Role of oxygen in wound healing: a review of evidence

• **Objective:** To review the evidence regarding the influence of oxygen as an intrinsic factor on cutaneous wound healing.
• **Method:** A literature search was performed using Ovid and the Cochrane Database with the search terms: 'Wound healing', 'Oxygen', 'Collagen', 'Angiogenesis', 'Inflammation' and 'Surgical Site Infection'. Human and animal studies were included if relevant and examined for methodological quality.
• **Results:** There are no meta-analyses of the use of oxygen in wound healing and only two randomised controlled trials (RCTs). Studies vary in methodological quality. The majority of the data comes from animal models. In total 1568 studies on wound healing and oxygen were found.
• **Conclusion:** Oxygen is vital throughout wound healing, especially in the inflammatory and proliferative phases. Research suggests that patient supplementation with oxygen could enhance bacterial killing and angiogenesis, reduce surgical site infection rates and increase wound tensile strength, facilitating improved healing.
• **Conflict of interest:** None.

oxygen; wound healing; inflammation; collagen; surgical site infection

A.C. Chambers,[1] BM BS, Foundation Doctor; **D.J. Leaper**,[2] MD ChM FRCS FACS FLS, Visiting Professor;
1 Western General Hospital, Edinburgh, UK;
2 Department of Dermatology and Wound Healing, Cardiff University, UK.
Email: adam.chambers@nhs.net

Following injury, wound perfusion and oxygenation are critical for acute inflammation and optimal wound healing. They can be compromised by many factors, both local (such as disrupted local vasculature following wounding, or relative hypoxia due to the increased oxygen demands of normal healing) and systemic (for instance, pre-existing co-morbidities, which can affect the oxygen and nutrient supply to injured tissues).[1] Tissue hypoxia (defined as oxygen levels below 30mmHg) can impact greatly on all phases of the wound healing process.[2]

Since the 1960s, oxygen and reactive oxygen species (ROS) have been studied to discern the roles they play in the different phases of wound healing. Early research by Hunt and colleagues[3] recognised that oxygen was vital both for granulation tissue formation (through aerobic cell respiration) and as a cofactor during the hydroxylation of proline and lysine during collagen synthesis. The same team went on to show that the tensile strength of experimental wounds was directly proportional to the partial pressure of atmospheric oxygen.[4]

A clinical guideline has been published by the National Institute of Health and Clinical Excellence (NICE) to address the issue of surgical site infections (SSIs).[5] With their considerable morbidity and mortality, SSIs are a major economic cost to the NHS. They increase the burden of wound management, sometimes causing further complications (that can require additional surgeries) and they have a huge impact on quality of life.

In 1984, Knighton et al.[6] showed that in an animal model, higher inspired concentrations of oxygen reduced the extent of infection following intradermal bacterial inoculation. In the early respiratory burst of the inflammatory phase, oxygen is a crucial substrate for the production of ROS by neutrophils,[1] whereas in the later proliferative phase of wound healing, the absence of oxygen plays a role in angiogenesis — hypoxia initiates neovascularisation and the induction of vascular endothelial growth factor (VEGF).[7]

This review examines the evidence of oxygen's influence on wound healing since these early experiments, and aims to give a balanced view of both hypoxia and hyperoxia throughout the different phases of wound healing.

Oxygen and inflammation

Neutrophils are active in the early inflammatory phase of healing.[8] They produce ROS, which destroy micro-organisms in a process named the 'respiratory burst'.[9] ROS are especially important during the inflammatory phase of wound healing.[1]

An *in vitro* experiment by Allen et al. showed the concentration of atmospheric oxygen to be directly proportional to neutrophil oxygen consumption during the respiratory burst.[10] Temperature, pH and glucose concentration were all tightly controlled, although the experiment was not blinded and gave no data of inferential statistics. The researchers found no quantitative differences between neutrophils isolated from serum and those isolated from wounds, which enhanced the experiment's external validity. Interestingly, neutrophil oxygen consumption failed to plateau, which suggests that it may be possible to enhance neutrophils' antibacterial activity, using high concentrations of inspired oxygen, clinically.

