**Reactive oxygen species (ROS) and wound healing: functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process**

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**Running title:** Reactive oxygen species and wound healing

**Abstract**

Reactive oxygen species (ROS) play a pivotal role in the orchestration of the normal wound healing response. They act as secondary messengers to many immunocytes and non-lymphoid cells, which are involved in the repair process, and appear to be important in coordinating recruitment of lymphoid cells to the wound site and effective tissue repair. ROS also possess the ability to regulate the formation of blood vessels (angiogenesis) at the wound site and the optimal perfusion of blood into the wound healing area. ROS act in host defence through phagocytes which induce an ROS burst onto pathogens present in wounds, leading to their destruction, and during this period excess ROS leakage into the surrounding environment has further bacteriostatic effects. In light of these important roles of ROS in wound healing, and the continued quest for therapeutic strategies to treat wounds in general and chronic wounds, such as diabetic foot ulcers, venous and arterial leg ulcers and pressure ulcers in particular, manipulation of ROS represents a promising avenue for improving wound healing responses when they are stalled. This article presents a review of the evidence supporting the critical role of ROS in wound healing and infection control at the wound site, and some of the new emerging concepts associated with ROS modulation and its potential in improving wound healing are discussed.

**Key words:** reactive oxygen species, wound healing, host defence, wound infection

**Introduction**

A wound may be described as the sequel of damage to an epithelial surface and its underlying connective tissues which may be complicated by underlying excessive tissue damage, underlying pathology and poor tissue perfusion and oxygenation. They can be categorised into two main types:

**Acute wounds**, which heal normally through optimal haemostatic and inflammatory cascades with tissue repair and regeneration after surgery, burns or trauma within a time frame of approximately 30 days;

**Chronic wounds**, which do not heal in normal time frame because of a disruption of these phases (discussed below) and persistent underlying pathologies, especially infection.

Our ageing population, combined with an increased increase in cardiovascular and neurological diseases and diabetes, means that there is an increasing number of patients presenting with these chronic and often complicated wounds [1-3]. Consequently, manipulation of obtunded healing processes in these individuals is required to reduce the morbidity, mortality, hospital resource and economic cost currently associated with this increasing health burden [4, 5].

Optimal wound healing, the process observed in an acute wound, has been divided into four chronologically overlapping phases which follow platelet exposure to collagen and extracellular matrix (ECM): (i) vasoconstriction and coagulation, collectively leading to haemostasis, ii) acute inflammation, iii) cellular proliferation and iv) wound remodelling (for detailed description of these phases see [6, 7]. In brief, coagulation leads to the formation of platelet thrombus and a fibrin clot, followed by an acute inflammatory response which gives early protection against contaminating bacteria, comprised first by the recruitment of pathogen-destroying phagocytic neutrophils and later through macrophages. Both platelets and macrophages in the wound area release growth factors and pro-inflammatory cytokines, which regulate lymphoid cell-mediated antimicrobial defence, as well as keratinocyte, endothelial and fibroblast activation. Mast cells also release histamine and other mediators which cause surrounding vessels to become permeable to the immunocytes, already present in the wound, to potentiate their effects. Thereafter, a complex proliferative phase begins, characterised by the deposit of new ECM and the deposition of collagen and fibronectin by fibroblasts and their differentiated counterparts, the myofibroblasts. Angiogenesis is enhanced by endothelial cell division, triggered by vascular-promoting growth factors, and leads to the formation of tissue granulation and the final stages of wound healing, including re-epithelialisation and tissue remodelling. During the tissue remodelling phase, fibroblast-rich granulation tissue is gradually replaced with a relatively acellular scar and cross-linked collagen molecules give the scar tensile strength that approaches that of intact skin. During this process keratinocytes also stimulate fibroblasts to synthesise growth factors, in a paracrine manner, which triggers both cell types to proliferate [6-8].

