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Reactive Oxygen® made easy

Introduction

Reactive oxygen species (ROS) are part of the body's antimicrobial response and have a major role in wound healing. Members of the ROS family include O_2^-, O_2^{-2}, H_2O_2. The term Reactive Oxygen® refers to the antimicrobial mechanism for SurgihoneyRO™. SurgihoneyRO™ is an antimicrobial wound gel that delivers precisely controlled and sustained, therapeutic levels of Reactive Oxygen® to the wound bed. SurgihoneyRO™ has been shown to have fast-acting antimicrobial action, and the ability to prevent biofilm formation, eradicate mature biofilm and kill almost all of the 12 multi-drug resistant bacteria listed in a World Health Organization (2017) report, including all those commonly found in chronic wounds.

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SETTING THE SCENE

Wound infection is a common complication of wounds, significantly delaying wound healing. Infection negatively affects quality of life for patients, families and carers, and increases the risk of loss of limb and life (International Wound Infection Institute [IWII], 2016). Chronic wound infection can be financially costly to healthcare organisations and to patients, in terms of prescriptions, time off work etc.

Guest et al (2015) estimated that the annual NHS cost of managing wounds and associated co-morbidities is £5.3 billion, 78% of which is for managing chronic wounds. Wound chronicity is often suggestive of infection or biofilm (Box 1). It is difficult to calculate prevalence and economic cost of chronic wounds as there is currently no universally accepted diagnostic standard (Gardner et al, 2009). However, for surgical site infection, which has a well-established definition, the mean cost per patient is €5800, based on European data and mean additional length of stay of 11 days (Posnett et al, 2009).

Costs associated with chronic infection and biofilm include hospital stays and antibiotics (Filius and Gyssens, 2002; Gottrup et al, 2013). Antibiotic and antimicrobial misuse is frequent among those with infected and uninfected wounds, especially among vulnerable patients in long-term care facilities (Nicolle, 2014). Antibiotic misuse increases the risk of antimicrobial resistance – an increasing global issue (World Health Organization [WHO], 2016).

Box 1. What's what? Biofilms

A wound biofilm is a dynamic community of diverse microbes that develop on or near the wound surface. It is associated with persistent inflammation and wound chronicity (Bjarnsholt et al, 2006; James et al, 2008; IWII, 2016). Biofilms develop quickly after initial colonisation and are difficult to eradicate by surface irrigation or superficial debridement (Swanson et al, 2014). Biofilm-encased bacteria may show increased resistance to traditional antibiotics and biocides, and appear to allow the bacteria to resist antimicrobial agents and host defences in the wound environment (Cutting et al, 2016).

There is much debate on whether biofilms are visible to the naked eye; however, studies estimate that 60–100% of non-healing chronic wounds have a biofilm present (World Union of Wound Healing Societies [WUWHS], 2016). Clinicians should assume that all non-healing, chronic wounds that have not responded to standard care have a biofilm (WUWHS, 2016), and treatment should be targeted towards this.

INFECTION AND BIOFILM MANAGEMENT

Determining infection

If infection is suspected following holistic assessment, there are established visual signs and symptoms to diagnose wound infection, such as pain, malodour, inflammation and erythema, (IWII, 2016). In some cases, microbiology testing may be appropriate, e.g. wound culture or swabbing (Levine technique), needle aspiration or tissue biopsy. Tissue biopsy is the 'gold standard', but costly and uncomfortable for the patient, and should only be performed in an appropriate health care setting by a trained professional. Hand-held real-time imaging technologies are also available to visualise bacteria, e.g. MolecuLight i/X (MolecuLight, Toronto, Canada).

Wound bed preparation

Principles to promote a healthy wound bed include therapeutic wound cleansing, disruption of biofilm and removal of necrotic, non-viable tissue through wound debridement (IWII, 2016). Debridement is one of the most important treatment strategies against biofilms, but does not

