

Role of oxygen in wound healing

Acute wounds are initially hypoxic. This state triggers the diffusion of oxygenated plasma from the surrounding intact tissue to the hypoxic area, and sets in train processes resulting in oxidative killing, angiogenesis and collagen synthesis

hypoxia; oxygen gradient; reactive oxygen species; growth factors; supplemental oxygen

Research has identified the importance of oxygen in all aspects of the healing process. It helps to promote angiogenesis and collagen synthesis, ensure effective leucocyte and fibroblast functioning, and produce growth factors and reactive oxygen species (ROS). This overview examines the evidence on the role of oxygen in acute wound healing.

Hypoxia

Hypoxia is inevitable in wounded tissue,¹ and delivery of oxygen to the wound site is essential to promote healing. Physiological disorders where normal circulation and oxygen levels are disrupted, such as diabetes, can lead to impaired healing.

Following disruption to local blood vessels as a result of tissue injury, cell metabolism and oxygen consumption is increased,² producing a hypoxic wound.^{2,4} However, the hypoxia then acts as a stimulus to tissue repair by creating an oxygen gradient between the hypoxic tissue at the wound site and the nearby perfused unbroken tissue.⁵ It is this oxygen gradient that is thought to promote the diffusion of oxygen to the hypoxic tissue.

The first event in the wound healing process is haemostasis, during which vasoconstriction and coagulation take place. Once haemostasis is established, the inflammatory phase is initiated. Vasodilation occurs and increased capillary permeability allows enzymes, a variety of cell (including leucocytes) and oxygen to reach the wound site and continue the healing process.³

Throughout healing, wounds are generally hypoxic at the centre, with an increasing oxygen gradient towards the intact tissue.^{6,8} The tissue oxygen tension (the balance between oxygen perfusion and tissue consumption of oxygen⁹) ranges from 0–20mmHg at the wound centre to 60–70mmHg at the periphery. The arterial oxygen tension (partial pressure of oxygen in the blood or tissue) is approximately 100mmHg.^{6,8} This hypoxic state lasts until angiogenesis is complete and blood supply is restored at the end of the proliferative phase.

Niinikoski,¹⁰ one of the first to research this topic, proposed that the higher the cellular oxygen con-

sumption at the wound site, the steeper the gradient driving the diffusion of oxygen from the oxygenated plasma to the wound cells. This theory is still supported today.^{11,12}

Reactive oxygen species

The main events of the inflammatory response are the elimination or control of invading bacteria and the removal of debris.¹³ Initial work conducted in the 1950s and followed up in the 1980s demonstrated a dramatic increase in leucocyte oxygen consumption during phagocytosis.^{14–16} Not all of the oxygen is used for energy as some is converted into highly reactive ions and reactive oxygen species (ROS), including superoxide ions and hydrogen peroxide.⁵ This process is known as respiratory burst.

The production of these ROS is crucial for oxidative killing⁴ — the destruction of bacteria. During this process, superoxides break down the bacterial membranes and leucocytes produce hydrogen peroxide. However, it is thought that the production of superoxides decreases in a hypoxic environment, such as is found within even acute wounds. It has been suggested that oxygen tensions below 30–45 mmHg are most critical in this respect; many parts of a wound bed are within this range.^{5,17}

However, this contradicts a previous *in vitro* study by Gabig et al.¹⁸ that investigated the effect of oxygen tension and pH on neutrophil respiratory burst. They observed that superoxide production in hypoxic conditions showed minimal decline until oxygen concentrations dropped to below 1% (7.6mmHg). This suggests that neutrophils can continue to produce a respiratory burst and bactericidal activity even in severely hypoxic conditions.

