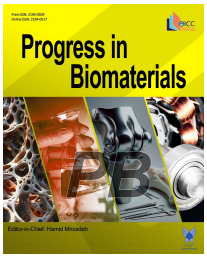

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The Hidden Glory of Nature in Burn Wound Treatment: Electrospinning Gelatin/PVA Nanofibers Loaded with Methanolic Clove Extract for Burn Wound Healing

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Original Research

Abstract:

This study focused on the design and fabrication of a composite nanofibrous dressing based on gelatin and polyvinyl alcohol (PVA) loaded with clove methanolic extract using the electrospinning technique. The scaffolds were cross-linked with glutaraldehyde vapor and characterized for their morphological, chemical, and mechanical properties by scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and tensile testing. The results demonstrated that the fabricated dressings exhibited a uniform nanofibrous structure, suitable mechanical strength, and high-water absorption capacity. Moreover, the incorporation of clove methanolic extract significantly enhanced the antibacterial activity of the dressing, particularly against Gram-positive bacterial strains, and accelerated the healing process of burn wounds in an animal model. These findings suggest that this biocompatible and antibacterial dressing holds great potential as an innovative and effective therapeutic approach for burn wound management.

Keywords:

Wound Dressing; Bourn wound; Clove methanolic extract; Electrospinning; Nanofiber; Antibacterial activity; Wound healing

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1. Introduction

The wound healing process is a complex biological process consisting of four stages: hemostasis, inflammation, proliferation, and remodeling. Initially, bleeding is stopped by blood clotting, infection is cleared by immune cells, new tissue is formed, and eventually the wound closes. Several factors such as oxygen deficiency, infection, burns, aging, hormones, stress, diabetes, obesity, drugs, and diet can alter the healing process and lead to the development of acute wounds. Effective treatment of acute wounds requires an understanding of these factors (Guo and DiPietro, 2010). Clove extract (Syzygium aromaticum), which is rich in eugenol, flavonoids, and tannins, possesses strong antibacterial and anti-inflammatory properties. This extract has the capability to promote wound healing and can be used as a natural, safe, and effective treatment for antibiotic-resistant infections (Rizal Arief et al., 2024). The bioactive compounds in clove extract inhibit pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli*. Phenolic compounds reduce oxidative stress and inflammation, aiding in the regeneration of skin cells. Studies have shown that adding clove extract to sutures reduces infection, facilitates healing, and does not cause toxicity, thus presenting a natural solution for improving wound dressings and sutures. Clove and its main active compound, eugenol, possess strong anti-inflammatory and antibacterial properties. Eugenol effectively reduces inflammation by regulating key cellular pathways such as NF-κB and MAPK, and by inhibiting pro-inflammatory cytokines. This results in decreased swelling and tissue damage in experimental models. Additionally, clove exhibits significant antibacterial effects, making it useful in combating infections. The synergistic actions of eugenol and other flavonoids in clove contribute to its potent anti-inflammatory and antimicrobial activity, supporting its traditional and therapeutic use in managing inflammatory conditions and bacterial infections (Vicidomini et al., 2021). The electrospun cellulose acetate fibers impregnated with clove extract exhibit remarkable an-

tibacterial and antibiofilm properties, effectively inhibiting infection-causing bacteria and accelerating wound healing. Combining this extract with other oils like cinnamon produces a synergistic effect, enhancing antibacterial activity and increasing healing speed by up to 65%. This innovative approach holds great potential as a natural and efficient alternative for advanced wound dressing design in treating infected wounds (Parham and Kharazi, 2022). Rapid and effective healing of acute wounds, especially those with severe bleeding, requires dressings that can stop bleeding, prevent infection, and support tissue regeneration. Electrospun nanofibrous dressings, which can mimic the skin's extracellular matrix and also provide drug delivery, have high potential in this field. The combination of biocompatible polymers such as gelatin and polyvinyl alcohol (PVA) not only provides mechanical strength but also creates a suitable environment for the skin. The uniform nanofibrous structure, high water absorption, and targeted drug delivery capability make these dressings an innovative solution for treating acute wounds (Pandey et al., 2023). Traditional dressings have limitations such as limited exudate absorption capacity and lack of controlled drug delivery, which restrict their applications. Nanofiber technology, especially electrospinning of natural polymers like gelatin, chitosan, and alginate, offers a novel solution. These biocompatible dressings, with a structure similar to the extracellular matrix, provide superior exudate absorption, gas exchange, controlled drug release, and cell growth compared to conventional dressings, opening new avenues for managing chronic wounds. However, challenges such as improving mechanical strength and enhancing biological functionality still remain (Bombin et al., 2020). Polyvinyl alcohol (PVA) is a synthetic biodegradable and biocompatible polymer that, due to its suitable hydrophilicity and mechanical strength, is considered an appropriate choice for nanofibrous wound dressings. PVA provides a long-lasting nanofibrous matrix that retains moisture and gradually releases antibiotics, facilitating wound healing and inhibiting microbial infection through controlled drug release (Kenawy et al., 2023). In recent years, the design of novel industrial biomaterials for the improvement of skin wounds, especially infected and chronic wounds, which is a major focus in tissue engineering, has shown a growing trend. Synthetic polymers such as gelatin, chitosan, and PVA are widely used due to their biocompatibility, hydrophilicity, and ability to form porous three-dimensional networks with suitable water retention capacities that mimic the natural extracellular matrix and facilitate cell growth and proliferation. The addition of active compounds such as natural extracts, honey, or zinc oxide (ZnO) nanoparticles enhances antibacterial activity, elasticity, and overall bioactivity. Freeze-thaw and electrospinning techniques enable the fabrication of hydrogels and nanofibrous dressings with reproducible porosity, high water absorption, and controlled drug release. Increasing the concentration of PVA improves mechanical stability and is more suitable for rapid cell growth. ZnO nanoparticles reduce water absorption and slow degradation, while bilayer nanofiber-hydrogel constructs synergistically enhance mechanical strength, stability, and biocompatibility. Gelatin/PVA hybrid dressings, with or without bioactive molecules, provide a superior platform for microprocedural wound coverage and dermal tissue engineering with optimal mechanical strength, biocompatibility, biodegradability, and infection resistance. Other studies conducted on plants have shown that copper carboxylate nanoparticles synthesized using the plant Allium eriophyllum Boiss (CuNPs@A. eriophyllum) exhibit significant inhibitory effects on the growth of all tested bacteria and fungi at higher concentrations. These nanoparticles demonstrated antimicrobial efficacy comparable to or even surpassing that of standard antibiotics, suggesting their potential as effective antimicrobial agents and suitable alternatives to conventional drugs (Khan et al., 2024; Kim et al., 2020; Mahnama et al., 2017; Nasiri et al., 2022; Shamloo et al., 2021; Sun et al., 2022; Tennakoon et al., 2024; Zhao et al., 2022). Healing large and deep wounds remains a clinical challenge because natural skin cannot be fully restored, and skin grafts are limited due to donor site availability and side effects. Skin tissue engineering, using biodegradable and biocompatible dressings to mimic the bilayer of dermis and epidermis, is a novel approach. Gelatin, a collagen derivative, is a suitable material for the dermal layer dressing because it is biocompatible, hemostatic, and water-absorbing. It forms a nanofibrous matrix that facilitates the growth of skin cells by mimicking the extracellular matrix. However, gelatin alone is mechanically weak and is therefore combined with synthetic polymers to enhance its strength (Budai-Szűcs et al., 2021; Chi et al., 2022; Wiegand et al., 2016). In this study, a scaffold was fabricated using a dual-nozzle electrospinning technique, employing the natural polymer gelatin and the synthetic polymer polyvinyl alcohol (PVA). The resulting scaffold exhibited biocompatibility and sufficient mechanical strength, making it suitable for application in burn wound treatment. Considering previous studies on natural extracts, known for their effective antibacterial resistance, clove methanolic extract was incorporated. The structure of the electrospun scaffold was characterized by scanning electron microscopy (SEM), and drug distribution within the scaffold was evaluated using Fourier-transform infrared spectroscopy (FTIR). Additionally, both *in vitro* and *in vivo* assessments were conducted to evaluate the wound dressing's performance on burn wound healing, showing promising results.

2. Materials and methods

Materials

Gelatin (Type B, powder, Bioreagent, suitable for cell culture) was purchased from Sigma-Aldrich (bovine skin source). Polyvinyl alcohol (PVA) was also obtained from Sigma-Aldrich. Indian clove (Eugenia caryophyllata), belonging to the Myrtaceae family, was supplied in a semi-crushed form by the Medicinal Plant Research Institute of Jihad University. Pure glutaraldehyde (50% aqueous solution) was used as a crosslinking agent. Bacterial strains *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were employed for antimicrobial testing. NRF2 gene reagents were utilized as required. Mueller-Hinton agar and Mueller-Hinton broth media were prepared for bacterial culture. Phosphate-buffered saline

(PBS) with pH 7.4 was used in all relevant experiments.

Clove (syzygium aromaticum) extract preparation

Ten grams of powdered clove buds were weighed and transferred into 100 mL of methanol. It was placed under maceration for 48 hours on a magnetic stirrer for the exhaustive extraction of active components. The solution was filtered through several layers of sterile gauze and filtered using Whatman filter paper for a clear and homogeneous extract. The extract was filtered and placed in a petri dish and left in the sterile hood for 24 hours to allow the solvent to dry up totally, obtaining a dry concentrated extract.

