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Design of PLGA nanofiber wound dressing enriched with *Echinacea purpurea* extract and *in vitro* characterization

Busranur Senol[1]*, Ayse Malbora[1], Mehmet Emin Senol[1], Zehra Gul Morcimen[2]

[1]Ege University, Graduate School of Natural and Applied Sciences, Department of Biomedical Technologies, 35040, İzmir, Turkey
[2]Ege University, Graduate School of Natural and Applied Sciences, Department of Bioengineering, 35040, İzmir, Turkey.

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Corresponding Author
Tel.: +905051259195
E-mail: busrafit94@gmail.com

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Abstract

Electrospun nanofiber wound dressings potentially provide a highly suitable environment for healing compared to traditional wound dressings, which often fail to meet all the requirements of the wound healing process. This study aimed to develop PLGA nanofibers incorporating silver nanoparticles containing *Echinacea* extract for use as wound dressings; to perform their characterization tests, and evaluate their wound healing efficacy using *in vitro* models. *Echinacea* silver nanoparticles were synthesized using a green synthesis method. PLGA, silver nanoparticle containing PLGA (AgNP/PLGA), 1%, 2%, 3%, *Echinacea* silver nanoparticle containing PLGA (Ec-AgNP/PLGA) nanofibers were produced by electrospinning method. The nanofibers were characterized by SEM, FTIR, XRD and antibacterial analysis and evaluated using an *in vitro* wound scratch test. The average zone diameters of Ec-AgNP/PLGA nanofibers against the *E. coli* and *S. aureus* bacteria were found to be 13.5±0.3 mm. The antioxidant capacity analysis of the *Echinacea* extract used in the study was evaluated by the *DPPH* (2,2-diphenyl-1-picrylhydrazyl) method and the percentage of *DPPH* radical removal was calculated as 46.8% and the ascorbic acid equivalent was determined as 35.5 µM. The results demonstrated that *Echinacea* significantly accelerated wound healing due to its therapeutic and antioxidant properties.

Introduction

The skin is a fundamental part of the human body, serving as the largest organ and the first line barrier of defense against the environment (Mühlstädt et al., 2012). It protects internal tissues from mechanical damage, microbial infection, ultraviolet (UV) light and extreme temperatures. Injuries occur due to the loss of the skin's normal integrity, structure, and function due to a physical, chemical or mechanical agent (Latenser et al., 1991). When the skin structure is compromised, all its functions need to be restored as soon as possible to maintain the homeostasis of the organism. Therefore, the wound healing process begins immediately after the skin injury (Mühlstädt et al., 2012). Wound healing is a complex process divided into four main stages: hemostasis, inflammation, proliferation and tissue regeneration (Sun et al., 2014). As a result, various treatment methods are applied depending on the type of wound. Common treatments include creams, ointments, gauze, antibiotics, and analgesics. In addition to these, emerging methods such as stem cell therapy, photothermal, photodynamic therapy, drug carrier systems and synthetic, semi-synthetic and natural modern wound dressings are being explored (Arif et al., 2021). An ideal wound dressing should provide oxygen and water vapor permeability, absorb exudate, adhere to the wound surface, prevent bacterial invasion, be elastic, easy to apply, easy to sterilize (Arif et al., 2021).

Nanofiber wound dressings produced by electrospinning have gained prominence among modern wound dressings in recent years. Electrospun nanofiber wound dressings offer a highly favorable environment for healing compared to traditional bandages, which often fail to meet all the requirements of the wound healing process (Jayaraman et al., 2004).

These nanofiber dressings feature a highly porous structure with high gas permeability and biocompatibility and biodegradability, making them highly desirable. When the properties of the polymer used in nanofiber production are combined with these characteristics, optimal conditions for wound healing can be achieved (Ahmad, 2022). Biodegradable and biocompatible synthetic polymers have widespread use in medicine. Polymers such as poly-glycolic acid (PGA), poly-lactic acid (PLA), polyethylene glycol (PEG), PCL, PLGA from the polyester family have attracted considerable attention in the biomedical field because of their favorable properties (Küçükturhan, 2012). They are widely used in surgical suture production, wound dressing production, tissue engineering applications, and drug delivery systems. Cytotoxic products may occur in the environment as a result of the degradation of some synthetic polymers, and this restricts they're *in-vivo* use. In order to minimize these limitations, it is more appropriate to use them together with cell-compatible natural therapeutic components and other natural or synthetic polymers (Terzopoulou et al., 2022). The PLGA (Poly (lactic acid-co-glycolic acid)) polymer used in this study is a biodegradable material known for its high biosafety, biocompatibility and sustained-release properties. For this reason, it is widely used in drug delivery systems and tissue engineering applications (Vijayan et al., 2019).

Echinacea purpurea (L.) Moench, a medicinal and aromatic plant, possesses significant therapeutic properties including immune system enhancement, infection prevention and pain relief. Rich in vitamins A, C, E, B2, selenium and various flavonoids, *Echinacea* exerts a strong effect on the immune system, largely due to its polysaccharide components (Ciganović et al., 2023). Studies have demonstrated it has antibacterial, antiinflammatory and antioxidant effects (Ciganović et al., 2023). Additionally, the European Medicines Agency states that preparations made from the above-ground parts of *Echinacea* are traditionally used to alleviate skin disorders and minor wounds (European Medicines Agency, 2005).

Silver has long been utilized in wound care and continues to be a popular active ingredient in wound treatment today. It is available in various forms and exhibits broad-spectrum antimicrobial activity (Puccetti et al., 2023). Recent advances in nanotechnology have brought silver nanoparticles (AgNPs) into the spotlight, as their unique antimicrobial properties make them promising candidates for wound healing applications (Puccetti et al., 2023). The green synthesis of AgNPs using plant extracts or microorganisms and their antimicrobial activities have been widely investigated. Plant-mediated synthesis of AgNPs is a widely adopted technique due to the availability of various plants and their ease of handling (Huq et al., 2022). The synthesis of bioactive AgNPs using plants is more stable and efficient due to the biomolecules present in plant extracts.