Despite this, it is now generally accepted that all wounds are initially hypoxic and that ROS production by phagocytes, and thus oxidative killing, is dependent on local oxygen tension.^{2,7,12} In other words, oxidative killing cannot take place at the wound site unless there is adequate oxygen perfusion, which as stated above is related to the oxygen gradient. Indeed, various studies have identified the importance of an adequate oxygen supply in preventing infection.^{16,19,20}

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Excessive production of ROS can result in tissue injury. This can cause cellular protein and DNA damage, inducing cell death through apoptosis or necrosis.²¹

In a literature review, Juránek and Bezek²³ concluded that ROS, when present in low concentrations, play an important role in cell signalling and re-establishing haemostasis. This is supported by other authors, who also suggested that low concentrations of ROS stimulate the release of growth factors and angiogenesis.^{7,12,22} In recent years it has been suggested that hypoxia stimulates initial ROS production, while oxygen is required to sustain it. Accordingly, chronic hypoxia is unable to maintain the process.^{12,22,23}

Most research on ROS has used *in vitro* or animal wound models as, clearly, human cellular responses are difficult to measure *in vivo*.²¹ For this reason, clinical studies have been restricted to observing the effect of supplemental oxygen on the frequency of wound infection. Indeed, this has been the focus of most of the more recent studies on the relationship between oxygen diffusion and the prevention of infection. Evaluation of these studies is beyond the scope of this paper.

Growth factors

Growth factors can stimulate wound healing through various mechanisms.²⁴ For example, transforming growth factor-beta (TGF- β) stimulates procollagen and fibronectin production²⁵ and vascular endothelial growth factor (VEGF) enhances angiogenesis.²⁶ Research has demonstrated that the production of some growth factors can be upregulated by either hypoxia or hyperoxia.

Falanga et al.²⁷ and Siddiqui et al.²⁸ investigated the production of TGF- β 1 by human dermal fibroblasts in hypoxic conditions.

Falanga et al.²⁷ took fibroblasts from the dorsal forearm of healthy volunteers and grew them in standard oxygen conditions until almost confluent. Cultures were then exposed to either a hypoxic atmosphere (2% or 14mmHg oxygen) or standard oxygen culture conditions (15% or 94mmHg oxygen at the cell surface) for various time periods up to 72 hours. (Atmospheric air contains approximately 21% oxygen).

Secretion of TGF- β 1 was ninefold higher after 72 hours exposure to 2% oxygen, compared with 15% oxygen. Similarly, TGF- β 1 messenger RNA (mRNA) levels were eightfold higher after 72 hours exposure to 2% oxygen when compared with 15% oxygen ($p < 0.05$).

The authors concluded that low oxygen tension upregulates synthesis of TGF- β 1 by human dermal fibroblasts, increasing the secretion of this peptide. Fibroblasts migrate to the wound a few days after initial wounding when the area is still hypoxic.

These results suggest this environment may initiate collagen production.

In contrast, Siddiqui et al.,²⁸ investigated the effect of chronic hypoxia on human dermal fibroblasts cultured from skin biopsies of healthy participants. Cells grown in a hypoxic environment (1% oxygen) were compared with those grown in standard culture conditions (20% oxygen). Chronic hypoxia was defined as more than six passages (the culture was serially subcultured six times) in the 1% environment. Acute hypoxia was not defined.

Cell proliferation was three times slower following exposure to 1% oxygen when compared with 20% oxygen, and chronic hypoxia resulted in a 3.1-fold decrease in TGF- β 1 mRNA.

The investigators concluded that a chronic hypoxic environment affects the production of TGF- β 1 from dermal fibroblasts in a different manner to an acutely hypoxic one: acute hypoxia stimulates processes such as growth factor production, whereas chronic conditions inhibit or eliminate them. Neither study has since been replicated.

More recent work by Falanga et al.²⁹ on hypoxia and TGF- β 1 concentrated on the transcriptional activity (the making of RNA from DNA) of this peptide. The TGF- β 1 was produced from fibroblasts of the dorsal forearm of healthy volunteers. They found that acute hypoxia may act as a stimulus for collagen synthesis, with TGF- β 1 playing a critical role.

Patel et al.⁸ studied the expression of VEGF by human keratinocytes *in vitro* when exposed to 20% and 90% normobaric oxygen, and hyperbaric oxygen (90% oxygen at 3ATA [atmospheres absolute]). The cells were placed in culture plates and put into three separate incubators; a hyperbaric incubator was used to achieve a pressure of 3ATA. Exposure to 90% normobaric oxygen resulted in limited cell growth when compared with 20% normobaric oxygen, while there was virtually no growth with 90% normobaric oxygen at 3ATA. However, mRNA analysis suggested that oxygen therapy (normobaric and hyperbaric) induced VEGF expression by keratinocytes. The authors thus proposed that, while hypoxia can initiate angiogenesis, oxygen is required for the continued release of VEGF, thereby sustaining the process.