**The role of oxygen and its radicals in cellular homeostasis**

Oxygen (O2) is the essential substrate required for high mitochondrial-driven ATP yields and, in the context of wound healing, it supplies the increased amount of energy required for tissue renewal. Radical derivatives of O2,known as ROS are also paramount to this process, as they act as secondary messenger signalling molecules. The term ‘ROS’, applies to molecules containing O2, but which have been reduced with added electrons to become a highly reactive, radical format. Well-known members of the ROS family of molecules are superoxide anion ∙O2-, peroxide ∙O2-2, hydrogen peroxide H2O2, hydroxyl radicals ∙OH, and hydroxyl OH-ions. Endogenous cellular ROS can arise from mitochondrial oxidative phosphorylation during ATP production, from the endoplasmic reticulum, or from a class of enzymes known as oxidoreductases. ROS ‘steal’ electrons from other nearby molecules, via an oxidation reaction, which damages the structure of the latter. When it comes to the role of ROS in cellular homeostasis, there is evidence to suggest that:

(i) aberrantly low levels of ROS induce cell cycle arrest (i.e. are cytostatic)

(ii) basal ROS levels maintain normal cell functioning and homeostasis

(iii) increased amounts induce a number of transcription factors to drive a cell-mediated defence response

(iv) excessive ROS induction activates pro-apoptotic proteins for subsequent induction of cell death, and in extreme cases cellular necrosis [9, 10].

In addition, ROS regulate vascular constriction (vasoconstriction) and vascular relaxation (vasodilation). However, radical forms of O2 containing nitrogen, such as nitric oxide (NO), regulate the latter. NO belongs to a group of radicals known as reactive nitrogen species (RNS) which are also generated as part of normal physiology following NO oxidation. NO spontaneously reacts with a number of molecules including molecular O2, ROS, transition metals and thiols or, to yield nitrosyl-metal complexes, S-nitrosothiols, N2O3, NO2-, and ONOO-.NO conversely also acts as an antioxidant defence against ∙O2- and this with peryoxynitrate (ONOO-)constitutes the major RNS in biological systems. During the wound healing response a number of cells utilise these radicals, including platelets, macrophages, fibroblasts, endothelial cells and keratinocytes [11] (Figure 1).

**Antioxidant-mediated regulation of cellular ROS levels**

The homeostatic control of cellular ROS levels (redox state) is the role of a specialist group of proteins known as antioxidants. The antioxidants are a system of proteins designed to remove the deleterious effects of ROS and they do so by donating their own electrons, thus preventing them from capturing electrons from other important molecules, such as DNA, proteins and lipids. Known members of the antioxidant system are Thioredoxin-1 (Trx-1) and -2 (Trx-2), glutathione (GSH) related glutathione s-transferases (GSTs), superoxide dismutase (SOD), glutathione peroxidases (GPx), NADP(H) quinone oxidoreductase (NQO1), catalase, epoxide hydrolase, heme oxygenase-1 (HO-1), UDP-glucuronosyl transferases (UGTs) and glutamylcysteine synthetase. In addition to proteinaceous ROS control, cells also utilise non-enzymatic metabolites which are small antioxidant molecules, such as vitamin C, vitamin E, β-carotene, glutathione, coenzyme Q, bilirubin, α-tocopherol, nicotinamide adenine dinucleotide phosphate (NADPH) and urate. Moieties with a metal ion capable of oxidation/reduction reactions, such as transferrin and ferritin, possess an increased ROS-scavenging capability [12, 13]. Oxidative stress may be assessed by examining the oxidised format of GSH, known as GSSG, and also by screening for redox-sensitive kinases such as ASK-1, p38 and JNK and also, transcription factors such as NF-κB and particularly AP-1 [14, 15]. In light of the fact that so many proteins respond to oxidative stress, cells have evolved to utilise ROS not only to signal but also to warn of potential cell pathophysiology.

**Oxidative stress and impaired wound healing**

In this review, the role of ROS in wound healing is discussed together with the potential manipulation of ROS as a promising therapeutic avenue. Our aim was to focus on the positive effects that ROS can exert in the wound healing process and to discuss relevant strategies for ROS-based enhancement of the process.