Evaluation of minimum inhibitory concentration and minimum bactericidal concentration

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antibacterial agent at which no visible bacterial growth occurs. The MIC determination was carried out using the broth microdilution method. A 96-well polystyrene microplate was used to prepare different concentrations of clove extract through serial dilution. The extract concentrations ranged from 5 mg/mL to 0.00976 mg/mL. Mueller-Hinton broth was prepared by dissolving 1.5 g of powder in 50 mL of distilled water. The mixture was transferred to an Erlenmeyer flask and stirred at room temperature until fully dissolved and homogeneous. The prepared medium was then autoclaved for two hours to sterilize it and make it ready for use. In the first step, 95 µL of Mueller-Hinton broth was evenly distributed into wells 1 to 9 of the 96-well microplate. In the second step, 100 µL of clove extract was added to wells 1 to 9 through serial dilution. Then, 5 µL of bacterial suspension standardized to 0.5 McFarland was added to each well. Wells 11 and 12 served as positive and negative controls, respectively. After incubation, wells showing no bacterial growth along with their adjacent wells were subcultured on agar plates and incubated for 24 hours to determine the MIC and minimum bactericidal concentration (MBC) (Al-Ameedi and Nahi, 2019; Faujdar et al., 2020; Esmaeili et al., 2020; Hussein et al., 2022).

Preparation of electrospinning solutions

The 20% gelatin solution was initially prepared by mixing 1 g of gelatin powder with 5 mL of 80% acetic acid. According to previous studies, gelatin, being a natural protein, dissolves poorly in water alone and does not achieve a suitable quality for electrospinning; therefore, it is usually dissolved in mild solvents such as acetic acid. This acid breaks and unfolds the three-dimensional structure of gelatin protein, resulting in better dissolution of gelatin in the solution. Using acetic acid as a solvent also improves the electrical conductivity of the solution, which is of great importance in the electrospinning process and leads to the production of nanofibers with better morphology and uniform distribution. Additionally, an appropriate viscosity for electrospinning is achieved. The solution was magnetically stirred at 600 rpm for 1 hour at room temperature until homogeneous. For the preparation of the clove extract-loaded solution, 0.5% powdered methanolic clove extract was added to the gelatin solution and magnetically stirred at 100 rpm for 24 hours to achieve complete dissolution of the extract. Furthermore, a 10% polyvinyl alcohol (PVA) solution was prepared by adding 0.5 g of PVA powder to 5 mL of distilled water and stirring at 80 °C and 600 rpm for 2 hours until completely dissolved. The good solubility of PVA is due to the presence of hydroxyl (-OH) groups in its molecular structure, which makes it highly hydrophilic and easily dissolvable in water. This hydrophilicity makes PVA a suitable polymer for preparing aqueous polymer solutions used in processes such as electrospinning. The use of aqueous PVA solution in electrospinning is important since its high-water solubility allows for the formation of a homogeneous solution with optimal viscosity, resulting in better quality and uniform morphology of the produced nanofibers and improving the electrospinning process. The drug was dissolved in a natural polymer solution for controlled release, while PVA was used through a separate nozzle to enhance mechanical strength. The dual-nozzle electrospinning system was employed to prevent drug encapsulation and ensure effective drug distribution (El-Maati et al., 2016; Gu et al., 2009; Yao et al., 2019).

Surface morphology and diameter of fiber analysis

To measure the surface structure and diameter of the electrospun samples and obtain high-magnification, high-resolution microscopic images, a TESCAN MIRA3 scanning electron microscope (SEM) (made in the Czech Republic) was used. Samples were cut into 2×2 cm^2 fragments and coated with a thin layer of gold to enhance the nanofibers' conductivity, then mounted on a special holder. Subsequently, SEM was employed to examine the morphology and uniformity of the nanofibers.

FT-IR analysis of prepared nanofiber

For analyzing the structure and identifying the chemical bonds of gelatin- and PVA-based nanofibers and wound dressing loaded with clove methanolic extract powder, FTIR spectroscopy was carried out on a Nicolet 800 instrument using the attenuated total reflectance (ATR) method at room temperature between 400 to 4000 cm^{-1} (Borges-Vilches et al., 2022).

Crosslinking of nanofiber wound dressing

The wound dressings were subjected to a crosslinking process by exposure to 50% glutaraldehyde vapor for 4 hours. In this process, the amino groups present in the polymer chains react with the aldehyde groups of glutaraldehyde, forming stable covalent bonds that create a strong three-dimensional network. This network structure enhances mechanical strength, long-term stability, and resistance of the dressings against dissolution or swelling. Consequently, the mechanical stability and structural integrity of the nanofibrous wound dressings are significantly improved (Borges-Vilches et al., 2022; Shahid et al., 2022; Yao et al., 2019).

Mechanical properties of electrospun nanofibers

Uniaxial tensile testing was performed to examine the mechanical characteristics of the electrospun nanofibers using an Instron 5967 universal testing machine (Instron GmbH, Darmstadt, Germany). Rectangular-shaped electrospun

nanofiber samples of 1 cm width and 3 cm length were cut and stuck in a correct paper frame. These tests were conducted at a 5 mm/min crosshead rate using a 100 N load cell. Young's modulus, tensile strength, and break elongation were obtained from the tensile stress-strain curves. Three repeats were conducted on each electrospun nanofiber sample in each test and were averaged (Sadri et al., 2015).

Swelling and water absorption analysis

The cross-linked samples were initially cut into 1 × 1 cm^2 pieces and their weights were measured. The samples were then placed in Falcon tubes filled with distilled water. The tubes were incubated at 37 °C for predetermined time intervals of 1, 3, 6, 12, 24, and 48 hours. After each time interval, the samples were removed and weighed again. All measurements were performed in triplicate to ensure reproducibility of the results (Ahmadi et al., 2021).

$$\text{Water Absorption} = \frac{m_1 - m_0}{m_0} \times 100$$

where m_0 weight of the dry sample and m_1 wet sample.

Evaluation of *in vitro* degradation

The cross-linked samples were cut into 1 × 1 cm^2 pieces and their initial weights were recorded. The samples were then immersed in PBS solution at pH 7.4 and incubated at 37 °C. At predetermined time intervals (3, 6, 12, 24, 48, and 72 hours), the samples were removed from the solution, and after complete drying of the remaining PBS, their weights were measured again. The degradation rate of the scaffold was evaluated at each time point by calculating the percentage of weight loss using the following equation (Mansourian et al., 2014).

$$\text{Weight loss } (\%) = \frac{W_i - W_t}{W_i} \times 100$$

where W_i and W_t represent the initial weight of the wound dressings before and after the degradation, respectively.

Analysis of antibacterial activity

Petri dishes. The target bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, were adjusted to a 0.5 McFarland standard and uniformly streaked on the surface of the agar using a sterile swab. Electrospun nanofiber samples loaded with methanolic clove extract were cut into 1 × 1 cm^2 pieces and placed on the inoculated agar plates. The plates were then incubated at 37 °C for 24 hours. After incubation, the inhibition zones around the nanofiber samples were measured, and the antibacterial efficacy against Gram-positive and Gram-negative bacteria was compared.

Macroscopic evaluation of burn wound area using image J

To evaluate the burn wound area macroscopically, digital photographs of the wounds were taken on days 0 (immediately after burn induction), 3, 7, 10, 14, 17 and 21 post-burns using a high-resolution digital camera (Canon EOS 600D, Japan) at a fixed distance of 20 cm perpendicular to the wound surface under consistent lighting conditions. A millimeter-scale ruler was placed adjacent to the wound in each image for calibration purposes. The wound area was measured using Image J software (version 1.53, NIH, USA). Each image was first calibrated based on the scale marker, and the wound margin was outlined manually using the polygon selection tool. The wound area was then calculated in square centimeters (cm^2). All measurements were performed in triplicate by two independent observers blinded to the experimental groups, and the average values were used for statistical analysis. Wound contraction rate (%) was calculated using the following formula:

$$\text{Wound contraction } (\%) = \frac{\text{Initial wound area} - \text{Wound area on day x}}{\text{Initial wound area}} \times 100$$

Statistical analysis

The healing rate was statistically analyzed using GraphPad Prism using two-way ANOVA with Tukey's post hoc test.

Microscopic evaluation of burn wound

The wound healing efficacy of the prepared nanofibers was evaluated using a full-thickness model in 30 adult male rats. The animals were randomly divided into 6 groups, with 5 rats in each group. For general anesthesia, a combination of ketamine (70 mg/kg, 5%) and xylazine (6 mg/kg, 2%) was injected intraperitoneally. A circular full-thickness wound with a diameter of 1 cm was then created on the dorsal skin of the rats; this wound was induced using a burn device for 5 seconds to produce a second-degree burn, damaging both the epidermis and dermis. The nanofibrous wound dressings were secured onto the wounds using elastic adhesive bandages. Animals from each group were euthanized on days 7, 14, and 21 post-treatments, and the skin samples were harvested and immediately fixed in 10% neutral buffered formalin (pH = 7.26) for 48 hours to prepare them for further analyses. The fixed tissue samples were then processed, embedded in paraffin, and sectioned to 5 µm thickness. Finally, the sections were stained with hematoxylin and eosin (H&E). The histological slides were evaluated by two independent reviewers, using light microscopy (Olympus; Olympus, Tokyo, Japan) in a double-blind fashion. Inflammatory cell infiltration, fibroplasia (collagen content) was assessed in different groups, comparatively. Magnification ×400 was employed to count different cells and calculation was repeated in five fields. Finally, the average number of each criterion for these fields was then recorded. Epithelialization was assessed, on 10 and 20 DPI (dots per inch), semi-quantitatively on 5-point scores: 0 (without new epithelialization), 1 (25%), 2 (50%), 3 (75%), and 4 (100%) epithelialization. For these parameters, the results were validated by a comparative analysis of two independent observers blinded to the treatment groups.

