

PERSPECTIVE ARTICLE

Wound healing essentials: Let there be oxygen

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ABSTRACT

The state of wound oxygenation is a key determinant of healing outcomes. From a diagnostic standpoint, measurements of wound oxygenation are commonly used to guide treatment planning such as amputation decision. In preventive applications, optimizing wound perfusion and providing supplemental O₂ in the perioperative period reduces the incidence of postoperative infections. Correction of wound *p*O₂ may, by itself, trigger some healing responses. Importantly, approaches to correct wound *p*O₂ favorably influence outcomes of other therapies such as responsiveness to growth factors and acceptance of grafts. Chronic ischemic wounds are essentially hypoxic. Primarily based on the tumor literature, hypoxia is generally viewed as being angiogenic. This is true with the condition that hypoxia be acute and mild to modest in magnitude. Extreme near-anoxic hypoxia, as commonly noted in problem wounds, is not compatible with tissue repair. Adequate wound tissue oxygenation is required but may not be sufficient to favorably influence healing outcomes. Success in wound care may be improved by a personalized health care approach. The key lies in our ability to specifically identify the key limitations of a given wound and in developing a multifaceted strategy to specifically address those limitations. In considering approaches to oxygenate the wound tissue it is important to recognize that both too little as well as too much may impede the healing process. Oxygen dosing based on the specific need of a wound therefore seems prudent. Therapeutic approaches targeting the oxygen sensing and redox signaling pathways are promising.

The clinical application of O₂ to wound healing occurs at many levels: diagnostic, preventive, and therapeutic. From a diagnostic standpoint, measurements of wound oxygenation (transcutaneous O₂ measurements or TCOM) are commonly used to guide treatment planning such as amputation decision.^{1–6} In preventive applications, optimizing wound perfusion and providing supplemental O₂ in the perioperative period reduces the incidence of postoperative infections.^{7–9} Correction of wound *p*O₂ (partial pressure of oxygen in the wound tissue) may, by itself, trigger some healing responses.^{10–18} More importantly, approaches to correct wound *p*O₂ favorably influence outcomes of other therapies such as responsiveness to growth factors and acceptance of grafts.^{10,19,20} This leads to the concept of correction of wound hypoxia as adjunct to other therapeutic modalities.^{14,21} Although the case for therapeutic approaches aimed at correcting wound tissue hypoxia is compelling, outcomes in the wound clinics have been inconsistent. The objective of this review article is to concisely address some of the fundamental and emergent concepts in tissue O₂ sensing and response with the goal to illuminate salient complexities and perform critical analysis of what should help improve clinical outcomes in response to O₂-based therapeutics.

WOUND ISCHEMIA AND HYPOXIA

Vascular complications commonly associated with problematic wounds are primarily responsible for wound

ischemia. Limitations in the ability of the vasculature to deliver O₂-rich blood to the wound tissue leads to, among other consequences, hypoxia. Hypoxia represents a reduction in oxygen delivery below tissue demand, whereas ischemia is a lack of perfusion, characterized not only by hypoxia but also by insufficient nutrient supply. Hypoxia, by definition, is a relative term. It is defined by a lower tissue partial pressure of oxygen (*p*O₂) compared with the *p*O₂ to which the specific tissue element in question is adjusted to under healthy conditions *in vivo*. Depending on the magnitude, cells confronting hypoxic challenge either induce an adaptive response that includes increasing the rates of glycolysis and conserve energy or suffocate to death.²² Generally, acute mild to moderate hypoxia supports adaptation and survival. In contrast, chronic extreme hypoxia leads to tissue loss. While the tumor tissue is metabolically designed to thrive under conditions of hypoxia,²³ hypoxia of the wound primarily caused by vascular limitations is intensified by coincident conditions (e.g., infection, pain, anxiety, and hyperthermia) and leads to poor healing outcomes.^{24,25}

Three major factors may contribute to wound tissue hypoxia: (i) peripheral vascular diseases (PVDs) garroting O₂ supply, (ii) increased O₂ demand of the healing tissue, and (iii) generation of reactive oxygen species (ROS) by way of respiratory burst and for redox signaling (Figure 1). Other related factors such as arterial hypoxia (e.g., pulmonary fibrosis or pneumonia, sympathetic response to pain, hypothermia, anemia caused by major blood loss, cyanotic

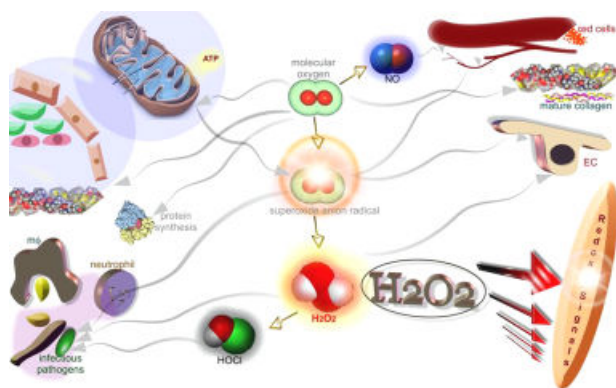


Figure 1. Significance of molecular oxygen and its derivatives in wound healing. In its molecular form, oxygen is required for oxidative metabolism-derived energy synthesis, protein synthesis, and the maturation (hydroxylation) of extracellular matrices such as collagen. Molecular oxygen is also required for NO synthesis, which in turn plays a key role in the regulation of vascular tone as well as in angiogenesis. In a wound setting, large amounts of molecular oxygen are partially reduced to form reactive oxygen species (ROS). ROS includes oxygen free radicals such as superoxide anion as well as its nonradical derivative hydrogen peroxide (H_2O_2). Superoxide anion radical is the one electron reduction product of oxygen. NADPH oxidases represent one major source of superoxide anion radicals at the wound site. NADPH oxidases in phagocytic cells help fight infection. Superoxide anion also drives endothelial cell signaling such as required during angiogenesis. In biological tissues, superoxide anion radical rapidly dismutates to hydrogen peroxide—either spontaneously or facilitated by enzymes called superoxide dismutases. Endogenous hydrogen peroxide drives redox signaling, a molecular network of signal propagation that supports key aspects of wound healing such as cell migration, proliferation, and angiogenesis. Neutrophil-derived hydrogen peroxide may be utilized by myeloperoxidase to mediate peroxidation of chloride ions resulting in the formation of hypochlorous acid (HOCl), a potent disinfectant.

heart disease, high altitude) may contribute to wound hypoxia as well. Depending on factors such as these, it is important to recognize that wound hypoxia may range anywhere from near-anoxia to mild–modest hypoxia.^{26,27} In this context, it is also important to appreciate that point measurements²⁸ performed in the wound tissue may not provide a complete picture of the wound tissue biology because it is likely that the magnitude of wound hypoxia is not uniformly distributed throughout the affected tissue especially in large wounds. This is most likely the case in chronic wounds presented clinically as opposed to experimental wounds, which are more controlled and homogeneous in nature. In any single problem wound presented in the clinic, it is likely that there are pockets of near-anoxic as well as that of different grades of hypoxia (Figure 2). As the weakest link in the chain, tissue at the near-anoxic pockets will be vulnerable to necrosis, which in turn may propagate secondary tissue damage and infection. Pockets of extreme hypoxia may be flooded with hypoxia-inducible angiogenic factors but would fail to functionally vascularize because of insufficient O_2 that is necessary to fuel the

