An intact layer of skin is the first defence mechanism of the body to avoid the entry of potentially harmful microorganisms and foreign bodies. This means it is crucial that the body is able to repair any wound that might occur, to prevent further damage and protect the rest of the body.

Wound healing is a complex and highly regulated process that begins directly after wounding and continues for varying amounts of time depending on the extent of damage. It is categorised into stages (Rawat et al., 2012; Reinke and Sorg, 2012):

- The coagulation stage, where the blood clots preventing bleeding.
- The inflammatory stage, where white blood cells remove bacteria and debris and stimulate the migration of cells for wound healing.
- The proliferative stage, where granulation tissue is formed and collagen synthesis occurs.
- The remodelling stage, where the connective tissue is reorganised to strengthen the wound.

Chronic wounds - However, in some cases wounds can become chronic and take longer than three months to heal, or never heal at all. These wounds often get stuck in certain phases of wound healing, for example they can remain in the inflammatory phase too long, or there can be an upset in the balance of collagen degradation and regeneration.

Chronic wound bacteria and antimicrobial resistance

Antibiotic resistant bacteria are becoming an increasing problem both in hospitals and the community. The most high profile of which being methicillin-resistant *Staphylococcus aureus* (MRSA). In a large population of bacteria exposed to an antibiotic, some are naturally resistant due to spontaneous mutations. These will survive and reproduce, creating a population of resistant bacteria, as in natural selection. Antibiotic
misuse has led to a greater selection pressure for antibiotic resistance, leading to the spread of resistant strains, and the development of strains resistant to multiple antibiotics has limited the number of alternative antibiotics that can be used to treat the infection, so it is at risk of becoming chronic or fatal. As such, alternatives are actively being researched.

Some wounds, such as burn wounds can become infected and cause secondary problems. In some cases, wounds become chronic due to bacterial colonisation. These bacteria promote inflammation, thus prolonging the inflammatory stage of wound healing, causing further damage to the area by increasing the number of inflammatory cells to the area. Oftentimes, bacteria associated with chronic wounds form biofilms, large, dense communities of bacteria or fungi that adhere to the wound surface. Biofilms are especially hard to treat with antibiotics, as they have numerous defence mechanisms. They produce a sticky polysaccharide layer called extrapolymeric substances that surrounds the community, acting as a barrier to the antibiotic molecules. Even if the antibiotic can penetrate the polysaccharide layer, it is unlikely to kill those deep within the biofilm, which regrow after the outer layers are killed off. Biofilms are therefore very hard to treat, so research is ongoing into alternative antimicrobials for these chronic infections.

Essential oils and other natural products have been used as antiseptics and antimicrobials for years; and a plethora of oils have been studied in scientific experiments with the hope of using them as alternatives to antibiotics. One application
that is promising is their use for treating biofilms and antibiotic resistant wound infections, delivered topically or in wound dressings.

Wound dressing studies

Antimicrobial Resistance

Orange essential oil vapour effectively inhibited MRSA and vancomycin-intermediate-resistant *S. aureus* (VISA) strains in an *in vitro* dressing model, where 40µl neat essential oil was dropped onto a sterile dressing, which was used to cover a petri dish inoculated with the bacterial test strain (without contact so only the vapour was having the effect). The plates were incubated for 24 hours and the inhibition of the bacteria was analysed. Orange essential oil vapour was found to successfully inhibit the growth of all of the tested strains. The study also investigated the bactericidal effects of the essential oil on the bacterial test strains when infecting keratinocytes (skin cells). Compared to untreated controls, the addition of 0.1% orange oil to MRSA-infected keratinocytes decreased the number of live MRSA cells by 99% in 1 h and in VISA strain 99.9% reduction was observed in 1 h. After 3 h viable *S. aureus* cells were not detected in the 0.2% orange oil treatment. These concentrations did not show any cytotoxic effect on the human skin keratinocyte cells *in vitro*. This provides preliminary evidence to suggest that orange essential oil could be a safe and useful addition to wound dressings as a complementary or alternative therapy to control antibiotic resistant bacteria-infected wounds (Muthaiyan et al., 2012).