Ethical Considerations:

All animal experiments were performed in accordance with the ethical guidelines of the Tehran University of Medical Sciences Animal Care and Use Committee, and this study was approved under ethical code [700/672].

3. Results

Analysis MIC/MBC clove methanolic extract

The findings showed that the methanolic extract of clove has strong inhibitory and bactericidal effects against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The minimum inhibitory concentration (MIC) was 0.03125 mg/mL for *Staphylococcus aureus* and 2.5 mg/mL for *Pseudomonas aeruginosa*, indicating higher sensitivity of the Gram-positive bacterium. The minimum bactericidal concentration (MBC) was 0.625 mg/mL for *Staphylococcus aureus* and 5 mg/mL for *Pseudomonas aeruginosa* (Fig. 1). Previous studies have demonstrated that clove extract exhibits significant antibacterial activity at concentrations of 50 to 100 µg/mL against *Staphylococcus aureus* and *Escherichia coli*. This activity is stronger against Gram-positive bacteria such as *Staphylococcus aureus* compared to Gram-negative bacteria like *Escherichia coli*, which is attributed to differences in their cell membrane structures. The presence of an outer protective membrane in Gram-negative bacteria limits the penetration of active compounds. Similarly, reports have highlighted high antibacterial activity of plant extracts such as the ethanolic extract of Tamarindus indica against Gram-positive bacteria compared to Gram-negative strains. Higher concentrations of the methanolic extract were also required to improve antibacterial activity against *Pseudomonas aeruginosa*, due to the more complex and resistant cell wall structure of this Gram-negative bacterium. Therefore, the differing sensitivities of bacteria to the methanolic clove extract align with previous research findings, and the clove extract shows high potential as a natural antibacterial agent, especially against Gram-positive bacteria such as *Staphylococcus aureus*. In a study, clove oil demonstrated strong antibacterial effects against MRSA at concentrations of 20 and 40 µL/mL, with no change in the inhibition zone when combined with imipenem. Concentrations below 20 µL/mL showed much lower inhibitory effects. The MRSA strain was resistant to most antibiotics but remained sensitive to linezolid, vancomycin, and minocycline. The minimum inhibitory concentration (MIC) of clove oil was 2.5 µL/mL, and the minimum bactericidal concentration (MBC) was 5 µL/mL. At concentrations of 5 µL/mL and above, no bacterial colonies formed on mannitol salt agar, indicating strong bactericidal activity (Alanazi et al., 2022; Alghamdi, 2023; El-Maati et al., 2016; Ghaly et al., 2023).

Characterization of electrospun nanofibers

In this study, neat PVA nanofibers were initially produced with an average diameter of 227.5 ± 31.9 nm, exhibiting uniform and relatively thin morphology. Subsequently, gelatin (GEL) nanofibers were observed with an average diameter of 268.3 ± 37.2 nm. The PVA and gelatin blend showed a higher average diameter of 270.7 ± 83.3 nm, likely due to increased viscosity and intermolecular interactions between the two polymers, which influenced the electrospinning pro-

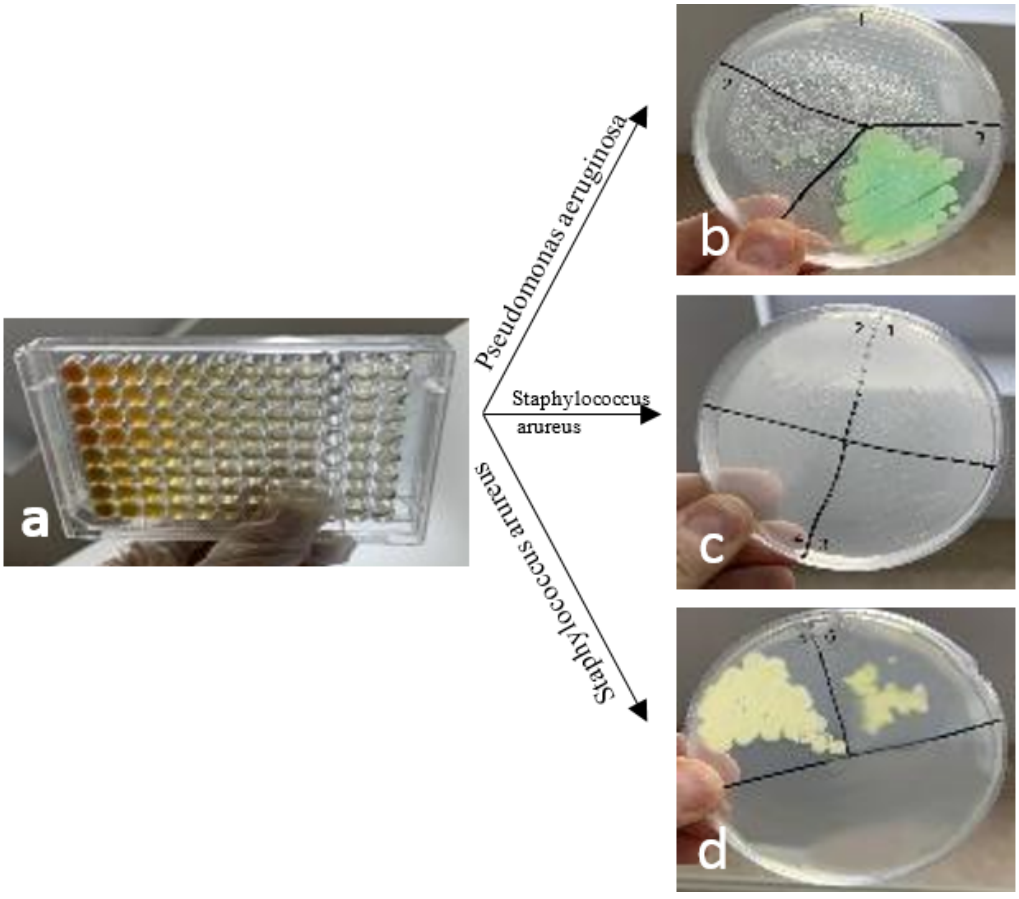

Figure 1. Antibacterial activity of clove methanolic extract: (a) Minimum Inhibitory Concentration (MIC) against *Staphylococcus aureus* and *Pseudomonas aeruginosa*; (b) Minimum Bactericidal Concentration (MBC) against *Pseudomonas aeruginosa*; (c, d) Minimum Bacteria.

cess and fiber formation.The addition of clove extract to gelatin increased the average nanofiber diameter to 250.6 ± 24.4 nm compared to pure gelatin, indicating a rise in solution viscosity and morphological changes. The final PVA/gelatin blend with clove extract presented the largest average diameter of 292.7 ± 44.4 nm, confirming the increase in viscosity and morphological alterations caused by the combination of all three components (Fig. 2). According to these results, optimizing polymer blends and additives to fabricate nanofibers with suitable and uniform diameters is crucial and can significantly enhance applications in tissue engineering and biological wound dressings. The findings of this study are consistent with previous reports and confirm the pivotal role of compositional and processing parameters in controlling nanofiber morphology (Aran et al., 2021; Doostan et al., 2023).

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the samples provide clear evidence for chemical interactions and compatibility between the components of the wound dressing, i.e., PVA, gelatin, and clove phenolic extract. A broad band for the stretching vibration of hydroxyl (O–H) and amine (N–H) groups with intense hydrogen bonding between the polymer chains and extract compounds that is responsible for compatibility and interaction between the components. These hydrogen bonds can help improve the uniformity of the structure and physical properties of the wound dressing. The peak at 2927 cm^{-1} caused by C–H stretching vibrations of alkyl groups (CH_2 and CH_3) reveals the presence of a common carbon skeleton for the three components. This suggests that clove extract organic compounds are well embedded within the polymer matrix and are integrated into the general wound dressing structure. The 1650 cm^{-1} band is attributed to the C=O stretching of the residual acetate groups of PVA and referred to as the amide I band (C=O stretching of protein amide groups) for gelatin. It may also be due to the stretching of the carbonyl (C=O) group of ketones, aldehydes, or esters in the clove extract. Within the composite system, this region reflects the secondary protein structure of the gelatin and the presence of the carbonyl groups of the extract. Changes in peaks here can indicate chemical interaction and cross-link formation between components that would ultimately enhance structural strength and stability. Finally, the peak at 1091 cm^{-1}, which is associated with C–O stretching, is observed in ether/ester or acetate groups of PVA, cross-links and amide groups of gelatins, and ether, ester, or amine groups of the clove extract. The region suggests possible cross-linking and chemical interaction among the components, which can improve the mechanical and chemical properties of the wound dressing. Overall, the FTIR results demonstrate a nice compatibility and effective interaction between PVA, gelatin, and clove extract that can lead to the development of wound dressings with enhanced properties and broad-spectrum applications (Fig. 3) (Ahlawat et al., 2019; Balaji et al., 2016; Razzaq et al., 2021).

