Review article

Potential of plant secondary metabolite-derived polymers to enhance wound healing

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Abstract

There is a global epidemic of non-healing wounds. Chronic inflammation, overexpression of pro-inflammatory cytokines, oxidative stress and bacterial infection are implicated in delayed wound healing. Natural extracts are a rich source of bioactive molecules called plant secondary metabolites (PSMs) that include terpenes and phenols. These molecules may facilitate wound healing through their antioxidant, anti-inflammatory, and antibacterial activity. After briefly outlining the process of wound healing and how it is compromised in chronic wounds, this review focuses on investigating how PSMs-derived polymers may improve wound healing. Best methods for incorporating PSMs into wound dressings are reviewed and critically compared. The exiting body of literature strongly suggests that PSMs-derived polymers incorporated into wound dressings could have clinical value in aiding wound healing.

Statement of significance

Chronic wounds are developed by the persistence of inflammation, oxidative stress and infection. Chronic wounds affect the worldwide population, by reducing quality of life of patients with significant cost to healthcare systems. To help chronic wounds to heal and overcome this burden, materials with anti-inflammatory, antioxidant and antibacterial properties are required. Plant secondary metabolites (PSMs) are volatile materials that have all these properties. PSMs-derived polymers can be fabricated by the appropriate polymerization techniques. The present review provides an overview of the state-of-the-art of the wound healing mechanisms of PSMs. Current developments in the field of PSMs-derived polymers are reviewed and their potential use as wound dressings is also covered.

1. Introduction

Dressings have been applied to wounds since ancient times and their evolution has been constant [1]. Wound dressings have many functions such as absorbing exudate, retaining moisture, decreasing microbial contamination, and potentially delivering wound healing agents to the wound milieu. A large range of different types of wound dressings has been developed, including films, hydrogels, foams, hydrocolloids, alginates, gauzes, hydro-fibers and tissue-engineered skin, among others [2-5]. Gauzes, for example, are the most common wound dressings used to protect the wound from external contamination and maintain appropriate levels of moisture. Tissue-engineered skin has been proposed to support cell proliferation, migration and differentiation, and to reduce the risk of infection to accelerate wound healing [6].

Chronic wounds reduce the quality of life of patients and represent a burden to healthcare systems worldwide [7]. Chronic wounds affect the quality of life of approximately 2% of the global population [8]. In developed countries like the USA, the total Medicare expenses for all types of wounds range from 3.8% to 13.3% of the total budget [9]. In Wales wounds account for 5.5% of the National Health System (NHS) spending [10]. Developing countries, too, spend ~3% of their total healthcare expenditure on chronic wounds [11]. Therefore, there is an urgent need to develop novel wound dressings that not only promote wound healing but also help reduce healthcare costs associated with delayed wound healing.

The incorporation of plant secondary metabolites (PSMs) in wound dressings is an emerging topic of research that is driven by their wound healing properties. PSMs have been proposed to

inhibit inflammation, promote cell proliferation and wound remodeling [12]. PSMs have been included in wound dressings through topical application or by polymerization techniques, such as plasma polymerization [13], electrospinning [14], solution casting [15], and encapsulation [16]. Nevertheless, the incorporation of PSMs into wound dressings remains a relatively novel approach to promoting wound healing and limiting wound infection that has strong potential to address some of the persisting challenging of wound management.

In this review, we briefly discuss the mechanisms involved in wound healing, biofilm formation, antibiotic resistance and their impact on wound healing. We summarize the properties by which PSMs have been proposed to promote wound healing, with a focus on their antioxidant and anti-inflammatory properties. The review also describes methods by which PSMs have been or may be incorporated in wound dressings.

2. Wound healing

Normal wound healing is usually described by three overlapping stages, namely hemostasis/inflammation, proliferation, and remodeling/maturation. These stages are regulated by a cascade of different factors and mediators [17, 18]. In the first stage, hemostasis is triggered by constriction of blood vessels to stop blood loss. Platelets aggregate to form a fibrin network or clot. Different types of cells such as neutrophils, monocytes, keratinocytes and fibroblasts migrate to the wound site. Neutrophils kill bacteria and degrade damaged cells. Monocytes transform into macrophages and engulf debris, bacteria and remaining neutrophils. Macrophages stimulate fibroblasts to form granulation tissue. They also stimulate endothelial cells to promote re-epithelialization and angiogenesis. In the proliferation/reconstruction phase, fibroblasts continue to form granulation tissue over the wound site and transform into myofibroblasts to promote contraction of the wound edges. The last stage, remodeling and maturation start with apoptosis and the removal of dead cells. Collagen deposition starts to build up leading to contraction of the wound edges until the scar and epidermis mature and the wound finally heals (Fig. 1 A)) [19].

Wound healing is an overlapping process of various stages, during the first stage, hemostasis and inflammation occur, activating vasoconstriction, aggregating platelets, promoting pro-inflammatory signals and free radicals. In the second stage of proliferation, cells start to proliferate promoting angiogenesis and the formation of collagen. In the last stage, remodeling/maturation, the epidermis, and scar matures and wound contracts. (Fig. 1 B)) A comparison between cell composition within the normal and impaired wounds. In acute wounds,

the wound healing process occurs without any interruption, whereas in impaired wounds, the wound healing process is delayed by different factors such as persistent inflammation, oxidation, and/or infection.

If any of the above elements fail, the wound can become chronic. A chronic wound is essentially a non-healing wound. Chronic inflammation, oxidative stress, bacterial infection and a deficit of cell proliferation have been implicated in impaired wound healing (Fig. 1 B)) [20]. Chronic inflammation contributes to the formation of excess reactive oxygen species (ROS), causing further molecular damage and tissue injury [21]. ROS promote inflammation and the formation of free radicals (e.g., nitride oxide (NO), superoxide (SO), anion (O_2*-), hydrogen peroxide (H_2O_2) and hydroxyl (OH*)) and the release of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, tumor necrosis (TNF)-α and interferon (IFN)-γ. Antioxidant and free radical scavengers attenuate inflammation by downregulating pro-inflammatory cytokines through the inhibition of ROS [22].