However, it is important to note that in addition to the positive influence that low ROS levels can have on wound healing (discussed below), excessive ROS production leads to oxidative stress that can have detrimental effects on wound healing. Elevated and sustained ROS have been detected *in vivo* and have been associated with impaired wound repair in chronic, non-healing wounds [16]. At the molecular level, in addition to ROS-mediated transcription that can lead to sustained pro-inflammatory cytokine secretion and induction of matrix metalloproteases, excessive ROS and RNS can directly and indirectly (via proteolysis activation) modify and/or degrade ECM proteins and also cause impaired dermal fibroblast and keratinocyte function [17]. In fact, it is clear that the precise balance between low *versus* high levels of ROS is critical in determining functional outcome: low levels of ROS are essential in stimulating effective wound healing [18], whereas excessive ROS release results in cellular damage and impaired wound repair [19]. One approach to manipulate ROS indirectly, as a wound healing strategy, could be to manipulate the antioxidant system instead. Interestingly, N-acetyl cysteine (NAC), a well characterised thiol-containing antioxidant decreases ROS levels and favours the formation of NO and has been suggested as a highly promising entity for the enhancement of wound healing [11, 20].

The negative impact of sustained, high ROS levels in wound healing and its clear importance in chronic wounds, as well as the prevention of excessive ROS levels and the utilisation of the antioxidant system as a strategy to improve stalled wound healing have been extensively reviewed in the literature [12, 21] and are not within the scope of this review.

**ROS and protection from wound infection**

As mentioned above, ROS (and also RNS) play an integral role in host defence, particularly during wound healing, as phagocytic neutrophils and macrophages utilise their reactive and destructive properties (Figure 1). These cells are able to engulf bacteria within a phagosome and this triggers four NADPH oxidase cystolic subunits (p47, p67, p40 and Rac2) in proximity with a membrane subunit (cytochrome b558). An intense uptake of O2, known as the respiratory burst, occurs whereby NADPH reduces molecular O2 in the phagosome to either ∙O2- or H2O2; this creates lethal levels of ROS (and RNS) which can destroy an engulfed pathogen [22]. As phagocytic macrophages and neutrophils destroy invading microbes in their phagosomes, they release high concentrations of H2O2 and this stunts growth of adjacent, contaminating bacteria [23, 24]. Bacteriostatic effects of H2O2 in *E.coli* have been shown at concentrations between 25–50 μM, but concentrations exceeding 500 μM were needed to eradicate the bacteria [25]. Importantly, therefore, the ability of professional phagocytes to secrete H2O2 into the extracellular milieu, rather than solely retaining it in the phagosome, means that ROS can exert their antimicrobial properties throughout the wound area. It has also been demonstrated that a H2O2 gradient is formed around the wound margin, reaching a distance of approximately 100 to 200 µm, and supports this concept [26]. Further support of this notion can be seen in individuals who have defects in their respiratory burst process and are prone to bacterial and fungal infection, particularly in the lungs, in X-linked recessive chronic granulomatous disease (GCD). GCD sufferers have mutations in the NADPH oxidase system, thereby phagocytic cells cannot generate ∙O2- to help fight infectious challenges [27]. Moreover, as removal of infectious agents is essential in preventing complicated, chronic wounds, GCD patients are prone to poor wound healing [28].

An intense uptake and use of O2 during healing leads to tissue hypoxia and levelsof O2 left available influence the tissue repair response, as rapidly dividing cells involved in the healing process also require a greater supply to produce enzymes and proteins [20]. Furthermore, as the vascular supply is constricted in the early stages of the wound healing response (mediated by ROS release), this further contributes to lack of O2 bioavailability and the overall hypoxic state. Low partial pressures of blood O2 indeed reduces the rate of the mitochondria energy production and also, many endogenous enzymes that utilise O2 (mainly Nox members) deplete ROS from the area. This may deplete O2 levels to an extent that ROS mediate responses are affected as its generation relies upon O2 presence [29]. The resulting wound hypoxia, typify the poor wound healing observed in diabetic patients, particularly those with diabetic foot ulcers (DFU) [1]. Long-term damage by poorly regulated glucose levels can also lead to diabetic microvascular vascular damage, which causes further reduced blood perfusion in lower limb extremities. Furthermore, as tissue suffers from poor hypoxiathere is impaired ROS mediated angiogenesis and vasculogenesis, thus more exacerbating the problem in highly complicated wounds [1]. The consequences of lack of O2 have been thoroughly demonstrated at the molecular level; for instance the expression of α-smooth muscle actin, the main component of the myofibroblast cytoskeleton, decreases when there is a reduced supply of O2  [30]. Thus, collectively, normal wound healing requires not only O2,but also its radical format, ROS.