Mechanical properties of electrospun nanofiber mats

This section presents the results of strength tests for various samples, including pure gelatin (GEL), gelatin mixed with clove extract (GEL + clove extract), pure polyvinyl alcohol (PVA), a mixture of polyvinyl alcohol and gelatin (PVA + GEL), and a ternary mixture of polyvinyl alcohol, gelatin, and clove extract (PVA + GEL + clove extract). Each test was performed in triplicate, and the average strength along with the standard deviation were calculated. The gelatin sample with clove extract (GEL + clove extract) showed an average strength of 0.89 ± 0.03, indicating low strength with little variation in the data. Samples containing polyvinyl alcohol exhibited higher strength; the average strength of these samples was 1.60 ± 0.21, reflecting the favorable mechanical properties of this polymer. The mixture of polyvinyl alcohol and gelatin with clove extract (PVA + GEL + clove extract) showed better strength compared to pure gelatin, with an average strength of 0.80 ± 0.12. These changes are mainly attributed to the reinforcing role of polyvinyl alcohol in improving the mechanical structure of gelatin. Overall, the results highlight the importance of polyvinyl alcohol as a reinforcing agent that effectively reduces the mechanical weaknesses of gelatin (Fig. 4 (a,b)). In this study, the elongation percentages of different samples were also evaluated. The gelatin sample with clove extract (GEL + clove extract) had an elongation of 3.4%, while the pure polyvinyl alcohol (PVA) sample showed the highest elongation at a significant value of 176.6%, indicating the high flexibility and elasticity of this polymer. Furthermore, the blend of polyvinyl alcohol, gelatin, and clove extract (PVA + GEL + clove extract) showed an elongation of

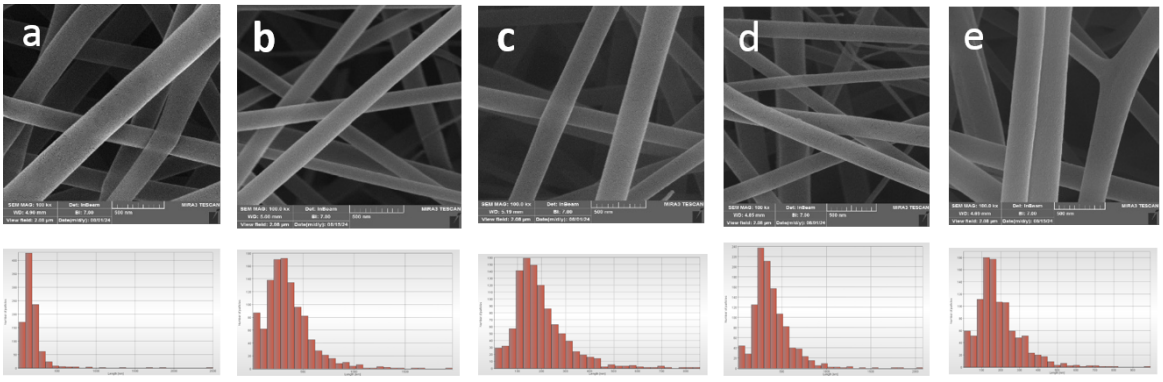

Figure 2. SEM images of electrospun nanofibers with different compositions: (a) PVA, (b) gelatin, (c) GEL + clove extract, (d) PVA + GEL, (e) PVA + GEL + clove extract.

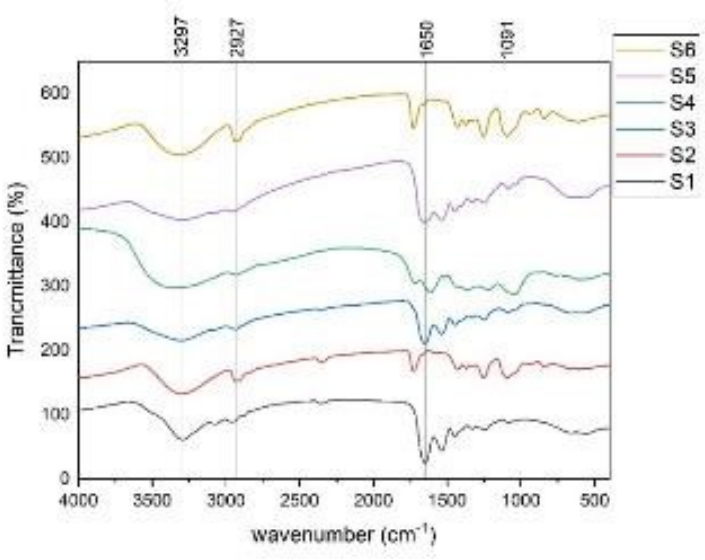

Figure 3. FTIR spectra of the samples: S1 = GEL; S2 = PVA; S3 = PVA + GEL; S4 = Clove extract; S5 = GEL + Clove extract; S6 = PVA + GEL + Clove extract.

4.8% (Fig. 4 c). The results clearly indicate that polyvinyl alcohol plays a key role in enhancing the elasticity of the samples. Appropriate elongation indicates optimal flexibility of the materials, which allows better conformity of the dressing to the natural movements of the skin and limbs. This feature is very important for wound dressing applications because it prevents tearing or detachment of the dressing under repeated stretching. Additionally, materials with suitable elasticity can absorb and distribute mechanical stresses, which helps reduce damage to wound tissue cells and accelerates the healing process. The combination of high elasticity and sufficient strength enables protection of the wound site and maintenance of necessary mechanical stability throughout the treatment period, preventing penetration of harmful external agents. Also, flexible dressings that conform well to the skin surface can reduce friction and skin irritation, thereby decrease inflammation and create a favorable environment for wound healing.

Water absorption behavior analysis

The high swelling of PVA dressings indicates a high capacity for water absorption, which in turn effectively absorbs the excessive exudate in burn wounds; this feature is very important for managing exudative wounds. The rapid swelling rate on the first day (about 40%) and the continuous increase up to about 90% within 48 hours demonstrate the ability of the PVA dressing to maintain moisture and prevent the wound environment from drying out, which can provide favorable conditions for tissue repair. However, very high swelling may lead to premature saturation of the dressing, which reduces its further absorption capacity and increases the need for more frequent dressing changes. This issue should be considered in clinical applications to ensure timely dressing replacement and prevent accumulation of saturated exudate. In contrast, the lower swelling of gelatin dressings and moderate swelling of composite dressings (PVA + GEL and PVA + GEL + Clove extract) indicate a balance between the absorption capacity for exudate and maintaining the structural stability of the dressing, which may be beneficial for long-term wound management. These dressings have an adequate absorption capacity without saturating rapidly (Fig. 5). Overall, correlating swelling data with the physiological properties of the wound indicates that each type of dressing can be suitable depending on the

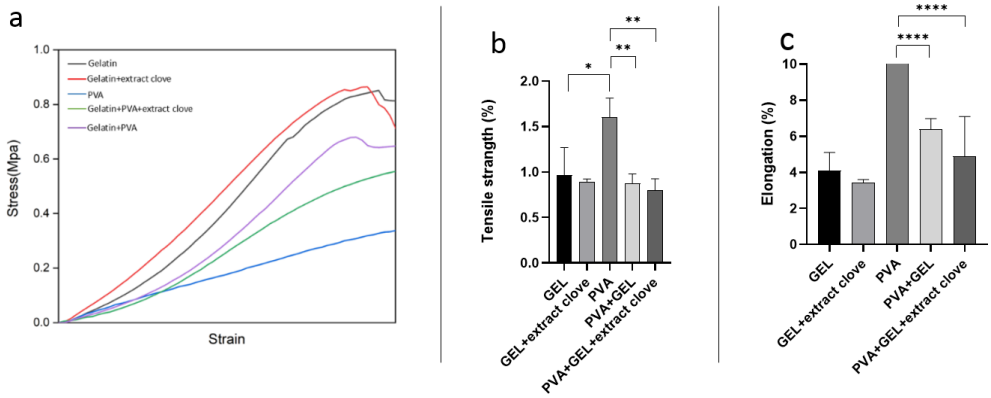

Figure 4. (a): Stress-strain curves (b): tensile strength (%) (c): Elongation (%).

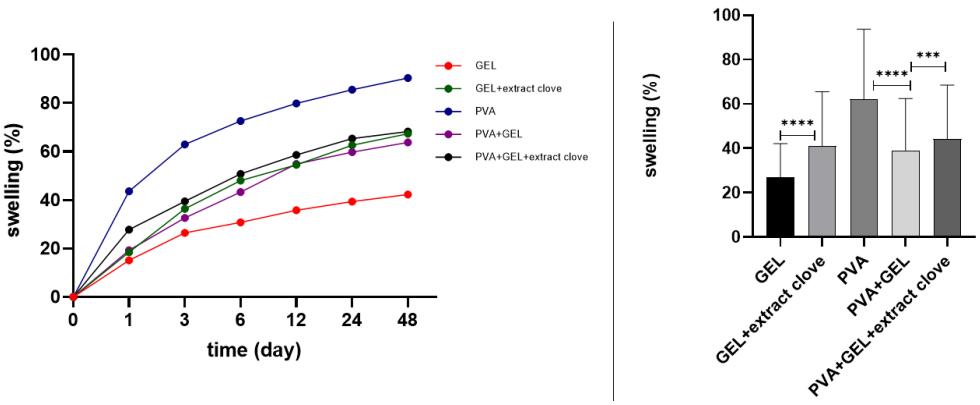

Figure 5. Water absorption evaluation.

intensity of exudate and the duration required for treatment, and the choice of dressing should be based on clinical needs and the specific conditions of the wound. In one study, the average swelling percentage of nanofibers was reported as 86.88%, indicating a high-water absorption capacity of these nanofibers. This property is vital for absorbing wound exudate and maintaining dryness of the wound surface. Additionally, contact angle tests indicated high hydrophilicity of the nanofiber surfaces, which can enhance cell adhesion and facilitate the wound healing process (Amer et al., 2022; Soleiman-Dehkordi et al., 2024).

