Antimicrobial and healing-promoting properties of animal and plant oils for the treatment of infected wounds

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ABSTRACT

Chronic wounds are a serious medical problem both in the hospital and community setting. The healing of chronic wounds is often compromised by colonisation of different bacterial pathogens leading to life-threatening infections. Bacterial infections are a critical contributing factor to chronic wounds and can lead to biofilm formation and inhibition of innate inflammatory responses, including the reduction of acute inflammation.

Concerningly, the overuse of systemic antibiotics and the use of traditional therapeutics, including topical antimicrobials – iodine, chlorhexidine and silver – have both greatly contributed to the development of a global increase in antimicrobial resistance. This has therefore led to a renewed interest in natural and alternative antimicrobial treatment strategies in wound care for the treatment of infected wounds.

This review summarises the pre-clinical and clinical evidence that exists for the use of natural remedies, namely essential and animal oils, as adjunctive therapeutic approaches for the treatment of infected wounds. It also discusses novel approaches in nanotechnology that are being used for the development of natural remedies aimed at improving the healing of infected chronic wounds.

INTRODUCTION

Delayed healing in chronic wounds changes the normal microbiota of the wound, leading to colonisation by more virulent microbial pathogens and subsequent infection; this is often described as a continuum spectrum from contamination to colonisation to infection[1]. Generally, when more than 10^5 pathogenic organisms/gram of tissue colonise the wound, the wound is considered infected and localised infection develops as bacteria use different mechanisms

to overcome the host immune responses[2]. Subsequently, if bacteria overcome the host immune responses and enter the surrounding tissue and blood vessels, producing a systemic host response, an infection is classified as systemic infection or sepsis[3]. In chronic wounds, infection is most commonly poly-microbial, although this changes over time. In early wound infection, the majority of organisms present are gram-positive, with *Staphylococcus aureus* (being most common. Later in wound infection, Gram-negative organisms, including *Escherichia coli* and *Pseudomonas* species make up the majority of the organisms present[3].

Management of wound infection is essential to reduce the presence of bacteria and promote healing[4]. Generally, current treatments involve cleaning the wound, treating the infection with antimicrobials[1,2], and applying wound dressings[3]. Topical application of currently used antimicrobials – including iodine, chlorhexidine and silver – aids in reducing the presence of pathogenic bacteria in the wound. However, many of the common antimicrobials have cytotoxic effects on mammalian cells when used at the concentration required to clear the pathogenic bacteria[1]. In cases where infection has spread, systemic antibiotics are also used to treat infections that may clinically present as sepsis, cellulitis or abscess formation[2]. Unfortunately, this overuse of systemic antibiotics has given rise to the development of antimicrobial resistance and wound 'super bugs' such as methicillin-resistant *S. aureus* (MRSA) which pose a serious threat to the community[5]. With drug resistance as a growing problem, choosing the appropriate treatment for wound infection is therefore becoming increasingly difficult[6]. Drug-resistant bacterial strains like MRSA are increasing the need for new antimicrobial products in both community and hospital settings. Consequently, natural remedies, including essential and animal oils, are gaining more interest clinically.

Essential oils are natural oils which are typically obtained by distillation and have the characteristic odour of the plant from which they are extracted[7]. Indeed, many natural essential – as well as animal oils – have been reported to improve healing in various pre-clinical models of animal wound healing[4,8,9], and some have shown strong antimicrobial effects against the most common pathogenic bacteria present in clinical chronic wound infections[10–12].

Natural remedies, including essential and animal oils such as tea tree oil and emu oil, have historically been used in wound care as safe therapeutic agents with strong antimicrobial properties. However, while these oils could be a potential adjunctive therapy for the management of difficult to treat hospital-acquired infections with multidrug-resistant strains, it is important to acknowledge that antimicrobial activity of these oils is not suitable as a stand-alone option for treating serious microbial infections of chronic wounds. This is mainly due to variations in the terpenic composition of these oils leading to variable differences in antimicrobial activity[7]. Here we summarise evidence for the principal essential and animal oils used as natural remedies for the treatment of infected wounds. We also discuss the latest approaches in the development of adjunctive therapies using nanomedicine comprising natural remedies for the treatment of wound infection and improved tissue regeneration.

METHODS

Databases (PubMed, Medline, Scopus and Ovid) were searched for all publications between 1997 and 2019 using the search terms 'natural oils', 'plant oils' and 'animal oils' combined with 'wound healing' and 'wound infection'. This was used to select the panel of plant and animal oils with healing and antimicrobial properties as a primary focus for this review. Inclusion criteria were English language articles identifying the key search terms. We further hand-examined references of selected key papers to find additional sources of information outside our search terms. The search strategy included using broad keywords and synonyms concerning wound healing and infection, with further information relating to the animal and plant oils obtained by adding 'wound healing', 'wound infection' and 'antimicrobial' to the type of oil being searched, as well as 'clinical trials' and types of common pathogens – these are listed in Table 1. Papers relating to *in-vitro*, bacterial, animal and human studies were all included.