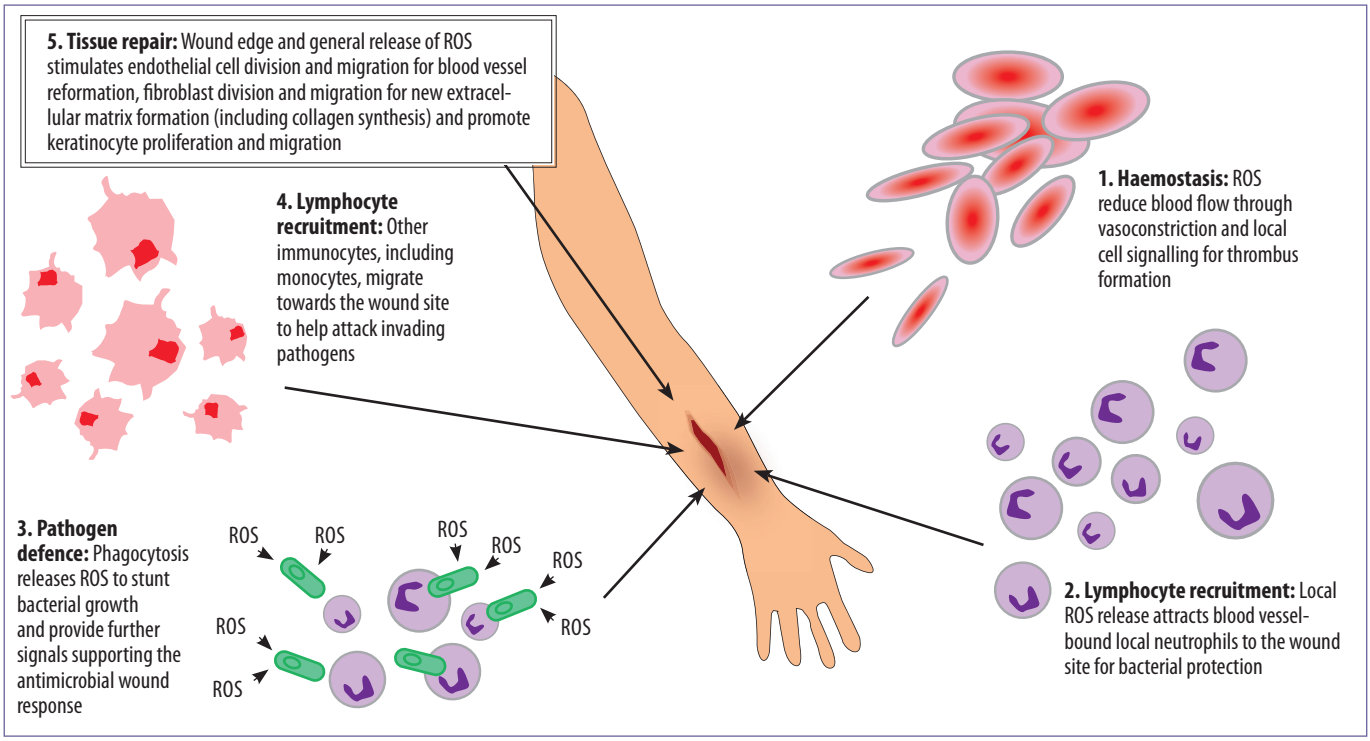

Figure 1. Roles of reactive oxygen species (ROS), e.g. H_2O_2, in wound healing (adapted from Dunnill et al, 2017)

necessarily remove all biofilm (Schultz et al, 2017). Adequate analgesia should be administered prior to debridement. A longer term review of analgesia should be considered for the duration of antimicrobial therapy to ensure patient comfort and tolerance throughout the treatment phase.

Antimicrobial agents

Despite a lack of evidence in the literature, broad-spectrum systemic antibiotic therapy is often used indiscriminately until healing occurs for an infected or suspected to be infected wound Abbas et al, 2015). Repeated courses of antibiotic therapy potentiate the selection of resistant bacteria and increase the risk of antibiotic-resistant infections, impacting negatively on costs and patient outcomes (Gottrup et al, 2013).

Timely use of topical antimicrobial agents to the wound bed is critical to prevent and manage infection locally, avoid unnecessary use of systemic antibiotics, and reduce the risk of antimicrobial resistance (Lipsky and Hoey, 2009). While there are many antimicrobial products available. Products with a sustained release of antimicrobial agent at low concentrations to minimise toxicity, but still able to destroy or inhibit bacterial and fungal growth, are recommended (IWII, 2016).

AN ANTIMICROBIAL AVENUE: REACTIVE OXYGEN SPECIES

The term reactive oxygen species (ROS) describes highly reactive molecules containing reduced oxygen or oxygen atoms with extra electrons. Members of the ROS family include superoxide anion O_2^- , peroxide O_2^{-2}, hydrogen peroxide H_2O_2.