Request Permissions
© MA Healthcare Ltd. Downloaded from magonlinelibrary.com by 130.014.124.023 on February 16, 2018.
Use for licensed purposes only. No other uses without permission. All rights reserved.

In vivo human evidence comes from a study of 130 surgical patients, in whom the rate of SSIs (classified as infection in the wound, up to 30 days postoperatively) had a statistically significant, inversely proportional relationship with subcutaneous wound oxygen tension ($p<0.03$).[11] Due to the variety of cases, the external validity of this result was high, but reliability was limited as there was no control over the use of supplemental oxygen, prophylactic antibiotics or fluids given post-operatively. However, despite these methodological flaws, this result is hard to ignore.

Further clinical evidence comes from two double-blind randomised controlled trials (RCTs).[12,13] In the first of these studies, by Greif et al., 500 patients were given either 30% or 80% oxygen (via mask) intra-operatively and for two hours post-operatively. SSI rates (classified as wound infections in the first 15 days post surgery) were found to be significantly lower in the 80% oxygen group ($p<0.01$). The original study design aimed to collect data on 1000 patients, but the trial was stopped early due to the marked statistical difference between groups and the ethical implications of not offering all patients the best treatment.[12]

The second study, by Belda et al.,[13] had similar results in 300 colorectal patients. SSI rates were statistically significantly reduced, from 24.4% in the 30% group to 14.9% in the 80% group ($p=0.04$). This is remarkably similar to Greif et al.'s results, which is surprising given that the predicted SSI rates in Belda's study were nearly double those expected in Greif's. Belda's study differed further, as oxygen was inhaled for an extra 4 hours postoperatively, and there was no mention that patients were always adequately oxygenised, determined using pulse oximetry in the UK, which is recommended in the NICE SSI guideline.[5] We do not know the extent to which the 15-day cut off reduced the reported incidence of SSIs compared with a 30-day cut off (as seen in previous research).[9] Additionally, it remains unclear how a postoperative FiO_2 of 0.8 was achieved in Belda's study, as even using a re-breathing mask, the maximum possible FiO_2 is 0.6.

Interestingly, a more recent clinical study from Denmark has refuted these findings.[14] This large, double blinded RCT found no statistically significant difference in SSI rates between patients inhaling 0.8 FiO_2 and those inhaling 0.3 FiO_2 during abdominal operations and for two hours post-operatively ($p=0.64$). However, it is possible that the trial was

References

1 Gordillo, G., Sen, C. Revisiting the essential role of oxygen in wound healing. Am J Surg. 2003; 186: 3, 259–263.

2 Kulikovsky, M., Gil, T., Mettanes, I. et al. Hyperbaric oxygen therapy for non-healing wounds. Isr Med Assoc J. 2009; 11: 8, 480–485.

3 Hunt, T.K., Zederfeldt, B., Goldstick, T.K. Oxygen and healing. Am J Surg. 1969; 118: 4, 521–525.

4 Stephens, F.O., Hunt, T.K. Effect of changes in inspired oxygen and carbon dioxide tensions on wound tensile strength: an experimental study. Ann Surg. 1971; 173: 4, 515–519.

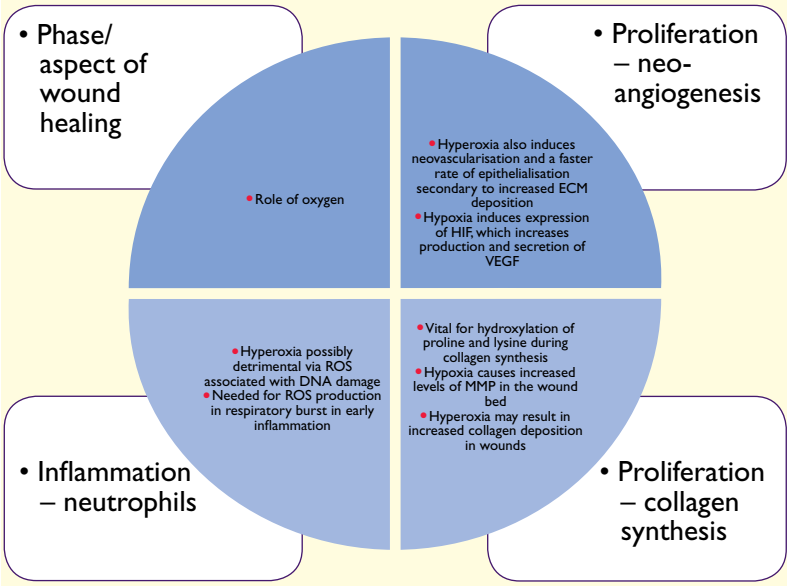
5 NICE. CG74 Surgical site infection: NICE guideline. NICE, 2001. [Available at: http://egap.evidence.nhs.uk/CG74/ Last accessed: 22/2/11]