THE USE OF ESSENTIAL OILS IN WOUND HEALING AND INFECTION

Natural plant oils, or essential oils, are commonly used as an inexpensive option for skin care[13]. Essential oils, also referred to as volatile natural mixtures, are secondary plant metabolites extracted from the flowers, seeds, leaves and fruit of plants[3,6]. These natural oils are, primarily made up of unsaturated hydrocarbons termed terpenes, polyphenols and fatty acids and, together, these components are responsible for the medicinal, culinary and fragrant uses of some plants[14]. Many essential oils have been reported to possess antimicrobial properties in *in-vitro* assays[7], in pre-clinical rodent models of wound repair[14,15], and in human clinical trials[11,12] primarily attributed to polyphenols and terpenes, (primarily monoterpenes), eugenol, cinnamaldehyde, carvacrol, and thymol[11]. However, the composition of essential oils from the same plant species varies based on a variety of factors, including differences in growing conditions, in storage and processing conditions, and in the method of oil extraction[7,16]. This variation between oils of the same type can alter the effectiveness of the oil at treating wound infections. Additionally, it is important to acknowledge that further differences in essential oils can occur due to oil degradation by various factors including oxidation, heating and light[6,17].

Essential oils, for example tea tree, lavender, oregano and clove oil, have also been reported to have properties that could improve healing and reduce infection in wounds[3,9]. These properties have been demonstrated in both *in-vitro* cell and bacterial assays[18] as well as in pre-clinical rodent wound

	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*	Effect on Healing	References
Tea tree oil	Inhibits at 0.5% v/v Agar dilution method	Inhibits at >5.0% v/v Disc diffusion assay	Inhibits at 0.25% v/v Agar dilution method	Inhibits at 0.5% v/v Agar dilution method	Increases macrophage numbers Reduces tissue oedema Accelerates wound closure	**Inhibitory concentration** Hammer *et al.*, 1999 (Agar dilution method) Kavanaugh and Ribbeck, 2012 (Disc diffusion assay) **Healing** Edmondson *et al.*, 2011 (Human clinical trial) Lee *et al.*, 2014 (Human clinical trial) Flores *et al.*, 2015 (Wistar rats)
Lavender oil	Inhibits at 1.0% v/v Agar dilution method	Inhibits at >5.0% v/v Disc diffusion assay	Inhibits at 0.25% v/v Agar dilution method	Inhibits at 0.25% v/v Agar dilution method	Enhanced TGF-β expression Increases collagen synthesis Accelerates wound closure	**Inhibitory concentration** Hammer *et al.*, 1999 (Agar dilution method) Kavanaugh and Ribbeck, 2012 (Disc diffusion assay) **Healing** Mori *et al.*, 2016 (Sprague-Dawley rats)
Clove oil	Inhibits at 0.25% v/v Agar dilution method	Inhibits at >5.0% v/v Disc diffusion assay	Inhibits at 0.25% v/v Agar dilution method	Inhibits at 0.12% v/v Agar dilution method	Decreased inflammation and reduced odour of infected wounds Increased fibroblast migration Accelerates wound closure Promotes angiogenesis Prevents scaring	**Inhibitory concentration** Hammer *et al.*, 1999 (Agar dilution method) Kavanaugh and Ribbeck, 2012 (Disc diffusion assay) **Healing** Akhmetova et al., 2016 (Human clinical trial) Alam et al., 2017 (Wistar rats) Singh *et al.*, 2018 (Balb/c mice) Li *et al.*, 2019 (Balb/c mice)
Emu oil	No effect Disc diffusion assay	No data	No effect Disc diffusion assay	No effect Disc diffusion assay	Improves keratinization, fibrillogenesis and wound regeneration Protects against oxidative damage Increases mature hair follicles around wound	**Inhibitory concentration** Riley and Carson 1999 (Disc diffusion assay) **Healing** Politis and Dmytrowich, 1998 (Wistar rats) Rodrigues *et al.* 2012 (Wistar rats) Afshar *et al.*, 2016 (Balb/c mice)
Ostrich oil	Reduces bacterial count at 2.00% w/w Balb/c mice	Reduces bacterial count at 2.00% w/w Balb/c mice	No data	No data	Reduces inflammation Increases fibroblast proliferation and collagen synthesis Promotes angiogenesis Accelerates dermal wound maturation	**Inhibitory concentration** Farahpour *et al.*, 2018 (Balb/c mice) **Healing** Farahpour *et al.*, 2018 (Balb/c mice)
Crocodile oil	Bacterial growth reduced by half at 10% v/v Bacterial culture broth	No data	No data	Bacterial growth reduced by half at 5% v/v Bacterial culture broth	Improves epidermal organisation Improves hair follicle formation Improves collagen deposition Accelerates wound closure	**Inhibitory concentration** Buthelezi *et al.*, 2012 (Bacterial culture broth) **Healing** Li *et al.*, 2012 (Wistar rats) Li *et al.*, 2017 (Wistar rats)