ROS are naturally occurring in the body. Basal ROS levels maintain normal cell functioning and homeostasis (Trachootham et al, 2008), while increased amounts induce a cell-mediated antimicrobial defence response (Shen and Pervaiz, 2009).

ROS are part of the innate immune response, and play a critical role in wound healing and infection control at the wound site (Dunnill et al, 2017). They are released within minutes of epithelial injury, signalling rapid recruitment of leukocytes (Stone and Collins, 2002). ROS directly damage microbes that invade tissues (Lau et al, 2008). In wound healing, ROS are secondary messengers to many immunocytes and non-lymphoid cells, and regulate angiogenesis, haemostasis and tissue repair (Dunnill et al, 2017) (Figure 1).

REACTIVE OXYGEN®

Reactive Oxygen® is the antimicrobial mechanism for SurgihoneyRO™. In contact with moisture, SurgihoneyRO™ releases Reactive Oxygen®.

In vitro studies have shown that Reactive Oxygen® at close to naturally occurring levels:

- stimulates proliferation of human fibroblasts and vascular endothelial cells (Murrell et al, 1990; Stone and Collins, 2002)
- stimulates angiogenesis via vascular endothelial growth factor (VEGF) signalling (Cho et al, 2001)
- promotes keratinocyte cell migration and proliferation (Loo et al, 2011).

A controlled and sustained level of H_2O_2 that is non-harmful and non-toxic to surrounding tissue, is a therapeutic opportunity for wound care (Box 2). SurgihoneyRO™ does this through the release of Reactive Oxygen®.

WHAT IS SURGIHONEYRO™?

SurgihoneyRO™ is an antimicrobial wound gel that has been bioengineered to provide a consistent level of antimicrobial activity through the production of precisely controlled levels of Reactive Oxygen® (equivalent to what happens in the body's natural defence systems). When the honey carrier is activated by moisture Reactive Oxygen® is released at a sustained and therapeutic level to the wound bed. The charged oxygen atom 'steals' electrons binding to proteins in the bacterial wall, cytoplasm, and nuclear content of the cells. The bacterial cell walls rupture, resulting in cell death (Figure 2).

What is the difference between SurgihoneyRO™ and medical-grade honeys?

Medical-grade honey is an established topical treatment for wounds and burns due to its antimicrobial and wound healing promotion activity. It provides rapid autolytic debridement, reduces odour, and decreases wound-related pain and bio-burden (Wounds UK, 2013). Honey works in a different way to antibiotics – it is hypersomolar, restricting the availability of water to bacteria and dehydrating bacteria by osmosis.

Natural honeys vary hugely in their antimicrobial properties and there is little consistency in antimicrobial effects because there can be no control over nectar source, sugar content and other contaminants in entirely natural products. Many medicinal honeys are based on Manuka honey, which is one of the more antimicrobial natural honeys. Different mechanisms of action have been suggested for the antimicrobial effects of honey, but the main ones are the high-sugar concentration, H_2O_2, the 1,2-dicarbonyl compound methylglyoxal (MGO), the cationic antimicrobial peptide bee defensin-1 and the low pH (Kwakman et al, 2011). Some patients may experience momentary stinging or a drawing sensation when using honey therapies due to its autolytic debriding properties and the drawing of fluids by osmosis away from the wound (Zbuchea, 2014).

SurgihoneyRO™ has all the natural healing-inducing, desloughing and tissue regenerative properties of the honey carrier, but it has been bioengineered to deliver precisely controlled level of Reactive Oxygen®. When SurgihoneyRO™ comes into contact with moisture, Reactive Oxygen® is instantly released and sustained for over 72 hours (Dryden et al, 2014a).

Box 2. History of hydrogen peroxide in wound healing

3% hydrogen peroxide solution has been used as an antiseptic since the 1920s. Historically, the therapeutic dosage used in wound care was 300 times higher than the naturally occurring dose, and was indiscriminate between healthy and unhealthy tissue. Therefore, hydrogen peroxide solution is no longer recommended in wound care except in low-resource, developing nations, where alternative, contemporary antiseptics are not always available (IWII, 2016).

