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INVITED REVIEW

Influence of oxygen on wound healing

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Abstract

Oxygen has an important role in normal wound healing. This article reviews the evidence concerning the role of oxygen in wound healing and its influence on the different stages of wound healing. The evidence reviewed has demonstrated that improving oxygenation may be helpful in limiting wound infection, although there is a lack of good quality studies on the role of oxygen in the proliferative phase and in reepithelialisation. Overall, the relationship between oxygen and wound healing is complex. Knowledge of this aspect is important as many treatment modalities for refractory wounds are based on these principles.

Introduction

The process of wound healing consists of the partially overlapping phases of haemostasis, inflammation, proliferation, epithelialisation and remodelling (1), within which each step requires oxygen (2). The role of oxygen in wound healing has been studied extensively since the 1960s when Hunt *et al.* (3) identified that adequate wound oxygenation could enhance formation of granulation tissues and synthesis of collagen. Oxygen is essential for the production of adenosine triphosphates (ATPs) and other biological energy sources via various metabolic cycles in cellular respiration (4). Furthermore, sufficient oxygenation is especially important for cell proliferation, bacterial defence, angiogenesis, collagen synthesis and epithelialisation (5). The latter are important for proper cellular function, especially during wound healing when there is an increased demand for reparative processes where sufficient tissue oxygenation is required to maintain high energy levels (6). This article reviews the evidence concerning the role of oxygen in wound healing and its influence on different stages of wound healing.

Inflammatory phase

The inflammatory phase of wound healing starts immediately after wounding and may last up to 1 week (1). Bacterial killing by phagocytosis is an important element, which depends on a high partial pressure of oxygen. After engulfing the pathogen, respiratory burst occurs. By transferring electrons from nicotinamide adenine dinucleotide phosphate (NADPH), NADPH oxidase in the neutrophil membrane produces superoxide, which combines with oxygen molecules and undergoes further changes to produce reactive oxygen species (ROS) (7). ROS subsequently mediates bactericidal killing (8). Common ROS include peroxide anion (HO_2^-), hydroxyl ion (OH^-), superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) (9).

Allen *et al.* (10) performed an *in vitro* experiment using neutrophils from blood of healthy volunteers and wounds of two patients undergoing mastectomy. The bacterial killing capacity of the neutrophils was measured by oxygen consumption using a Clark-type oxygen polarograph. The authors found that the concentration of atmospheric oxygen was directly proportional to neutrophil oxygen consumption during the respiratory burst, with other confounders such as temperature, pH and glucose concentration being tightly controlled. The half-maximal velocity (Km) for the NADPH oxidase with oxygen as a substrate was 40–80 mmHg. Clinically, the resistance to infection with reference to the neutrophil activity was expected to be critically impaired by wound hypoxia, but became more efficient with an increase in the tissue oxygen partial pressure, rising up to very high levels (500–1000 mmHg).

This study demonstrated that oxygen tension was an important factor affecting the respiratory burst and antimicrobial

Key Messages

- oxygen has an important role in normal wound healing
- this article reviews the evidence concerning the role of oxygen in wound healing and its influence on different stages of wound healing
- overall, the relationship between oxygen and wound healing is complex

effects of neutrophils, which may in turn affect infection rates and wound healing. However, the study was neither blinded nor randomised, which may limit its reliability and external validity.

Hopf *et al.* (11) conducted a prospective study of 130 surgical patients to determine whether subcutaneous wound oxygen tension could predict the development of wound infection. The authors found that the subcutaneous wound oxygen tension was inversely proportional to the risk of infection as predicted by an anticipated Study on the Effect of Nosocomial Infection Control (SENIC) score, a result that was also statistically significant ($P < 0{\cdot}03$). This suggests that improving wound oxygen tension may reduce the risk of infection and subsequently promote wound healing. The validity of the SENIC index as a predictor of surgical wound infection risk has been well verified (12); therefore, this was an objective and comprehensive measurement of infection risk, which makes the result more reliable. However, since other potential confounders, such as supplemental oxygen or prophylactic antibiotics, were not controlled, the overall reliability of the study may be questionable.