and infection models[14,15,19] and human clinical studies[11,12]. Other oils, such as emu oil, have yet to demonstrate antimicrobial properties; however, in the experimental setting, topically applied emu oil has been demonstrated to decrease inflammation, improve epidermal keratinisation, and increase the number of mature hair follicles around the wound in pre-clinical animal models of wound repair[8]. Indeed, bacterial and mammalian cell *in-vitro* assays and pre-clinical animals models have demonstrated that a mixture of essential oils with potent antibacterial and healing-promoting properties could provide the optimal approach to treat infected wounds using natural remedies[20]. Additionally, different pre-clinical animal studies have shown that both oral and topical administration of natural oils have benefits in promoting wound healing by accelerating recovery from the inflammatory phase of wound repair, while some essential oils have synergistic effects against pathogenic wound bacteria in combination with recommended antibiotics[15,21,22]. Table 1 summarises the minimum inhibitory concentrations (MICs) of selected essential and animal oils discussed in this review against the four major pathogenic bacteria normally present in chronic wounds and their subsequent effects on wound healing.

Oil from *Melaleuca alternifolia* (tea tree) has been used by Indigenous Australian people for skin infections and wound healing for thousands of years[18]. Today, tea tree oil and its components have been integrated into various skin care products[3]. Tea tree oil is comprised of a complex mixture of terpenes and hydrocarbons, with the most active components being terpinen-4-ol and 1,8-cineole, giving tea tree oil its antimicrobial, antifungal and anti-inflammatory properties[3,17]. The antimicrobial properties of tea tree oil have been shown to inhibit *E. coli* and *S. aureus* in *in-vitro* bacterial assays with minimum inhibitory concentrations of 0.25% and 0.5% respectively[7]. Additionally, tea tree oil inhibits *Pseudomonas aeruginosa* biofilms in *in-vitro* bacterial assays with minimum inhibitory concentration of 5%[23].

In the context of clinical wound healing, its biological effects are mediated via an enhanced innate immunity response, attributed to its ability to activate monocyte differentiation into pathogen-killing macrophages and reduce tissue oedema[11,12,24]. Importantly, it has been shown that using water-miscible tea tree oil (3.3%) solution as an aspect of the wound dressing cleansing regimen at each dressing change is effective against MRSA and can improve the healing of

human infected wounds[12,25,26]. In addition, tea tree oil is as effective as the standard antibiotic treatment mupirocin in clearing MRSA infection; it has been demonstrated to clear MRSA infection in 41% of patients where no mupirocin resistance was present, while mupirocin – the "gold standard antibiotic treatment" – cleared MRSA infection in 49% of patients[25]. This tea tree oil preparation hence allowed previously non-healing infected human wounds to heal[12] and significantly reduced MRSA colonisation while promoting healing when added to wounds after saline wash and before dressing application[26]. Additionally, previously reported problems of wound maceration and colonisation by Gram-negative bacteria did not occur in this study[12].

Another essential oil, lavender oil, is used predominantly in aromatherapy and can be found in a number of cosmetic and therapeutic settings. Historically, lavender oil was used by ancient Romans and Greeks for its antibacterial and antifungal properties post-burn injuries and insect bites[27]. The properties of lavender oil are highly dependent on plant source[3], resulting in different compositions; however, the main constituents of lavender oil include linalool and linalyl[3,15,27], both of which have marked anti-inflammatory and antimicrobial properties[15]. Lavender oil comprises four main lavender types – *Lavandula latifolia*, *Lavandula angustifolia, Lavandula stoechas* and *Lavandula intermedia.* Lavender oil from different lavender types has been used for different clinical applications, including as an abortifacient, a diuretic and for the treatment of headaches and insomnia[27]. Two variations of *L. angustifolia* (French lavender and Tasmanian lavender) have been shown to inhibit *S. aureus* activity at 1% in *in-vitro* bacterial studies, with studies also showing *L. angustifolia* to have a minimum inhibitory concentration of 0.25% against *E. coli* and *Candida albicans* and an inhibitory concentration >5% for *P. aeruginosa*[7,23]. Additionally, topical application of only 1% of *L. angustifolia* oil has been demonstrated to improve cutaneous healing in Sprague Dawley rats with 10mm diameter excisional wounds, significantly increasing TGF-β expression, fibroblast proliferation, collagen synthesis and accelerated granulation tissue formation[15]. Lavender oil treatment also increased the myofibroblast population, resulting in increased wound contraction and reduced wound area compared to untreated wounds of Sprague Dawley rats demonstrating healing-promoting properties[15].