6 Knighton, D.R., Halliday, B., Hunt, T.K. Oxygen as an antibiotic. The effect of inspired oxygen on

Request Permissions

© MA Healthcare Ltd. Downloaded from magonlinelibrary.com by 130.014.124.023 on February 16, 2018.
Use for licensed purposes only. No other uses without permission. All rights reserved.

Figure 1. The role of oxygen in wound healing



ROS=reactive oxygen species; ECM=Extracellular matrix; HIF= hypoxia-inducible factor; VEGF=vascular endothelial growth factor; MMP=matrix metalloproteinase

infection. Arch Surg. 1984; 119: 2, 199–204.

7 Sen, C.K., Khanna, S., Babior, B.M. et al. Oxidant-induced vascular endothelial growth factor expression in human keratinocytes and cutaneous wound healing. J. Biol Chem. 2002; 277: 36, 33284–33290.

8 Hart, J. Inflammation 1: its role in the healing of acute wounds. J Wound Care. 2002; 11: 6, 205–209.

9 Sarsour, E., Maneesh, K., Chaudhuri, L. Redox control of the cell cycle in health and disease. Antioxid Redox Signal. 2009; 11: 12, 2985–3011.

10 Allen, D., Maguire, J., Mahdavian, M. et al. Wound hypoxia and acidosis limit neutrophil bacterial killing mechanisms. Arch Surg. 1997; 132: 9, 991–996.

11 Hopf, H.W., Hunt, T.K., West, J.M. et al. Wound tissue oxygen tension predicts the risk of wound infection in surgical patients. Arch Surg. 1997; 132: 9, 997–1004.

12 Greif, R., Akça, O., Horn, E.P. et al. Supplemental perioperative oxygen to reduce the incidence of

underpowered to find a small statistically significant risk reduction. This study suggested that a short duration high FiO_2 is not detrimental to respiratory function, an adverse effect that was previously considered following sub-group analysis in Greif et al.'s study.[12]

Further research is needed to determine if excessive oxygen causes uncontrolled release of ROS. A study of hyperbaric oxygen in the treatment of a variety of chronic wounds,[15] found concomitant antioxidant and hyperbaric therapy to increase the percentage of healing wounds compared with placebo. However, it also showed that cells in an environment with high levels of ROS become sensitised to oxidants, with less oxidative damage to DNA over time. It is unknown if high levels of ROS in acute wounds inflict the same level of DNA damage and whether or not supplementation with normobaric oxygen might be harmful.

Further evidence is needed to elicit which FiO_2 is most beneficial to wound healing, and the duration for which supplemental oxygen should be inspired for maximum benefit.

Oxygen and angiogenesis

In the early 1980s, research on neo-angiogenesis highlighted the importance of hypoxia and a wound-tissue hypoxic gradient.[16,17] It is now understood that hypoxia promotes angiogenesis via hypoxia-inducible factor 1 (HIF-1) and dependent expression of pro-angiogenic growth factors such as VEGF.[18]

Paradoxically, a more recent study has found wound hypoxia to be detrimental throughout all the phases of wound healing, including angiogenesis, while hyperoxia seems to confer benefit.[19] This animal study by Hopf et al. examined the effects of hypoxia and hyperoxia on neovascularisation in gel plugs implanted under the skin of mice. They found significant increases in angiogenesis in hyperoxic wounds ($p<0.05$) while observing a significant decrease of angiogenesis in hypoxic wounds ($p=0.001$). Reliability was low, however, as hypoxic wounds were also treated with VEGF, impregnated into the implanted gel. It is also of note that all significant results came from wounds that were treated with hyperbaric (2.0, 2.5 and 3.0 ATA) and not normobaric 100% oxygen.