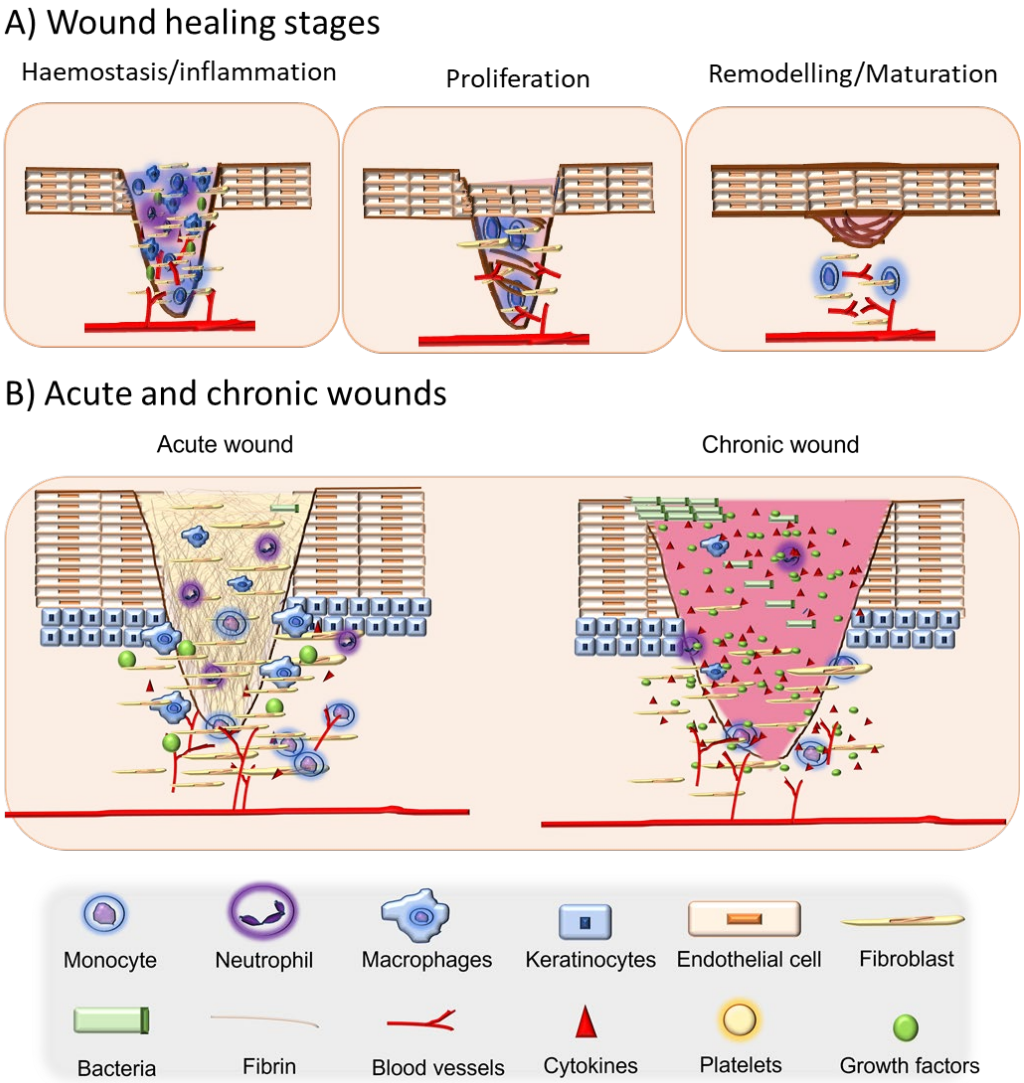

Fig. 1. A) Wound healing is an overlapping process of various stages, during the first stage, haemostasis and inflammation occur, activating vasoconstriction, aggregating platelets, promoting pro-inflammatory signals and free radicals. In the second stage of proliferation, cells start to proliferate promoting angiogenesis and the formation of collagen. In the last stage, remodeling/maturation, the epidermis, and scar matures and wound contracts. B) A comparison between cell composition within the normal and impaired wounds. In acute wounds, the wound healing process occurs without any interruption, whereas in impaired wounds, the wound healing process is delayed by different factors such as persistent inflammation, oxidation, and/or infection.

Wound infection occurs when bacteria, viruses or other microorganisms evade the immune system of the host and colonize the wound, leading to pain, wound contamination, slow re-epithelization and bad odor [21]. An infected wound compromises the general health of the patient. The overgrowth of bacteria within the wound may evolve into a structure community, *i.e.* the biofilm, which is held together by extracellular polymeric substances and is attached to a wound bed [23].

3. Antibiotic resistance and biofilms

Even though the discovery of antibiotics has changed medicine and immeasurable human lives have been saved, the effectiveness of antibiotics has been diminished due to bacterial survival ability. Bacteria can ensure its survival through a variety of genetic mutations and its ability to form highly resistant communities namely biofilms.

Antibiotic resistance (AR) and biofilms play a major role in chronic wounds, approximately 60% of chronic wounds are associated with AR and biofilms. In the United States alone, AR affects nearly 3 million people and more than 35,000 people die as a consequence of developing antibiotic resistant infections [24]. AR can be developed by the repeatedly administration of antibiotics whereas biofilm infection is more likely to be developed in diabetic patients due to metabolic dysfunction, dysregulation of inflammatory and immune responses. Therefore, to overcome AR and biofilms-infected wounds is important to know what they are and how to treat them.

AR arise when a bacterium evolves through chromosomal mutation, or by the acquisition of mobile genetic element (free-floating RNA). AR bacteria also eliminate antibiotics through free efflux pumps. Additionally, plasmid and bacteriophages transfer genes to the bacterial

population through horizontal gene transfer (HGT). HGT has two main mechanisms, conjugation and transduction. In conjugation, AR genes are transferred allowing bacteria to evolve resistance whereas during transduction, bacteriophages transfer AR genes to bacteria (Fig. 2, A)) [25]. Other mechanism of bacterial survival is the development of biofilms. Biofilms are microbial congregations generally formed by single or multispecies of microorganisms including bacteria, fungi and protists enclosed within a matrix of extracellular polymeric substances (EPS) adherent to an abiotic or biotic surface [26, 27]. EPS has a major role in biofilm formation, develop and survival. EPS protects microorganisms by providing a barrier against different factors, such as hostile environments, antibiotic resistance and host's immune response. It sustains intracellular interactions through a process called quorum sensing (QS). QS benefits microorganisms within the biofilm by enhancing access to nutrients and increasing resistance to antibiotics. Biofilm formation is often described in four steps, namely surface attachment, microcolony formation, biofilm maturation, dispersal and detachment [27]. In *Caulobacter crescentus*, a model microorganism widely used to study biofilm formation, mechano-sensing of the surface is induced by its flagellar motor that acts as a sensor for stimulating polysaccharide adhesin and surface adherence [28, 29]. Bacterial cells aggregate and proliferate in the wound. Subsequently, biofilms mature with ongoing bacterial proliferation (Fig. 2, B)) [30], when biofilms enter to this stage it became more difficult to eradicate and the potential to develop a chronic wound increase.