Angiogenesis

While it is known that angiogenesis helps restore blood flow to injured tissue,⁵ it is still unclear whether hypoxia or oxygen is required to initiate this process. The accepted theory is that, while hypoxia is required to initiate angiogenesis, increased oxygen levels are needed to create the oxygen gradient that stimulates and maintains it.^{7,8} Vascular endothelial growth factor enhances angiogenesis, and its relationship with oxygen has already been discussed.

Most of the research on this area comprises animal studies, most probably due to the difficulty of controlling confounding factors in humans and in recreating the complexity of *in vivo* interactions in *in vitro* studies. However, the relevance of animal studies to the human healing process is debatable, so the results should be treated with caution.

Knighton et al.³⁰ investigated the rate and density of capillary growth in wounds on the ears of rabbits following inhalation of 12%, 20%, 40% and 70% oxygen concentrations. Unfortunately, the paper did not explain why these concentrations were chosen, nor did it state how long the animals were exposed to these oxygen levels. An ear chamber (a small flat circular object) was implanted into surface of the skin on the ventral surface of each ear. The chamber was then covered with a close-fitting cover to stop any atmospheric oxygen from reaching the wound surface. Capillary density was measured by counting the number of capillaries present on a tracing taken of the vascular pattern that developed over the rabbit ear chamber. In addition, the percentage of the surface of the ear chamber covered with capillaries was calculated using photographs and tracings. From this, the rate of vessel growth was calculated.

Inspiration of 40% oxygen resulted in significantly slower vessel growth than exposure to the lower oxygen concentrations on day 1, but a sharp increase in the rate of angiogenesis from days 2–4, although this difference was no longer significant. Capillary density was significantly higher at the end of the experiment in the rabbits who inhaled 40% and 70% oxygen compared with the lower oxygen concentrations; unfortunately, the time scale for this was not specified. Capillary growth was arrested when the chamber covers were removed, exposing the hypoxic area to atmospheric oxygen.

Knighton et al.³⁰ suggested that these results demonstrate that an oxygen gradient is required for angiogenesis to take place, and that capillary growth will stop if this gradient is removed. They also proposed that inhalation of oxygen affects the rate and density of capillary growth, with higher oxygen concentrations resulting in more efficient angiogenesis overall.

Knighton et al. used an animal model so that they could manipulate the oxygen concentrations and control the wound environment.

Hopf et al.³¹ used a mouse subcutaneous wound model to investigate the effect of oxygen on angiogenesis by exposing the mice to oxygen concentrations of 13% (hypoxic), 21% (normoxic, the control group), 100% (hyperoxic) and 100% at 2ATA, 2.5ATA and 3ATA (all hyperoxic and hyperbaric). Mice in the 13% and 21% groups were exposed constantly, while those in all of the hyperoxic groups were exposed for 90 minutes twice daily in order to mim-

ic hyperbaric oxygen therapy. Half the mice in all groups were given supplemental VEGF. Angiogenesis was assessed microscopically in the laboratory.

Results after seven days showed that angiogenesis increased significantly in all hyperoxic groups and was inhibited in the hypoxic group. No significant angiogenesis occurred in the non-VEGF supplemented control group.

These results demonstrate that angiogenesis in wounds requires oxygen and Hopf et al.³¹ appear to have confirmed their hypothesis that hyperoxia accelerates angiogenesis while prolonged hypoxia inhibits it. Unfortunately, the sample sizes were not specified and the quality of the blood vessels was not assessed, so these results must be interpreted with caution.

Even though animal models are never a direct illustration of human wounds (for example, Cho et al.³² found that human wounds contain greater levels of oxidants than rat wounds), Hopf et al.³¹ discussed the relevance of their results to the clinical environment and suggested their study reflects the hypoxic environment and impairment of angiogenesis commonly observed in both acute and chronic wounds.

However, further human *in vitro* research is needed to provide a more thorough understanding of the underlying mechanisms.