Alginate dressings, which are extensively used in wound care, were impregnated with eucalyptus essential oil by incorporating the alginate fibres with the essential oil during the spinning process. The addition of the eucalyptus essential oil significantly increased the antimicrobial activity of the dressing against *S. aureus*, suggesting that this could be a useful addition to wound care. The authors also noted the anti-inflammatory activity of eucalyptus essential oil, which would also be a useful for wound healing (Khajavi et al., 2013).

Gelatine films impregnated with thymol were also tested for their antimicrobial activity against *S. aureus*, *P. aeruginosa*, *Escherichia coli* and *Bacillus subtilis*, common wound pathogens, using simple *in vitro* tests. All bacterial test species were inhibited with 1% thymol in gelatine film, with *S. aureus* and *B. subtilis* being the most susceptible. 1% thymol inhibited over 50% of these two species, 4% thymol inhibited over 50% of *E. coli* and 8% inhibited over 50% of *P. aeruginosa* (Kavoosi, Dadfär and Purfard, 2013). As such this could be a useful addition to wound dressings, however the levels required to inhibit *P. aeruginosa* may be too high to be tolerated by the patient, so further research would need to be carried out.
Geranium essential oil was tested for antimicrobial activity against Gram-negative bacteria isolated from wounds. Gram-negative bacteria often infect wounds late in the course of chronic wound degeneration, and are often resistant to antibiotics, making the wound hard to treat. Geranium was found to inhibit the growth of all 5 wound isolates \textit{in vitro} at concentrations ranging from $3.0\mu l/ml$ to $10.5\mu l/ml$ (0.0105ml in 1 ml of solvent). The antimicrobial activity of geranium against these strains did not correlate with that of the antibiotics, to which they were resistant, suggesting that geranium could be used as alternative treatment for Gram-negative wound infections. The anti-inflammatory properties of geranium could also be a useful addition for wound care (Sienkiewicz et al., 2014).

Patchouli, tea tree, geranium, lavender essential oils and a grapefruit seed extract called Citricidal were used singly and in combination to assess their antibacterial activity against \textit{S. aureus}, MRSA and epidemic MRSA (EMRSA). A dressing model constructed of four layers of dressings: the primary layer consisted of either Jelonet or TelfaClear with or without Flamazine; the second was a layer of gauze, the third a layer of Gamgee and the final layer was Crepe bandage. The oil combinations were placed in either the gauze or the Gamgee layer. This four-layered dressing was placed over the inoculated agar plate, incubated and the zones of inhibition measured. No anti-bacterial effects were observed when Flamazine was smeared on the gauze in the dressing model. When Telfaclear was used as the primary layer in the dressing model compared to Jelonet, greater zones of inhibition were observed. A combination of Citricidal and geranium oil showed the greatest anti-bacterial effects against MRSA, whilst a combination of geranium and tea tree oil was most active against the \textit{S. aureus} (Edward-Jones et al., 2004).

A recent study investigated the antimicrobial activity of tea tree oil and silver ions against wound microorganisms, alone and in combination. These materials were encapsulated within liposomes, which would improve the delivery when used in the wound dressings. The encapsulated materials were tested \textit{in vitro} against \textit{P. aeruginosa} and \textit{S. aureus} and all exhibited antimicrobial activity. Tea tree oil and silver ions were found to act synergistically against both test species, reducing the quantities of each needed to inhibit or kill the bacteria, which may help avoid potential side effects when used \textit{in vivo}, thus could be potential candidates for use in alternative wound dressings (Low et al., 2013).