**Hydrogen peroxide and its role in wound healing *in vivo***

H2O2 is the main second messenger in wound healing responses and its levels are regulated at the wound edge by local antioxidant release, SOD, GPx, and phospholipid hydroperoxide glutathione peroxidase; this has been well demonstrated in models of mouse-based cutaneous injury [31]. In fact, the overall evidence for H2O2 having an integral role in stress/inflammatory responses and the subsequent tissue/neuron repair process is striking. The functional role of H2O2 is due to some of its fundamental properties; it is easily synthesized, easily degraded, it is present within all types of cells, it has a longer half-life than radical ROS and, most importantly, its small uncharged molecule allows it to diffuse freely through membranes and tissues. Moreover, it does not react indiscriminately with neighbouring molecules like other radicals tend to do [31-34]. A 10 μM concentration of H2O2 has been demonstrated to act as a chemo-attractant to inflammatory cells and this was shown to be without any dependence on blood bound signalling components [35] (Figure 1). H2O2 also stimulates the proliferation of human fibroblasts and vascular endothelial cells within a comparable concentration range [36, 37]. 500 µM H2O2 was found to stimulate the production of macrophage inflammatory protein (MIP)-1α which is a chemotactic ligand to mononuclear phagocytes, neutrophils, eosinophils, basophils and lymphocytes [35, 38]. It has also been shown that 100 µM H2O2 stimulates angiogenesis via vascular endothelial growth factor (VEGF) signalling [39] and is chemotactic to keratinocytes; low levels of H2O2 also promote keratinocyte cell migration and proliferation [40]. Using zebra fish wounded dorsal fins, it has been shown that H2O2 released by dual oxidase (DUOX) mechanism, spreads at the wound margin at a decreasing concentration gradient within minutes of epithelial injury and that this signals the rapid recruitment of leukocytes [26]. In addition, H2O2 was shown to be the key danger signal for haemocyte (*Drosophila* macrophage) recruitment to the wound, and that this also occurred via a DUOX mediated mechanism [41]. It was further demonstrated in *Drosophila* that calcium, released from the wound edge, travels distally in waves and that these trigger the release of H2O2 for haemocyte attraction [42]. Studies using *C. elegans* imply that the mitochondria may also play a role in wound healing by producing mitochondrial ROS (mROS), with the trigger for this demonstrating as Ca2+ [43].

**Therapeutic strategies based on modulation of ROS function**

Topical application of ROS intermediates, which are converted into biologically available O2, such as H2O2, tetrachlorodecaoxide, and benzoyl peroxide have been suggested as wound healing enhancement products, based on positive results from in vitro experiments [44]. H2O2 infused cream has also been tested *in vivo* for this purpose in Guinea pigs with ischemic ulcers. An increased blow flood to the wound occurred with use of H2O2-infused cream and was attributed to angiogenesis; specifically this effect was observed between 7-21 days post-trauma. Interestingly, undamaged (control) tissues treated with H2O2 also had an increased blow flow, but this was less than the ischaemic ulcers, thus emphasising that H2O2 is only one of many factors which might dictate the wound healing response. Importantly, there was no clinical evidence that the ischaemic ulcers showed improved healing related to increased perfusion. The addition of H2O2 in PBS has also been tested in mice with excisional wounds at concentrations of 10mM and 166mM to determine whether different oxidative stress levels produce biphasic effects. 166mM H2O2 delayed wound closure but towards the latter stages of healing it was equivalent to the control, showing a rapid yet late response with no improvements in angiogenesis. The delayed response did not appear to be attributed to oxidative (or nitrosative) damage. 10mM H2O2 showed minor effects on wound closure and, as found by Tur and colleagues (1995), improved angiogenesis was observed at comparable H2O2 concentrations, suggesting this could help improve the reparative response. By contrast, 166mM H2O2 appeared to be a stronger signal for recruitment of neutrophils to the wound site, but neither concentration affected macrophage recruitment compared with controls [44]. In summary, H2O2 may help the wound response via many mechanisms but it remains unclear whether levels that induce cell based reparative response would also be bactericidal (or bacteriostatic) at the wound site, which would most probably offer a clear advantage in the overall restoration of the damaged tissue.