Many strategies have been implemented to fight biofilms, from aggressive and intensive antibiotic administration to cold plasmas, ultrasounds, electromagnetic currents and photothermal techniques. However, the administration of antibiotics is generally ineffective producing antibiotic-resistance (AR) and the mentioned techniques are difficult to implement *in vivo* and require expensive equipment [31]. There is wide evidence on the use of PSMs against AR bacteria and biofilms [32]. The mechanisms of how PSMs can contribute to wound healing including antibacterial mechanisms are discussed in the following sections.

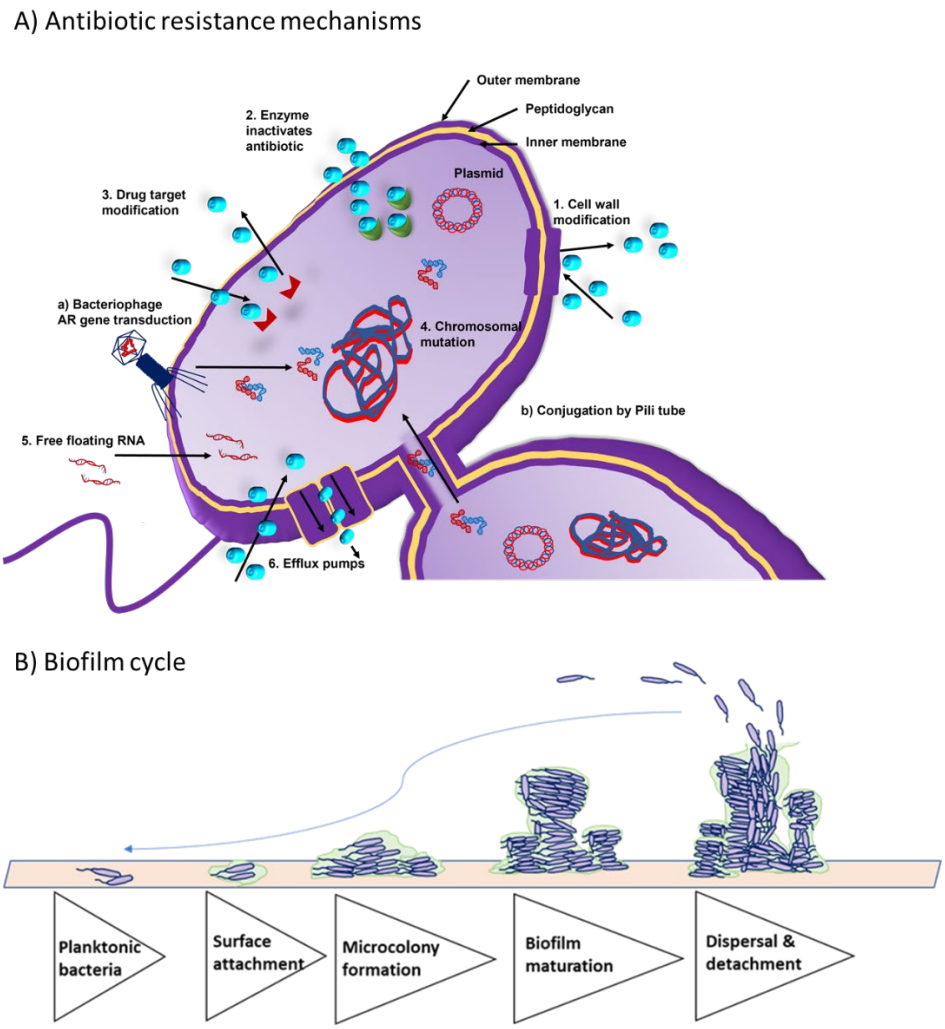

Fig. 2. A) Representation of antibiotic resistance mechanisms in a gram-negative bacterium: 1. Cell wall modification, 2. Antibiotic inactivation by enzymes, 3. Drug target modification, 4. Chromosomal mutation by a) Bacteriophage AR gene transduction and b) conjugation by pilus tube, 5. Free-floating RNA and 6. Efflux pumps. B) Biofilm cycle: Planktonic bacteria move freely around the surface, then bacteria attach to the surface, and a micro colony formation of biofilm starts, biofilm grows and maturates d), then bacteria detach and disperse along the surface.

4. Mechanisms by which PSMs may promote wound healing

PSMs, such as those found in essential oils and herb extracts, are bioactive molecules with strong wound healing potential. Terpenes and phenols in particular display biological activity that is highly relevant to wound healing [33], namely anti-oxidant, anti-bacterial, anti-fungal and anti-inflammatory properties [33-39].

4.1 Antioxidant and anti-inflammatory effects of PSMs

Antioxidants act as physical barriers that prevent ROS generation or ROS access to important biological sites. The inhibition of ROS reduces inflammation by preventing the activation of pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6) and pro-inflammatory pathways (e.g., NF-kb, MAPK), see (Fig. 3 A), B)). The antioxidant and anti-inflammatory properties of PSMs are listed in **Table 1**.

In a study by Cheng *et al.* [40], the monocyte/macrophage-like cells (RAW264.7 cells) were pre-treated with oregano essential oil (OEO) for 12h and subsequently incubated with lipopolysaccharide (LPS) for 12h. It was shown that LPS increased mRNA levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 (Fig. 3 C)), whereas OEO inhibited the mRNA expression of the pro-inflammatory cytokines. Furthermore, OEO-treated cells showed decreased production of inflammatory mediators in the AKT, MAPKs and NF-κβ pathways. Additionally, oxidative stress activator Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 was inhibited in OEO treated cells [41]. In other studies, essential oils from *Origanum vulgare* collected at different locations of Tunisia were reported to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. These effects were correlated with the percentage of phenolic compounds present in the oils collected from the different regions [42]. Different extracts of oregano with high content of carvacrol showed inhibition of ROS and NO production in an LPS activated macrophage cell line (RAW 264.7 cells) [43-45]. Additionally, oregano extract promoted downregulation of cyclooxygenases activity (COX-1 and COX-2) and reduced mitochondrial dehydrogenase activity [43-45]. Kivrak *et al.* [46], recently published a detailed report of the antioxidant activity of different types of lavender and lavandin extracts with high content of linalyl acetate and linalool. It was shown that the extracts rich in linalyl acetate and linalool were more effective in scavenging of DPPH, ABTS and β-carotene. This result suggests the potential antioxidant properties of *Lavandula angustifolia* and its extracts.