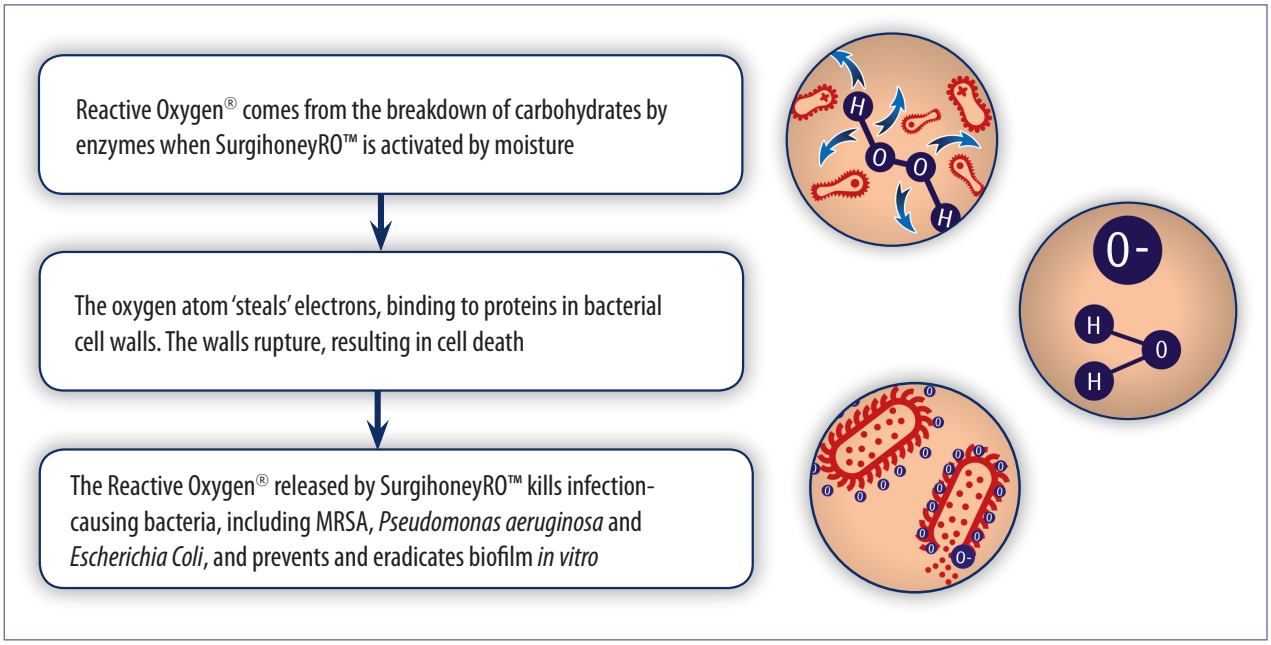

Figure 2. SurgihoneyRO™ mode of action

Table 1. Summary of *in vivo* clinical studies using SurgihoneyRO™

Reference	Name of paper	Summary of results
Dryden et al, 2014b	Using antimicrobial Surgihoney to prevent caesarean wound infection	All women presenting for caesarean section were offered SurgihoneyRO™ as a single application wound dressing at the end of the procedure to assess its effect on surgical site infection (SSI) rate. The SSI rate was compared to the infection rate in the 9 months prior to the evaluation. Thirty days later, a single application of SurgihoneyRO™ dressing had reduced SSI rate by 60.33% from a rate of 5.42% (*n*=590) to 2.15% (*n*=186).
Dryden et al, 2014c	The use of Surgihoney to prevent or eradicate bacterial colonisation in dressing oncology long vascular lines	The study population consisted of oncology patients with central intravenous lines who were receiving outpatient chemotherapy. SurgihoneyRO™ was applied to the line exit site to assess its effects on bacterial colonisation in long lines. There were 30 patients in each study arm – with or without SurgihoneyRO™. In the SurgihoneyRO™ arm, two patients with existing line site colonisation were cleared of bacterial colonisation and none acquired colonisation during the study period. In the non-treatment arm, 6 patients were colonised at the line site prior to screening or during the evaluation and colonisation was maintained throughout the evaluation.
Dryden et al, 2016	A multi-centre clinical evaluation of reactive oxygen topical wound gel in 114 wounds	Over a mean duration of 25.7 days treatment, a multi-centre clinical evaluation of 114 wounds (ulcers, surgical wounds and trauma wounds) in 104 patients showed: ■ 100% of wounds improved during the evaluation ■ 100% of wounds reduced in size ■ 21% of wounds healed ■ 2.6% of patients reported stinging during the evaluation. There was a reduction in wound pain, exudate production, devitalised tissue and wound bacterial load, assessed by a reduction in slough and necrotic material. Of the 40 wounds swabbed, 39 showed a reduction in bacterial load, confirming the antimicrobial activity of SurgihoneyRO™.

