCL-ZnO and PCL-ZnO-Ag (Figure 4). In the FTIR spectrum of pure PCL, several characteristic peaks are observed: prominent C–H stretching vibrations at 2865 and 2945 cm^{-1} related to the methylene groups of the PCL chain, and bands in the range of 1150–1260 cm^{-1} , particularly near 1238 and 1160 cm^{-1} , attributed to C–O–C stretching vibrations of the ester linkages[24, 34]. In the spectrum of pure PCL, a strong and characteristic absorption band is observed at 1720 cm^{-1} , corresponding to the stretching vibration of the ester carbonyl group (C=O). This peak is sharp and intense, typical of semi-crystalline PCL. Upon incorporation of ZnO nanoparticles, the carbonyl peak exhibits a slight red shift to approximately 1718 cm^{-1} in the PCL-ZnO spectrum. Interestingly, with the addition of both ZnO

and Ag nanoparticles in PCL-ZnO-Ag, the carbonyl band shifts further to about 1715 cm^{-1} . These downward shifts in wavenumber show increasing interaction between the ester carbonyl groups and the metal oxide nanoparticles, likely through coordination interactions or hydrogen bonding.

In addition to the carbonyl region, the C–O–C asymmetric and symmetric stretching vibrations, normally seen at 1237 cm^{-1} and 1157 cm^{-1} in PCL, also exhibit slight shifts upon nanoparticle incorporation. In PCL-ZnO, these peaks are observed at approximately 1239 cm^{-1} and 1160 cm^{-1} , while in PCL-ZnO-Ag, they are slightly more broadened and shift further to 1241 cm^{-1} and 1164 cm^{-1} . These changes suggest interfacial interactions with the metal nanoparticles.

3-3 Wettability test

The water contact angle of the fabricated nanofibrous webs composed of PCL, PCL-ZnO, and PCL-ZnO-Ag is depicted in Figure 6. The PCL nanofibrous web was hydrophobic, as evidenced by its high contact angle of 109.9° . Upon incorporating ZnO nanoparticles into the PCL nanofibrous web, the water contact angle of the PCL-ZnO sample increased to 122.5° . This rise in contact angle can be attributed to the insolubility of ZnO nanoparticles in water, resulting in a higher water contact angle for the PCL-ZnO nanofibrous web[36]. The introduction of Ag nanoparticles to the PCL-ZnO sample led to a decrease in the contact angle, resulting in a value of 119.5° . This finding aligns with the results reported in other studies, demonstrating the consistent effect of Ag nanoparticles on the contact angle of nanofibrous webs[27]. Thomas *et al.* demonstrated a reduction in the water contact angle of Ag-incorporated PCL nanofibers, with the value decreasing from 93° to 73° . This decrease in the contact angle can be attributed to the increased surface hydrophilicity of the PCL nanofibers upon adding Ag nanoparticles[37]. Here, adding ZnO into PCL increased the water contact angle, and then adding Ag into PCL-ZnO decreased it. So, Ag nanoparticles can improve wettability in the electrospun nanofiber structure.

The antibacterial activity of the ZnO and Ag-incorporated PCL samples was assessed using the disc diffusion method,

observing their inhibitory effects against Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria. The obtained results are presented in Table 3 and Figure 7. As shown in Figure 6, the PCL nanofibers exhibited no activity against both tested bacteria. In contrast, the PCL-ZnO nanofibrous webs demonstrated significant antibacterial activity against both *E. coli* and *S. aureus*. The antibacterial effects of PCL-ZnO nanofibrous webs can be attributed to two main mechanisms. Firstly, ZnO nanoparticles release zinc ions (Zn^{2+}) in the surrounding environment, allowing these ions to enter bacterial cells and disrupt essential cellular processes, eventually leading to bacterial death. Additionally, ZnO nanoparticles generate reactive oxygen species (ROS), such as superoxide radicals (O_2^-), hydroxyl radicals (OH^-), and hydrogen peroxide (H_2O_2), upon exposure to moisture or light. These ROS are highly reactive and can damage bacterial cell membranes and biomolecules, resulting in cell death [38]. On the other hand, the inhibitory activity of the tested bacteria for the PCL-ZnO-Ag sample is higher than PCL-ZnO sample. The synergistic effects of ZnO and Ag nanoparticles contribute to a broader spectrum of antibacterial activity, making these nanofibers highly effective against both Gram-positive and Gram-negative bacteria. According to Hu *et al.* report [30], Ag nanoparticles can induce bacterial death by destroying the cell wall or membrane, disturbing protein synthesis and processing, preventing DNA replication, and disturbing the antioxidative system. The antibacterial effect against *E. coli* bacteria was higher than *S. aureus*. The biocompatible nature of ZnO and Ag nanoparticles further enhances the appeal of these antibacterial nanofibers for biomedical applications.