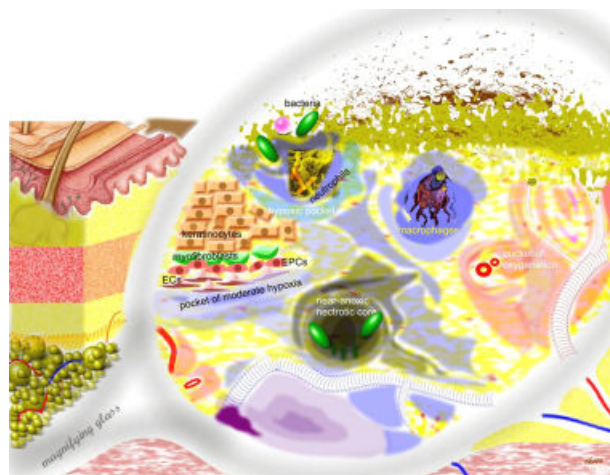


Figure 2. Heterogeneous distribution of oxygen in the wound tissue: hypothetical pockets of graded levels of hypoxia. Structures outside the illustrated magnifying glass represent the macro tissue structures. Objects under the glass represent a higher resolution. Shade of black (anoxia) or blue represents graded hypoxia. Shade of red or pink represents oxygenated tissue. Tissue around each blood vessel is dark pink in shade representing regions that are well oxygenated (oxygen-rich pockets). Bacteria and bacterial infection are presented by shades of green on the surface of the open wound.

repair process. Indeed, uncontrolled expression of vascular endothelial growth factor (VEGF) and its receptors leads to insufficient skin angiogenesis.²⁹ Whether cells in the pockets of extreme hypoxia are O_2 -responsive is another concern. Even if such cells may have passed the point of no return in the survival curve, correction of tissue oxygenation is likely to help clean up the dead or dying tissue^{30,31} and replace the void with proliferating neighboring cells. Pockets of moderate or mild hypoxia are likely to be the point of origin of successful angiogenic response as long as other barriers such as infection and epigenetic alterations are kept to a minimum.

WOUND HYPOXIA: THE IMBALANCE BETWEEN LIMITED SUPPLY AND HIGH DEMAND

Limited supply: PVDs

PVD can affect the arteries, the veins, as well as the lymph vessels. The most common and important type of PVD is peripheral arterial disease (PAD), which affects about 8 million Americans. The ankle brachial pressure index represents a simple noninvasive method to detect arterial insufficiency within a limb. Arterial diseases, especially those associated with diabetes, represent a major complicating factor in wound healing. PAD is the only identifiable etiology in approximately 10% of leg ulcers.³² In an ischemic limb, peripheral tissues are deprived of blood supply as PAD progresses causing tissue loss, ulcers, and gangrene.

Venous insufficiency, on the other hand, is the root cause of most leg ulcers.³³ Chronic venous insufficiency,

characterized by the retrograde flow of blood in the lower extremity, is associated with changes in the venous wall and valves generally caused by inflammatory disorders induced by venous hypertension and associated fluid shear stress. Factors causing arterial hypoxemia may also limit O₂ supply to the wound tissue. Compromised pulmonary health,³⁴ loss of hepatic function,^{35,36} hemodialysis,³⁷ anemia,^{38,39} altitude hypoxemia,⁴⁰ nitroglycerin therapy,⁴¹ nasal packing,⁴² critical illness,⁴³ pain,⁴⁴ and hypothermia^{45,46} are some examples of conditions associated with arterial hypoxemia. Vasoconstricting drugs may contribute to tissue hypoxia as well.⁴⁷

High demand: increased demand of the healing tissue

Mitochondrial respiration is responsible for more than 90% of O₂ consumption in humans. Cells utilize O₂ as the final electron acceptor in the aerobic metabolism of glucose to generate ATP, which fuels most active cellular processes such as during wound healing.⁴⁸ Increased energy demand of the healing tissue leads to a hypermetabolic state wherein additional energy is generated from oxidative metabolism increasing the O₂ demand of the healing tissue.^{49–52} ATP thus generated powers tissue repair. At the injury site, extracellular ATP may be contributed by platelets and other disintegrating cells. Extracellular ATP liberated during hypoxia or inflammation can either signal directly to purinergic receptors or, after phosphohydrolytic metabolism, can activate surface adenosine receptors. Purinergic signaling may influence numerous aspects of wound biology including immune response, inflammation, vascular, as well as epithelial biology. ATP may be immunostimulatory or vice versa depending on extracellular concentrations as well as on expression patterns of purinergic receptors and ecto-enzymes.⁵³ Extracellular ATP induces receptor activation in epithelial cells. ATP, released upon epithelial injury, acts as an early signal to trigger cell responses including an increase in heparin-binding epidermal growth factor (EGF)-like growth factor shedding, subsequent transactivation of the EGF receptor and its downstream signaling, resulting in wound healing.⁵⁴ ATP released from the injured epithelial cells is now known to also turn on NADPH oxidases,⁵⁵ the activity of which is critically required to produce the redox signals required for wound healing.^{19,56,57} Human endothelial cells are rich in purinergic receptors and therefore responsive to extracellular ATP as well.⁵⁸ ATP induces endothelium-dependent vasodilation.⁵⁹ Both ATP as well as adenosine regulate smooth muscle and endothelial cell proliferation.⁶⁰ Recognizing that hypoxia limits ATP synthesis in the ischemic wound tissue, therapeutic ATP delivery systems have been studied for their effect on wound healing.⁶¹ While these approaches may compensate for the deficiency of ATP per se in the ischemic wound tissue, they will fail to address the other essential functions of O₂ and its derivatives in wound healing as discussed below.

Absolute requirements for O₂ arise in several points along the angiogenic sequence. For instance, all vessels require a net or sheath of extracellular matrix (ECM), mainly collagen and proteoglycans, to guide tube formation and resist the pressures of blood flow. Conditions for collagen deposition and polymerization can be created only if molecular O₂ is available to be incorporated into the structure

of nascent collagen by prolyl and lysyl hydroxylases. Without the obligatory extracellular, hydroxylated collagen, new capillary tubes assemble poorly and remain fragile.^{62–64} This has a convincing clinical correlate in scurvy, i.e., ascorbate deficiency. Scurvy may result from insufficient intake of ascorbate, which is required for correct collagen synthesis in humans. Ascorbate is required for the posttranslational hydroxylation of collagen that enables the matured collagen molecules to escape to the extracellular space and provide the necessary tensile strength.⁶⁵ In scurvy, the collagenous sheath cannot form because, under ascorbate-deficient conditions, collagen cannot be hydroxylated. Consequently, new vessels fail to mature. Older vessels weaken and break, and wounds fail to heal.⁶² In this context, it is important to recognize that the collagen hydroxylation process requires molecular oxygen. Thus, even under ascorbate-sufficient conditions collagen may fail to mature if there is insufficient supply of oxygen to the tissue. Collagen deposition proceeds in direct proportion to *p*O₂ across the entire physiologic range, from 0 to hundreds of mmHg. The *K_m* for O₂ for this reaction is approximately 25 and the *V_{max}* is approximately 250 mmHg, suggesting that new vessels cannot even approach their greatest possible rate of growth unless the wound tissue *p*O₂ is high.⁶⁶ Angiogenesis is directly proportional to *p*O₂ in injured tissues.⁶³ Hypoxic wounds deposit collagen poorly and become infected easily, both of which are problems of considerable clinical significance.^{67,68}