SurgihoneyRO™ has been shown to:

- have fast-acting antimicrobial action (Dryden et al, 2014a)
- prevent and disrupt biofilm formation (Halstead et al, 2016a; 2016b)
- eradicate mature biofilm (Davies et al, 2018)
- reduce pain and inflammation (Dryden et al, 2016)
- kill a wide range of multi-drug resistant (MDR) bacterial strains (Thomas and Westgate, 2018a; 2018b).

SURGIHONEYRO™ ACTIVITY AGAINST MULTI-DRUG RESISTANT BACTERIA

The WHO (2017) published a list of 12 families of antibiotic-resistant "priority pathogens" that pose a threat to human health. SurgihoneyRO™ is the first published, antimicrobial wound dressing that has been tested against the 12 priority MDR bacterial strains, and has been shown to be effective against almost all these organisms (Thomas and Westgate, 2018b).

The WHO priority pathogen list includes four MDR bacterial strains commonly found in chronic wounds: carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *Pseudomonas aeruginosa*, *VRE* and meticillin-resistant *Staphylococcus aureus* (MRSA). Treatment with SurgihoneyRO™ antimicrobial wound gel was shown to kill these MDR bacterial strains, plus *Streptococcus pyogenes*, *S.aureus*, *Corynebacterium minutissimum*, *Staphylococcus epidermidis* and *Escherichia coli*. SurgihoneyRO™ presents a viable treatment option where MDR organisms are suspected or where the risk of emerging MDR organisms is high (Thomas and Westgate, 2018a).

SurgihoneyRO™ has been successfully used for infection prevention, eradication of MDR bacterial strains and prevention of surgical site infections and intravascular line care *in vivo* (Table 1; Dryden et al, 2014b; 2014c; 2016).

SURGIHONEYRO™ ACTIVITY *IN VITRO*

Early use of SurgihoneyRO™ on infected wounds can have a positive impact on bio-burden and biofilm, sparing conventional antibiotic use, and supporting infection control (Dryden et al, 2014a; 2014b; 2014c; Halstead et al, 2016a). *In vitro* studies have shown:

- SurgihoneyRO™ has been shown to be effective at reducing dispersal of pre-formed biofilms of 16 clinically relevant wound pathogens *in vitro*, and also has anti-biofilm activity (Halstead et al, 2016a).
- SurgihoneyRO™ is rapidly active *in vitro* against all Gram-positive and Gram-negative bacteria tested, including MDR bacterial strains. It has been shown to be more active than other honeys against MDR bacterial strains (Figure 3), and is comparable with synthetic antiseptics, such as iodine (Dryden et al, 2014a).