Table 3 Mean diameter of the zone of inhibition in mm after 24 hours

Samples	<i>E. coli</i> (G-)	<i>S. aureus</i> (G+)
Positive control (Ampicillin)	25.3 ± 0.6	14.1 ± 0.4
PCL	5.0 ± 0.0	5.0 ± 0.0
PCL-ZnO	9.2 ± 0.3	7.5 ± 0.3
PCL-ZnO-Ag	11.6 ± 0.4	8.8 ± 0.3

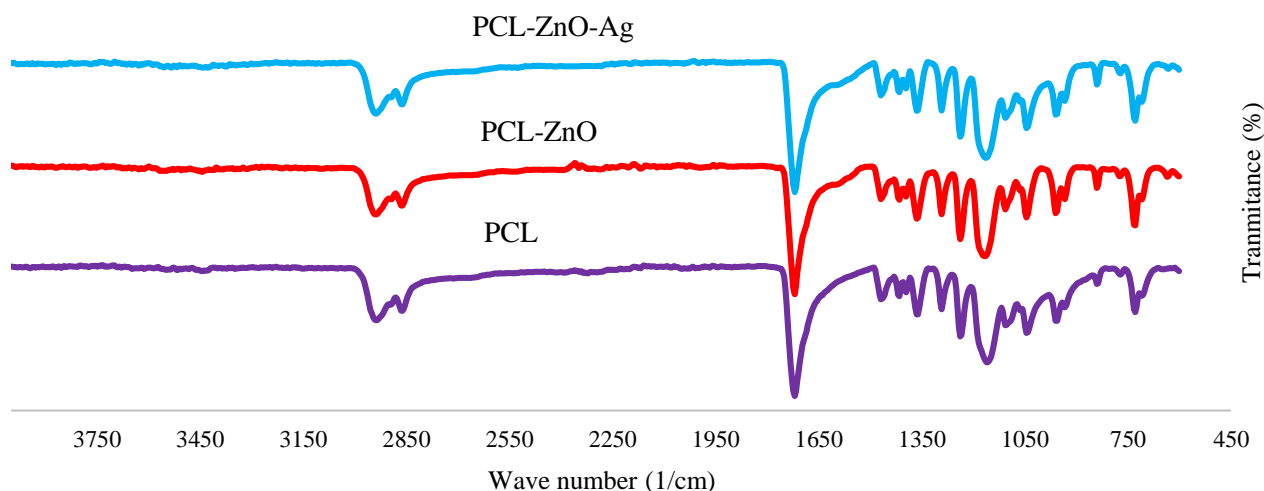


Fig. 4 Infrared spectrum of PCL, PCL-ZnO, and PCL-ZnO-Ag samples

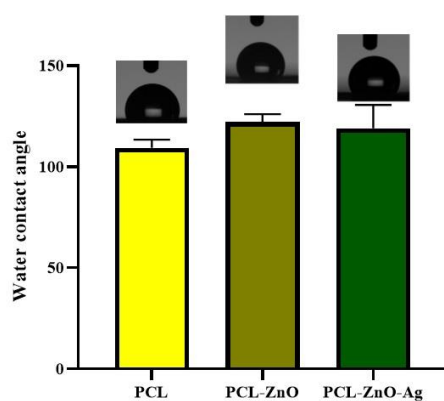


Fig. 5 Water contact angle of PCL, PCL-ZnO and PCL-ZnO-Ag sample

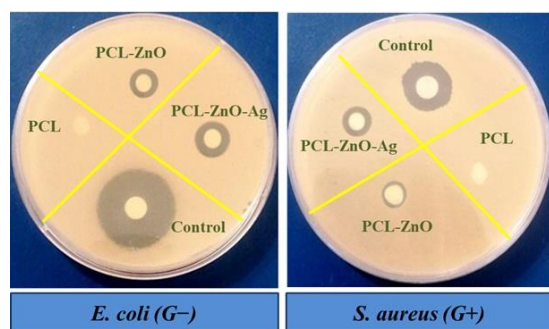


Fig. 6 Antibacterial activity of PCL, PCL-ZnO and PCL-ZnO-Ag against *E.coli* (a), *S. aureus* (b) showing inhibition zone

Since the PCL-ZnO-Ag webs showed uniform fiber diameter distribution with less average nanofibers diameter and excellent antibacterial activity, they can be used for wound dressing applications.

4 CONCLUSIONS

Producing nanofibers containing PCL and its composites with ZnO and ZnO-Ag has yielded structures with diverse applications. The fabricated nanofibers exhibited smooth structures without cracks. Elemental composition analysis confirmed the presence of ZnO and Ag nanoparticles in the PCL samples, indicating successful incorporation during the electrospinning process. Wettability tests revealed that the water contact angle of the PCL-ZnO sample decreased to 119.5° due to the addition of Ag, as compared to PCL-ZnO alone. This suggests that combining ZnO and Ag nanoparticles improved the surface hydrophilicity of the nanofibers in comparison to the PCL-ZnO sample. Moreover, the nanofibers with ZnO and Ag composites exhibited enhanced antibacterial activity against *E. coli* and *S. aureus* bacteria.

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