In vitro degradation test

The percentage degradation of various polymeric wound dressings over time (0 to 72 hours) is shown in the figure. According to the results, degradation of all wound dressings during the study period is evident, but the rate and extent of degradation at the end vary widely among the different samples. The gelatin (GEL) wound dressing alone exhibits the fastest degradation rate, reaching over 50% by hour 72. This indicates fairly rapid biodegradability of gelatin under the studied conditions. The PVA wound dressing also shows a high degradation rate, with approximately 45% degradation by hour 72. Its degradation profile in the initial phase (up to about hour 12) is similar to gelatin, but afterward it degrades slightly slower than gelatin. The degradation rate of the GEL dressing with clove extract is slower than pure gelatin and even PVA in the early stages (up to hour 24), degrading approximately 38% by hour 72. This suggests that addition of clove extract to the gelatin scaffold significantly reduces the degradation rate, possibly due to a protective effect or polymer structure modification. The composite PVA + GEL wound dressing has a moderate degradation profile. Its degradation up to hour 24 is equivalent to that of pure gelatin and PVA but gradually reduces to about 42% by hour 72. This indicates a modulating effect of the two polymers on the overall degradation rate. Finally, the PVA + GEL + clove extract wound dressing shows a degradation pattern quite similar to PVA + GEL but with slightly less degradation towards the later period (hour 72), reaching about 45%. This implies that clove extract still retains its retardant effect on degradation even in the presence of PVA, although this effect is weaker compared to pure gelatin (Fig. 6). These results indicate that degradation of polymeric scaffolds depends on the polymer type used and the presence or absence of clove extract. Gelatin and PVA alone degrade at higher rates, and addition of clove extract to gelatin clearly slows degradation. This variation in degradation rates is particularly important in various applications, especially tissue engineering, where precise control over dressing degradation timing is required to match the tissue regeneration process. Controlling the amount and rate of dressing degradation helps clinicians optimize dressing change intervals and prevent accumulation of saturated

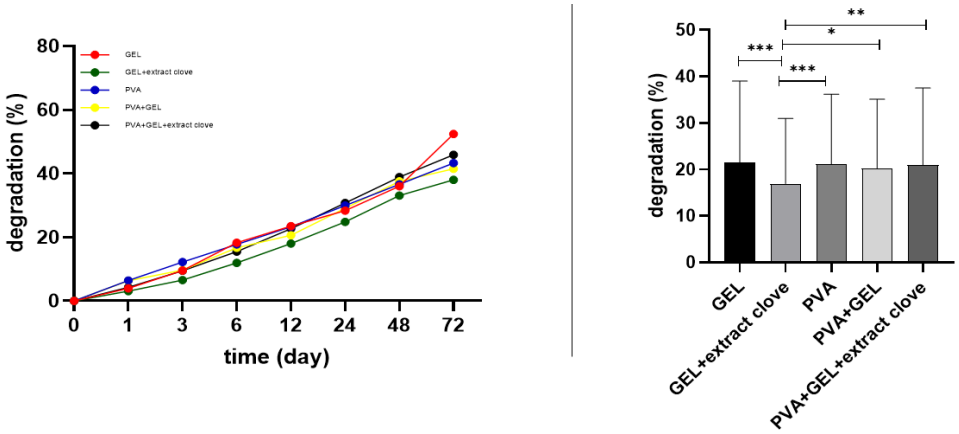

Figure 6. Assessment of degradation in PBS.

exudates that can lead to infection. Moreover, controlled degradation synchronized with tissue regeneration is critical in tissue engineering to ensure the dressing degrades in harmony with tissue healing (Borges-Vilches et al., 2022; Maleki et al., 2024).

Antibacterial activity of electrospun nanofibers

The incorporation of antibacterial systems has a positive impact on the tissue regeneration process, as it effectively inhibits the growth and activity of pathogenic microorganisms. The results demonstrate the comparative antibacterial efficacy of the PVA + Gel nanofibrous mat with clove extract and without it against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The presence of an inhibitory zone around the nanofiber samples indicates the antimicrobial properties of the nanofibrous mat. For the negative control, nanofibrous mats without clove extract were used for comparison (Fig. 7 (a,b)). Based on the results and the evaluation of MIC and MBC tests, the methanolic clove extract exhibits higher efficacy against Gram-positive bacteria. The inhibitory halo around the nanofibers is clearly observable. The average antibacterial activity of the nanofibrous mat with clove extract against Gram-positive bacteria is approximately 1/96 cm, while against Gram-negative bacteria it is around 1/56 cm. This difference highlights the positive effect of using methanolic clove extract. The low standard deviation of both groups (approximately ± 0.1 cm) reflects the reproducibility and precision of the experiment. In a study the clove essential oil-containing nanofibers (with an average diameter of 154 ± 35 nm), demonstrated significant antibacterial, anti-inflammatory, and antioxidant activities. The encapsulation efficiency was 87.6%, with 79% release observed in an acidic environment. Nanofibers with clove essential oil exhibited larger and more effective inhibition zones against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* compared to blank nanofibers (e.g., $36.2 \pm 3.5\%$ vs. $23.55 \pm 5.27\%$ for *Staphylococcus aureus*). These results indicate a high potential of these

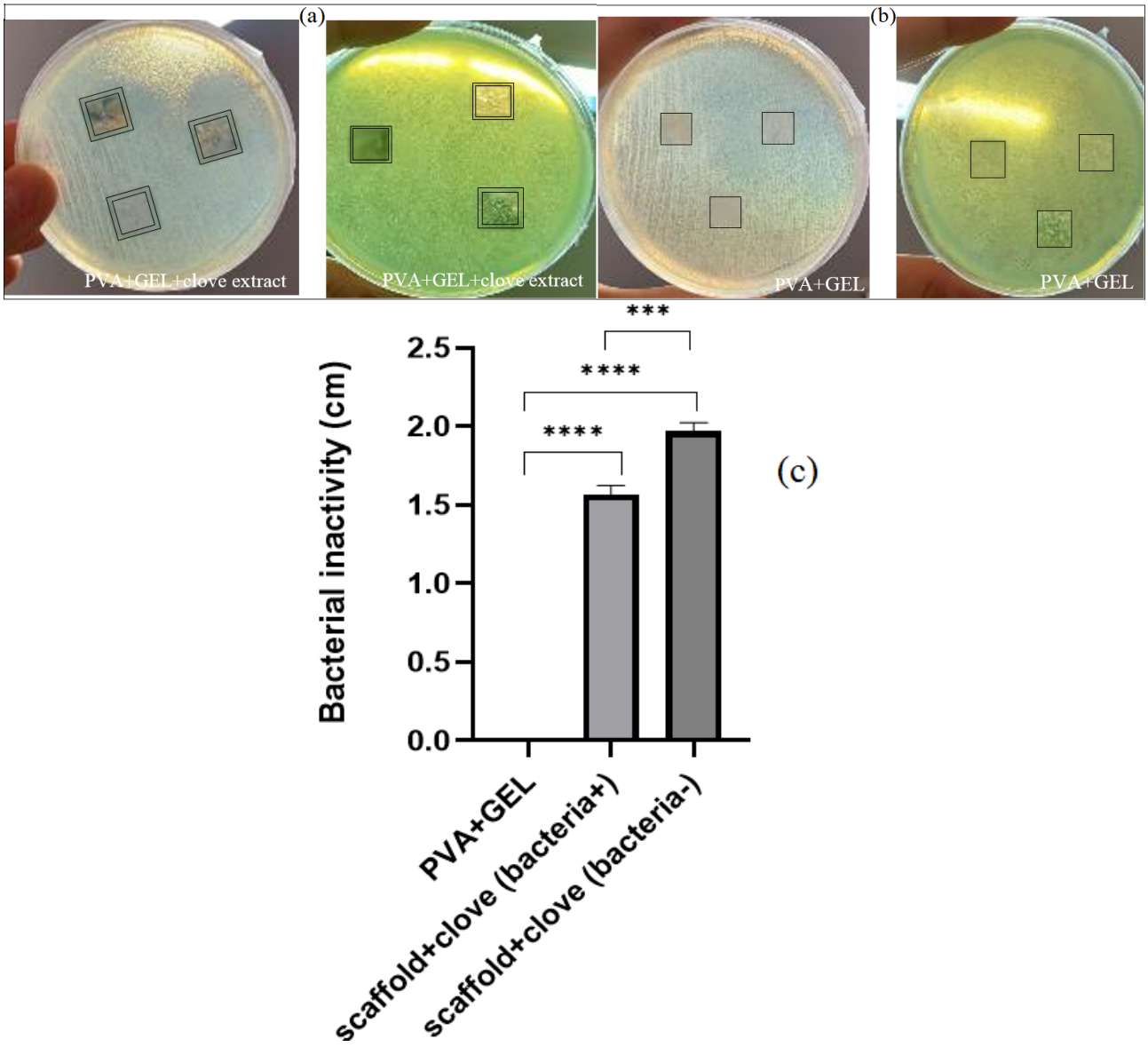

Figure 7. (a) Inhibition of *Staphylococcus aureus* and *Pseudomonas aeruginosa* growth by nanofibrous mat with clove extract. (b) Nanofibrous mats without clove extract. (c) Statistical analysis of the antibacterial properties of polymeric nanofibers PVA + GEL and PVA + GEL + clove extract.

nanofibers to enhance wound healing by reducing inflammation and providing antioxidant protection (Fig. 7 c) (Mouro and Gouveia, 2024; Hameed et al., 2021).