Collagen synthesis

Fibroblasts synthesise collagen fibres, which create a scaffold for the healing process. Collagen, being a triple-stranded helix, is an unusual protein.⁵ Hydroxyproline is essential in stabilising the triple helix, and when its levels are low, the helix can unwind.³³ Hunt and Pai³⁴ believed that fibroblasts can only produce collagen if sufficient oxygen is present. This was supported by the study on TGF- β 1 by Siddiqui et al.²⁸ discussed above.

Niinikoski¹⁰ was the first to investigate the effect of oxygen supply on wound healing. He conducted a range of experiments in rats, and the results of experiments investigating scaffold formation showed that inhalation of 35–70% oxygen significantly increased the tensile strength of healing wounds.

Hunt and Pai³⁴ attempted to determine whether changes in oxygen levels in rabbits alter the rate and density of collagen synthesis. Cylinders were implanted into six rabbits, and the resulting wounds were left to heal in the air for 20 days. Two rabbits were placed in a 45% (hyperoxic) oxygen environment, two in air (normoxic) and two in 14% oxygen (hypoxic) until day 25.

The results suggest that exposure to the hyperoxic environment accelerated collagen synthesis and this was closely related to arterial oxygen tension, possibly with the oxygen gradient, as discussed earlier, playing an essential role. However, their investigation on the effect of hypoxia was inconclusive as, by

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their own admission, experimental variability and the small sample numbers reduced the reliability of the results. The initial lack of a moist wound healing environment may also have affected the results.

Mehm et al.³⁵ studied the effect of oxygen on murine fibroblast proliferation and collagen synthesis *in vitro* and found that both were optimised at oxygen levels approximately twice those of normal tissue. They exposed cells to 1788mmHg, 722mmHg, 160mmHg, 80mmHg, 38mmHg (normal tissue levels) and 15mmHg partial pressures of oxygen for four days. Maximum cell growth was seen in cultures exposed to partial pressures of under 80mmHg; the highest collagen production was in the 80mmHg and 38mmHg groups. These results demonstrate that hyperoxia over 80mmHg can be detrimental to fibroblast proliferation and collagen synthesis. Mehm et al. suggested this was because the higher levels are toxic to cells.

Following research in rabbits using tissue oxygen tension (ptO₂) to predict leakage of colonic anastomoses,³⁶ Sheridan et al.³⁷ proceeded to investigate this in humans. Collagen is key to anastomotic healing.³⁷ They used a Clark electrode to measure the tissue oxygen tension of the colon in 50 patients undergoing colonic resection and anastomosis. Patients were administered standardised 33.3% oxygen. The 10% of patients with significantly lower tissue oxygen tension all experienced anastomotic leakage (p<0.01), despite receiving 33.3% oxygen. Furthermore, the tissue oxygen tension in those experiencing leakage dropped to 50% of that of the pre-resection value and was less than 20mmHg in most cases, suggesting that good tissue oxygen tension levels are required to prevent dehiscence in anastomotic healing, probably due to the effect of oxygen on collagen synthesis.

Most of the studies on this area have been per-

formed in animals. However, recent research is now using *in vitro* molecular techniques to investigate the effect of oxygen on collagen synthesis — an example being the study by Falanga et al.²⁹

Conclusion

Oxygen is essential for successful wound healing. However, hypoxia is a normal occurrence in all wounds and is required to stimulate processes such as the release of growth factors and angiogenesis, and to create an oxygen gradient. To sustain the healing process, the tissue oxygen tension needs to be significantly higher in the surrounding tissue than at the centre of the wound.

Some studies have suggested that too much oxygen can be detrimental to healing — for example, in relation to collagen synthesis — although in clinical practice supplemental oxygen is used to prevent complications of wound healing, such as infection and dehiscence.

The effectiveness of normobaric versus hyperbaric oxygen therapy has not been discussed here in any detail. Given the apparent benefits of oxygen and the importance of the oxygen gradient, this area requires further study.

Much of the research has been conducted either *in vitro* or in animal models. It is difficult to measure cellular responses and not always possible to control for confounding factors in humans. However, *in vitro* studies do not allow for the cell-to-cell interaction that can occur *in vivo*, and human healing processes are complicated by many varying factors including disease and nutrition.

More clinical trials are therefore needed to assess the optimum oxygen levels required for effective wound healing. This will in turn determine the best intervention for chronic and ischaemic wounds and encourage rapid healing of acute surgical wounds. ■

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