Glucose oxidase has been shown to have the ability to generate ROS [45] and, after being embedded into a dressing, was tested on diabetic rats with full thickness wounds. An initial increased production of ROS was found in the wound fibroblasts (at 3-7 days) but, as with other studies [44], eventually the wound demonstrated a rapid response even without intervention. Tied with an early increase in ROS by glucose oxidase, an increase in NO was observed as well as changes in SOD, GSH and catalase antioxidant expression. The latter were assessed by enzymatically-assaying, wound sample homogenates and these findings provided evidence that antioxidants also play a key role in the redox regulation of wound repair. Importantly, wounds showed visual indication of faster closure, whilst immunohistochemical staining showed differentiation of keratinocytes and collagen formation [46]. Therefore, ROS appear to play an integral role in the diabetic rat wound response. However, these studies could have provided more direct, functional evidence for ROS-mediated effects by the inclusion of an antioxidant, such as NAC.

Honey has been extensively studied for its potential as a wound healing product and Jull and colleagues (2015) performed a systematic review of all related evidence. Overall there was not enough quality evidence to advocate its use as a generic entity, possibly due to the heterogeneous nature and individual differences of the wounds studied. However, the review concluded that honey demonstrated greater efficacy for partial thickness burns and for post-operative wounds compared with conventional treatments [47]. The main active ingredient(s) in honey, which may aid wound healing, remain unknown but may include: glucose oxidase (GOX), methylglyoxal (MGO), bee defensin-1 or H2O2, which are believed to contribute their effects mostly as antimicrobial agents [45, 47]. A bio-engineered type of honey has been developed, marketed as Surgihoney®,which not only has the antimicrobial effects of honey, but can also generate high levels of ROS in the form of H2O2. The release of H2O2 was shown to be necessary for the antimicrobial activity of the honey and also may aid the wound response through its secondary messenger effects, such as cell proliferation and immunocyte recruitment, [48, 49] although further studies are needed to support this. The efficacy of Surgihoney® as an antibacterial, and as a potential promoted of wound healing, was tested using a pilot study of 30 patients who had intravenous catheters being used to deliver chemotherapy. The skin insertion sites were randomised to be treated with Surgihoney® dressings or an anti-microbial dressing. Two patients with existing infection had resolution of catheter/insertion site infection and no recurrence, whereas six patients with an existing infection did not have resolution following use of the anti-microbial dressing comparator. The incidence of bacteraemia was also decreased compared with previous months when Surgihoney® dressings were used. These findings support the promise of using *Surgihoney*® as a ROS-dependent antibacterial agent, although further studies are needed to provide evidence for the potential of Surgihoney® for the reduction of wound site infection, or improvement of wound healing [50].

Some studies have explored the application of an electrical field to stimulate the directional migration of fibroblasts towards the wound site using charged particles known as galvanic particles. Studies have found that zinc micro-particles, combined with copper specks to intact skin, enhanced keratinocyte ROS production and reduced IL-1α, IL-2, NO and TNF-α pro-inflammatory cytokine secretion [51, 52]. The latter *in vitro* studies utilised a model to apply galvanic zinc copper particles onto an artificial epidermis covered with dermal fibroblasts. The results demonstrated specifically that fibroblast migration was ROS-mediated and driven by the BMP/Smad pathway [51]. In *vivo* studies may be able to further evaluate the potential behind this technology, additionally how and when it could be applied, in respect of its anti-inflammatory effects.