Table 1. Examples of anti-inflammatory effects of PSMs.

PSMs	Inflammatory agent	Model	Mechanism of action	Reference
Oregano oil Carvacrol Thymol	Fe^{2+}	SH-SY5Y cells	↓ TNF-α[a)], IL-1β[b)], IL-6 ↓MAPK[c)]/JNK[d)]-NF-κβ[e)]	[47]
	LPS[f)]	RAW 264.7 cells	↓ TNF-α, IL-1β, IL-6 ↓ MAPK, NF-κβ, AKT[g)] ↓ NOX2[h)], ROS[i)]	[40]
	LPS	Monocytes THP-Cells	↓ Phosphor- NF-κβ ↓TLR4[j)]	[48]

Lavender oil Linalool	Croton oil	Mice, edema model	↓Edema formation ↓MPO[k)] ↓Nitric oxide	[49]
	LPS, TLR/4, TLR2/4	Human monocytes	↓ IL-1β, IL-6, IL-10	[50]
Tea Tree oil Terpinen-4-ol			↓ NF-κβ, MAPK	

Abbreviations: a) Tumor necrosis factor-alpha, b) interleukin, c) mitogen-activated protein kinase, d) c-Jun N-terminal Kinase, e) nuclear factor kappa light chain enhancer of activated B cells, f) lipopolysaccharide, g) protein kinase b (Akt), h) nicotinamide adenine dinucleotide phosphate oxidase isoform 2, i) reactive oxygen species, j) toll-like receptor, k) myeloperoxidase.

In a study by Huang *et al.* [48], human monocyte (THP) cells were first activated with LPS and subsequently treated with lavender essential oil (LEO). Protein levels of phosphor-NF-κβ and membrane toll-like receptor 4 (TLR4) were increased by LPS stimuli and decreased after treatment with LEO. Linalool and linalyl acetate are the major components of LEO. The authors also reported an increase in the expression of the cytoprotective heat shock protein (HSP)-70 after LEO and LPS treatment, however, there were no changes in HSP70 expressions between the treatment groups. The authors speculated that this result could be associated with the contribution of signaling molecules produced by both treatments. However, this pathway is not well elucidated yet [48]. The influence of tea tree oil (TTO) and terpinen-4-ol was evaluated on human monocytes cell line [50]. Monocytes were treated with LPS, TLR/4, and TLR2/4 activators. Terpinen-4ol showed major activity on the reduction of pro-inflammatory cytokines, specifically on levels of TNF-α, IL-1β, IL-6, and IL-10 by the inhibition of NF-κβ, p38, and ERK MAPK pathways. These pro-inflammatory markers were not inhibited by pure TTO, suggesting the anti-inflammatory potential of terpinen-4ol.