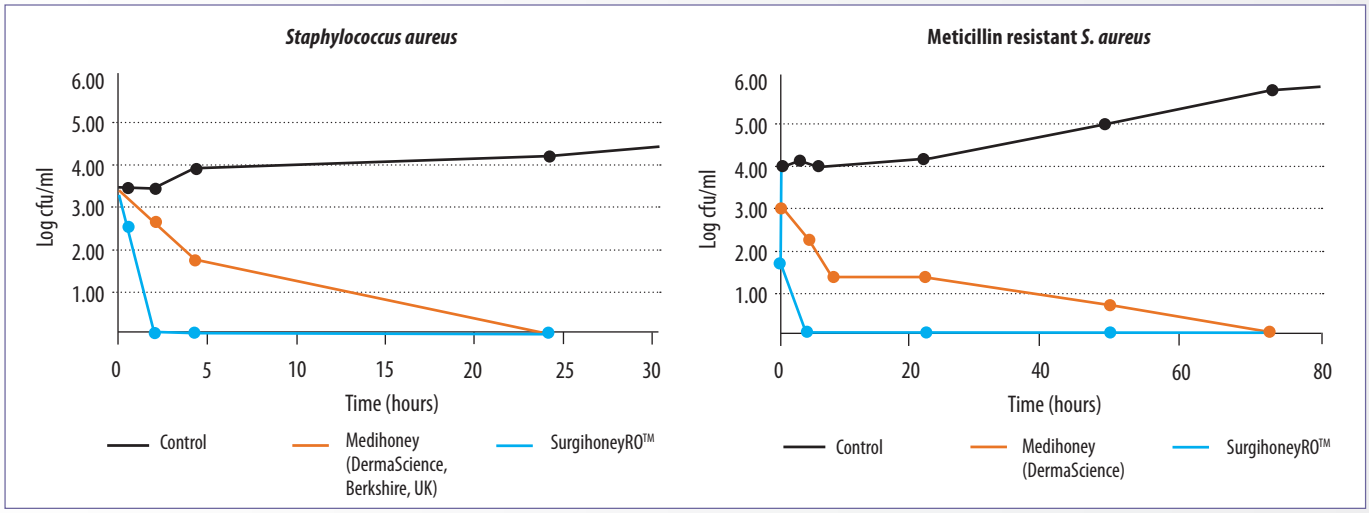

Figure 3. Time-kill curves of *S Staphylococcus aureus* and MRSA show the speed of cidal activity to be extremely rapid. After 30 minutes the bacteria had fallen 1,000 fold, with all bacteria killed within 48 hours (Dryden et al, 2014a)

- Halstead et al (2016b) investigated the ability of SurgihoneyRO™ and comparators to prevent biofilm formation of 16 isolates *in vitro*. SurgihoneyRO™, two medical-grade honeys and five other honey or silver antimicrobial dressings were compared. All honey products were antimicrobial and able to prevent the formation of biofilms, but SurgihoneyRO™ was most potent, with efficacy at lower dilutions than medical-grade honeys for five isolates, and equivalent dilutions for a further six. Additionally, SurgihoneyRO™ was superior in antimicrobial potency to three commercially available honey dressings.
- The abilities of SurgihoneyRO™ and a cadexomer iodine dressing to disrupt pre-formed 72-hour mixed species biofilms were compared. No viable *P. aeruginosa* or *S. aureus* were recovered after 24 hours of treatment, suggesting the treatments were comparable as they could both treat pre-formed biofilms (Davies et al, 2018).

WHAT IS SURGIHONEYRO™ INDICATED FOR?

SurgihoneyRO™ is indicated for all stages of wound healing in a variety of acute and chronic wounds, e.g. ulcers, surgical wounds, trauma wounds, cuts, abrasions and burns, donor and recipient sites, infected wounds, and wounds with suspected biofilms. It can be used to prevent and treat low-grade localised infection (Dryden, 2016). It is also safe to be used on patients with diabetes under appropriate medical supervision.

Public Health England's (2017) guidance on management and treatment of common infections in primary care recognised SurgihoneyRO™ as a treatment for venous leg ulcers to reduce bacterial load and infection. It states that using SurgihoneyRO™ could cut unnecessary antibiotic prescribing.

HOW TO USE SURGIHONEYRO™

Cleanse the wound as per local protocols, and apply a 2mm layer SurgihoneyRO™ directly to the wound bed. Alternatively, SurgihoneyRO™ can be applied to an inert wound contact or secondary dressing then placed on the wound bed. Select an appropriate secondary dressing for exudate level and to maintain moist wound healing environment.

It is recommended that SurighoneyRO™ antimicrobial therapy is applied every 72 hours (3 days) and more frequently in critical wound infections and heavily exuding wounds to maximise effect. For critically infected wounds, it is recommended that SurgihoneyRO™ should be applied daily, then reduced to alternate days, then to every 3 days over the first 2 weeks (subject to individual clinical scenario). If there is no improvement in the wound condition and microbial status, use should be reviewed. SurgihoneyRO™ is a single patient, multi-use product that once opened may be used for up to 28 days.

SurgihoneyRO™ may be used under compression bandaging for up to 7 days; however, the potency will decrease after day 3. With a low therapeutic dose of H_2O_2, there are no contraindications associated with long-term use, and it is non-toxic to healthy tissue. It may be used on children,

pregnant and lactating women. Side effects are mild and self-limiting, including some reports of stinging on application, which is a common side effect with honey-based products (Zbuchea, 2014).

SUMMARY

Reactive Oxygen® has a pivotal role in infection management and wound healing. *In vitro* studies have shown that at close to naturally occuring levels, Reactive Oxygen® activates an antimicrobial response, and stimulates angiogenesis and tissue repair. The Reactive Oxygen® released by SurgihoneyRO™ is a broad-spectrum antimicrobial against wound-relevant MDR bacterial strains, and has been shown to prevent and disrupt biofilm. SurgihoneyRO™ presents one option against the growing global issue of antimicrobial resistance.

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