Hyperbaric O2 is a form of clinical therapy which increases blood O2 levels and may benefit healing, in stalled-healing or chronic wounds, through several potential mechanisms. Blood vessel damage results in local tissue hypoxia in wounds and a greater diffusion gradient is generated between the underlying tissue and its adjacent damaged counterpart. Tissue repair requires the energy-driven, mitotic-regeneration of cells which is fuelled primarily by anaerobic respiration. The immune system activation is also critically dependant on cell regeneration, such as clonal expansion of antigen recognising immunocytes; phagocytic cells utilise a burst of ROS during pathogenic destruction, during which time they adsorb large amounts of O2. Hyperbaric therapy could potentially increase systemic levels of ROS, which act as secondary messengers for the proliferation, angiogenesis and differentiation of crucial active wound-repairing cells, including stem cells [53]. A number of elegant studies by Thom and colleagues have demonstrated that ROS can be critical in the mobilisation of progenitor (bone marrow) cells to the site of the wound [54, 55]. Moreover, ROS-mediated transcription factor activation leads to the secretion of growth factors for further autocrine/paracrine signalling of wound repair processes. The most promising benefits of hyperbaric O2 therapy appear to be in diabetic patients with chronic foot ulcers (DFUs). A meta-analysis has found that it offers statistically significant short-term (though no long term) benefits [56]. A second meta-analysis, which focused on surgical wounds and trauma, found an increased skin graft survival and that healing of traumatic injuries was improved following hyperbaric therapy. Finally, a systematic review focusing on 8 studies of acute wounds, but with the exclusion of diabetic patients, found that hyperbaric therapy was beneficial in treating a range of wound types (for a detailed review on relevant clinical trials see [57]. Collectively, based on *in vitro*, *in vivo* and clinical evidence, hyperbaric O2 therapy appears to offer many biological advantages which are linked to the wound healing process. Given the complexity of different wound types it will be necessary to further assess its suitability in robust, well-powered, randomised clinical trials, and the practicalities of its use, including how often, how long and when it is indicated, as well as its financial, social and socio-economical suitability. Moreover, despite the clear importance of O2 in wound healing and therapeutic promise of hyperbaric O2 therapy, concern has been expressed over the possibility that excessive O2 may lead to augmented oxidative stress, thereby counteracting the potential benefits of ROS [50]. Furthermore, excess ROS may limit NO availability and thereby decrease perfusion and access of essential wound healing factors, including that of O2 itself [13, 58].

PDGF is a growth factor secreted initially by platelets during the early wound healing process and is approved by the FDA for the treatment of DFUs. A study in rats, with excisional skin wounds, investigated the oxidative events that occur after skin trauma following either untreated, chitosan or chitosan and PDGF-treated groups. Differential levels of NO were observed in rats supplemented with PDGF compared with the other groups, specifically lower levels being observed on days 3 and 7 post-wounding. Furthermore, levels of lipid peroxidation, GSH and ascorbic acid significantly varied, so PDGF is at least one factor which maintains ROS homeostasis at the wound site for effective healing [59]. The importance of PDGF in this context is additionally supported by a biological observation which showed that catalase and the antioxidant N-acetylcysteine can block the response of cultured smooth muscle cells to platelet-derived growth factor (PDGF), which induces chemotaxis and DNA synthesis, mediated by H2O2 [60]. In agreement, wound healing is impaired in mice lacking adequate PDGF receptors or ligands [61]. Moreover, *in vivo* studies suggest that PDGF stimulates neutrophil, macrophage, fibroblast and endothelial cell chemotaxis as well as stimulating induction of tissue repair molecules [62]. In summary, it appears understanding the PDGF-ROS link may be beneficial to understanding its potential for wound healing enhancement.