Syzygium aromaticum (clove) or clove oil also has anti-oxidative, antibacterial, anti-inflammatory and analgesic properties[14,21,28,29] that have been used in herbal medicine to treat cuts and burns, to provide pain relief from toothache, and to treat tooth infections[29]. Clove oil can be highly cytotoxic to human skin cells *in-vitro,* with 54–73% of the cytotoxicity being attributed to eugenol[29]. Eugenol is the main component in clove oil, making up 71–90% of the oil and is primarily responsible for its antimicrobial properties[21,28]. Eugenol is generally regarded as safe and is often added to food for its spicy flavour[28,29]; however, at a concentration of 0.06%, eugenol can become highly cytotoxic to human skin cells[29]. Despite this cytotoxicity, eugenol at a concentration of 0.001% has been shown to have a protective effect on skin photoaging[28]. Clove oil has also been shown to improve wound healing in pre-clinical animal models of wound healing[14,21], and exhibits antibacterial activity in *in-vitro* bacterial assays against *E. coli and S. aureus,* with minimum inhibitory concentrations of 0.25% for both, while its effects on *C. albicans* are observed at 0.12% and against *P. aeruginosa* at >5%[7,23]. Interestingly, application of clove oil significantly enhanced the therapeutic effectiveness of Fluconazole drug and eradication of dual-species biofilms (*C. albicans*/ *S. aureus*) in *in-vitro* bacterial assays compared to antibiotics alone[30]. Indeed, *in-vitro* bacterial studies focused on understanding how clove oil exerts its antibacterial activity showed that clove oil could increase the cell wall permeability of gram-positive *S. aureus* and lead to inhibition of bacterial DNA and protein synthesis[31].

In pre-clinical wound healing studies, the effect of clove oil (eugenol) has also been attributed to accelerated healing of diabetic wounds via its effects on reduced lectin-like oxidised low-density lipoprotein receptor 1/ NF-κB induced dysfunction in endothelial cells and promotion of angiogenesis[32]. Additionally, incorporation of clove oil into a wound dressing has been shown to improve wound healing via increased fibroblast migration and deposition of collagen that resulted in significantly decreased scar formation in pre-clinical models of wound repair in mice[14]. Clinically, topical applications of clove oil have been suggested as an effective strategy for controlling odour of chronic wounds and for decreasing inflammatory responses post-infection[33].

THE USE OF ANIMAL OILS IN WOUND HEALING AND INFECTION

Animal oils have been used in various traditional medicines, for example, crocodile oil has been used as an ointment for burns and scalds in traditional Chinese and Southeast Asian medicine[34,35], and emu oil has been used by Indigenous Australian people to aid wound regeneration[18,36]. Similar to essential oils, the composition of animal oils can vary with different environmental conditions, such that the same species of animal from different areas can have variations in compositions[8]. However, unlike essential oils, animal oils such as emu oil require less refining and are more readily metabolised[36]. While the majority of research has focused on the use of essential oils in wound healing applications, the antibacterial and healing properties of animal oils are now also gaining attention as potential sources of novel antimicrobials.

Oil from the flightless bird *Dromaius novaehollandiae* (emu) was used by Indigenous Australian people to treat burns, scrapes, and to accelerate wound healing[18,36] and is still used today as a complementary medicine to enhance healing[8]. Emu oil is collected from the subcutaneous and retroperitoneal fat of the emu, consisting primarily of fatty

acids, including omega-9 (oleic acid; 42%), omega-6 (linoleic acid; 21%), palmitic acid (21%), and omega-3 (α-linolenic acid; 1%)[37]. The anti-inflammatory properties of emu oil have been shown to reduce clinical indicators of disease, decrease tissue inflammation, and promote intestinal repair in various clinical inflammatory conditions, including gastrointestinal disorders[37–40] and polyarthritis[36].