High demand: increased production of reactive species

Phagocytic NADPH oxidases

Sbarra and Karnovsky's 1959 discovery of the leukocyte oxidase⁶⁹ in phagocytes came into limelight in the late 1970s, when the pioneering works of Bernard Babior linked the explosive production of superoxide ions (O₂^{•-}) by leukocyte oxidase to bacterial killing.⁷⁰ During phagocytosis of microbial intruders, professional phagocytes of our innate immune system increase their O₂ consumption through the inducible activity of NADPH oxidase (NOX) that generates O₂^{•-} and H₂O₂. These oxygen-derived metabolites give rise to yet other ROS that are potentially antimicrobial but which may also cause damage by destroying surrounding tissue and cells. NADPH oxidase, catalyzing the deliberate production of ROS by cells, has been extensively investigated in phagocytes (neutrophilic and eosinophilic granulocytes, monocytes, and macrophages).⁷¹ Exposure of these cells to any of a large number of stimuli activates a "respiratory burst," caused by an activation of the plasma membrane-bound NADPH oxidase (NADPH + 2O₂ → NADP⁺ + 2O₂^{•-} + H⁺). The O₂^{•-} then rapidly dismutates to H₂O₂. Approximately 98% of the O₂ consumed by wound neutrophils is utilized for respiratory burst.²⁴ NADPH oxidase supports macrophage survival⁷² and enables dead cell cleansing by phagocytosis.⁷³ Appropriate infection management may therefore spare precious O₂ at the wound site, which would otherwise be utilized via respiratory burst.⁷⁴ Overt infection poses the risk of intensifying wound tissue hypoxia.

The NOX of "professional" phagocytic cells transfers electrons across the wall of the phagocytic vacuole, forming O₂^{•-} in the lumen. It is generally accepted that this

system promotes microbial killing through the generation of ROS and through the activity of myeloperoxidase.⁷⁵ In response to bacterial infection, the neutrophil NADPH oxidase assembles on phagolysosomes to catalyze the transfer of electrons from NADPH to O₂, forming O₂^{•-} and derivative ROS. The active oxidase is composed of a membrane-bound cytochrome (e.g., gp91phox and p22phox) together with three cytosolic phox proteins, p40phox, p47phox, and p67phox, and the small GTPase Rac2, and is regulated through a process involving protein kinase C, mitogen-activated protein kinase, and phosphatidylinositol 3-kinase.^{76,77} In the resting cell, two of the subunits, p22phox and gp91phox, are located in the membrane, and the remaining components are present in the cytosol. The electron-carrying components of the oxidase are located in gp91phox.^{78–81} The NADPH-binding site is generally regarded to be in gp91phox as well, but there is some evidence that it may be in p67phox. The catalytic subunit gp91phox, dormant in resting cells, becomes activated by assembly with cytosolic regulatory proteins. When the oxidase is activated, p47phox is phosphorylated at specific sites, and the cytosolic components together with Rac2 migrate to the membrane to assemble the active oxidase.¹⁹ Mutations in p47phox are a cause of chronic granulomatous disease, an immune-deficient condition characterized with impaired healing response.^{82,83} Rac2 mutation is another factor responsible for impaired human neutrophil NADPH oxidase function, low O₂^{•-} generation, and compromised wound healing.⁸⁴ The concentration of O₂ necessary to achieve half maximal ROS production (the K_m) is in the range of 45–80 mmHg, with maximal ROS production at pO₂ at > 300 mmHg.⁵⁴ Thus, the maximal effects of respiratory burst-dependent wound infection management can only be achieved through the administration of supplemental O₂ to attain wound pO₂ levels beyond those encountered when breathing room air.⁸⁵ This also explains why the state of wound tissue oxygenation is a sensitive indicator for the risk of infection in surgical patients.^{8,9,86,87}

Oxygen free radicals and reactive derivatives: a paradigm shift and emergence of redox signaling

In the 1980s, oxygen free radicals drew much attention in biomedical research. Limitations in methodological approaches to sensitively detect and monitor the extremely short-living reactive species clouded a true appreciation of the significance of oxygen-derived free radicals and reactive species in health and disease. The paradigm that emerged was too simple to be meaningful in its complete sense. The primary identity of free radicals was that they were destructive to biological tissues, and that approaches to antagonize free radicals, i.e., antioxidants, are helpful.^{88–96} Based on this crude preliminary concept, numerous clinical trials testing the efficacy of antioxidants were hastily started and the results were understandably disappointing.^{97–101} Lack of consideration of a very important aspect of free radical biology that started to crystallize only in the late 1990s proved to be very expensive in many ways. Work during the mid-late 1990s led to the recognition that at very low levels, oxygen-derived free radicals and derivative species such as H₂O₂ may serve as signaling messengers.^{102–104}

The field of redox signaling was thus born^{102,105–107} with a dedicated international peer-reviewed journal (<http://www.liebertpub.com/ars>). Today, the concept that reactive derivatives of O₂ may serve as signaling messengers has revolutionized cell biology^{108–123} and has led to the concept of redox-based clinical therapeutics.^{124–129}

Nonphagocytic NADPH oxidases

Given the traditional bad and ugly image of oxygen free radicals and its derivatives, few would have imagined that even nonphagocytic cells of the human body have a dedicated apparatus to generate ROS. In 1999, the cloning of Mox1 marked a major progress in categorically establishing the presence of distinct NADPH oxidases in nonphagocytic cells.¹²³ Mox1 or p65Mox was described as encoding a homolog of the catalytic subunit of the O₂^{•-}-generating NADPH oxidase of phagocytes, gp91phox. Mox1 messenger RNA is expressed in colon, prostate, uterus, and vascular smooth muscle, but not in peripheral blood leukocytes. Later, Mox1 was renamed as NOX1 referring to NADPH oxidase.¹³⁰ Over the last years, six homologs of the cytochrome subunit of the phagocyte NADPH oxidase were found: NOX1, NOX3, NOX4, NOX5, DUOX1, and DUOX2. Together with the phagocyte NADPH oxidase itself (NOX2/gp91(phox)), the homologs are now referred to as the NOX family of NADPH oxidases. Activation mechanisms of these enzymes and tissue distribution of the different members of the family are markedly different. The physiological functions of NOX family enzymes include host defense, posttranslational processing of proteins, cellular signaling, regulation of gene expression, cell differentiation, and renewal of precursor cells.^{131–135} NOX enzymes also contribute to a wide range of pathological processes. NOX deficiency may lead to immunosuppression, lack of otoconogenesis, or hypothyroidism. Increased NOX activity also contributes to a large number of pathologies, in particular cardiovascular diseases and neurodegeneration.¹³⁶ Thus, optimal generation of O₂^{•-} is required to sustain healthy living.