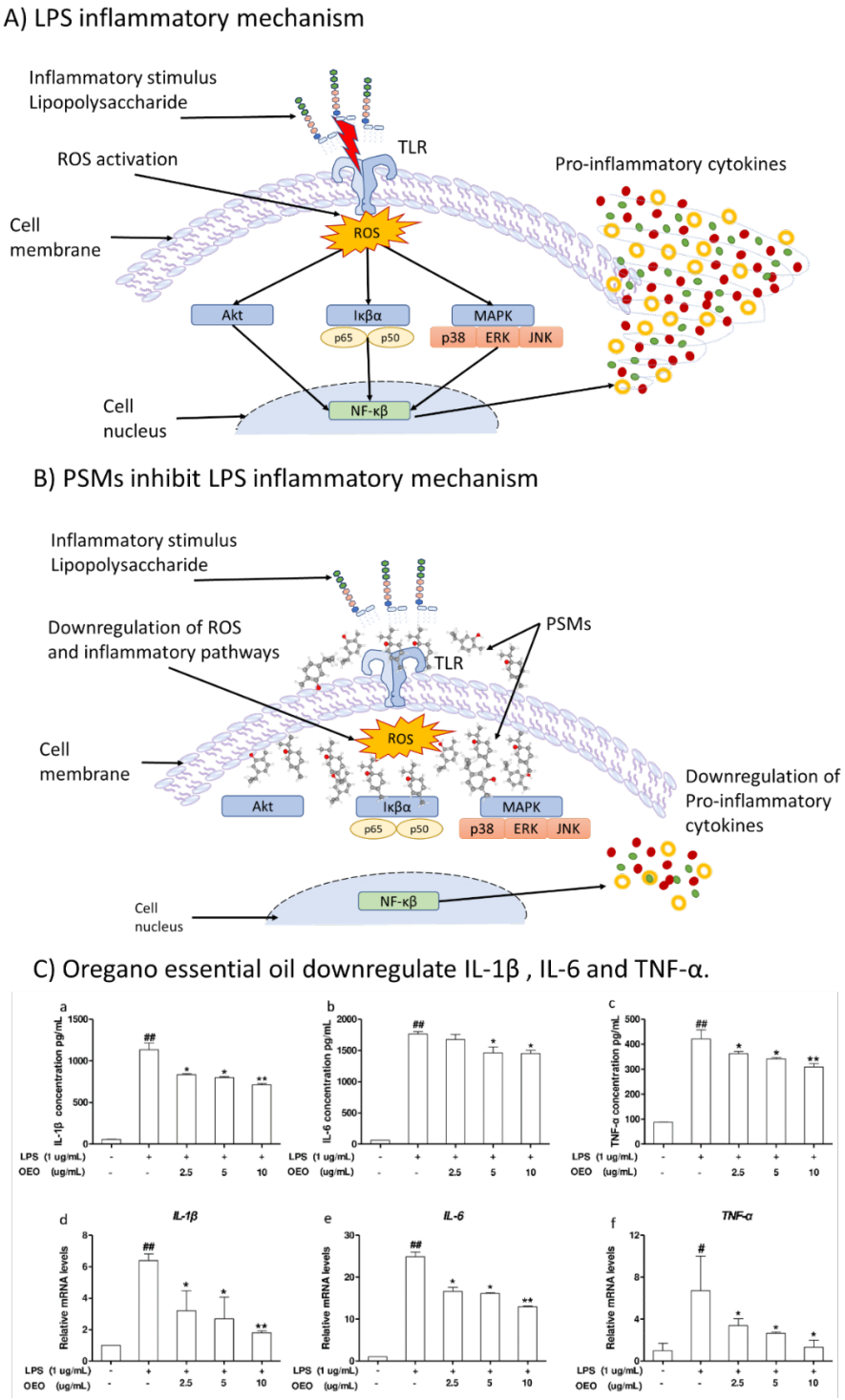

Fig. 3. A) Schematic representation of inflammatory and oxidant mechanisms induced by LPS. B) Anti-inflammatory and antioxidant mechanisms promoted by PSMs. C) Oregano essential oil (OEO) inhibits LPS induced mRNA levels of pro-inflammatory cytokines expression in murine macrophage cells (RAW264.7 cells). (**a**–**c**) Cells were pretreated with OEO for 12 h, then incubated with 1 μg/mL LPS for 12 h. (**d**–**f**) Cells were pretreated with OEO for 12 h, then incubated with 1 μg/mL LPS for 1 h. Reproduced under terms of the CC-BY license [40]. Copywrite 2018. Chuanshang Cheng, Yi Zou, and Jian Peng, published by MDPI.

4.2 Antibacterial effects of PSMs

The antibacterial properties of PSMs are attributed to their wide variety of phenolic monoterpenes [51]. PSMs cause damage to the bacteria cell membrane, interrupt quorum sensing, thus inhibiting biofilm formation. PSMs also inhibit ATP generation, disturb ion transport and kill bacteria. Studies assessing the antibacterial properties of PSMs include comparisons of the effects of different types of PSMs against different bacteria strains (Table 2). PSMs have also been reported to have antifungal properties against *Candida albicans*, *Candida tropicalis* and *Candida parapsilosis* [52-58]. PSMs have been reported to inhibit proliferation of multidrug resistance pathogens [59, 60]. Monoterpenes derived from oregano, tea tree, and clove oils (carvacrol and thymol, terpinen-4-ol, and eugenol, respectively) act as antibacterial agents against a wide variety of pathogenic bacteria. These monoterpenes attack the cell membrane causing leakage of various substances, such as ions, ATP, nucleic acids and amino acids. The mechanism of OEO against bacterial infection of different pathogens, including Methicillin-resistant *Staphylococcus aureus* (MRSA), proposes that OEO destroys bacterial cell membrane causing leaking of Na^+, K^+ and irreparable damage to bacteria. Additionally, recent studies have shown that when carvacrol reaches bacterial DNA alters its genetic composition preventing it from replication, transcription and translation [61-64]. Terpinen-4-ol induced leakage of K^+ ions from *E. coli* and inhibition of respiration in exponential and stationary phase cell suspensions of *E. coli* [65]. Eugenol increased permeability in the cytoplasmic membrane of *Salmonella typhi*, affecting membrane-embedded proteins and inducing the inhibition of the respiratory system and alteration of ion transport activities of bacterial cells (Fig. 4 A)) [66]. The antibacterial mechanism of PSMs is highly effective against bacterial colonization, biofilm formation and AR bacteria (Fig. 4 B)) [67].

Polo *et al.* reported the synthesis of calcium phosphate microparticles grafted with vanillin essential oil (VEO) at different concentrations [68]. (Fig. 4 C)) shows healthy *E. coli* on uncoated commercially available bone regenerator material, Surgibone ®, (Fig. 4 D)-F)) shows antibacterial properties of this material enhanced with VEO against *E. coli*. Moreover, thymol/carvacrol was loaded into polythioether nanoparticles via thiol alkene photopolymerization in miniemulsion [69]. (**Fig. 4, G)**) represents the loading of thymol/carvacrol **H)** shows the multifunctional monomers used to generate the nanoparticles and SEM images of them, and **I)** shows the antibacterial properties of the material at different concentrations of carvacrol/thymol against various bacterial strains. Overall, these results suggest the enhancement of antibacterial properties when including PSMs in different materials.

Among wound healing properties of PSMs, antibacterial properties have been the most studied. While most of the studies are on the wound healing applications of PSMs as essential oils, our interest is focused on the potential use of PSMs in medical devices as a polymer film for wound healing purposes.