Greif *et al.* (13) conducted the first randomised controlled trial (RCT) recruiting 500 patients undergoing colorectal surgery to examine whether peri-operative supplemental oxygen reduced the incidence of wound infection. Patients were given either 30% or 80% oxygen during their operation, up to two hours postoperatively. The wound infection rate during the first 15 days postsurgery was halved in the 80% oxygen group compared with the control group ($P < 0{\cdot}01$). The anaesthetic procedures and surgery were standardised and similar in both groups, which limited potential confounding effects. Moreover, supplemental oxygen was given via endotracheal tubes and non-rebreathing masks to all patients, with subcutaneous oxygen tension ($PscO_2$) and arterial oxygen partial pressure being measured and documented throughout the process. This ensured correct delivery of the planned oxygen concentration to the different groups and hence improved the validity of the study. However, in contrast to the study by Hopf *et al.* (11) who used a validated tool, in this study a wound was considered to be infected only when there was culture-positive pus. Therefore, the incidence of wound infection may have been underestimated as not all infected wounds would have fulfilled this criterion.

These *in vitro* and *in vivo* studies demonstrated a reduction in the rates of infection with increasing wound oxygenation. Subsequent trials designed with similar objectives demonstrated more heterogeneous results.

A recent RCT conducted by Thibon *et al.* (14) recruited 434 patients to study the effects of hyper-oxygenation with inspired oxygen of 80% compared with 30% oxygen on the frequency of surgical site infection (SSI) within 30 days postoperation in patients who had undergone routine abdominal, gynaecologic and breast surgery. Using a computer-generated allocation list, 226 and 208 patients were randomised into the 80% and 30% inspired oxygen groups, respectively. These oxygen fractions were administered only during intubations, and only the anaesthesiologists were aware of the group to which the patients had been allocated. The baseline characteristics were similar in both groups. In contrast to Greif *et al.*, (13) this study found no statistically significant difference in the outcome between the two groups.

In this study, the researchers utilised the National Nosocomial Infection System (NNIS) risk index to evaluate SSI, which was different from the one used in the previous two trials. The different results of these studies maybe due to the different types of operations performed on the subjects, the different methods of oxygen administration, or the different ways of result assessment. Moreover, the authors did not measure $PscO_2$ or arterial oxygen partial pressure, which may limit the validity of the findings.

Recently, Kao *et al.* (15) conducted a systematic literature review and Bayesian meta-analysis to determine whether peri-operative supplemental oxygen reduced SSI in patients undergoing surgery. The authors identified a total of eight suitable RCTs to be included in the review and found a 77–85% chance of reduction in SSI with hyperoxia of up to 80% oxygen. Furthermore, they confirmed a higher probability of benefit of hyperoxia in patients with colorectal operations, with an 86–92% chance of reduction in SSI in this patient subset. However, the treatment hazards of hyperoxia were not studied, which may limit its use in clinical practice despite the demonstrated potential benefits in SSI reduction and wound healing.

Proliferative phase

The proliferative phase starts approximately 4–5 days after wounding and may last for a number of weeks (16). It consists of angiogenesis, formation of granulation tissue and extracellular matrix (ECM) and reepithelialisation, processes which Schreml *et al.* (5) suggested required oxygen to progress.

Angiogenesis

Angiogenesis is stimulated by both hypoxia and ROS (17). Hypoxia initiates angiogenesis by activating the transcription factor hypoxia-inducible factor (HIF)-1a, which in turn upregulates vascular endothelial growth factor (VEGF), the major growth factor of angiogenesis (18). Paradoxically, a review by Chambers and Leaper (19) suggested that VEGF expression is linked to ROS. Sen *et al.* (20) suggested that hyperoxic conditions, for example, by increasing local ROS, could induce a higher degree of angiogenesis.