Acute inflammation following injury is the site for abundant production of ROS by phagocytic NADPH oxidases. As inflammation resolves and phagocyte count at the wound site falls, several aspects of healing such as cell proliferation and migration are supported by redox signaling where low-level ROS produced by nonphagocytic oxidases serve as messenger molecules.⁵⁷ The critical significance of the NADPH oxidases in wound healing is rapidly unfolding. As discussed previously, NADPH oxidase-deficient mice and humans suffer from impaired healing. As an integral part of the healing response, wounding induces H₂O₂ production.⁵⁶ This response is also conserved in plants.¹³⁷ Wound fluid from healing tissues contains the highest concentration of H₂O₂ compared with all other bodily fluids.^{56,138} Of note, selective decomposition of H₂O₂ at the wound site using catalase overexpression approaches impairs the healing process demonstrating the key significance of H₂O₂ in wound healing.⁵⁶ Importantly, catalase-dependent decomposition of H₂O₂ generates O₂ as end-product. Thus, molecular O₂ is not sufficient if NADPH oxidase-dependent O₂ consumption and redox signaling is impaired. How redox signals may contribute to

tissue repair has been recently reviewed elsewhere^{57,139} and is beyond the scope of this article. In the context of this article, it is important to appreciate that redox signals are generated at the cost of tissue O₂. Thus, tissue hypoxia will limit redox signaling and disable the function of several growth factors (e.g., platelet-derived growth factor [PDGF], VEGF, keratinocyte growth factor, insulin-like growth factor, transforming growth factor- α) and numerous molecular mechanisms (e.g., leukocyte recruitment, cell motility, integrin function), which rely on redox signaling.^{57,139,140}

Collagen deposition provides the matrix for angiogenesis and tissue remodeling. Maturation of collagen is O₂ dependent. Of the O₂-dependent enzymatic processes, the rate of collagen synthesis is reflected by the rate at which prolyl hydroxylation occurs.¹⁴¹ Collagen synthesis is half-maximal (K_m using Michaelis–Menton equation) at a pO_2 of 20–25 mmHg,^{66,142} with V_{max} at levels approaching 250 mmHg. This represents levels of O₂ availability that exceeds the pO_2 normally present in the wound tissue and suggests that adequate wound tissue oxygenation is crucial to support collagen synthesis and maturation. Indeed, increasing wound oxygenation results in increased collagen deposition and tensile strength.^{143–145}

Nitric oxide (NO) synthases

NO is widely recognized as a major signaling messenger that drive numerous aspects of (patho)physiology.^{146–149} O₂ consuming NO synthases (NOS) catalyze NO formation from the amino acid L-arginine. The reaction of NOS with O₂ is fast and takes place within several steps.¹⁵⁰ NOS are known to catalyze more than one reaction: the NO-producing reaction is considered to be the coupled reaction, and the uncoupled reactions are those that produce ROS, such as O₂^{•-} and H₂O₂.¹⁵¹ The key significance of NO in wound healing has been reviewed elsewhere.^{152,153} In the context of this article, it is important to note that O₂ is often the overlooked substrate in NO synthesis. To date, there has been little consideration of the role of O₂ tension in the regulation of NO production associated with wound healing. Tissue O₂ tension is known to significantly alter endogenous NO production in articular cartilage where the tissue pO_2 is comparable to that of ischemic wounds.¹⁵⁴ The preliminary observation that hyperbaric oxygen (HBO) therapy may significantly increase local wound NO levels is therefore understandable.¹⁵⁵ Once generated, the biological significance of NO also depends on the tissue oxygenation status.¹⁵⁶ As NO gas-based therapies are being considered for healing wounds clinically, it is important to recognize that NO can block mitochondrial function by interacting with the cytochrome *c* oxidase (complex IV) of the electron transport chain in a manner that is reversible and in competition with O₂. Concentrations of NO too low to inhibit respiration can trigger cellular defense response mechanisms. Inhibition of mitochondrial respiration by NO at low O₂ concentrations can cause so-called “metabolic hypoxia” and divert O₂ toward other oxygen-dependent systems. Metabolic hypoxia refers to a state wherein although O₂ is available the cell is unable to utilize it for respiration.¹⁵⁷ Such a diversion reactivates prolyl hydroxylases and thus accounts for the

prevention by NO of the stabilization of the hypoxia-inducible factor (HIF). When NO inhibits mitochondrial respiration under hypoxia, it prevents mitochondria from depleting local oxygen, enabling the continued hydroxylation and degradation of HIF-1 α , thus leading to a situation in which the cell may fail to register hypoxia. Furthermore, in a wound setting where O₂^{•-} production is highly active, NO is likely to generate peroxynitrite that can affect the action of key enzymes, such as mitochondrial complex I, by S-nitrosation.¹⁵⁷ NO-based wound therapeutics should be designed in light of these complexities.

The stability of HIF, and therefore its ability to drive HIF-dependent gene transcription, is differentially regulated by NO under conditions of normoxia and hypoxia. While NO stabilizes HIF under normoxia, the effect is exactly opposite under conditions of hypoxia.¹⁵⁸ Under conditions of normoxia, NO may attenuate the ubiquitination of HIF-1 α and thus abrogate binding of von Hippel-Lindau (pVHL) to HIF-1 α .¹⁵⁹ Ubiquitination of HIF would not take place if HIF is not hydroxylated by prolyl hydroxylase domain enzymes (PHDs). Indeed, NO inhibits PHD activity. Fe²⁺ coordination by NO seems to be the explanation for how NO inhibits PHDs. The stabilization of HIF under normoxia is also explained by the induction of HIF-1 α synthesis by NO.¹⁶⁰ Although speculative, different redox-active products, derived from chemically distinct NO donors, use divergent transmission systems to stabilize/express HIF-1 α .¹⁶⁰ Under conditions of hypoxia, NO and its derivatives inhibit hypoxia-induced HIF-1 α accumulation.¹⁵⁸ In light of the observation that NO attenuates PHD activity under normoxia to stabilize HIF-1 α , raises the question whether PHD activity is regained under conditions of hypoxia–NO coexistence. An affirmative answer to this question came from the observation that oxygen-dependent death domain of HIF-1 α , which accounts for protein stability, is needed for NO and its derivatives to reverse hypoxic HIF-1 α stabilization.¹⁶¹ Several mechanistic hypotheses have been proposed to explain how NO impairs accumulation of HIF-1 α under hypoxia.¹⁵⁸ The scenario gets even more complicated in a wound setting where both phagocytic as well as non-phagocytic NADPH oxidases generate copious amounts of superoxide anion radicals.^{56,138} Furthermore, hypoxic tissues are known to generate more ROS. The HIF system has revealed an unexpectedly direct connection between molecular oxygen, superoxide, and NO in achieving or attenuating responses to hypoxia. The reaction between O₂^{•-} and NO represents a primary biochemical path in vivo.¹⁶² Flux rates of NO and O₂^{•-}, as well as the presence of antioxidant enzymes, can modulate HIF-1 α stabilization.¹⁵⁸ Understanding the multiple signals, which have the potential to deliver a flexible and controlled response to hypoxia, will be critical to develop therapeutic maneuvers. Thus, a clear appreciation of the specific wound tissue redox environment⁵⁷ becomes critically important in the context of planning NO-based therapeutics.