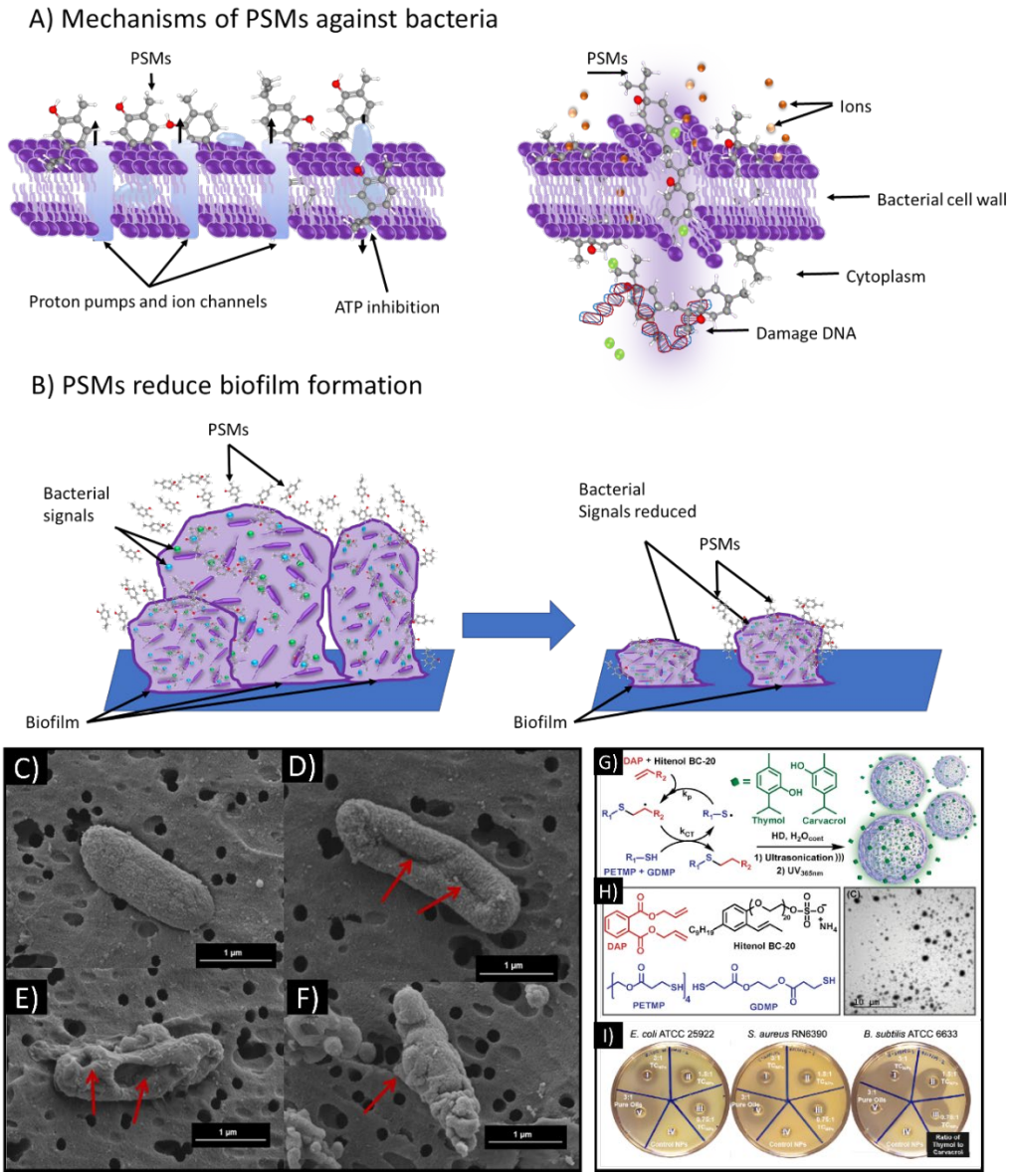

Fig. 4. Mechanisms of PSMs against bacteria and biofilms. **A)** PSMs disturb ions exchange and ATP process, induce membrane permeability, DNA damage, and leakage of ions. **B)** PSMs reduces biofilms by interrupting bacterial signaling and quorum sensing. **C)-F)** Field emission scanning electronic microscopy (FESEM) images show the antibacterial properties of commercially available bone regenerator material (Surgibone ®) uncoated **C)** and coated with different concentrations **D)-F)** of vanillin essential oil. Reproduced with permission [68]. Copywrite 2018. Elsevier. **G)** Antimicrobial thymol/carvacrol-loaded polythioether nanoparticles **H)** various multifunctional monomers used to generate polythioether

nanoparticles via thiol alkene photopolymerization in miniemulsion **I)** Antimicrobial activity of nanoparticles loaded with different ratios of carvacrol and thymol. Reproduced with permission [69]. Copywrite 2016, Wiley.

Table 2. Plant secondary metabolites and their activity against different types of bacteria

Plant secondary metabolites	Pathogens	Reference
Eugenol	*Candida albicans*	[53-57, 66]
	Escherichia coli	
	Enterobacter aerogenes	
	Proteus vulgaris	
	Salmonella typhi	
	Staphylococcus aureus ATCC25923	
	Enterococcus faecalis ATC29212	
	Escherichia coli ATCC25922	
	Candida albicans ATCC90028	
Oregano oil (carvacrol and thymol)	*Candida albicans*	[63, 64, 70, 71]
	Candida krusei	
	Candida tropicalis	
	Candida dubinensis	
	Pseudomonas aeruginosa	
	Bacillus cereus	
	Escherichia coli	
	Salmonella thypi	
	Yersinia enterocolitica	
	Staphylococcus aureus	
	Listeria monocytogenes	
	Enterococcus faecalis	
Thyme oil	Candida albicans	[56, 57, 63, 71]
	Staphylococcus aureus	
	Enterococcus faecalis	
	Escherichia coli	
	Pseudomonas aeruginosa	
	Salmonella typhi	
	Yersinia enterocolitica	
	Staphylococcus aureus	
	Listeria monocytogenes	
	Enterococcus faecalis	
Lavender oil (linalyl acetate)	*Staphylococcus aureus* ATCC25923	[57, 62, 71]
	Enterococcus faecalis ATC29212	
	Escherichia coli ATCC25922	
	Candida albicans ATCC90028	
	Candida albicans	
	Staphylococcus aureus	
	Enterococcus faecalis	
	Escherichia coli	
	Pseudomonas aeruginosa	

	Salmonella typhi *Yersinia enterocolitica* *Staphylococcus aureus* *Listeria monocytogenes* *Enterococcus faecalis*	
Tea tree oil	*Staphylococcus aureus* ATCC25923 *Enterococcus faecalis* ATC29212 *Escherichia coli* ATCC25922 *Candida albicans* ATCC90028 *Escherichia coli* *Candida glabrata* *Herpes simplex* virus type 1 (HSV-1) *Methicillin-resistant Staphylococcus aureus* (MRSA) *Pseudomonas aeruginosa*	[65, 72, 73]

5. Incorporation of PSMs in wound dressings

Wound dressings technology involves the incorporation of novel materials by different techniques, including plasma treatments, electrospinning, dip coating and sputtering into the dressings to accelerate the healing process. These dressings can act as drugs carriers, scaffolds and tissue regenerators. Radiofrequency plasma-enhanced chemical vapor deposition (RF-PECVD) is a polymerization technique that can easily integrate PSMs vapors into wound dressings. The retention of PSMs properties along with a low temperature of deposition and its relatively easy control are the main features of RF-PECVD polymerization. A previous study utilized the RF-PECVD plasma thin film preparation method from terpinen-4-ol and found that the antibacterial properties of terpinen-4-ol were successfully retained and were effective against *Staphylococcus aureus* [74]. In similar studies, antibacterial terpinen-4-ol thin films were fabricated with pulsed plasma polymerization and the resulting films were effective against *Pseudomonas aeruginosa* (ATCC-589), as seen in (Fig. 5) [13, 75].Like carvacrol, the antibacterial properties of terpinen-4-ol are related to their lipophilicity. It works by penetrating bacteria cell wall and cytoplasmic membrane, causing structural damage and intracellular loss. Lipophilicity of essential oils permeates cytoplasmic membrane causing bacterial cell damage and death [76]. The retention of antibacterial activity of pristine 1,8-cineole was achieved by plasma polymerization. 1, 8-cineole thin films were effective against *S. aureus* and *E. coli* [77]. Thus, the above studies suggest that plasma polymerization is a robust method to incorporate PSMs in wound dressings. However, more studies of the wound healing properties of these polymers are needed.