Ciganović et al. (2023) investigated the wound healing effect of *E. purpurea in vitro* using *E. purpurea* extracts and glycerol. In this study, cells created in a wound scratch model were exposed to different ratios of *E. purpurea* extracts and glycerol. Hanks' Balanced Salt Solution (HBSS), was used as a negative control group to monitor the wound closure process for 48 hours. Model wounds of HaCaT cells treated with *E. purpurea* and glycerol extracts tended to decrease over time. At the end of 48 hours, it was stated that the scratch surface in the cell layer treated with this extract nearly fully closed. These findings indicate that *E. purpurea* has excellent wound healing activity. Zhai et al. (2009) also investigated the wound healing effect of *Echinacea* with another method in another study. Using a different approach, mice were orally fed *Echinacea* and the healing status of the wounds opened on the skin was observed. It was determined that the wound healing speed of mice fed *Echinacea* was significantly shortened compared to the mice in the control group. In addition, it was determined that *Echinacea* had a healing effect under stress conditions that negatively affect wound healing. In a recent study similar to our study, Fahimirad et al. (2023) investigated the *in vivo* wound healing ability of double-layered Polycaprolactone/Polyvinyl alcohol-Chitosan lactate electrospun nanofiber containing *E. purpurea* extract. According to the results of the research, they determined that nanofiber wound dressings containing *E. purpurea* extract and chitosan lactate showed antioxidant potential up to 88.28% without any cytotoxic effect on the viability of human dermal fibroblast cells and resulted in 98.1% wound closure on the 15th day of wound treatment.

In this study, it was aimed to obtain ideal wound dressing properties by combining the superior properties of PLGA polymer, the advantages of its nanofiber structure, and the therapeutic effects of *Echinacea*. One major advantage of the electrospinning method is that various active substances can be easily added to the material during the production phase. Therefore, in addition to PLGA in our study, we used *Echinacea* extract, which supports cell renewal with its natural content and has anti-inflammatory and antioxidant properties. In the literature, there is a wound dressing study in which *Echinacea* extract was used together with chitosan, PCL and PVA polymers, similar to our study (Fahimirad et al., 2023). This study was also determined to be successful in terms of wound healing. However, the variety of polymer materials used is high. No study has been found in the literature on nanofiber structured PLGA wound dressings produced by electrospinning using *Echinacea* extract. Although *Echinacea* is widely used in many areas, its wound healing effect has increased in recent years and is open to discovery.

Within the scope of the study, silver nanoparticles synthesized using *Echinacea* extract (Ec-AgNPs) were synthesized from *Echinacea* extract via a green synthesis

method. These Ec-AgNPs were then incorporated into PLGA solution and PLGA (Ec-AgNP/PLGA) was produced by electrospinning method and the applicability of this wound dressing was evaluated through *in vitro* characterization.

Materials and Methods

Preparation of *Echinacea* extract, antioxidant activity analysis and green synthesis of Ec-AgNPs

The *Echinacea purpurea* plant used in the study was supplied by Pariza Medicinal Plants. The supplied part includes dried flower parts. Silver nitrate ($AgNO_3$) used in the production of Ec-AgNPs together with *Echinacea* extract in the study was supplied by Carlo Erba company (CAS number 423957). To prepare the extract, dried *Echinacea* flower parts were mixed with distilled water and allowed to steep for 24 hours. For the $AgNO_3$ solution prepared as 0.1 M for the production of additional AgNP, 8.44 g of $AgNO_3$ was added to 500 ml of distilled water, and mixed with a magnetic stirrer (Gilik, 2021). The plant extract was used as a reducing agent in the production of AgNP with green synthesis. $AgNO_3$ serves as the Ag^+ source. The biomolecules in the plant extract reduce Ag^+ ions to Ag^0 ions, and thus the production of silver nanoparticles occurs. As a result of the measurements taken with the help of a UV spectrophotometer, it was determined that a ratio of 5:4:1 (extract/$AgNO_3$/distilled water) could be used for the production of additional AgNP (Öktemer, 2021). The solution prepared according to the determined ratio was covered with aluminum foil to protect it from light exposure and left to stand for 24 hours. During this time, it was expected that the silver ions would settle to the bottom as a result of the reduction reaction and a color change from brown to gray would occur in the solution. The color change indicates that the nanoparticles were successfully produced (Gecer et al., 2022). At the end of 24 hours, the resulting solution was centrifuged to ensure that the silver nanoparticles completely settled to the bottom. The settled part was transferred into a glass Petri dish and placed in an incubator to dry. After drying, Ec-AgNPs were obtained by scraping from the petri dish. The size of the produced nanoparticles was measured with the Zetasizer device (Malvern ZS) and crystal structure characterization was carried out by XRD analysis.

The antioxidant activity of the *Echinacea* plant extract as a natural component in wound dressing production was evaluated before use. The antioxidant activity analysis results were determined based on two values. The first of these is the calculation of ascorbic acid equivalent. The other is the calculation of *DPPH (%)* removal. The *DPPH (%)* removal value is the capacity of the antioxidants in the substance to inhibit *DPPH* free radicals. Liquid *Echinacea* extract was dissolved in methanol for analysis and *DPPH* solutions were prepared. The solution was incubated at 30 ° C incubator in the dark for 30 min. The absorbance was measured using a UV-VIS spectrophotometer. The measurements were evaluated considering the reference solution known to be antioxidant. The UV-VIS spectrophotometer was used in the wavelength range of 515-528 nm, where the reference solution gave maximum absorbance (Seyhan, 2019). As a result, *DPPH (%)* removal value and ascorbic acid equivalent (µM) of *Echinacea* extract were determined.