THE NORMOXIC SETPOINT AND OXYGEN SENSING

Cellular O₂ homeostasis is tightly maintained within a narrow range (“normoxia”) due to the risk of oxidative

damage from excess O₂ (hyperoxia), and of metabolic demise from insufficient O₂ (hypoxia). The vast majority of the current literature focuses on the sensing of hypoxia, and the work on hyperoxic sensing is limited. Both hypoxia and hyperoxia are relative terms. They refer to a state of oxygenation that departs from the normoxic setpoint, i.e., the pO₂ to which cells or tissues are adjusted to under basal conditions.¹⁶³ For any given cell or tissue, normoxic setpoint represents that state of oxygenation where the cell or tissue does not report hypoxia neither do they induce hyperoxia-induced cell signaling or manifest overt oxygen toxicity. It is likely that this setpoint would represent a range of pO₂, the span of which might depend on the tissue in question. Any change of O₂ ambience exceeding that span would result in the switching on of a hypoxic or hyperoxic response. In the finest of scales, such response would be detected in the molecular scale such as HIF stabilization or hypoxia response element (HRE) transactivation for hypoxia and say p21 induction for hyperoxia.^{164,165} In a relatively coarser scale, oxygen-sensitive changes in cellular phenotype may be noted. Of note, different organs of the body have different normoxic setpoints. While the lung and arterial vasculature represent the high end, organs such as the liver have very low basal pO₂. pO₂ ranges from 90 to below 3 torr in mammalian organs under normoxic conditions with arterial pO₂ of about 100 torr or ~14% O₂.¹⁶⁶

Hypoxia sensing

Hypoxia sensing and response is activated upon exposure to a state of oxygenation that is lower than the pO₂ to which the cells or tissue is adjusted to under basal conditions. This response cascade is centrally important in coping with the challenge of O₂ deficiency. Hypoxia response has been mostly studied in transformed and tumor cells. It is important to recognize that findings from such cells may not be directly applicable to nontransformed primary cells that are involved in wound healing.¹⁶⁷ Hypoxia is a hallmark of all ischemic diseases but is also noted under several physiological processes where exposure to a dynamic state of oxygenation is an integral component. During early pregnancy, trophoblast differentiation occurs in an environment of relative low O₂ tension, which is essential for normal embryonic and placental development.¹⁶⁸ O₂ supply to the human embryo in the first trimester is tightly controlled, suggesting that too much O₂ may interfere with development. Relative to maternal tissue pO₂, the embryo is normally in a state of partial hypoxia.^{169,170} Thus, hypoxia sensing and response is not only implicated in ischemic disease conditions but is also required for development where a changing state of oxygenation seems to serve as a cue for successful development. Whether this is nature's approach to quality check each healthy birth for the ability of the new born to cope with ischemic diseases later on in their lives may be viewed as a matter of interesting speculation.

Hypoxia sensing and response mechanisms may be broadly classified into two general categories: HIF-dependent and HIF-independent. Extensive discussion of these pathways is beyond the scope of this article and the readers are referred to excellent review articles.^{171–173}

HIF-dependent pathways

The basic helix–loop–helix (bHLH) proteins form a large superfamily of dimeric transcriptional regulators that are found in organisms from yeast to humans and function in critical developmental processes. One basis for the evolutionary classification of bHLH proteins is the presence or absence of additional domains, of which the most common are the PAS, orange, and leucine-zipper domains. PAS domains, located carboxy-terminal to the bHLH region, are 260–310 residues long and function as dimerization motifs. They allow binding with other PAS proteins, non-PAS proteins, and small molecules such as dioxin. The PAS domain is named after three proteins containing it: *Drosophila* Period (Per), the human aryl hydrocarbon receptor nuclear translocator (Arnt), and *Drosophila* Single-minded (Sim). HIFs belong to the bHLH–PAS family of environmental sensors that bind to canonical DNA sequences called HREs in the promoters or enhancers of target genes.¹⁷⁴ HIF is able to direct transcription from either of two transactivation domains, each of which is regulated by distinct mechanisms. The O₂-dependent asparaginyl hydroxylase factor-inhibiting HIF-1 α (FIH-1) is a key regulator of the HIF C-terminal transactivation domain, and provides a direct link between O₂ sensing and HIF-mediated transcription. Additionally, there are phosphorylation and nitrosylation events reported to modulate HIF transcriptional activity, as well as numerous transcriptional coactivators and other interacting proteins that together provide cell and tissue specificity of HIF target gene regulation.¹⁷⁵

HIF-1 consists of a constitutively expressed subunit HIF-1 β and an oxygen-regulated subunit HIF-1 α (or its paralogs HIF-2 α and HIF-3 α). The transcriptional role of HIF is primarily dependent on the stabilization of HIF-1 α or its paralogs under hypoxic conditions. Under O₂-replete conditions HIF-1 α is very labile.¹⁷⁶ Molecular O₂ targets HIF for degradation by posttranslational hydroxylation at specific prolyl residues within the α subunits. Hydroxylation at two prolyl residues within the central degradation domain of HIF-1 α increases the affinity for the pVHL E3 ligase complex by at least three orders of magnitude, thus directing HIF- α polypeptides for proteolytic destruction by the ubiquitin/proteasome pathway. Because the HIF hydroxylases have an absolute requirement for molecular O₂ this process is suppressed in hypoxia allowing HIF- α to escape destruction and activate transcription.

The O₂-sensitive PHDs and the asparagines hydroxylase (FIH) regulate the transcriptional activity of HIFs.¹⁷⁵ The unusual high *K_m* of PHDs for oxygen allows small changes in the oxygen supply to affect enzyme activity, which makes this system an ideal oxygen sensor. In hypoxia, FIH-1 hydroxylation of Asn803 within the C-terminal transactivation domain does not occur and HIF-1 α fails to form a fully active transcriptional complex. Thus, HIF prolyl hydroxylation regulates proteolytic degradation of HIF whereas HIF asparaginyl hydroxylation modulates interaction with transcriptional coactivators. These hydroxylations are catalysed by a set of non-heme Fe(II)- and 2-oxoglutarate (2-OG)-dependent dioxygenases. During catalysis, the splitting of molecular O₂ is coupled to the hydroxylation of HIF and the oxidative decarboxylation of 2-OG to give succinate and CO₂. The von Hippel-

Lindau tumor suppressor gene product, pVHL, functions as the substrate recognition component of an E3-ubiquitin ligase, which targets the O₂-sensitive α -subunit of HIF for rapid proteasomal degradation under normoxic conditions and as such plays a central role in molecular O₂ sensing.

Stabilization of HIF under hypoxic conditions is followed by nuclear localization where HIF may bind to DNA sequences and other transcriptional regulators to influence gene expression (Table 1). The passage of transcription factors, e.g., HIF-1 α into the nucleus through the nuclear pore complex is regulated by nuclear transport receptors. Therefore, nucleocytoplasmic shuttling can regulate transcriptional activity by facilitating the cellular traffic of transcription factors between both compartments.¹⁷⁷

Shortly after the cloning of HIF-1 α , a closely related protein, HIF-2 α (also known as endothelial PAS protein, HIF-like factor, HIF-related factor, and member of the PAS superfamily 2), was identified and cloned.¹⁷⁸

HIF-2 α regulates erythropoietin production in adults.¹⁷⁹ HIF-1 α functions as an upstream player in the p21-mediated growth arrest of keratinocytes.¹⁸⁰ Thus, HIF may antagonize certain aspects of skin repair. Negative pressure wound therapy, known to be effective in healing wounds clinically, is known to antagonize the stabilization of HIF-1 α .¹⁸¹ HIF-dependent pathways for survival and vascularization can function under conditions where hypoxia is moderate and not extreme. As long as there is a threshold level of oxygenation sufficient to sustain life, HIF-dependent survival responses may benefit wound healing.^{182–184} Near-anoxic hypoxia, often noted in problem wounds,^{26,27} is not compatible with life or tissue repair.