In vitro cell viability assay

In the first 24 hours, the decrease in cell viability percentage is due to changes in the environmental conditions and physical location of the cells, which leads to cellular stress and their initial adaptation to the new culture environment. This process involves changes in cell attachment to the substrate surface, responses to new physical and chemical conditions, and metabolic activity adjustments. It is temporary and considered a normal response in cell culture experiments. At 48 hours, cell viability increased in all groups. The highest cell viability was observed in the PVA + GEL + clove extract group at 93.9%. The clove extract and PVA + GEL groups also showed increased viability at 90.9% and 88.7%, respectively. This indicates greater cellular adaptation to the samples over time and recovery from the initial cytotoxic effects. At 72 hours, the increasing trend in cell viability continued. The PVA + GEL + clove extract group again showed the highest viability at 98.2%. The clove extract and PVA + GEL groups exhibited 93.8% and 90.9% viability, respectively. These findings confirm that clove extract combined with PVA and gelatin (PVA + GEL + clove extract) had the highest cell compatibility among the groups (Fig. 8). This trend generally indicates excellent biocompatibility and even potential positive effects of the clove extract when incorporated into the polymer matrix. Results of the MTT assay demonstrate that adding clove extract to the PVA + GEL formulation significantly improves cell biocompatibility, and this blend has the minimum cytotoxic effect on cells in the long term.

Evaluation of cell adhesion and spreading in the presence of clove methanolic extract

Surface wettability is a key factor in cell adhesion because it determines how well the surface can attract water and biological molecules. Surfaces with moderate to low wettability (relatively hydrophilic) tend to adsorb proteins related to cell adhesion, such as adhesion factors and extracellular matrix molecules, more effectively. This protein adsorption enhances the interaction between the scaffold surface and the cells, resulting in improved cell adhesion, spreading, and growth on the scaffold. On the other hand, if the surface is too hydrophobic or highly hydrophilic, protein adsorption and cell interactions decrease, leading to weaker cell adhesion. Therefore, modifying the scaffold's wettability by adding compounds like clove methanolic extract can create more optimal surface properties that enable better protein adsorption and consequently increase cell adhesion. In summary, changes in scaffold wettability facilitate enhanced cell interactions and are critically important for success in tissue engineering and wound healing applications. In the three upper images, i.e., the scaffolds without clove methanolic extract, cells appear scattered with weak attachment to the scaffold surface. The cells are not well spread, and cell-material as well as cell-cell interactions do not appear strong. The scaffold surface in these images looks relatively smooth and lacks dense cell layers. On the other hand, the three lower images, belonging to scaffolds treated with clove methanolic extract, show a remarkable density of cells. The cells have grown well on the scaffold surface and have formed stronger connections with both the scaffold and neighboring cells (Fig. 9). The presence of dense interconnected cell layers indicates improved cell adhesion in the presence of the clove methanolic extract. These findings demonstrate that clove methanolic extract contains compounds that enhance cell adhesion and spreading on the scaffold. This may be due to modifications in the scaffold's surface properties, upregulation of cell adhesion molecule expression, or other effects mediated by the extract's components. Such improvement in cell adhesion is very important in wound healing and tissue engineering, where optimal cell attachment to the scaffold is a major factor for new tissue formation and success in cell transplantation.

PCR test analysis

The mRNA expression of the NRF2 gene was measured in three groups: clove extract (TCP), the PVA + GEL group,

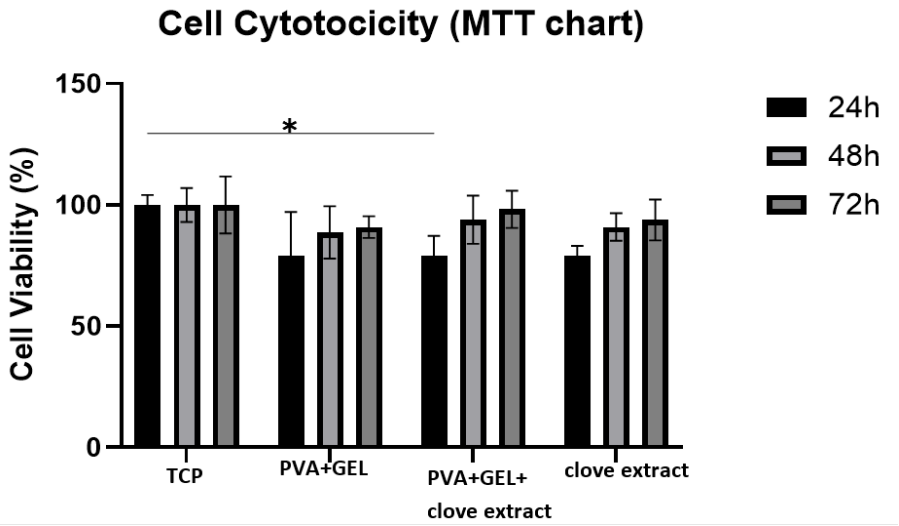

Figure 8. Analysis and comparison of scaffolds cell viability.

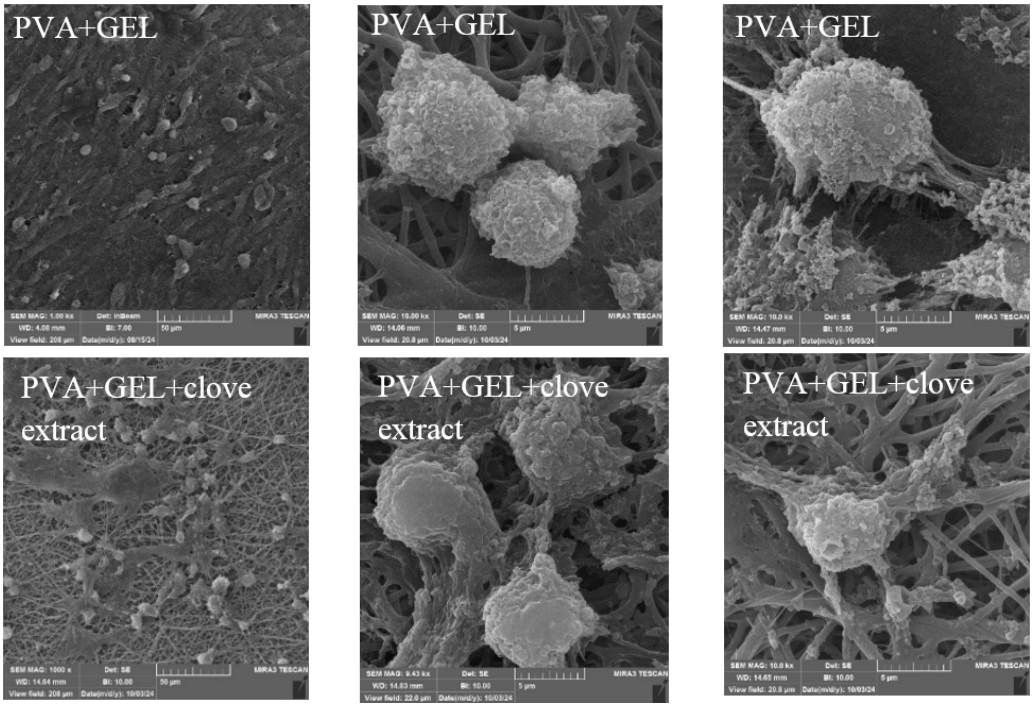

Figure 9. Microscopic imaging of cell adhesion at magnifications of 5 µm and 50 µm.

and the PVA + GEL + clove extract group. The results are expressed as $2^{\wedge} - \Delta CT$ values. In the control group (TCP), the $2^{\wedge} - \Delta CT$ value was 0.000022, representing the basal expression of the NRF2 gene. In the PVA + GEL group, this value increased to 0.00177, indicating a significant upregulation of gene expression compared to the control. The highest $2^{\wedge} - \Delta CT$ value was observed in the PVA + GEL+ clove extract group, with a value of 0.0103, reflecting a remarkable increase in NRF2 gene expression relative to the other groups. These findings suggest that PVA+GEL alone can induce NRF2 expression, and the addition of clove extract further enhances this activation. Therefore, the application of PVA + GEL, especially combined with clove extract, can serve as a potent activator of the NRF2 antioxidant pathway and holds great potential for biological and therapeutic applications (Fig. 10). According to previous research, Nrf2 is a key transcription factor that plays a crucial role in regulating the cellular response to oxidative stress and maintaining redox homeostasis. Its activation in injured tissues reduces oxidative stress by increasing the expression of protective cellular genes and antioxidant enzymes, while promoting cell proliferation and migration and inhibiting apoptosis. Nrf2 also contributes to angiogenesis by upregulating important growth factors such as VEGF, which are vital for neovascularization and wound healing. In chronic diseases such as diabetes, reduced Nrf2 activity leads to delayed wound healing, chronic inflammation, and impaired angiogenesis. Targeted activation of the Nrf2 pathway using natural and pharmaceutical compounds like sulforaphane, curcumin, and resveratrol can accelerate the healing of chronic wounds. However, prolonged and excessive activation of this pathway may result in adverse effects, including promotion of protumorigenic processes. Therefore, precise and controlled regulation of Nrf2 is essential for the development of safe and effective therapies (Udenni Gunathilake et al., 2017; Hiebert and Werner, 2019; Süntar et al., 2021; Wen et al., 2023).