Preparation of nanofiber solutions and production by electrospinning

The PLGA polymer used for nanofiber fabrication was supplied by Shenzhen Esun Industrial Co. and the lactic acid: glycolic acid ratio was 75:25 (CAS number: 26780-50-7). Commercially available pure silver nanoparticles (AgNP) were supplied by BRK Laboratory Materials Chemical Products. Dichloromethane (DCM), which was used to dissolve the PLGA polymer, was supplied by Carlo Erba (CAS number: 75-09-2). n,n-Dimethylformamide (DMF), a solvent with the formula C_3H_7NO, was used together with DCM to dissolve the PLGA polymer and was supplied by Carlo Erba (Cas number is 68-12-2).

For the production of nanofiber wound dressing using electrospinning method, the nanofiber solutions were prepared. For PLGA solution, DCM/DMF were mixed at 50:50 v/v ratio and then 20% w/v solid PLGA polymer was added to this mixture. The PLGA polymer was stirred with magnetic stirrer until it achieved a homogeneous consistency. The same procedure was followed for AgNP/PLGA solution where 1% of PLGA was added to AgNP and mixed until homogeneous. Additionally, 1%, 2% and 3% Ec-AgNP/PLGA solutions were prepared in a similar manner.

All solutions were drawn into separate syringes and placed in the electrospinning device, where a large number of nanofibers wound dressings were produced by varying different parameters. There are three important parameters to consider in the production of nanofibers by electrospinning. These are; solution flow rate, applied voltage value and the distance between collector syringes. Table 1 shows the applied parameter value ranges for each group.

Characterization tests

Extensive characterization analyses were conducted on the produced nanofiber wound dressings, including SEM, FTIR, XRD and antibacterial testing. Surface morphologies and fiber diameters of PLGA, AgNP/PLGA and Ec-AgNP/PLGA nanofibers produced with different parameters were examined with SEM images. Nanofiber diameter measurements were made using the images obtained using the ImageJ program.

A Perkin Elmer Spectrum Two FTIR device was used for FTIR analysis. Measurements were performed in the wavelength range of 600-4000 cm^{-1}. Chemical bond analyses of the produced PLGA, AgNP/PLGA and Ec-AgNP/PLGA nanofibers were performed with this analysis. The vibration frequencies of the chemical

bonds in the molecules of the nanofibers were measured and the peak changes originating from the nanoparticles in their content were compared with the peaks of pure PLGA.

The crystal structure morphologies of nanofibers containing nanoparticles were analyzed using XRD analysis. The XRD device in the Biosimilar Products Laboratory was used for the analysis. PLGA, AgNP/PLGA, Ec-AgNP/PLGA, and also produced powdered Ec-AgNPs were analyzed. The analysis was carried out at room temperature. Scanning was performed in continuous scan mode on the 2Theta/Theta axis between 2 and 65 degrees, with an increment of 2 degrees per minute (Khan et al., 2020). The three strongest peaks of the samples and the other peaks obtained were compared. Antibacterial activity test was performed by disk diffusion method. The analysis was performed Ege University in the Biological Analysis and Cell Culture Laboratory. 3 different samples of nanofibers, including PLGA, AgNP/PLGA and Ec-AgNP/PLGA, were analyzed in 2 replicates. *Escherichia coli* ATCC 25922 (*E. coli*) was used as gram-negative bacteria and *Staphylococcus aureus* ATCC 25923 (*S. aureus*) was used as gram-positive bacteria. Mueller Hinton Broth and Mueller Hinton Agar were used for the growth medium. For the analysis, bacteria stored in a medium containing 16% glycerol at -20 °C were seeded on Mueller Hinton Agar medium by streaking method and left for incubation (Zaidan et al., 2005). After the samples were placed in the petri dish, they pre-incubated for 15 minutes and incubated at 37 °C for 16-24 hours (Zaidan et al., 2005). (The samples were sterilized under double-sided UV light for 1 hour). After incubation, photographs of the zones formed by the sample were taken and the zone diameters were measured using ImageJ program.

In vitro wound scratch model

Human keratinocyte cells (HaCaT) were used to test the wound dressings. Cell culture was performed using DMEM high glucose medium. The medium was changed every two days, and when the cells covered 80% of the culture dish surface, passaged using 0.25% trypsin-EDTA. After determining that the cells maintained at least 80% of their viability, the cells were seeded into 24-well plates with a density of 1×10^5 cells per well to create a scratch model for human keratinocyte cells (Ker-Woon et al., 2015). After the cells seeded into the plates were filled to 100%, a scratch was made in the middle of the wells with a pipette to create a scratch wound model as specified in the literature. The wound closure from both sides of the scratch was observed over 48 hours with photographs taken every 3 hours starting from the 0- hour mark (Ker-Woon et al 2015). The images were analyzed using the ImageJ program, and one-way ANOVA was used to compare wound closure between groups.

Results and Discussion

Antioxidant activity analysis of *Echinacea* extract

In this study, the antioxidant capacity of *Echinacea* extract was evaluated based on the *DPPH (%)* removal value and the ascorbic acid equivalent, revealing its high antioxidant properties. The *DPPH (%)* removal value was calculated as 46.8% and the ascorbic acid equivalent was determined to be 35.5 µM. In a related study, the ascorbic acid and *DPPH (%)* removal values of *Echinacea* extracts of different species were evaluated, concluding that *Echinacea* species possess antioxidant capacity (Hu and Kitts, 2000). The literature findings and the results of this study corroborate each other, demonstrating that *Echinacea* exhibits antioxidant properties concerning the concentration of its bioactive components (Ciganović et al., 2023). Additionally, it has been reported that bioactive components in plants which confer antioxidant properties, support the AgNP synthesis reaction, facilitating easier production. The antioxidant properties of *Echinacea* also contributed to the successful production of Ec-AgNP via the green synthesis method (Huq et al., 2022).