Studying this same effect, Sander et al. examined acute wounds in rats treated with hyperbaric oxygen, finding that neovascularisation occurred statistically significantly earlier ($p<0.05$) compared with control animals, with faster rates of epithelialisation and wound closure.[20] This blinded study used an *in vivo* photographic method to measure vascularisation and would have been affected by hydration, cutaneous temperature and oxygenation of the blood, which reduces its reproducibility and the reliability of measurements. A method for overcoming such physiological variability and accurately measuring vascularity, is histological analysis of wound tissue. This has been used successfully in other studies.[19]

VEGF expression has previously been linked to ROS.[7] More recent work in patients with chronic wounds has shown that VEGF-β is significantly up-regulated by treatment with hyperbaric oxygen therapy ($p<0.05$).[21] VEGF-β appears to be regulated by ROS, as when patients are treated with α-lipoic acid (LA) at the same time, which acts as a biological antioxidant, scavenging ROS,[15] this result is reversed. It is unknown if this work translates to acute wounds.

A study by Trabold and colleagues has shown that VEGF synthesis is stimulated by increasing lactate levels in wounds, compared with control ($p<0.05$).[22] This study was not without its limitations — it used an animal model, all the wounds were incisional, and lactate production was artificial (from an implanted Polyglactin 910 woven mesh). We do not know if the levels of lactate in these experimental wounds match those seen clinically, which affects the external validity of the study. Nonetheless, this finding, thought to be independent of hypoxia,[19] highlights that there are other mechanisms of action within wounds, stimulating VEGF expression.

Oxygen and collagen

It has long been known that molecular oxygen is necessary for the hydroxylation of proline and lysine during collagen synthesis and that without it only proto-collagen, a functionally deficient variant of collagen, is produced.[3]

Request Permissions

© MA Healthcare Ltd. Downloaded from magonlinelibrary.com by 130.014.124.023 on February 16, 2018.
Use for licensed purposes only. No other uses without permission. All rights reserved.

This theory has been corroborated by evidence from Kan and colleagues,[23] who examined human fibroblasts in hypoxic wound conditions. They found a reduced amount of collagen in these wounds, which may be explained by a statistically significant increase ($p<0.001$) in matrix metalloproteinase-1 (MMP-1). This study was carried out *in vitro* on a small number of fibroblast populations. *In vivo* clinical data is limited, most likely due to the ethical considerations of enforcing hypoxic wound conditions in humans. This evidence suggests a possible new role of oxygen as an inhibitor of MMPs and hence, as a promoter of granulation tissue formation.

Oxygen supplementation has been examined with regard to the amount of collagen deposited in wounds. An *in vivo* study that used a rat model[24] showed that, compared with control, four weeks of hyperbaric oxygen therapy significantly increased fibroblast infiltration and collagen deposition in porous polyethylene blocks implanted beneath the skin ($p<0.05$). However, it is possible that this finding stems from a type 1 statistical error, as it was revealed by a sub-group analysis.

Human studies are less conclusive; Nakada and colleagues found that, in conjunction with basic fibroblast growth factor (bFGF), hyperbaric oxygen therapy increased the amount of collagen in healed wounds ($p<0.001$).[25] While this result is significant, it should be noted that the study was a case series, with no blinding of investigators or participants and no control groups. Without a control, we do not know if the result is due to hyperbaric oxygen alone, bFGF alone, or a combination of the two, although it seems unlikely that the result is solely due to hyperbaric oxygen, as seven of the patients had previously received hyperbaric oxygen therapy with no clinical improvement in their ulcers.