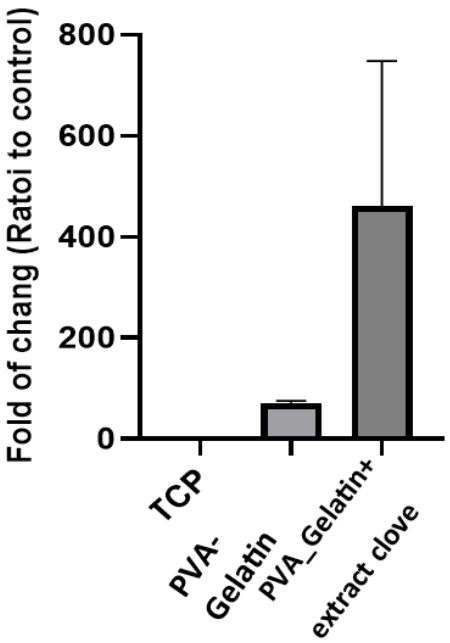

Figure 10. Evaluation of increased expression of the Nrf2 gene.

In vivo evaluation of methanolic clove extract

In the evaluation of the macroscopic results of *Staphylococcus aureus* infected wounds, the following results were obtained: On days 3, 7, 10, and 14, the healing rate in any of the groups was not statistically significant compared to the negative control group (Salami et al., 2021a). On day

17, the most statistically significant results were obtained. On this day, the PVA + Gel + Drug group showed a much faster healing rate ($P > 0.01$) compared to the negative control group. This comprised the most substantial result in this experiment. On day 21, there was a significant difference ($P > 0.05$) in the healing rate of PVA + Gel + Drug compared to the negative control group (Fig. 11 (d,a)). In the evaluation of the macroscopic results of Pseudomonas aeruginosa infected wounds, the following results were obtained: On days 3, 7, 10, 14, and 17 the healing rate in any of the groups was not statistically significant compared to the negative control group. On day 21, there was a significant difference ($P > 0.05$) in the healing rate of PVA + Gel + Drug compared to the negative control group (Fig. 12 (d,a)). Histopathological evaluation of the study groups infected with *Staphylococcus aureus* bacteria showed that the lesions in the Negative control group had granulation tissue formation and numerous inflammatory cell infiltration within 14

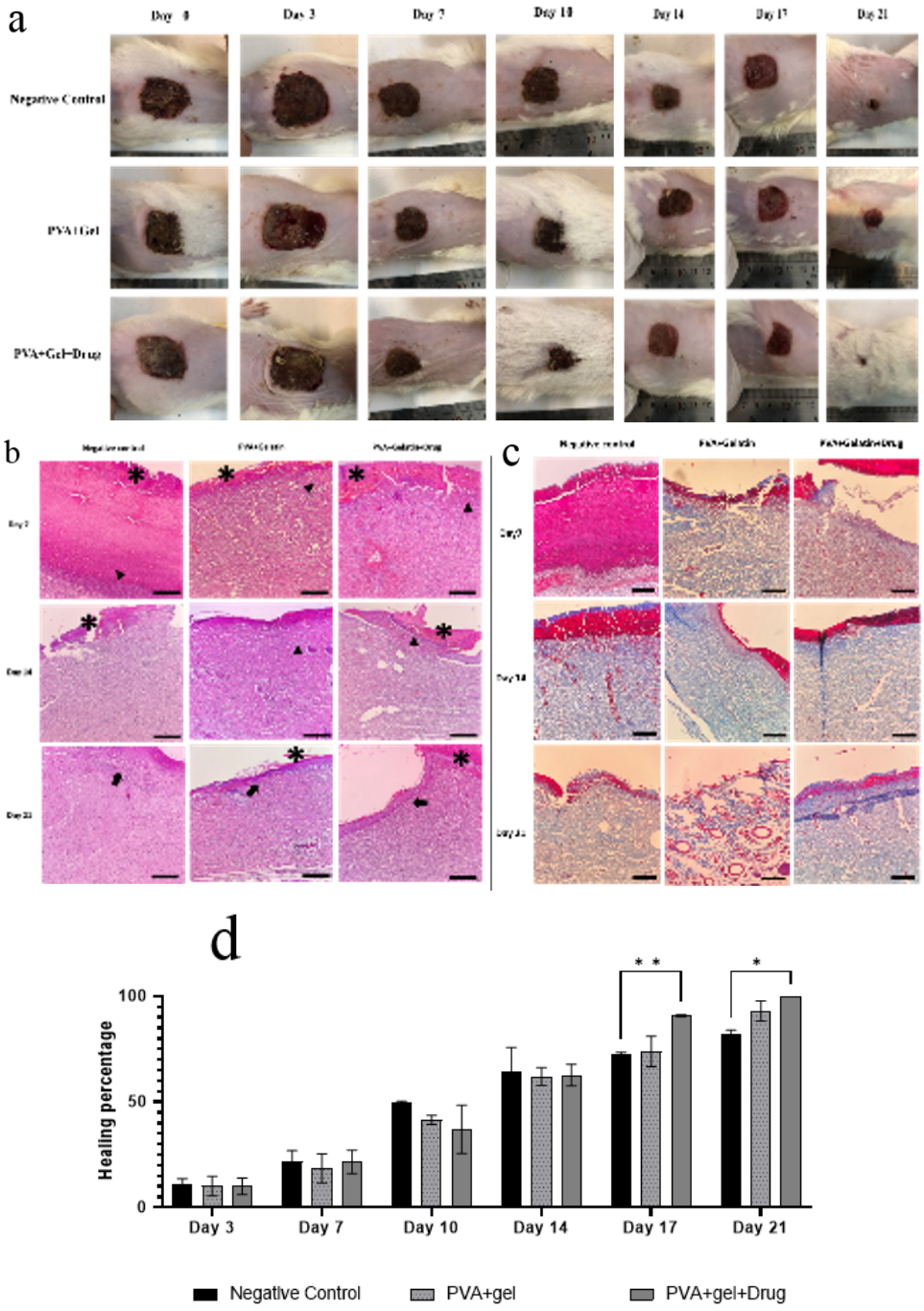

Figure 11. (a) Photographs of the wound surface infected with *Staphylococcus aureus* bacteria during 21 days of treatment in the treated groups. (b) H&E-stained histopathological sections of the healing wounds infected with *Staphylococcus aureus* at 7, 14 and 21 Days (Crusty scab is indicated by asterisks, re-epithelialization shown with arrows, triangles to indicate infiltration of inflammatory cells. The scale bar shows 200 microns). (c) Masson's trichrome stained histopathological sections of the healing wounds infected with *Staphylococcus aureus* at 7, 14 and 21 Days. The scale bar shows 200 microns). (d) Comparison of the percentage of healing of *Staphylococcus aureus* infected wounds and their significance compared to the negative control group during 21 days of treatment.

days. Furthermore, re-epithelialization did not begin until 21 days after injury. Histopathological evaluation of the negative control group at 21 days after the end of treatment showed that polymorphonuclear inflammatory cells (PMNs) had infiltrated and the epidermal layer had not yet formed. Micrographs of sections in the PVA + Gel group showed the wound surfaces at 7 and 14 days after treatment. At the early stage of the healing processes (day 7), the wounds in this group clearly showed inflammatory cell infiltration and granulation tissue formation. The inflammatory cells and granulation tissue did not disappear after 14 days. The re-epithelialization process was almost not completed at day 21 after treatment. PVA + Gel + Drug on days 7 and 14 showed no significant changes in terms of reduction of inflammatory cells and formation of epithelium layer compared to the negative control group. 21 days after treatment showed that the epithelialization process was being completed and inflammatory cells were reduced compared to the negative control group (Fig. 11 (b,c)). Histopathological evaluation of the study groups infected with Pseudomonas bacteria indicated that in the negative control group, granulation tissue formation and infiltration of numerous inflam-