HIF-independent pathways

Conservation of ATP under conditions of limited O₂ supply is a HIF-independent survival response that is not compatible with the energy-demanding healing process.⁴⁹ For example, HIF-independent hypoxic inhibition of protein synthesis and cell growth is mediated by (i) hypoxia-induced cellular energy depletion; (ii) mTOR inhibition via the AMP-activated protein kinase (AMPK)/TSC2/Rheb pathway; (iii) eEF2 inhibition mediated by AMPK; and (iv) induction of endoplasmic reticulum (ER) stress that leads to eIF2 α inhibition.¹⁸⁵ mTOR is a Ser/Thr kinase that integrates signals from growth factors and nutrients to increase ribosome biogenesis.¹⁸⁶ Upon hypoxic energy starvation, AMPK phosphorylates eEF2 kinase (eEF2K) on Ser398 and activates its kinase activity.¹⁸⁷ eEF2K then phosphorylates elongation factor eEF2 at Thr56, resulting in the inhibition of peptide elongation. mRNA translation is a critical component of cell growth and proliferation that is critically supported by eIF2 α . Hypoxia causes ER stress, which in turn inhibits eIF2 α .¹⁸⁵ Wound healing requires protein synthesis.^{188–190} Hypoxia causes global down-regulation of protein synthesis. Hypoxia-induced translational attenuation may be linked to ER stress and the unfolded protein response.¹⁹¹ The translational efficiency of individual genes is dynamic and changes with alterations in the cellular environment.¹⁹² Whereas changes in transcription can take hours to achieve, translational regulation is rapid and reversible.¹⁹³ Preferential translation of select mRNA is another hallmark of response to hypoxia. Roughly 2.5% of total cellular transcripts are preferentially translated, despite arrest of global protein synthesis, in response to sustained extreme hypoxia.¹⁹⁴ Taken together, while all these hypoxia responses

Table 1. Hypoxia-inducible factor-1 (HIF-1) target genes

Erythropoiesis/iron metabolism	Cell survival/proliferation	Angiogenesis	Vascular tone	Glucose metabolism	Matrix metabolism
EPO	IGF-2	VEGF	NOS2	HK1,2	MMPs
Tf	TGF- α	Leptin	HO1	LDHA	PAR/PAI
Tfr	ADM	TGF- β 3	ET1	PKM	Coll PHD
Ceruloplasmin	BNip3	EG-VEGF	ADM	PFKL	
	NIX		α_{1b}	PGK1	
	NDRG2			PFKFB3	
				GAPDH	
				GLUT1,3	
				ENO1	
				CA-9	
				ALD-A,C	
				AK-3	

α_{1b} , α_{1b} -adrenergic receptor; ADM, adrenomedulin; AK, adenylate kinase; ALD, aldolase; BNip3, Bcl-2/adenovirus E1B 19kD-interacting protein 3; CA, carbonic anhydrase; Coll PHD, collagen prolylhydroxylases; EG-VEGF, endocrine gland-derived VEGF; ENO, enolase; EPO, erythropoietin; ET, endothelin; GAPDH, glyceraldehyde phosphate dehydrogenase; GLUT, glucose transporters; HK1,2, hexokinase 1,2; HO, heme oxygenase; IGF, insulin-like growth factor; LDH-A, lactate dehydrogenase-A; MMP, matrix metalloproteinases; NDRG, N-Myc downstream-regulated genes; NIX, Nip 3-like protein X; NOS, nitric oxide synthase; PAR/PAI, plasminogen activator receptors and inhibitors; PGK1, phosphoglycerate kinase 1; PFKL, phosphofructokinase L; PKM, pyruvate kinase M; TGF, transforming growth factor; TF, transferrin; Tfr, Tf receptor.

represent important HIF-independent mechanisms of energy conservation that promote survival under low O₂ conditions, they are not compatible with the formation of new tissue as required during wound healing.

Intermittent hypoxia (IH)

O₂ sensing is no longer a unique property limited to chemoreceptors but is a common property of tissues.¹⁹⁵ The classic concept of IH has been markedly revised in light of our current understanding of O₂ sensing. IH, or periodic exposure to hypoxia interrupted by return to normoxia or less hypoxic conditions, occurs in many circumstances. Chronic intermittent hypoxia (CIH) is a common life-threatening condition that occurs in many different diseases, including sleep-disordered breathing manifested as recurrent apneas. Excessive ROS have been identified as one of the causative factors in a variety of morbidities.¹⁹⁶ In experimental models, CIH activates ROS-dependent responses that include (a) altered carotid body function, the primary chemoreceptor for sensing changes in arterial blood O₂; (b) elevated blood pressure; (c) enhanced release of transmitters and neurotrophic factors; (d) altered sleep and cognitive behaviors; and (e) activation of second-messenger pathways and transcriptional factors. Considerable evidence indicates elevated ROS levels in patients experiencing CIH as a consequence of recurrent apneas.¹⁹⁶ Recently, we evaluated the prevalence of obstructive sleep apnea (OSA) in the patient population of the OSU Wound Center. Between August 15 and September 30, 2007, 105 consecutive unscreened patients of the wound center completed a sleep screening questionnaire. In this representative sample of patients of the wound center, 51% either were diagnosed with, or were at very high risk for OSA. Forty-three percent of patients with chronic nonhealing wound were deemed at high risk for OSA.¹⁹⁷ Whether IH associated with OSA in chronic wound patients complicates wound healing warrants further investigation. Results of our survey may be explained by the association that many with chronic wounds are overweight due to metabolic complications (e.g., PAD and type II diabetes), and sleep apnea is more prevalent in overweight individuals. Merit of the hypothesis that sleep disorder may complicate wound healing is supported by the extensive literature identifying OSA as a causative factor underlying vascular disorders.^{198,199}

Hyperoxia sensing

O₂ got its name from “Principe Oxygene,” which means the acidifying principle. “Oxy” is from Greek, and means sharp or acid; “gen” is also from Greek, and means the origin of. Taken together, oxygen means “the origin of acid.” Joseph Priestly’s (1774) “dephlogisticated air”²⁰⁰ and Carl Scheele’s (1771) “fire air” were soon characterized by Antoine Lavoisier as pure respirable air.²⁰¹ Within decades of the first realization that oxygen is the element of life, Brizé-Fradin²⁰² noted in 1808 that “vital air” or pure oxygen would soon wear life out instead of maintaining it. That oxygen may be harmful to human health was first postulated in the late 19th century with Paul Bert’s work (1878) on oxygen sickness. Paul Bert’s work is regarded as one of the cornerstones of HBO medicine.²⁰³ He con-

cluded that to avoid harmful effects, oxygen should not be inhaled at a concentration above 60% at 1 ATA. Bert’s observation was extended through Michaeli’s theoretical considerations, Gerschman’s experimental verification, and finally caught the interests of biomedical scientists when in 1969 McCord and Fridovich demonstrated that a metalloenzyme produced H₂O₂ by combining O₂^{•-} with hydrogen.^{204,205} Today, H₂O₂ is widely known to function as a cellular messenger.^{108–123} Hyperoxia-inducible molecular biomarkers have been characterized^{164,165} enabling us to detect hyperoxic insult long before overt signs of oxygen toxicity and adverse clinical symptoms are manifested.²⁰⁶