In wound healing, emu oil has been shown to have a marked positive effect on skin regeneration by significantly increasing the number of mature hair follicles in the wound margins and improving dermal fibrillogenesis and collagen production compared to untreated controls in a pre-clinical murine model of wound repair[8]. The positive effects of emu oil have been attributed to its high oleic acid and linoleic acid composition which has been shown to increase TNF-α concentration and NF-κB activation at 1 hour post-wounding, and reduce IL-1 and IL-6 proinflammatory cytokine levels as well as NF-κB activation at 24 hours post-wounding in murine models of healing[8]. Additionally, emu oil effects on infected murine wounds have demonstrated protection against oxidative damage, a critical desired property for potential antimicrobial treatment[22]. These findings are in agreement with studies which showed that topical applications of linoleic acid on wounds of mice and rats improved the rate of wound healing, increased angiogenesis and reduced inflammation, while clinical treatment of human pressure ulcers with linoleic acid increased hydration and elasticity[41,42].

However, despite its positive effects on wound healing, emu oil has also been shown to delay healing in one pre-clinical wound healing study using mice with 1cm diameter burn wounds, by prolonging the inflammatory stage when applied immediately after wound formation[8]. Nevertheless, emu oil application still improved overall healing of the wound site. In that study, inflammatory cell density, primarily polymorphonuclear leukocytes, was increased in the emu oil groups on all days when compared with the control groups[8]. A separate pre-clinical study in Wistar rats with 6mm diameter excisional wounds showed that emu oil treatment improved wound re-epithelialisation when applied 24 hours post-wound formation, with no effect on wound contraction compared to control[43]. In that same study, an emu oil lotion comprised of emu fat and ultra-purified oil, vitamin E and botanical oil carrier resulted in almost a two-fold increase in wound contraction compared to untreated controls; an improvement also previously observed when application was delayed 48 hours compared to controls[43].

Struthio camelus (ostrich) has been farmed since the mid-1880s for feathers and hides, and more recently for meat and oil[18]. Ostrich oil mainly consists of oleic acid (30.39%), linoleic acid (17.32%), palmitic acid (32.11%), and palmitoleic acid (9.12%)[44]. This oil has been used in cosmetics and may have wound healing and anti-inflammatory activity[18]. The potential wound healing ability of ostrich oil has been tested in Balb/c mice with excisional wounds infected with *S. aureus* and *P. aeruginosa*[44]. Ostrich oil was mixed with an ointment base made from soft paraffin (90%), hard paraffin (5%) and lanolin (5%) such that the ointment contained either 2% or 4% ostrich oil. Compared to the ointment base, both 2% and 4% ostrich oil ointments decreased bacterial count and enhanced wound healing by reducing inflammation, increasing fibroblast proliferation and enhancing collagen deposition[44]. Additionally, compared to ointment base and mupirocin antibiotic treatment, ostrich oil ointment increased the number of blood vessels present in the wound by day 8 post-wound formation[44]. Ostrich oil treatment of *S. aureus* and *P. aeruginosa* infected wounds showed significantly decreased TGF-β and upregulated FGF-2, and VEGF expression leading to enhanced angiogenesis and fibroblast distribution which enhanced collagen biosynthesis and dermal maturation[44].

Another animal oil, crocodile oil, is rich in monounsaturated and polyunsaturated fats and has been used in ointments for burns and scalds in traditional Chinese and south east Asian medicine[34], and in Africa for skin rashes and to improve wound healing[35]. The oil from *Crocodylus siamensis* (Siamese crocodile) is mainly composed of oleic acid (30.47%), linoleic acid (11.74%), and palmitoleic acid (5.67%) and has been shown to accelerate healing in pre-clinical models of burn wound injury in Wistar rats compared to silver sulfadiazine treatment[34]. Additionally, studies have shown that Siamese crocodile oil can promote skin regeneration, collagen deposition and reduce scarring in second degree pre-clinical burn wound models in Wistar rats both as pure oil and when combined with a herbal ointment containing: *Arnebia euchroma*, root, dried; *Astragalus membranaceu*, root, dried; *Savia miltiorrhiza*, root, dried; *Sanguisorba officinalis*, root, dried; and *Borneolum syntheticum*[34,35]. This suggests the potential benefits of using a combination of essential and animal oils in the treatment of infected wounds, while the effect on skin scarring – which appears to be unique to crocodile oils – warrants further investigation in pre-clinical wound healing studies using large animals. Figure 1 provides a schematic diagram summarising the antimicrobial and anti-inflammatory effects of essential and animal oils as oral or topical treatments for infected wounds.