A double blinded RCT by Stotts and colleagues examined the effect of hydration on collagen formation in expanded polytetrafluoroethylene (ePTFE) tubes implanted into the upper arms of elderly, nursing home residents.[26] Patient hydration is known to augment subcutaneous tissue oxygen levels by increasing tissue perfusion.[27] However, Stotts' study found no significant differences between the treatment and control groups in either collagen levels ($p=0.84$) or subcutaneous tissue oxygen levels ($p=0.13$), indicating that in this elderly population there were other intrinsic factors controlling tissue oxygenation and subsequent collagen production.

surgical-wound infection. N Engl J Med. 2000; 342: 3, 161–167.

13 Belda, F.J., Aguilera, L., García de la Asunción, J. et al. Supplemental perioperative oxygen and the risk of surgical wound infection: a randomized controlled trial. JAMA. 2005; 294: 16, 2035–2042.

14 Meyhoff, C.S., Wetterslev, J., Jorgensen, L.N. et al. Effect of high perioperative oxygen fraction on surgical site infection and pulmonary complications after abdominal surgery: the PROXI randomized clinical trial. JAMA. 2009; 302: 14, 1543–1550.

15 Alleva, R., Nasole, E., Di Donato, F. et al. alpha-Lipoic acid supplementation inhibits oxidative damage, accelerating wound healing in patients undergoing hyperbaric oxygen therapy. Biochem Biophys Res Commun. 2005; 333: 2, 404–410.

16 Knighton, D.R., Silver,

Request Permissions

© MA Healthcare Ltd. Downloaded from magonlinelibrary.com by 130.014.124.023 on February 16, 2018.
Use for licensed purposes only. No other uses without permission. All rights reserved.

Further research using a younger *in vitro* human model is needed to clarify the role of supplemental oxygen in collagen formation in healing wounds.

Methods to increase wound oxygenation

As well as supplemental oxygen breathed at normal pressure, there are many methods for increasing tissue oxygenation, some with limited validation. Routine patient management, including the optimisation of hydration status and temperature, has been shown to improve tissue oxygenation as measured by transcutaneous partial pressure of oxygen ($TcPO_2$).[28] In this study, patients were excluded if there was a known history of vascular disease but not if they had other risk factors, such as diabetes or current smoking.

Another interesting route to supplement oxygenation is through the administration of liposomal haemoglobin vesicles, as has been done in mice with critically ischaemic tissue.[29] Wound healing has been found to significantly improve in mice supplemented with vesicle solution. Haemoglobin vesicles have a half-life of approximately 72 hours and histological examinations of the major organs has shown no damage. Further work is needed for efficacy and safety to be established in human models.

Hyperbaric oxygen therapy uses 100% oxygen administered at pressures of 2.5 ATM for durations of around 90 minutes per day.[2] Its use to enhance chronic wound healing is limited by the availability of facilities and tough criteria for treatment, considering its cost. Research on hyperbaric oxygen has shown that the oxygen carrying potential of the blood surpasses that of the maximum level of saturated haemoglobin; it is unknown if the same results can be obtained with normobaric high concentration oxygen.[2]

Finally, an animal model using pigs has shown that topically applied oxygen increases partial pressures of oxygen in the wound bed.[30] This study's method was limited in terms of the mechanism used to supply the wound with oxygen and the maximum wound depth to which oxygen is able to seep. At present, there are no valid clinical claims of its efficacy in wound healing, although more research may be expected.

Conclusion

Oxygen has a diverse and vital role in wound healing. Hyperoxia has been implicated in facilitating each phase of wound healing. It appears to be vital in the inflammatory phase, where increased pO_2 reduces wound infection rates via a neutrophil-ROS mechanism. Infection risk might, therefore, be minimised by ensuring that all surgical patients have supplemental oxygen delivered post-operatively. Given that body temperature and hydration status affect tissue oxygenation, these should also be closely monitored, with any deficits compensated for, to ensure optimal oxygenation.

Oxygen has a paradoxical role in angiogenesis, as the early growth of new vessels is stimulated by hypoxia. However, the later stages of angiogenesis are also stimulated by hyperoxia. By increasing the gradient between healthy tissue and the wound, angiogenesis is facilitated, with the benefits of hyperoxia passed onto the wound bed once a vascular network has been established, following stimulation by the steep oxygen gradient. This further suggests that optimal post-operative oxygen is important in facilitating enhanced wound healing.