Although marginal hyperoxic challenge may induce favorable responses,²⁰⁷ a state of tissue oxygenation that far exceeds the normoxic setpoint of a given tissue is a clear risk factor that deserves appropriate attention.²⁰⁸ In a wound with pockets of hypoxia ranging in magnitude from extreme to marginal (Figure 2), the goal should be to reestablish normoxia in the worst affected hypoxic pockets without exposing other parts of the wound tissue to such high levels of pO₂ that would antagonize healing by hyperoxia-induced growth arrest or simply overt oxygen toxicity. One needs to be cautious about too much of a good thing.²⁰⁹ Endothelial progenitor cells (EPCs) are essential in vasculogenesis and wound healing, but their circulating and wound level numbers are decreased in diabetes. Hyperoxia reverses the diabetic defect in EPC mobilization.²¹⁰ Moderate hyperoxia increases the appearance of new blood vessels in wounds.¹¹ In addition to inducing VEGF gene expression, moderate hyperoxia enhances the expression of VEGF_{121/165} proteins and facilitates the release of VEGF₁₆₅ from cell-associated stores.²¹¹ Among the factors that may oppose wound healing, extreme hyperoxia causes growth arrest^{212–215} and cell death by a mitochondria-dependent apoptosis pathway.^{171,216,217} In addition, extreme hyperoxia does pose the threat of oxidative stress.^{218,219}

Tuning the normoxic setpoint

When cells grown under standard culture conditions of 20% O₂ are moved to 5% O₂ ambience, hypoxia is reported by way of HIF-response elements. When the same cells are maintained at 5% O₂ over long periods of time, the O₂-sensitive molecular machinery undergoes adjustment such that the same cells no longer report hypoxia. Interestingly, if these cells are maintained under mild hyperoxic conditions, e.g., 30% O₂, and then brought down to 20% O₂ culture conditions they report hypoxia.¹⁶³ These simple observations establish two important points: (i) that it is not the actual pO₂ but the ΔpO₂ that seems to matter; and (ii) that the normoxic setpoint in a cell can be reset by the adjustment of O₂-sensing machinery that is capable of responding to changes in the O₂ ambience. In this simplified example, the machinery is represented by the PHD family of proteins, the expression of which is up-regulated under conditions of hypoxia and down-regulated under conditions of hyperoxia. This is noted not only *in vitro* but also *in vivo*. Here, although the example is limited to PHDs to keep the discussion simple, it is important to recognize that there are numerous other O₂-sensitive functions in a cell that would contribute to its overall response to any pO₂ outside the normoxic setpoint. Thus, the normoxic setpoint in a

biological cell is tunable. For example, under conditions of no change in ambient O₂ condition, a cell may be made to report hypoxia, as measured by HIF transactivation, simply by knock-down of the PHDs.¹⁶³ In response to down-regulated PHD1, cells not only report HRE-dependent gene expression but causes metabolic adaptations lowering tissue O₂ consumption.²²⁰ Conditional inactivation of PHD2 in mice is sufficient to activate a subset of HIF target genes, including erythropoietin, leading to striking increases in red blood cell production.²²¹ Tuning of the normoxic setpoint when the cells are exposed to modest changes in O₂ ambience seems to happen physiologically perhaps as an adaptive response. Comprehension of the pathways involved in such process should help us employ pharmacological and/or genetic approaches to therapeutically adjust the normoxic setpoint on an as needed basis. For example, moderate hypoxia is known to be a robust cue to initiate the angiogenic response. One can reap the angiogenic benefits of that knowledge by adopting therapeutic approaches that would lead to suppression of PHD function resulting in HIF stabilization and HRE-dependent transactivation. Indeed, this approach is being explored for wound therapies.

TISSUE OXYGENATION AND WOUND THERAPY

HIF PHD-directed wound therapeutics

The PHD inhibitor FG-4497 readily stabilizes HIF-1 α and subsequently drives the expression downstream of HIF target genes. FG-4497 is helpful in colitis perhaps by benefiting wound healing at the site of inflammation.²²² ECM is predominantly collagen, and the imino acids (Pro and HyPro) comprise 25% of collagen residues. The final step in collagen degradation is catalyzed by prolydase, the obligate peptidase for imidodipeptides with Pro and HyPro in the carboxyl terminus. Defective wound healing in patients with inherited prolydase deficiency is associated with histologic features of angiopathy, suggesting that prolydase may play a role in angiogenesis. Recently it has been demonstrated that prolydase inhibits PHD activity to induce HRE-dependent transactivation and facilitate angiogenic signaling.²²³ HIF-specific PHD inhibitors are being tried out for their efficacy in treating wounds. It is likely that such approaches to pharmacologically stabilize HIF will facilitate responses such as generation of angiogenic factors. Whether that response translates to functionally successful angiogenesis and improvements in wound closure will depend on whether other fundamental prerequisites such as a threshold level of tissue oxygenation is present to fuel the healing process. This is of particular concern for ischemic wounds that suffer from extreme chronic hypoxia. If hypoxia alone would have been sufficient to heal, all ischemic wounds would have undergone rapid healing. Clinical observation is exactly the opposite. The key here is to couple hypoxia-response signaling with conditions such as appropriate tissue oxygenation that could sustain the healing process. PHD inhibitors alone are not likely to yield favorable outcomes in extremely hypoxic wounds. Furthermore, it is important to note in this context that PHD inhibition may stabilize HIF but does not guarantee transcriptional function. Co-

substrate and cofactor requirements for Fe(II), ascorbate, and the Krebs cycle intermediate 2-OG, and inducible changes in the cellular abundance of three closely related HIF prolyl hydroxylases (PHD1–3) provide additional interfaces with cellular O₂ status that may be important in regulating the oxygen-sensitive signal. Although under conditions of acute hypoxia PHD inactivation supports tissue survival, recently it has been demonstrated that under conditions of chronic hypoxia PHD overactivation is necessary as a survival response.²²⁴ Chronic ischemic tissue overactivates all three isoforms of PHD to survive.²²⁴ The merit of PHD inhibition for the treatment of ischemic wounds involving chronic hypoxia warrants reconsideration in this new light.