As discussed above biofilms are a big issue in wound treatment. Several essential oils have been found to inhibit the formation and disrupt existing biofilms, however little research into essential oils in wound dressings as actually been formed. However, an
interesting study by Anghel et al. (2012) investigated the development of optimised coatings for wound dressings to prevent the formation of biofilms. The group created so-called 'hybrid phyto-nanostructures' comprised of a mixture of limonene or eugenol, which have strong antimicrobial properties, and magnetic iron oxide nanoparticles, to stabilize the essential oil components for a sustained release and help prevent bacteria adhering to surfaces to form a biofilm. They investigated the anti-biofilm activity against S. aureus and Pseudomonas aeruginosa, 2 bacterial species that are commonly found to infect wounds and form biofilms. The dressing containing limonene significantly reduced both the initial stage of biofilm formation and the development of a mature biofilm, as comparing with control, uncoated textile materials in both S. aureus and P. aeruginosa, which appeared not to be significantly different in susceptibility. The eugenol-containing dressing was also found to have significant antibiofilm activity, producing a pronounced biofilm inhibition on both S. aureus and P. aeruginosa at 24, 48 and 72 hours. The fact that anti-biofilm activity was observed at 72 h for both modified dressings demonstrates that the nanostructure reduced the volatility of the essential oil components without affecting their activity, suggesting that this could be a valuable method of coating wound dressings to prevent chronic, difficult to treat infections forming.

Healing effect of essential oils

Seabuckthorn is reputed for its healing properties in aromatherapy, and its leaves, fruits and seeds have been found to be a rich source of a large number of bioactive substances including flavonoids carotenoids (e.g. β-carotene and lycopene), vitamins (A, C, E and K), tannins, essential fatty acids and some essential amino acid. These bioactive substances reportedly lend to seabuckthorn’s antioxidant and anti-inflammatory activity, and its reported skin-healing activity. One investigation corroborated the wound-healing activity of sea buckthorn in experimentally-induced wounds in rats. Various concentrations of the oil were applied topically, twice daily for 7 days. Treatment with silver sulfadiazine ointment was used as control. The sea buckthorn treated group showed faster reduction in wound area in comparison with control (no treatment) and silver sulfadiazine-treated groups. The most effective concentration of the extract was 5.0% for burn wound healing. It’s mechanism of action was investigated, and concluded to be increased collagen synthesis and stabilization at the wound site, antioxidant activity and promotion of angiogenesis (creation of new blood vessels) (Uphadyay et al., 2011).
St. John’s Wort oil (*Hypericum perforatum*) was also found to significantly increase wound healing in experimentally induced burn wounds in rats compared to controls and silver sulfadine at days 10 and 17, suggesting that this oil may be useful as a complementary therapy in wound care (Peksen et al., 2014).

Hartman and Coetzee (2002) reported the effect of lavender and chamomile on wound healing in a five patients with chronic wounds of three to four months’ duration. The wounds were graded using the US National Pressure Ulcer Advisory Panel (NPUAP) guidelines based on depth and visual characteristics, and were treated with a 6% solution of two drops of lavender oil and one drop of German chamomile applied directly to the wound and added to the dressing. The wounds treated with the oils healed significantly quicker than the controls.

Dursun et al. (2003) investigated the impact of thyme oil on nitric oxide, an important inflammatory mediator in burns injury experimentally induced in rats. Their aim was to elicit any potential protective action of thyme oil on burns-induced nitric oxide production which could aid in healing. It was confirmed that nitric oxide was overproduced by the burn injury and found that thyme oil not only decreased the amount nitric oxide produced in response to burns injury but also facilitated wound healing. The oil’s thymol content may play a role in this wound-healing activity, as one investigation found that wounds dressed with dressings containing collagen-based films containing thymol showed significantly bigger wound retraction rates, at 7 and 14 days, improved granulation, and improved collagen density and arrangement during wound healing, compared to controls (Riella et al., 2012).

Wound-healing activities of aniseed, clove, cumin, fennel, laurel, lavender and melissa oils were investigated in one study. Topical treatment of incised wounds of rats with the lavender and laurel exerted the best wound tensile strength on day 10, with the values of 30.5 and 27.2%, respectively, while the rest of the essential oils did not display any significant healing effect. Circular excision wounds were also investigated, which allows the reduction in the wound area to be measured. This also revealed that lavender and laurel possess excellent wound-healing effect with a reduction of wound area by 39.05 and 42.22%. Notably, the groups treated with clove and cumin displayed delayed wound healing (Suntar et al., 2013).
References