An animal study by Hopf *et al.* (21) demonstrated stimulation of angiogenesis with hyperoxia. In their experiment, mice were administered a subcutaneous injection of an unsupplemented gel, gel with VEGF or with anti-VEGF antibodies. They were then maintained under various environments of 13% (hypoxia), 21% (normoxia) and 100% oxygen (hyperoxia) at 1 absolute atmosphere (ATA), 2 ATA, 2·5 ATA and 3 ATA. These gels were then explanted, sectioned and graded for the degree of angiogenesis. Angiogenesis was statistically significantly decreased in the hypoxic animals ($P = 0{\cdot}001$) but increased in the hyperoxic group ($P < 0{\cdot}05$) with unsupplemented gels compared with normoxic controls. The authors concluded that oxygen was required for angiogenesis. However, it was also found that this significant finding vanished with VEGF-supplemented gel in the hyperoxic mice under

1 ATA and 2 ATA. These findings suggested that the role of VEGF may dominate that of oxygen in angiogenesis under normoxic environments, while the role of oxygen may be significant only under hyperbaric conditions.

Another more recent animal study by Sander *et al.* (22) determined the effects of hyperbaric oxygen (HBO) on wound neovascularisation in mice. HBO treatment was provided to a 'non-impaired healing' group ($n = 8$) and a 'macrophage reduction' group ($n = 8$), with equal numbers of controls. The wounds were measured by photographic images, while neovascularisation was directly visualised and measured using intravital video microscopy and computerised planimetry. Measurement was assessed by blinded investigators unaware of the treatment groups. The results demonstrated that neovascularisation occurred earlier in the HBO treatment groups compared with controls, with faster rates of wound closure observed. This finding was statistically significant ($P < 0{\cdot}05$). However, photographic measurements rather than histological analysis were used in this study. Such methods may limit the reliability and reproducibility of results, as they may be affected by other confounders such as blood oxygenation, surrounding temperature and hydration status (19).

Extracellular matrix

Angiogenesis and ECM synthesis are interdependent processes (5). The new capillaries that form as a result of angiogenesis branch out and invade the surrounding matrix, which is then replaced by a new ECM produced and deposited by fibroblasts. This ECM consists of collagen fibres, proteoglycans, glycosaminglycans, fibrin, fibronectin and hyaluronic acid. Hydroxylation of proline and lysine is an important oxygen-dependent step in the production of collagen (6).

Tissue fibroblast growth and collagen biosynthesis are related to oxygen tension (23). Kan *et al.* (24) conducted an *in vitro* study on human fibroblasts. They found that hypoxia was responsible for delayed wound healing with a reduction in the amount of collagen in the wound, which was associated with an increase in MMP-1 synthesis. Moreover, Kang *et al.* (25) conducted another *in vitro* study on HBO treatment on human dermal fibroblasts. They found that daily HBO treatment at 2·0 atmosphere (ATM) selectively stimulated fibroblast proliferation after 7 days, together with an increase in basic fibroblast growth factor (bFGF) production.

A prospective RCT on humans ($n = 29$) was conducted by Hartmann *et al.* (26) to compare the accumulation of collagen in standardised wounds in patients who had abdominal operations and whose postoperative fluid replacement was decided either clinically or by measurements of $PscO_2$. Silicone rubber catheters were placed in the upper arm to measure $PscO_2$, while two tubes of expanded polytetrafluoroethylene (ePTFE) were implanted subcutaneously parallel to the silicone rubber catheter to measure the amount of collagen accumulated. They found that the group treated according to $PscO_2$ measurements received more fluid on the day of operation than the group treated according to clinical criteria ($P < 0{\cdot}05$); also, more collagen accumulated in their ePTFE tubes by day 7 ($P < 0{\cdot}05$). Collagen formation in healing wounds seems to be associated with improvement in $PscO_2$.

However, this trial studied collagen formation as a surrogate outcome, rather than studying wound healing directly, that is, by measuring wound size and depth. Abundant collagen formation secondary to improved tissue oxygenation or hydration may not be equivalent to improved wound healing as it is a complicated process involving other ongoing events, such as migration of various types of cells across ECM. Further studies are required to delineate this issue.