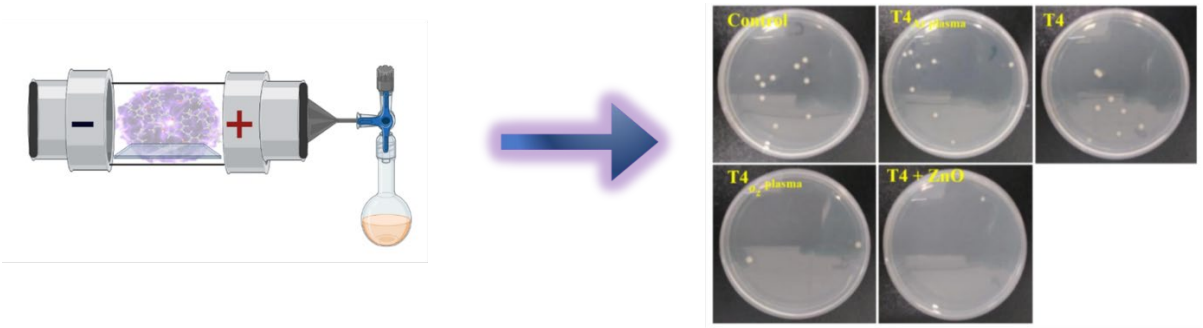

Figure 5. A) Scheme of plasma polymerization of PSMs, B) Shows bacterial inhibition of T4 PSM and ZnO nanoparticles. Reproduced under terms of the CC-BY license [75]. Copywrite, 2020. Avishek Kumar, Ahmed Al-Jumaili, Kateryna Bazaka, Peter Mulvey, Jeffrey Warner, and Mohan V. Jacob, published by MDPI.

On the other hand, electrospinning techniques have been widely used to fabricate micro and nanofibers from different natural sources, such as chitosan, alginate, collagen, cellulose, keratin and soy protein. Electrospinning can include synthetic and natural materials along the fibers; in encapsulated form or as nanoparticles [78]. Electrospun collagen hydrolysate nanofibers loaded with thyme or oregano presented antibacterial properties with potential use in wound dressings or cosmetics [79]. Wound dressings mats from polycaprolactone and polylactic acid (PCL/PLA) fibers were fabricated by electrospinning. Thymol was incorporated in the synthesis process and their anti-inflammatory and antibacterial properties were retained [80]. The antibacterial assays showed inhibition to S*taphylococcus aureus* and *Escherichia coli*. The results in the wound-closure assay (using male Wistar rat model) were significant for the samples containing thymol compared to the control (commercial gauze and Comfeel Plus® samples). Electrospun PCL/PLA with thymol accelerated wound closure more than 92% in 14 days, whereas gauze and Comfeel Plus® showed wound closure of 68% and 87%, respectively. Consequently, the promotion of cell growth and fibroblast cell formation occurred in an easier way than compared to the dry wound environment using gauze. The downregulation of pro-inflammatory cytokines is a key factor to prevent inflammation and stimulate healing progression. One example is the incorporation of thymol and tyrosol to electrospun PCL fibers. This study evaluated the anti-inflammatory properties on LPS-activated macrophages as is represented in (Fig. 6). The results shown expression of the pro-inflammatory mediators IL-1β and iNos. In addition, PCL/thymol reduced size of inflammed cells suggesting alleviation of the inflammatory response. These results suggest the use of these anti-inflammatory electrospun mats as wound healing dressings [81].

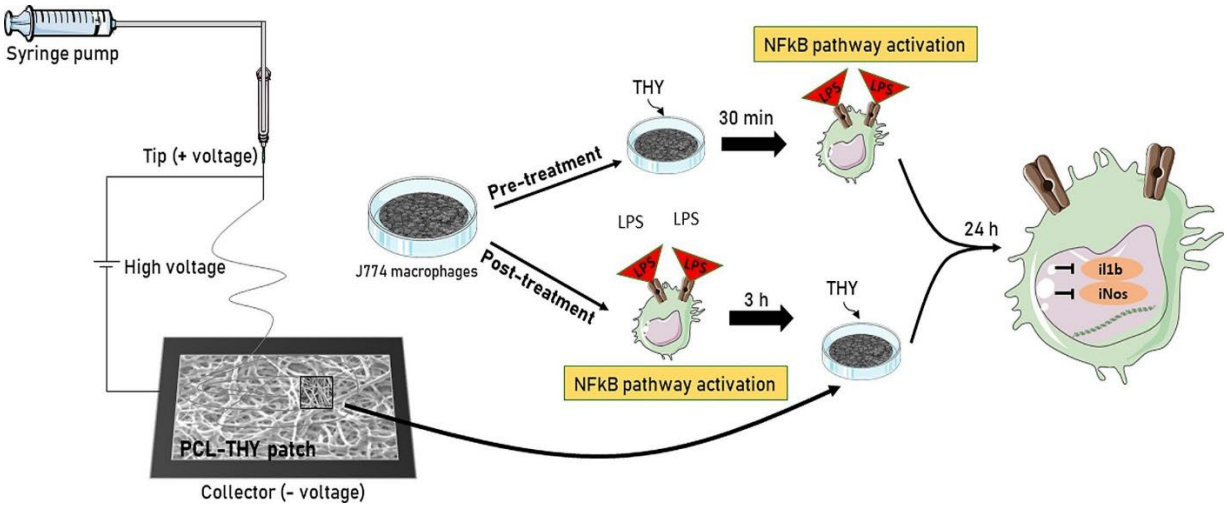

Figure 6. PSMs incorporated to electrospun for anti-inflammatory wound dressings. A) Shows a scheme of the PCL/Thymol electrospun mats that alleviate inflammation of LPS-activated macrophages through activation of NF-κβ pathway. Reproduced with permission [81]. Copyright 2021, Elsevier.