SEM image and nanoparticle size analysis

The surface morphologies of PLGA, AgNP/PLGA and Ec-AgNP/PLGA nanofiber wound dressings produced, under different parameters using electrospinning were examined through SEM images (Figure 1). Average fiber diameters were measured from these images; Table 2 presents the calculated fiber diameters of the nanofibers. The addition of AgNPs resulted in a reduction in fiber diameters, with the average fiber diameter of the AgNP/PLGA nanofibers calculated to be 315 nm. The fiber diameter distribution ranged from 210- 520 nm. Additionally, the incorporation of AgNPs introduced roughness into the fiber structure (Figure 1). In this study, a significant decrease was observed in fiber diameters with the addition of AgNPs to pure PLGA nanofibers. In the literature they compared spinning solutions, containing Ag with those free of Ag and noted a significant reduction in fiber diameters, attributed to increased conductivity in the Ag-containing solutions (Maleki et al., 2020). Another study examined three dosages (0.1, 0.5 and 1.0 wt%) of AgNPs in electrospun PLGA membranes and found that fiber diameters decreased as the amount of AgNPs increased (Wang et al., 2013). The findings of this study align with those reported in the literature.

In addition, particle size analyses of the produced Ec-AgNPs were measured with a Zetasizer device, and the average size was found to be 60±21.45 nm. The results support the successful production of nanoparticles.

FTIR analysis

FTIR analysis was conducted to characterize the chemical structure of nanofibers. Upon examining the

FTIR graphs, the most prominent peaks appeared at 1755 cm^{-1}, 1183 cm^{-1}, 1130 cm^{-1} and 1453 cm^{-1} spectra (Figure 2). In the case of Ec-AgNP/PLGA nanofibers, the addition of *Echinacea* nanoparticles caused a slight shift in the peaks, likely due to the physical interaction between the plant extract and the nanofiber. Since the interaction is physical rather than chemical, no additional FTIR spectrum peaks were observed (Uslu, 2020). In a previous study on PLGA/gelatin nanofibers, prominent peaks were similarly identified. Strong characteristic absorption bands were observed at approximately 1752 cm^{-1} for pure PLGA nanofibers. It was reported that the bands around 1182 cm^{-1} could be attributed to the stretching vibration of the C-O bond, the bands at 1182 cm^{-1} to the stretching of the C-O-C ether group, and the peaks at 1130 cm^{-1} and 1452 cm^{-1} to the C-O bond and the C-H bond of the methyl group of PLGA, respectively (Meng et al., 2010). Khalil et al. (2013) conducted FTIR analysis of PLGA polymer, PLGA nanofiber and PLGA nanofiber containing AgNP in their study and as a result, it was found that there was no change in the FTIR spectra regardless of whether the material was in the form of polymer, nanofiber or composite. The transmittance intensity of the PLGA nanofibers may have increased as a result of unsaturated groups resulted from the cross-linking reactions.

In this study, for pure PLGA nanofibers, the strong characteristic absorption bands at approximately 1755 cm^{-1} can be attributed to the stretching vibration of the C-O bond, while the bands at 1183 cm^{-1} likely result from the stretching of the C-O-C ether group. The peaks at 1130 cm^{-1} and 1452 cm^{-1} can be linked to the C-O bond and the C-H bond of the methyl group of PLGA, respectively. These findings are consistent with the characteristic FTIR peaks of PLGA reported in the literature (Figure 2). These results indicate that the materials added to the PLGA nanofiber do not cause a change in the chemical bond structure, the interaction is a physical interaction, so the peaks show values quite close to each other.

XRD analysis

The crystal structures were analyzed by XRD. Figure 3 shows the XRD graphs of PLGA, AgNP/PLGA, 1% Ec-AgNP/PLGA, and Ec-AgNPs, as well as the XRD graph of AgNP taken from the literature. When the graphs were examined, it was seen that the peaks for PLGA occurred at angles between 2θ= 15°-20°, for AgNP/PLGA at 2θ= 43.98°, for Ec-AgNP/PLGA at 2θ= 43.97°, and for Ec-AgNP at 2θ= 38.14°. In the XRD graph, the increase in peaks is directly proportional to the presence of crystalline structure in the material. The most intense peaks were observed in the Ec-AgNP graph in the crystalline structure. The group with the least intense peaks was the pure PLGA nanofiber group. An increase in peak intensity was observed as AgNPs were added to the material. In their study, Gecer et al. (2022) synthesized AgNPs using *Echinacea* extracts, in the XRD analysis of the synthesized *Echinacea* AgNPs, the diffraction peak angles were determined as 2θ= 38.1° and 44.3°. In our study, the most prominent peak angle in the graph of Ec-AgNP/PLGA nanofiber was determined as 2θ= 43.97°. This peak angle is parallel to the results obtained by Gecer et al. (2022). In addition, the graph with the highest peak intensity in our study belongs to Ec-AgNP. Ec-AgNP in powder form has a more crystalline structure compared to other amorphous structures. Therefore, the highest peak intensity is observed in this graph. In this respect, our results are consistent with the literature results (Kurul, 2019; Gecer et al., 2022). Based on the results, it can be said that Ec-AgNPs were produced successfully.

Antibacterial activity analysis

The antibacterial effects of the produced nanofibers were evaluated by measuring inhibition zone diameters for each sample (Table 3, Figure 4). The average zone diameters of Ec-AgNP/PLGA nanofibers against *E. coli* and *S. aureus* bacteria were found to be 13.5±0.3 mm. When comparing the average zone diameters and images, it was observed that Ec-AgNP/PLGA nanofibers exhibited the strongest antibacterial effect against both bacterial strains. Many phytochemicals found in *Echinacea* have been reported, including alkaloids, flavonoids, phenolic compounds and tannins and these substances have been found to have antimicrobial potential against various microorganisms (Ciganović et al., 2023). Among the many components mentioned, caffeic acid derivatives and other phenolic acids are among the most prominent. The most abundant caffeic acid derivatives in *E. purpurea* are cichoric acid and caftaric acid. Chicoric acid shows a variety of effects such as antiviral, antioxidant and antibacterial activities, which can help skin regeneration (Koriem, 2020). It is thought that the antibacterial effect comes from these components, as stated in the literature.