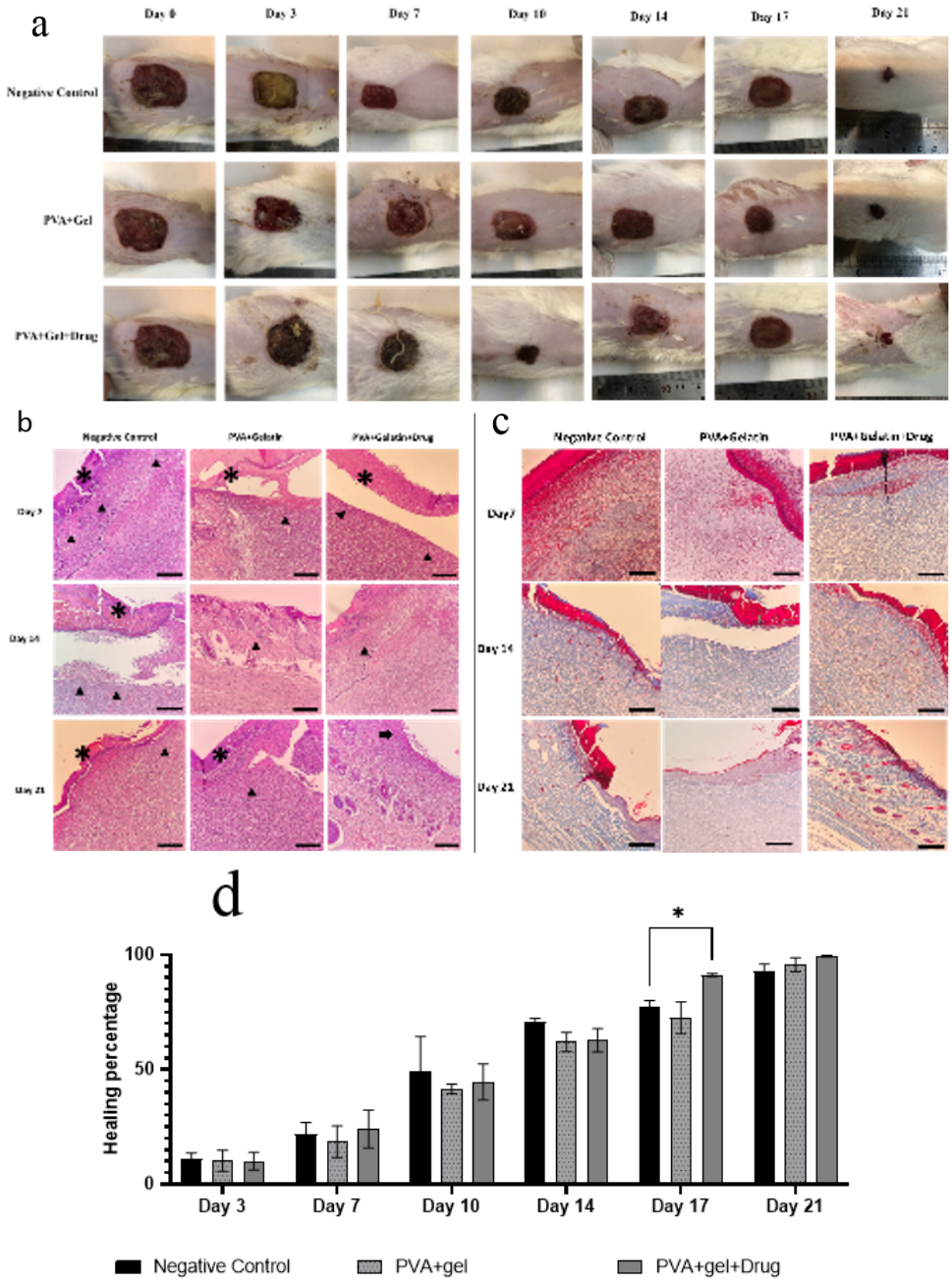

Figure 12. (a) Photographs of the wound surface infected with *Pseudomonas aeruginosa* bacteria during 21 days of treatment in the treated groups. (b) H&E-stained histopathological sections of the healing wounds infected with *Pseudomonas aeruginosa* at 7, 14 and 21 Days (Crusty scab is indicated by asterisks, re-epithelialization shown with arrows, triangles to indicate infiltration of inflammatory cells. The scale bar shows 200 microns). (c) Masson's trichrome stain histopathological sections of the healing wounds infected with *Pseudomonas aeruginosa* at 7, 14 and 21 Days. The scale bar shows 200 microns). (d) Comparison of the percentage of healing of *Pseudomonas aeruginosa* infected wounds and their significance compared to the negative control group during 21 days of treatment.

matory cells were observed on days 7 and 14. Evaluation on days 21 after the end of treatment showed that inflammatory polymorphonuclear cells (PMNs) had infiltrated and the epidermal layer had not yet formed. Sections in the PVA + Gel group, in the early stage of the healing process (day 7), the wounds of this group clearly showed inflammatory cell infiltration and granulation tissue formation. Inflammatory cells and granulation tissue did not disappear after 14 days. The re-epithelialization process was almost complete on day 21 after treatment. PVA + Gel + Drug showed no significant changes in terms of reduction of inflammatory cells and formation of the epithelium layer compared to the negative control group on days 7 and 14. Also, 21 days after treatment, it was shown that the epithelialization process was not completed and inflammatory cells were reduced compared to the negative control group, but their presence in the tissue was still evident (Fig. 12 (b,c)). In this study, the degradation of polymeric dressings was carefully evaluated over 72 hours, aligning with the dressing change schedule in the animal study, which was conducted every 3 days. This temporal alignment is crucial because controlled and appropriate degradation of the dressing within the three-day period can prevent the excessive accumulation of wound exudates and maintain optimal conditions for healing. Furthermore, histopathological assessments performed on days 7, 14, and 21 demonstrated that controlling the degradation rate of the dressings led to a significant reduction in inflammation and acceleration of the re-epithelialization process. This synchronization between the degradation rate of the dressing and the tissue healing process underlines the necessity of regular dressing changes every 3 days based on degradation timing to ensure treatment efficacy. Therefore, the obtained data reveal a direct relationship between dressing durability and degradation with biological responses in the animal model. This emphasizes the importance of designing dressings with controlled degradation in clinical applications and suggests that optimizing dressing change intervals based on precise knowledge of material degradation can create an environment conducive to tissue regeneration and burn wound healing. This approach represents an effective step toward developing smart dressings and optimizing clinical treatments for burns (Table 1) (Almasian et al., 2021; Du et al., 2024; Du et al., 2023; Fahimirad et al., 2023; Li et al., 2022; Salami et al., 2021b).

4. Conclusion

The findings of this study clearly demonstrated that the addition of clove methanolic extract to composite nanofibrous dressings significantly improved their key properties. The extract is naturally rich in bioactive compounds that can substantially enhance the wound healing process. Medicinal plants possess anti-inflammatory, antimicrobial, and antioxidant properties that help reduce inflammation, prevent infection, and stimulate tissue regeneration. These effective, safe, and cost-effective natural treatments serve as valuable complements to modern medical therapies in wound repair. Statistical data showed a significant increase in antibacterial activity, especially against Gram-positive bacteria such as *Staphylococcus aureus*, indicating a strong antimicrobial effect critical for infection control and providing a favorable environment for tissue regeneration. Furthermore, *in vivo* evaluations in an animal model demonstrated that these dressings significantly accelerated the healing of burn wounds compared to the control group. The study showed

Table 1. Summary of histological parameters in wound healing.

Re-epithelialization (Epithelial layer formation)	Granulation Tissue Formation	Inflammation (Number of inflammatory cells)	Day / Group
Not started	Prominent	High (extensive infiltration)	Day 7 Negative Control
Not started	Prominent	High (clear infiltration)	Day 7 PVA+Gel
Not started	Prominent	High (similar to control)	Day7 PVA+Gel+Drug
Low (delayed regeneration)	Granulation tissue maintained	Persistently high inflammation	Day 14 Negative Control
Low	Granulation tissue maintained	High inflammation	Day 14 PVA+Gel
Low	Granulation tissue maintained	High inflammation	Day14 PVA+Gel+Drug
Incomplete (epithelial layer not formed)	Granulation tissue persists	Slightly reduced inflammation	Day 21 Negative Control
Nearly complete	Granulation tissue reduced	Reduced inflammation	Day 21 PVA+Gel
Complete	Significantly reduced	Further reduced inflammation	Day21 PVA+Gel+Drug

that clove methanolic extract significantly improved antibacterial activity and wound healing, especially against Gram-positive bacteria. However, its effect on Gram-negative bacteria was weaker. Therefore, using other natural extracts with strong activity against Gram-negative bacteria in future work could enhance the overall antimicrobial efficiency of wound dressings and better manage resistant infections. Beyond this study, previous research highlights that treating diabetic foot ulcers (DFU) is highly challenging due to infections with drug-resistant bacteria. In this study, clove flower extract effectively inhibited the growth of the resistant bacterium Proteus mirabilis. Moreover, topical application of a hydrogel containing this extract in diabetic mice resulted in improved wound size, enhanced expression of genes related to wound healing and growth factors, reduced inflammation, and regulated apoptosis processes. Additionally, the oxidative state of the wound improved, and the microbial load decreased. Considering these results, clove flower extract could be a suitable alternative to antibiotics in diabetic foot ulcer treatment (Ali et al., 2022). Mechanical analyses confirmed that adding the synthetic polymer polyvinyl alcohol (PVA) increased the mechanical strength and tensile resistance of the dressings, contributing to better stability in the wound environment. Water absorption and swelling tests also validated the high absorption capacity of the composite dressings, a crucial feature for effectively managing wound exudates and maintaining an optimal moist environment for tissue repair. Another strength of this study is the optimal coordination between the degradation rate of the dressings and the wound healing process, ensuring the dressing degrades in harmony with tissue regeneration. This coordination prevents excessive accumulation of exudates and provides a conducive environment to accelerate wound healing. Overall, the results emphasize the importance of electrospun gelatin/polyvinyl alcohol nanofibrous dressings as an effective therapeutic platform for burn treatment. This study offers an innovative, evidence-based approach in skin tissue engineering, showing that dressings enriched with clove methanolic extract, due to their enhanced antibacterial properties-especially against Gram-positive bacteria-their efficacy in accelerating burn wound healing, and their optimal balance between dressing degradation and tissue repair, represent a highly promising option for treating complex wounds.

Authors contributions
All authors contributed equally to the conception, design, execution, and writing of this work. All authors read and approved the final manuscript.

Availability of data and materials
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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