Myofibroblasts are a specialised (differentiated) type of fibroblast which are integral in normal wound healing in relation to their ability to signal via growth factors, promote angiogenesis, effect wound contraction and synthesise new ECM. A protein known as Galectin-1 appears to play a central role in myofibroblast function and was discovered in the context of its role in malignant tumour progression. However, new understanding of its function could offer novel strategies to promote wound healing. Notably, it has been found that Galectin-1 induces ROS via NADPH oxidase (Nox) 4 and this is critical in the wound healing process as shown in mice injected with recombinant Galectin-1 protein. Although many signalling proteins were identified, it remains unclear exactly how the redox status was modified to enhance local wound healing [63], and further studies are likely to elucidate this (the strategies for ROS-based enhancement of the wound healing process described in this section are summarised in Table 1).

**Conclusions and future perspectives**

Accumulating *in vitro* and *in vivo* evidence is strongly suggestive of a positive, healing-enhancing role for ROS (mainly in the form of H2O2) at the wound site. Yet, notably, many of the models of investigation have examined acute wound repair responses where the situation is less complex than in a chronic or complicated wound. Acute wounds heal in an optimal time frame based on normal homeostatic controls; characteristics which are often severely dysregulated in chronic and complicated wounds. Moreover, there are even many types of acute wounds, ranging from skin trauma and surgical incision to burns. It may therefore be difficult, or even inappropriate, to draw clear conclusions which can be applied to chronic or more complex wounds based on the available evidence. Each wound type may also have its own idiosyncratic phenotype and may not respond to intervention as well as others, as demonstrated in the application of honey and in hyperbaric O2 therapy. Methodologies or technologies which harness the potential benefits of ROS, by promoting a more normal (acute-type) wound healing response, could be clinically tested and their efficacy reported to provide a greater understanding of which wounds respond best to which treatment. Clearly new treatments based on ROS modulation are promising, provided that ROS levels remain within non-toxic local concentrations and are applied for the correct, and appropriately timed, individual wound indication.

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**Figure legends**

**Figure 1 ROS and its role in wound healing**

The schematic diagram depicts the multiple roles of ROS during acute wound healing (note that this refers to homeostatic, not excessive, levels of ROS). 1. ROS are important in initial wound protection by reducing blood flow and local cell signalling for thrombus formation; 2. Local ROS release attracts blood vessel bound local neutrophils to the wound site for bacterial protection; 3. Phagocytosis releases ROS to stunt bacterial growth and provide further signals supporting the wound response; 4. Other immunocytes, including monocytes, migrate towards the wound site to help attack invading pathogens; 5. Wound edge and general release of ROS stimulates endothelial cell division and migration for blood vessel reformation, fibroblast division and migration for new ECM formation (including collagen synthesis) and promote keratinocyte proliferation and migration.

**Tables**

**Table 1 Summary of therapeutic strategies based on modulation of ROS function**

|  |  |
| --- | --- |
| **ROS-modulating**  **therapeutic approach** | **Evidence for positive physiological ROS effects on wounds** |
| Topical H2O2 (or related ROS intermediates) | Anti-bacterial, promotes O2 formation, increased angiogenesis, various immunocyte recruitments, keratinocyte proliferation and migration [35-40, 44] |
| Recombinant Glucose oxidase | Increased perfusion via NO, early facilitation of wound closure, keratinocyte differentiation and collagen formation, H2O2 related effects [46] |
| Honey | Anti-bacterial, immunocyte recruitment, H2O2 related effects [45-50] |
| Galvanic particles | Reduced inflammation, fibroblast migration H2O2 related effects [50, 52] |
| Hyperbaric O2 therapy | Reduced wound hypoxia thus better anabolism, efficient phagocytic respiratory bursts, H2O2 related effects [56,57] |
| Recombinant PDGF | Increased perfusion via NO, angiogenesis, neutrophil, macrophage, fibroblast, endothelial cell wound migration [59-62] |
| Recombinant Galectin-1 | Myofibroblast signalling and ROS release via NADPH oxidase, H2O2 related effects [63] |