In a related study, Burlou-Nagy et al. (2023) investigated the antibacterial effects of *Echinacea purpurea* on *E. coli* and *S. aureus,* reporting average zone diameters of 16.09 ± 0.25 mm for *E. coli* and 15.56 ± 0.36 mm for *S. aureus.* They concluded that *Echinacea* possesses significant antibacterial activity (Burlou-Nagy et al., 2023). The findings from this study, alongside those in the literature, confirm the antibacterial properties of *Echinacea* (Burlou-Nagy et al., 2023).

***In vitro* wound scratch model**

The materials used for cell culture are Gibco brand; Fetal Bovine Serum (FBS) (catalog number: 16000044), DMEM High Glucose (catalog number: 11965092), Penicillin Streptomycin (catalog number: 15140122) and Trypsin/EDTA 25% (catalog number: 25200072). *In vitro* cell culture experiments using human keratinocyte cells (HaCaT) were conducted with six different groups: control, pure PLGA, AgNP/PLGA, 1% Ec-AgNP/PLGA, 2% Ec-AgNP/PLGA and 3% Ec-AgNP/PLGA. Wound scratch

models were photographed every 3 hours, starting at hour 0 and observed over 48-hour period. Among the scratch models, complete wound closure was observed in the control, AgNP/PLGA and 1% Ec-AgNP/PLGA groups. However, no wound closure occurred in the pure PLGA, 2% and 3% Ec-AgNP/PLGA groups, and cell detachment was observed after a certain period (Figure 5, Figure 6).

In the control group, complete wound closure was achieved at the 27th hour, in the AgNP/PLGA group at the 24th hour, and in the 1% Ec-AgNP/PLGA at the 15th hour. Statistical analysis showed that the 1% Ec-AgNP/PLGA nanofiber group significantly accelerated wound healing compared to the other groups (Figure 7), ($P < 0.05$).

Echinacea contains high amounts of caftaric acid and cichoric acid from bioactive molecule groups. Caffeic acid derivatives such as caftaric acid and cichoric acid, which have high radical scavenging activity, have a good wound healing effect. These compounds increase collagen deposition and have a pronounced hyaluronidase inhibitory activity. Echinacea supports wound healing with these therapeutic properties (Ciganović et al., 2023). In the literature, a study on the *in vitro* wound healing effects of *Echinacea* evaluated wound closure percentages in scratch models using different concentrations (50, 100 and 200 µg/ ml) of *Echinacea* extracts. The study found that the 50 µg/ ml *Echinacea* concentration was the fastest healing group. Increasing the concentration of *Echinacea* did not accelerate wound healing, nor did it significantly improve wound closure. However, the group using 50 µg/mL *Echinacea* extract achieved approximately 92.55% wound closure and enhanced cell formation after 36 hours, compared to the other samples. In addition, in the same study, cytotoxicity cell viability tests were performed on human dermal fibroblast cells using different *Echinacea* concentrations (50, 100 and 200 µg/ml) and it was determined that the group with the maximum cell viability was the group using 50 µg/ml *Echinacea* extract (Burlou-Nagy et al., 2023). In our study, it is thought that increasing the amount of Ec-AgNP in the 2% and 3% Ec-AgNP/PLGA groups induced a toxic effect on the cells, thereby inhibiting cell proliferation. This finding aligns with the study by Burlou-Nagy et al. (2023), where higher concentrations of *Echinacea* negatively impacted wound healing. The increase in the amount of AgNP with *Echinacea* concentration in the 2% and 3% Ec-AgNP/PLGA groups may have limited and negatively affected wound healing in this group.

Additionally, the lack of wound closure in the pure PLGA group may be attributed to the incomplete evaporation of the solvents during the production phase, potentially causing toxicity and impairing cell growth.

Conclusion

In this study various types of nanofibers wound dressings were successfully produced and evaluated. SEM images revealed that the fiber diameters and structures were suitable for nanofiber applications. FTIR analysis confirmed that the chemical structures of the nanofibers closely aligned with the characteristic peaks of PLGA. The antibacterial activity tests demonstrated that the produced materials possess effective antibacterial properties. Additionally, the antioxidant capacity of the incorporated *Echinacea* extract was found to be high, as indicated by *DPPH (%)* removal and ascorbic acid equivalent measurements.

Both our data and literature sources suggest that *Echinacea* accelerates wound healing due to its antioxidant, antibacterial, and therapeutic properties. The 1% Ec-AgNP/PLGA nanofiber wound dressing exhibited 100% wound closure faster than the other groups. However, higher concentrations of *Echinacea* silver nanoparticles had a negative effect on wound closure, likely due to cellular toxicity.

In conclusion, nanofiber wound dressings containing silver nanoparticles and *Echinacea* extract show promise for use in wound healing applications.

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Author Contributions

BS: Data Curation, Formal Analysis, Investigation, Visualization and Writing – Original Draft Preparation; AM: Conceptualization, Project Administration, Writing – Review & Editing; MES: Investigation, Resources, Writing – Review & Editing; ZGM: Supervision, Project Administration, Validation, Writing – Review & Editing, Investigation.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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Table 1. Parameters used in electrospinning method.

Nanofiber Groups	**Solution Flow Rate *(ml/h)***	**Distance Between Collector Syringes *(cm)***	**Voltage *(kV)***
PLGA	0.3-0.4	13-15	20-24.6
AgNP/PLGA	0.4-0.5	13-15	20-25
Ec-AgNP/PLGA	0.2-0.4	15-17	17-25

Table 2. Fiber diameter distribution of nanofibers.

Nanofiber Type	Average Fiber Diameter	Fiber Diameter Distribution
PLGA	357±133.16 nm	220-615 nm
AgNP/PLGA	252±82 nm	105-350 nm
Ec-AgNP/PLGA	315±71.91 nm	225-500 nm

Table 3. Antibacterial analysis results.