NANOMEDICINE APPROACHES FOR DEVELOPMENT OF ADJUNCTIVE THERAPIES CONTAINING NATURAL ESSENTIAL AND ANIMAL OILS

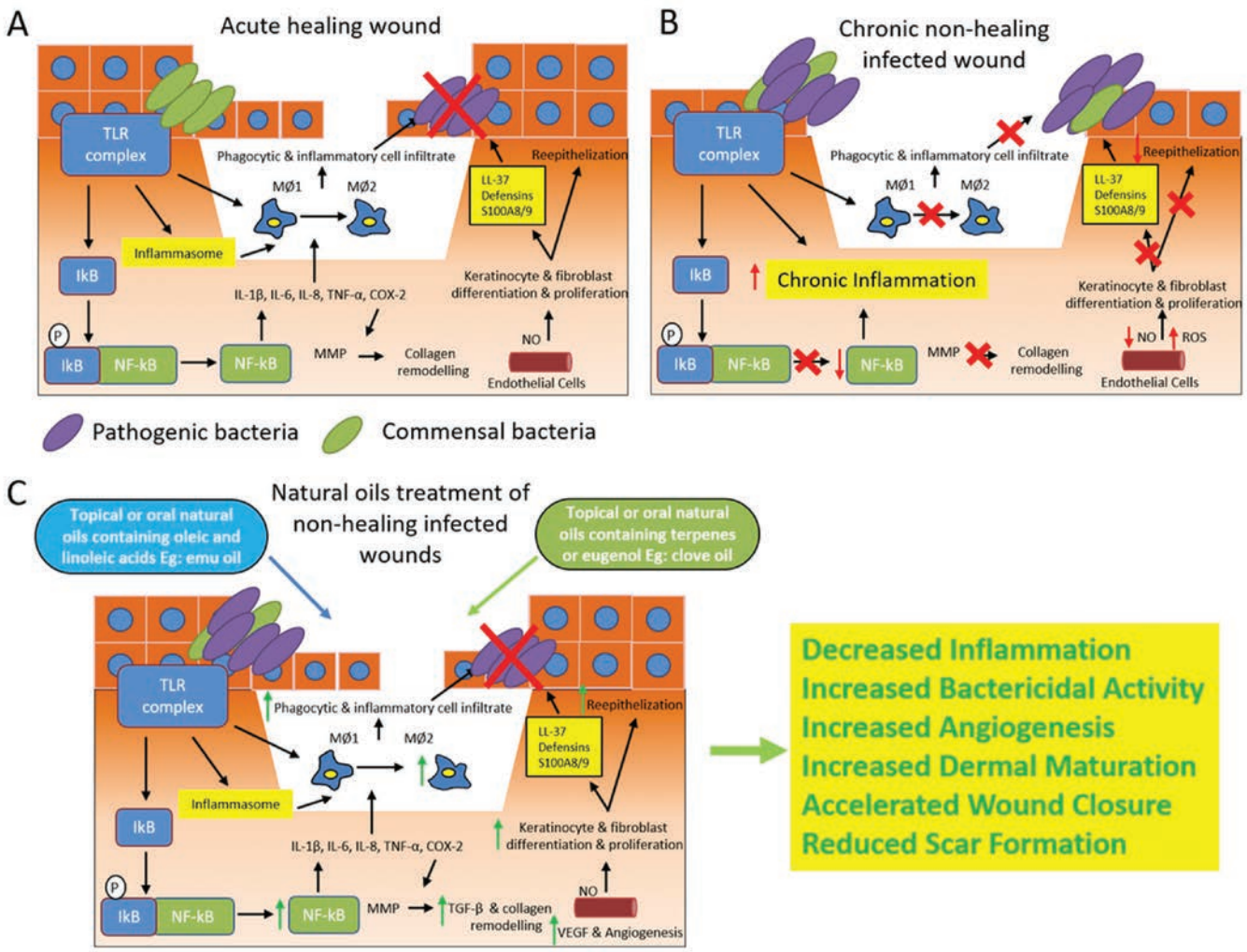
In order to develop effective adjunctive therapies containing natural essential and animal oils for the treatment of infected wounds clinically, the current main research focus is on improving the solubility and stability of these oils as well as developing different modes of delivery. Studies have shown that using different nanosystems that encapsulate these oils not only improves their solubility and stability, but also protects them from environmental factors that may cause chemical degradation while increasing their bioavailability and bioefficacy, especially antimicrobial and anti-inflammatory properties[8,17,21]. For example, a nano-

emulsion comprised of clove oil (1%), Triacetin (8%), Tween-80 (15%), labrasol (15%) and water (61%), administered orally each day for 21 days to albino Wistar rats was shown to improve wound closure of a 500mm^2 excisional wound compared to pure clove oil, the nano-emulsion without clove oil, and an untreated control[21]. Nanoparticles have the ability to protect and carry drugs or oils to specific targets in the body, enabling slower degradation, enhancing cutaneous penetration and improved treatment efficacy with lower systemic absorption[45]. Additionally, use of nanoparticles reduces the unwanted side-effects, while their incorporation into biodegradable scaffolds has demonstrated safer, longer and slower release of drugs/antibodies/silver ions to infected wounds, resulting in improved wound healing and decreased scarring[45]. The nanosystems used most commonly are either polymer-based (nanocapsules, nanoparticles, nanofibers, nanogels) or lipid-based (liposomes, solid lipid nanoparticles, nanostructured lipid carriers) and there is a wide range of literature on these approaches[45].