First and foremost it needs to be borne in mind that the overarching goal of oxygen therapy should be to correct wound hypoxia. While to some extent hyperoxia may be well tolerated by tissues, it would be prudent to avoid extreme hyperoxia.²²⁵ Although oxygen toxicity may not be imminently overt, an overdose of O₂ is likely to trigger molecular responses such as cell cycle arrest and epigenetic modifications,^{226,227} which would oppose healing. Second, approaches to keep a wound oxygenated over a longer period of time, as opposed to a few hours usually targeted in HBO therapy, should prove to be beneficial. In response to HBO, there is no sustained change in tissue O₂ tension much beyond the period of treatment.²²⁸

The most fundamental factors in wound care are fluid management, temperature management, pain control, increased arterial O₂ tension, the use of appropriate sterile techniques, and administration of prophylactic antibiotics.²²⁹ In addition, numerous cellular and molecular players are required to act in concert to successfully execute wound healing.^{230,231} While examining the efficacy of O₂ therapy in wound healing, it is critically important to recognize that O₂ cannot act in isolation. Oxygen therapy may be only expected to benefit in those cases where the remaining essential players are functional and hypoxia is the only rate-limiting factor. Thus, oxygen therapy is generally recommended as an adjunct to other forms of wound care.^{232,233}

HBO

HBO therapy represents an effective approach to bolster tissue O₂ levels⁵ and has been found to benefit wound healing under specific conditions.^{234–238} Importantly, HBO may potentially work synergistically with growth factors such as PDGF to improve the outcomes of ischemic wounds.²⁰ Because PDGF requires O₂-derived H₂O₂ for successful function, this finding is not surprising.²³⁹ HBO causes sharp elevation in tissue pO₂.^{240,241} The administration of two atmospheres of 100% O₂ for 2 hours may raise tissue pO₂ by 10–20-folds^{242,243} over the values under basal room air conditions. This systemic approach to oxygenate tissues seems to offer some unique potential advantages. HBO may increase bone marrow NO in vivo thereby increasing the release of EPC into circulation. EPC mobilization into circulation is triggered by hyperoxia through induction of bone marrow NO with resulting enhancement in ischemic limb perfusion and wound healing.^{244–246} HBO may also increase NO levels in perivascular tissues via stimulation of NOS. Exposures to 2.0 and 2.8 ATA O₂ stimulated neuronal (type I) NOS

(nNOS) and significantly increased steady-state NO concentration, but the mechanism for enzyme activation differed at each partial pressure. Enzyme activation at 2.0 ATA O_2 appeared to be due to an altered cellular redox state. Exposure to 2.8 ATA O_2 , but not 2.0 ATA O_2 , increased nNOS activity by enhancing nNOS association with calmodulin.²⁴⁷ Thus, dosing does seem to matter in HBO therapy. Yet, in the clinics HBO is applied in a standard format to all patients regardless of their individual needs. Could this be an important factor in explaining the less than satisfactory results that HBO is generally thought to have produced in clinical settings?²⁴⁸ When a flat dose of oxygen is provided to all wound patients, it is possible that the specific dose applied is successful in oxygenating the pockets of extreme hypoxia in some wounds. In these cases, beneficial outcomes should be expected to follow. In the same vein it may be hypothesized that for some other cases, the dose applied is excessive compared with the need of the wound. In these wound with pockets of more moderate hypoxia, the same dose of HBO may be excessive negating the beneficial effects of hypoxia. This is of outstanding interest because excessive oxygen is known to cause growth arrest and accelerate cellular senescence.^{249–251}

Because the ability to handle oxygen toxicity is dependent on the expression of genes encoding antioxidant proteins,^{252–259} it is possible that in some patients predisposed to oxidative stress the massive increase in tissue pO_2 following HBO results in molecular responses such as growth arrest,^{212–214,260} which may not manifest overt signs of oxygen toxicity but does resist wound healing. Another consideration in this regard would be the observation that a large fraction of chronic wound patients suffer from malnutrition.^{261–265} Such individuals are also known to be predisposed to oxidative stress and are limited in their ability to fend against oxygen toxicity.^{266–268} It is therefore reasonable to propose that chronic wound patients suffering from malnutrition are predisposed to HBO-induced oxidative stress. Taken together, such hypotheses would explain the inconsistent outcomes reported following HBO treatment^{269–272} and call for HBO dosing regimens where physicians would prescribe the target wound pO_2 . This approach would be consistent with the emerging concept of personalized healthcare²⁷³ and would require the design of new HBO devices fitted with the capability of real-time mapping of wound O_2 tension as can be made possible via technologies such as electron paramagnetic resonance spectroscopy.^{274,275}

Topical oxygen

Studies reported during the last 5 years renew interest in examining the significance of topical approaches to oxygenate cutaneous wounds as adjunctive therapy.^{1,14,18,276,277} Topically applied O_2 gas is able to modestly increase the pO_2 of the superficial wound tissue.²⁷⁷ In cases where hypoxia of the superficial wound tissue is a key limitation, topical oxygenation should prove to be helpful. Encouraging results obtained from the use of topical O_2 gas in both clinical^{1,18} as well as preclinical²⁷⁷ settings warrant serious consideration of this approach. Recently, perfluorocarbon droplets encapsulated in aqueous continuous phase has been used as topical O_2 emulsion

to treat experimental wounds. Results from this double-blind in vivo study demonstrate that topical approaches to oxygenate the wound significantly enhance the rate of epithelialization of partial-thickness excisional wounds and second-degree burns. Whether the emulsion was able to increase wound tissue pO_2 was not examined, however.²⁷⁶ Epithelial wound healing is improved by transdermal sustained-delivery treatment with 100% O_2 .¹⁴ A recent clinical study testing the effects of topical O_2 gas application on chronic wound presented clinically reports significant improvement in wound size. Interestingly, topical oxygen treatment was associated with higher VEGF expression in the wound edge tissue.¹⁸ Pure O_2 is known to induce VEGF.^{15,63,219} Findings of the study testing the effects of topical oxygen gas on chronic wounds are consistent with previous findings suggesting that topical treatment may induce wound angiogenesis.²⁷⁸ Randomized clinical trials testing the effects of topical oxygenation on wound outcomes are warranted.

HBO and topical oxygen approaches have several contrasting features. The systemic effects of HBO, both favorable as well as unfavorable, may not be expected with topical oxygen. Topical oxygenation can only modestly increase tissue pO_2 ²⁷⁷ and cannot match the large increases in tissue pO_2 typically noted in response to HBO.^{242,243} If the goal is to correct hypoxia of the superficial tissue, topical approaches should be helpful. However, if the goal is to achieve larger suprphysiological levels of tissue pO_2 , HBO would represent the approach of choice. An advantage of topical approaches is that they are portable and therefore applicable in a field or home setting. The cost advantage of topical oxygenation over HBO is another practical consideration.^{276,279,280}

SUMMARY

The etiology of chronic ischemic wounds is generally multifactorial of which hypoxia is a common factor in most cases. Primarily based on the tumor literature, hypoxia is generally viewed as being angiogenic. This is true with the condition that hypoxia be acute and mild–modest in magnitude. Extreme hypoxia, as commonly noted in problem wounds, is not compatible with life or tissue repair. Adequate wound tissue oxygenation is required but may not be sufficient to favorably influence healing outcomes. Success in wound care depends on a personalized health care approach. The key lies in our ability to specifically identify the key limitations of a given wound and in developing a multifaceted strategy to address those limitations. In considering approaches to oxygenate the wound tissue, it is important to recognize that both too little as well as too much may impede the healing process. Oxygen dosing based on the specific need of a wound therefore seems prudent. Therapeutic approaches targeting the oxygen sensing and redox signaling pathways are promising as well. Investment in bringing such capabilities to clinical practice should yield lucrative returns.

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