Nanofiber Type	**Average Zone Diameter *(E. coli)***	**Average Zone Diameter (*S. aureus)***
PLGA	8.5 ±0.1 mm	10.5 ±0.2 mm
AgNP/PLGA	11±0.0 mm	9.5±0.4 mm
Ec-AgNP/PLGA	13.5±0.3 mm	13.5±0.2 mm

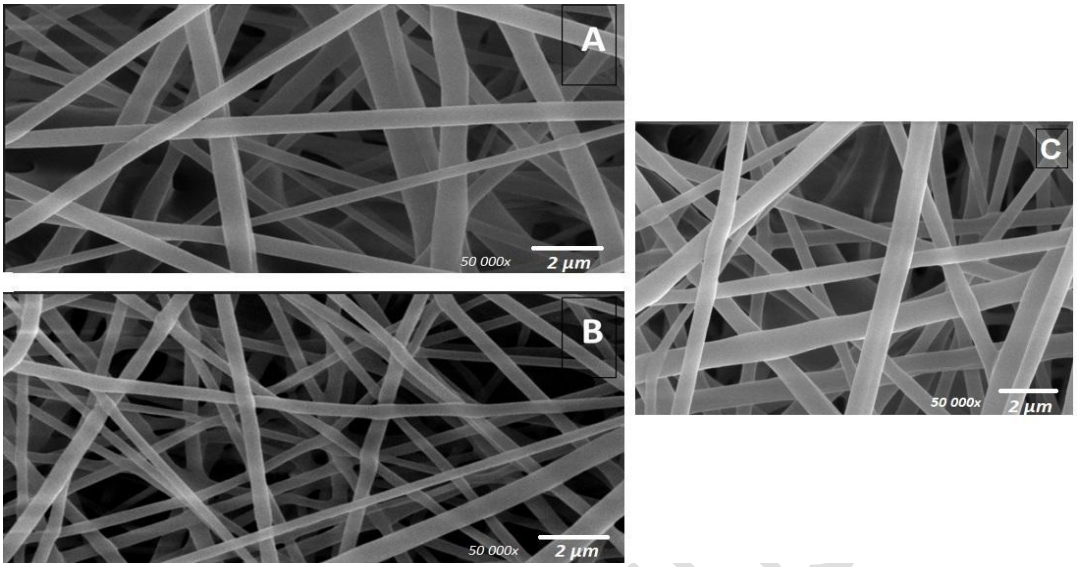

Figure 1. SEM images of nanofibers. (A) PLGA nanofibers, (B) Ec-AgNP/PLGA nanofibers, (C) AgNP/PLGA nanofibers.

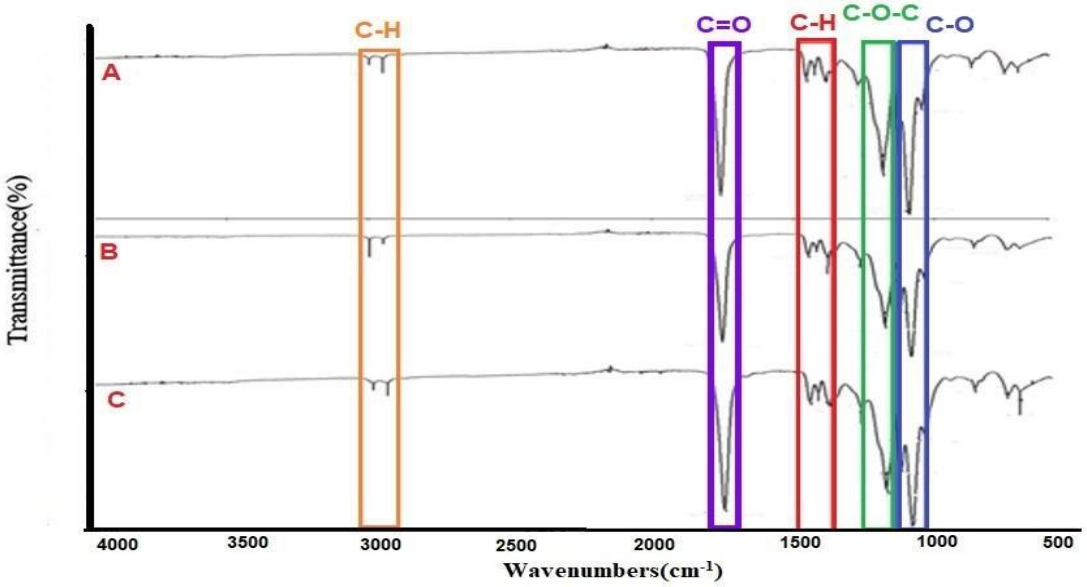

Figure 2. FTIR graphs of nanofiber structures A) PLGA nanofiber, B) AgNP/PLGA nanofiber, C) Ec-AgNP/PLGA nanofiber.