We know that oxygen plays a role in collagen synthesis but unfortunately, there appears to be confounding evidence surrounding what this role is exactly. Hypoxia and hyperoxia may affect the aesthetic result of the wound and it is possible that oxygen supplementation may improve tensile strength.

More work is needed to fully quantify the effects of oxygen on wound healing and determine the length of time for which oxygen supplementation is beneficial. Research is also warranted into the effects of increasing ROS concentration, following either normobaric or hyperbaric oxygen therapy, which might also determine whether or not these therapies have any detrimental as well as beneficial effects. ■

I.A., Hunt, T.K. Regulation of wound-healing angiogenesis-effect of oxygen gradients and inspired oxygen concentration. Surgery. 1981; 90: 2, 262–270.

17 Knighton, D.R., Hunt, T.K., Scheuenstuhl, H. et al. Oxygen tension regulates the expression of angiogenesis factor by macrophages. Science. 1983; 221: 4617, 1283–1285.

18 Forsythe, J.A., Jiang, B.H., Iyer, N.V. et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol. 1996; 16: 9, 4604–4613.

19 Hopf, H.W., Gibson, J.J., Angeles, A.P. et al. Hyperoxia and angiogenesis. Wound Repair Regen. 2005; 13: 6, 558–564.

20 Sander, A.L., Henrich, D., Muth, C.M. et al. In vivo effect of hyperbaric oxygen on wound angiogenesis and epithelialization. Wound Repair Regen. 2009; 17: 2, 179–184.

21 Alleva, R., Tomasetti, M., Sartini, D. et al. alpha-Lipoic acid modulates extracellular matrix and angiogenesis gene expression in non-healing wounds treated with hyperbaric oxygen therapy. Mol Med. 2008; 14: 3-4, 175–183.

22 Trabold, O., Wagner, S., Wicke, C. et al. Lactate and oxygen constitute a fundamental regulatory mechanism in wound healing. Wound Repair Regen. 2003; 11: 6, 504–509.

23 Kan, C., Abe, M., Yamanaka, M. et al. Hypoxia-induced increase of matrix metalloproteinase-1 synthesis is not restored by reoxygenation in a three-dimensional culture of human dermal fibroblasts. J Dermatol Sci. 2003; 32: 1, 75–82.

24 Dinar, S., Agir, H., Sen, C. et al. Effects of hyperbaric oxygen therapy on fibrovascular ingrowth in porous polyethylene blocks implanted under burn scar tissue: an experimental study. Burns. 2008; 34: 4, 467–473.

25 Nakada, T., Saito, Y., Chikenji, M. et al. Therapeutic outcome of hyperbaric oxygen and basic fibroblast growth factor on intractable skin ulcer in legs: preliminary report. Plast Reconstr Surg. 2006; 117: 2, 646–651.

26 Stotts, N.A., Hopf, H.W., Kayser-Jones, J. et al. Increased fluid intake does not augment capacity to lay down new collagen in nursing home residents at risk for pressure ulcers: a randomized, controlled clinical trial. Wound Repair Regen. 2009; 17: 6, 780–788.

27 Whitney, J.D. Supplemental perioperative oxygen and fluids to improve surgical wound outcomes: Translating evidence into practice. Wound Repair Regen. 2003; 11: 6, 462–467.

28 McPhail, I.R., Cooper, L.T., Hodge, D.O. et al. Transcutaneous partial pressure of oxygen after surgical wounds. Vasc Med. 2004; 9: 2, 125–127.

29 Plock, J.A., Rafatmehr, N., Sinovcic, D. et al. Hemoglobin vesicles improve wound healing and tissue survival in critically ischemic skin in mice. Am J Physiol Heart Circ Physiol. 2009; 297: 3, H905–910.

30 Fries, R.B., Wallace, W.A., Roy, S. et al. Dermal excisional wound healing in pigs following treatment with topically applied pure oxygen. Mutat Res. 2005; 579: 1-2, 172–181.

Request Permissions

© MA Healthcare Ltd. Downloaded from magonlinelibrary.com by 130.014.124.023 on February 16, 2018.
Use for licensed purposes only. No other uses without permission. All rights reserved.