Other examples are polymeric dressings from N-carboxybutyl chitosan, collagen/cellulose, and hyaluronic acid were loaded with Jucá fruit extract. The combination of Jucá fruit extract with the mentioned dressings downregulated proinflammatory cytokines TNF-α and IL-1 in LPS-stimulated macrophages [82]. Ajwain essential oil was incorporated in core-shell electrospun nanofibers resultin in antibacterial and wound healing potential. This nanofibers were tested in rat wound model infected with *S. aureus* bacteria. The results showed the wound healing potential to diminish bacterial infection and enhance wound closure. In addition, the histologial outcomes shown no inflammation and incresing collagen deposition as shown in (Fig. 7) [83]. Anti-inflammatory curcumin-loaded nanofibers have been developed. The remarkable contribution of this novel material is the reduction of inflammation through the partial inhibition of IL-6 in streptozotocin-induced diabetes mouse model. These results are promising for the future use of nanofibers in chronic wounds in patients with diabetes [84]. In other studies, peppermint essential oil (PEO) was loaded into nanostructured lipid carriers (PEO-NLC) and tested *In-vivo* using a mice model with an infected wound. The results have shown a significant reduction of bacteria and faster wound closure when compared to control [85]. Moreover, electrospun zein/clove essential oil membranes were deposited in situ and tested as a potential wound dressing in a Kunming mice model. These membranes exhibited good antibacterial properties and promote wound closure when compared to a control [86]. Antibacterial properties of TTO were retained in the chitosan nanofibers after electrospinning. The mechanical properties, permeability and breathability of the TTO-chitosan nanofibers were

enhanced with the addition of TTO, positively contributing to the overall performance of the dressing. This combination was also effective against different bacteria (*Staphylococcus aureus, Escherichia coli* and *Candida albicans*) suggesting the potential application of these fibers as nonwoven dressings with prolonged antimicrobial properties [87]. In other studies, antibacterial chitosan nanoparticles containing *Homalomena pineodora* essential oil, a Malaysian plant extract, were synthesized using ion gelation method. The polymer encapsulates the essential oil, controlling its diffusion from the core across its matrix. *Homalomena pineodora* oil/chitosan nanoparticles were efficient against bacteria (both gram-positive and gram-negative) and yeast. The release of the *Homalomena pineodora* oil medicinal effect lasted up to 3 days, suggesting the possible use of these nanoparticles as prolonged effect antibacterial wound dressings [88].

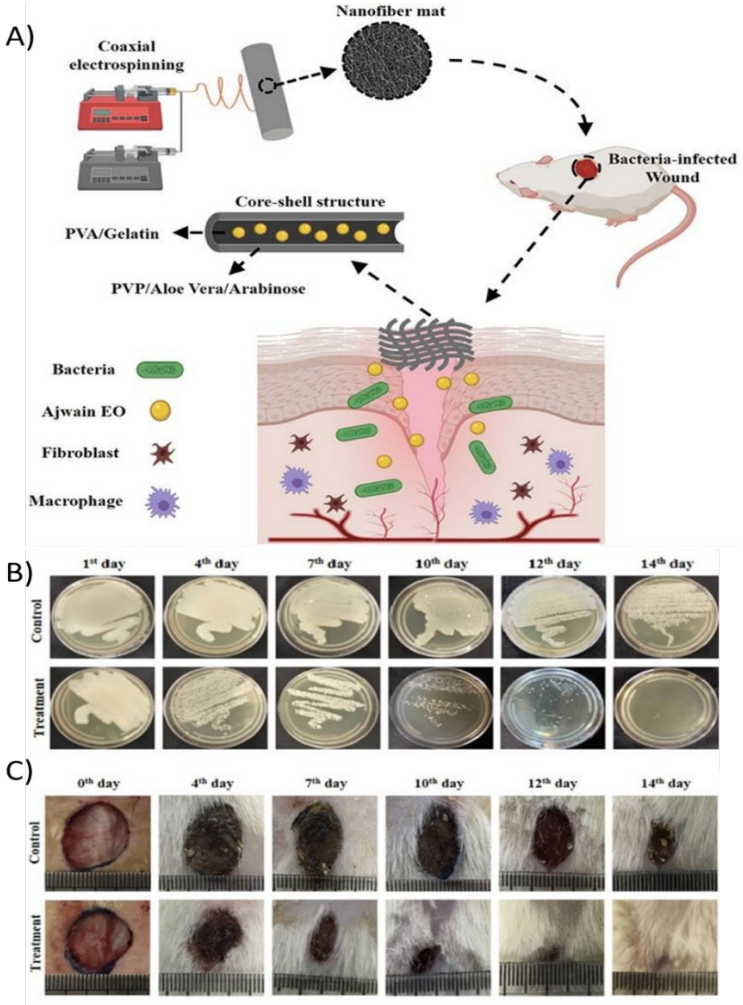

Fig. 7. Ajwain essential oils incorporated to nanofibers by electrospinning to accelerate healing in infected wounds in a rat model. **A)** Shows the scheme graphical abstract of the study. **B)** shows the antibacterial effect against *S. aureus* after 14 days. **C)** shows accelerated wound healing on infected wound in a rat model. Reproduced with permission [83]. Copyright 2021, Elsevier.

Conclusion

Delayed wound healing is promoted by chronic inflammation (upregulation of pro-inflammatory cytokines), oxidative stress (ROS generation) and bacterial infection (biofilm formation, antibiotic resistance). Downregulation of pro-inflammatory cytokines and ROS, as well as protection against bacterial infection and biofilms formation, are targets to achieve effective wound healing. It has been demonstrated that the main active compounds of PSMs have antioxidant, anti-inflammatory and antibacterial properties. The medicinal properties of PSMs can be retained by different methods such as electrospinning, micro and nano-encapsulation, plasma polymerization and laser evaporation. Despite the efforts to successfully integrate PSMs in wound healing materials, more clinical studies are needed to rigorously test these dressings' compounds. There is a need to study the efficacy of PSMs-derived polymers after a long storage period as these tend to auto-oxidase and may evaporate. The study of the toxicity of chronic use in human cells would be helpful to know the appropriate time of use in wound dressings. It also is important to consider the use of multicomponent oils e.g., tea tree oil or its major component, e.g., terpinen-4-ol. The use of multicomponent oils may prevent the development of resistance in bacteria, as multiple systems in the cell are targeted. On the other hand, the use of pure monomer ensures consistency and addresses seasonal and regional variability as it can occur with the multicomponent tea tree oil. The promising properties of PSMs plus the available processing techniques to incorporate them into polymers suggest their potential application in wound dressings.

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