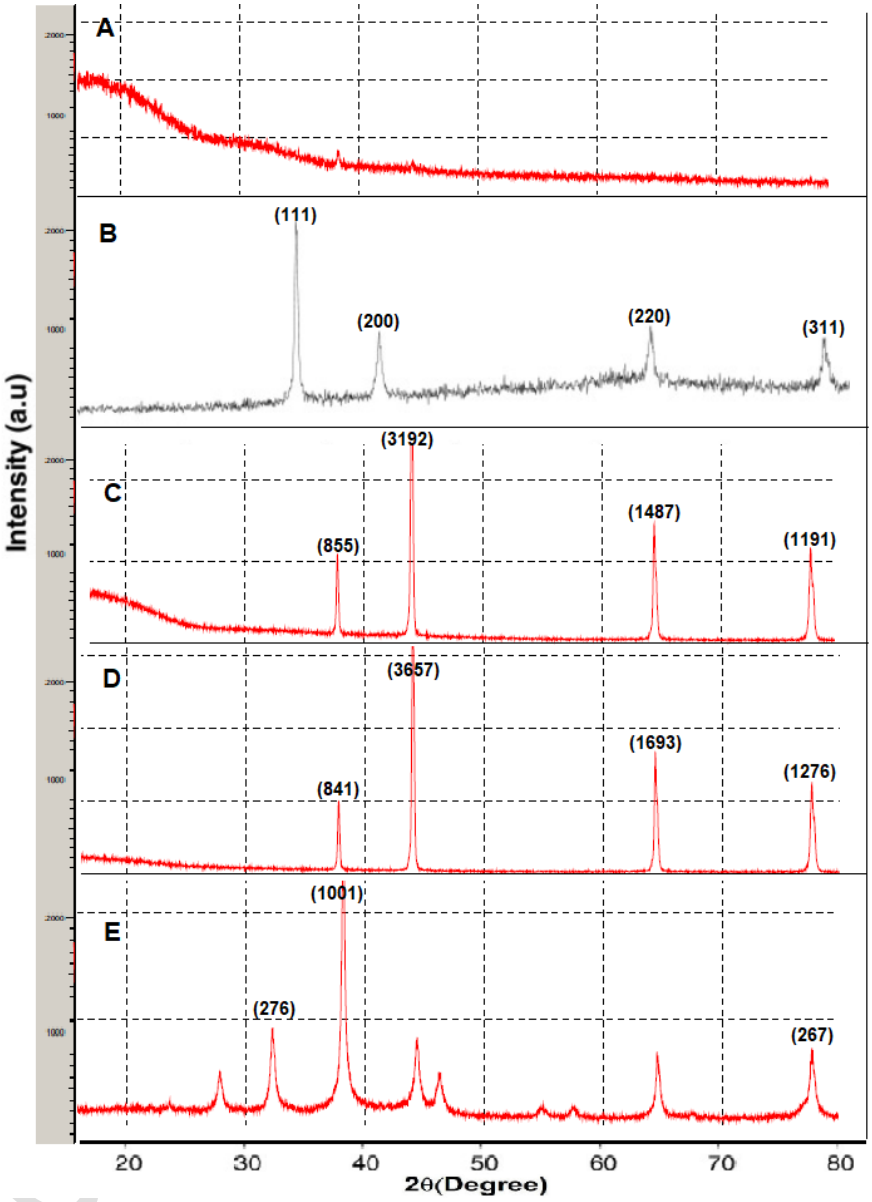

Figure 3. XRD graphs (A) PLGA, (B) AgNP (Chook et al., 2012), (C) AgNP/PLGA (D) Ec-AgNP/PLGA, (E) Ec-AgNP

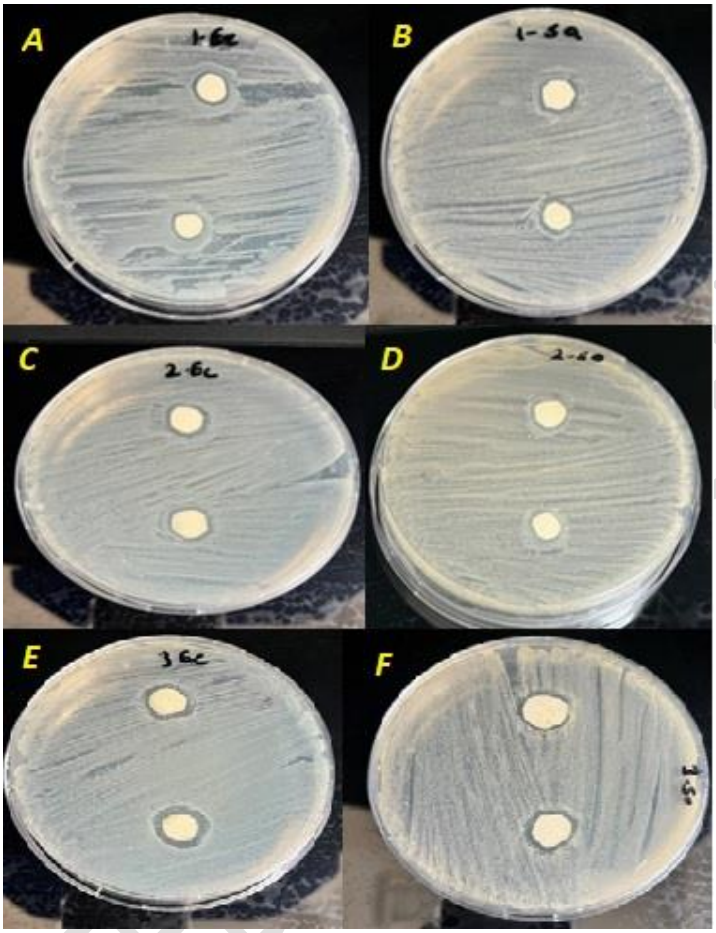

Figure 4. (A)PLGA, (C)AgNP/PLGA, (E) Ec-AgNP/PLGA nanofibers against *E. coli* bacteria; (B)PLGA, (D)AgNP/PLGA, (F) Ec-AgNP/PLGA antibacterial activity of nanofibers against *S. aureus* bacteria.