A recent systematic review of various rodent studies has revealed a significant amount of evidence on the use of essential oils as wound healing agents, and that their incorporation with chitosan, alginate, gelatin or collagen biopolymers results in active films or nanofibre dressings which have marked antioxidant, anti-inflammatory and antimicrobial activity[46,47]. For example, one study has shown that tea tree oil loaded into a chitosan hydrogel together with silver ions exhibited marked antimicrobial activity in *in-vitro* bacterial assays against *P. aeruginosa*, *S. aureus* and *C. albicans,* and further improved the antimicrobial activity by lowering the effective concentration of silver ions required, thereby reducing potential toxic effects on the healthy tissue surrounding the wound[48]. A similar *in-vitro* study using clove oil and chitosan hydrogel in bacterial assays has also reported promising anti-microbial and healing effects which were enhanced by delivering essential oil using nanotechnology approaches[17].

Additionally, studies using hydrogels containing nanocapsules and nanoemulsions of tea tree oil have demonstrated both antiedematogenic and promoted healing in rodent model of wound repair *in-vivo*[49]. In another study, dressings with either 5% clove oil or 10% sandalwood oil were applied

Figure 1. The antimicrobial and anti-inflammatory effects of essential and animal oils as oral or topical treatments for infected wounds.



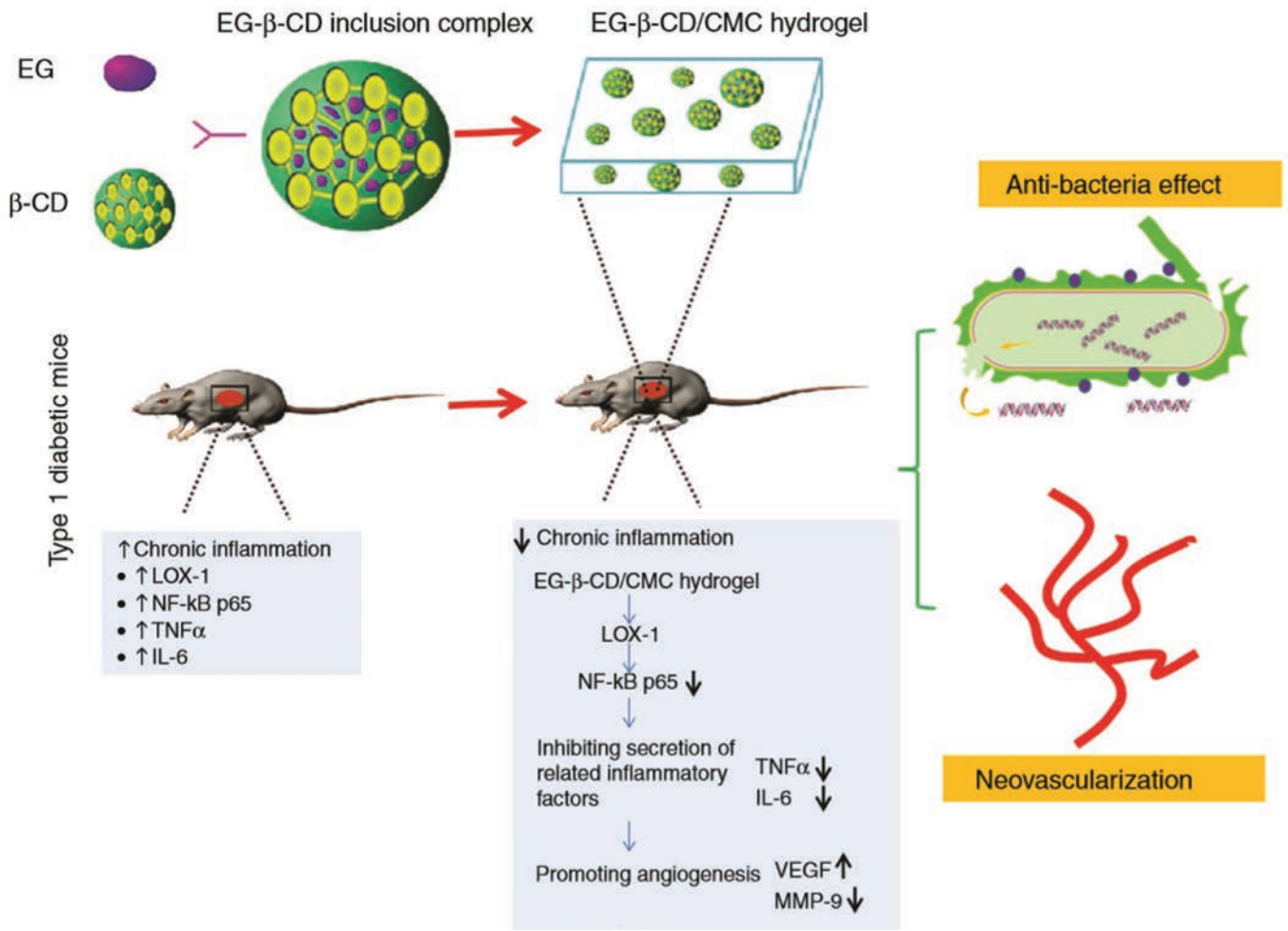
to 10x10mm^2 wounds on Balb/c mice for 21 days. By day 21, all mice treated with either oil had full wound closure, unlike mice given dressings without oil, again demonstrating the feasibility of using nanotechnology delivery systems to incorporate natural oils in hydrogels and/or dressings resulting in beneficial wound healing outcomes[14].