Nakada *et al.* (27) conducted a case series on seven patients with leg ulcers refractory to conventional therapy who were administered a combined therapy of HBO and human bFGF. HBO at 2 ATA for 90 minutes and spray treatment of bFGF to the ulcer bed, both daily, were prescribed for an average of 2·6 months (1·3–4·4 months). Biopsies of ulcers were obtained for histological examination as well as fibrous tissue measurement. Ulcers in five of the patients were completely healed and two showed a reduction in ulcer size macroscopically. Proliferation of connective tissue was found to be induced in the ulcer, with an increased amount of both collagen and non-collagenous proteins. However, the result could be questioned as no control group was recruited for comparison. The improvement could be due to HBO alone, bFGF alone, their combined effects, or other confounders. Moreover, no blinding of patients, medical practitioners and investigators was mentioned, which may impair the internal validity. In general, this study demonstrates the induction of connective tissue proliferation in ECM and enhanced wound healing by the combined effects of improved tissue oxygenation (with HBO therapy) and growth factor application (bFGF).

Reepithelialisation

Parallel to the formation of granulation tissue and underlying ECM, reepithelialisation is initiated to cover the wound surface by a layer of epithelium and is based on differentiation, proliferation and migration of epidermal keratinocytes from the margin of the wound (5).

O'Toole *et al.* (28) conducted an *in vitro* study on the motility of human keratinocytes subjected to either hypoxic (2% oxygen) or normoxic (20% oxygen) conditions, and demonstrated that keratinocytes migrated faster under hypoxic conditions on connective tissues, associated with the increased expression of lamellipodia proteins and collagenase, but decreased expression of laminin-5, which inhibits keratinocyte motility.

However, a more recent study by Loo and Halliwell (29) examined the effects of H_2O_2, one of the common ROS, on a keratinocyte-fibroblast co-culture model of wound healing. The re-epithelialisation rate was measured by taking images of the closure of wounds with a dissection microscope. In contrast to the study by O'Toole *et al.*, (28) the authors found that H_2O_2 increased keratinocyte proliferation and the rate of reepithelialisation.

These contradictory results may be due to the different culture models used as well as the different experimental settings. Oxygen may mediate activities of keratinocytes via other ROS, apart from H_2O_2, giving rise to different effects. An *in vivo* study, if practically possible, might be helpful in delineating such issues. Such information is important for future development of local treatments, such as local oxygen therapy or

various dressing materials, speeding up reepithelialisation of partial-thickness wounds or second-degree burns.

Conclusion

The evidence reviewed here has demonstrated that improving oxygenation may be helpful in reducing wound infection. However, as the majority of research has been undertaken only on surgical rather than other types of wounds, the external validity and generalisability of the studies are limited. Further research is required to identify the fraction of inspired oxygen that is most beneficial for wound healing, and the duration for which supplemental oxygen should be administered for maximum benefit.

There appears to be a lack of good quality human studies on the role of oxygen in the proliferative phase of wound healing, in particular concerning angiogenesis and formation of granulation tissues or ECM. Current evidence is mainly from *in vitro* or animal studies. However, the lack of *in vivo* clinical data is probably due to the ethical issues concerning induction of wound hypoxia in humans.

Moreover, no good quality *in vivo* or human studies are available concerning the relationship of oxygen and reepithelialisation. The studies and findings on this issue to date are contradictory and are mainly from *in vitro* studies. These experiments may oversimplify the situation and neglect the numerous possible interactions of keratinocytes in situ, such as the presence of other inflammatory cells, bacterial colonisation and granulation tissue. It may be similar to that of angiogenesis, where hypoxia plays a role in initiating the process, while adequate oxygenation is required for forming a healthy wound bed in order to complete reepithelialisation. Future studies, particularly *in vivo* ones, are surely required to fill this gap in the current knowledge base.

Overall, it seems the relationship between oxygen and wound healing is complex. Additional knowledge regarding this aspect is important as many treatment modalities for refractory wounds are based on these principles, including HBO, local oxygen therapy and other specific dressing materials.

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