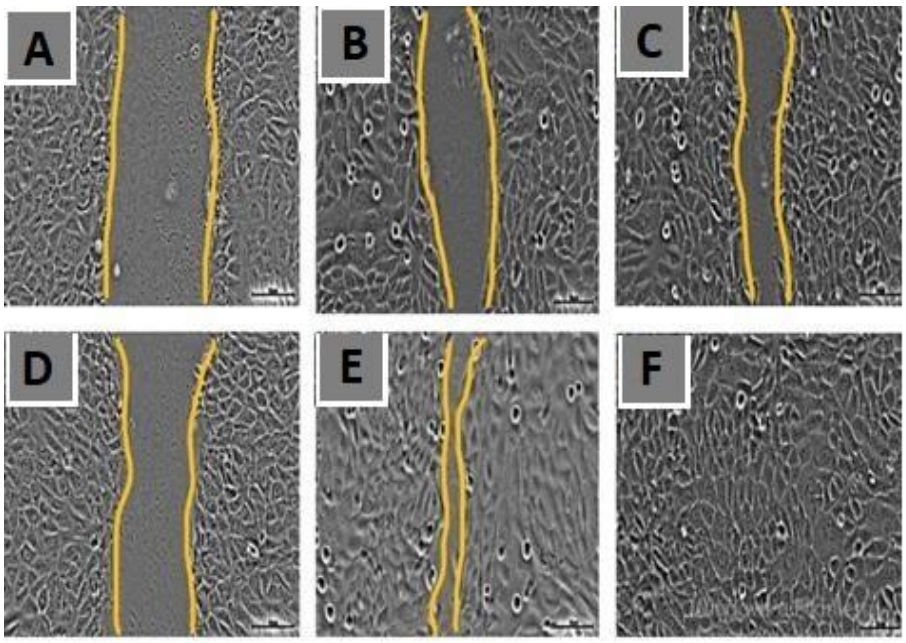

Figure 5. Microscope images showing wound healing in the control group and 1% Ec-AgNP/PLGA group at 0, 12, and 15 hours. (A) Control group at 0 hours, (B) Control group at 12 hours, (C) Control group at 15 hours, (D) 1% Ec-AgNP/PLGA group at 0 hours, (E) 1% Ec-AgNP/PLGA group at 12 hours, and (F) 1% Ec-AgNP/PLGA group at 15 hours.

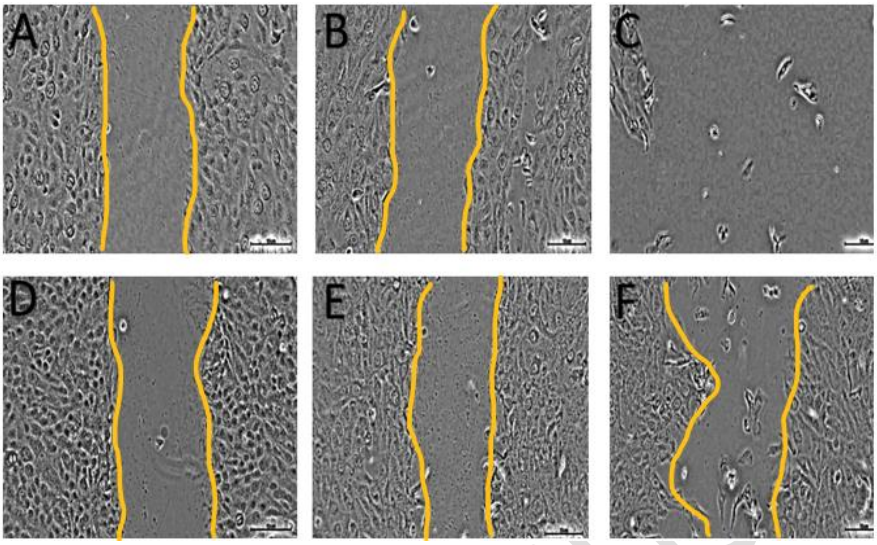

Figure 6. Microscope images showing wound healing in the 2% and 3% Ec-AgNP/PLGA groups at 0, 12, and 24 hours. (A) 2% Ec-AgNP/PLGA at 0 hours, (B) 2% Ec-AgNP/PLGA at 12 hours, (C) 2% Ec-AgNP/PLGA at 24 hours, (D) 3% Ec-AgNP/PLGA at 0 hours, (E) 3% Ec-AgNP/PLGA at 12 hours, and (F) 3% Ec-AgNP/PLGA at 24 hours.

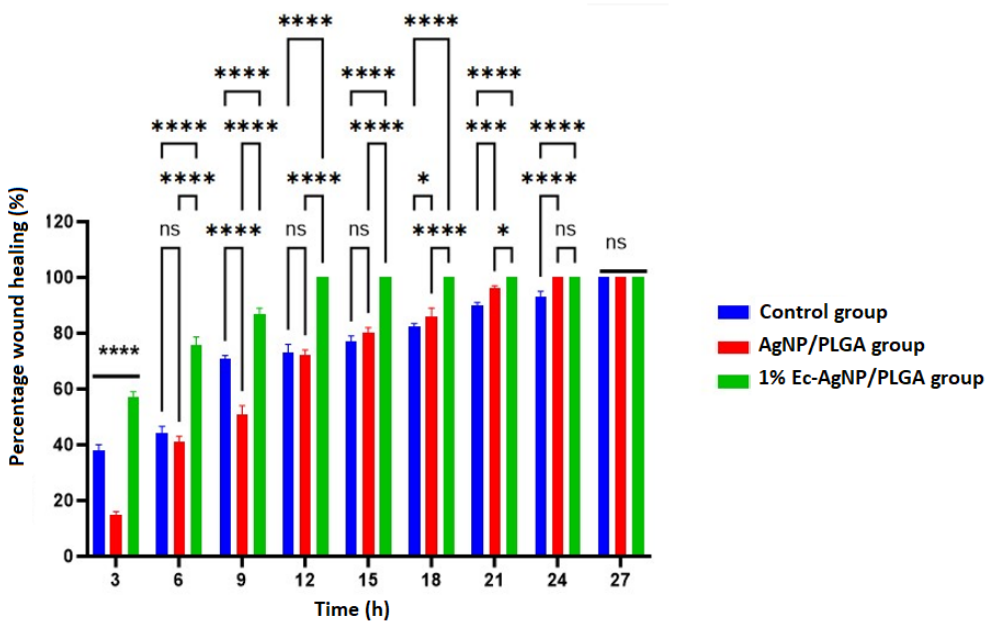

Figure 7. Percentage wound healing graph. *(**** P <0.0001, *** P <0.001, *P <0.05, ns P>0.05).*