A recent pre-clinical *in-vivo* study has demonstrated that solubility and stability of eugenol (the main component of clove oil) can be significantly enhanced by the creation of a bioactive carboxy-methylcellulose (CMC) hydrogel loaded with inclusion complexes of eugenol with β-cyclodextrin (β-CD) for accelerating healing in full thickness wounds in a diabetic mouse model[32]. Encapsulating the hydrophobic eugenol with β-CD into its cavity to form inclusion complexes overcame the issues of solubility and stability, allowing slow and sustained release of eugenol (EG) from EG-β-CD/CMC hydrogel into the infected diabetic wounds[32]. Subsequently, this allowed eugenol to reduce the chronic wound inflammation and promote healing by inhibiting the secretion of inflammatory factors (TNF-α and IL-6) and promoting angiogenesis by decreasing MMP-9 and increasing VEGF respectively[32]. This allowed eugenol to exert its antibacterial effect on *S. aureus* and *E. coli* while decreasing inflammation, promoting neovascularisation of the wounds, and consequently improving the rate of wound healing (Figure 2)[32]. Taken together, these studies clearly demonstrate the feasibility and rationale for using essential and animal oils as potential adjuvant therapies to counteract pathogens with multiple antibiotic resistance or to be used as enhancers of conventional therapy approaches. However, further clinical studies are required to demonstrate the optimal nanotechnology approaches by which we can safely deliver these compounds to wounds.

CONCLUSION

Natural remedies including essential oils and animal oils have a real potential to aid in the clinical management of chronic infected wounds. Used in place of, or alongside, traditional treatments, the antimicrobial properties of essential and animal oils have the potential for use where drug resistance makes selecting the ideal treatment near impossible. The high volatility and lipophilicity of essential and animal oils allows them to penetrate the cell membrane and exert biological

Figure 2. The antibacterial effect of eugenol on S. aureus *and* E. coli.



effects. Additionally, due to their physiochemical properties, oils prevent water loss and the invasion of microorganisms, hence assisting in reducing the rates of colonisation and subsequent wound infection[50].

Natural products obtained from medicinal plants have become an important alternative source of new substances for combating wound infections as, in addition to lowering costs, these compounds also have reduced toxicity against mammalian cells[22]. Indeed, the topical administration of essential and animal oils on open wounds is a low-cost alternative in the treatment of wounds; however, literature on this approach is relatively scarce.

It should also be noted that application of these oils on wounds has a different effect at different stages of wound healing. In the first phase, treatment with oils results in immunomodulatory effects which dampen the inflammation and oxidative stress while promoting antimicrobial activity. In the second phase, treatment with oils promotes re-epithelialisation, angiogenesis and development of granulation tissue while their application in the third phase of wound healing appears to alter fibroblast and keratinocyte growth, improve collagen deposition, and decrease early scar formation[20]. Consequently, these compounds present great potential for the development of new low-cost therapeutic approaches for wound repair. However, larger randomised clinical trials are required to further examine the efficacy of oils as natural remedies and adjuvant wound therapy as well as determining the optimal dose and safety of these compounds for wound treatment and, ultimately, the mechanisms by which they exert their beneficial effects.

CONFLICT OF INTERESTS

Authors declare no conflict of interest.

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CORRIGENDUM

Title: Study protocol: a pilot clinical trial of topical glyceryl trinitrate for chronic venous leg ulcer healing

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Volume 27, issue 3, page 131

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Correction: Methods section in Abstract.

The concentration of GTN ointment used in the study is indicated as 2% and should be 0.2%.

This error does not appear elsewhere in the publication (e.g. study design under methods section where the GTN concentration is correctly given as 0.2%).

The authors apologise for any confusion caused.