Oxygen in Wound Healing
Nutrient, Antibiotic, Signaling Molecule, and Therapeutic Agent

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KEYWORDS

• Oxygen • Hypoxia • Hyperbaric • Wound • Healing • Diabetes • Epithelium • Fibroblast

KEY POINTS

- With deeper scientific understanding of oxygen physiology, and with support from randomized, prospective clinical investigations, the judicious, individualized use of oxygen therapy in wound management may now be considered mainstream.
- Each of the most common categories of chronic wounds (arterial, venous, diabetic, pressure) become established or are perpetuated because of factors that limit oxygen delivery to the wound bed.
- At the low physiologic concentration of H_2O_2 (0.15%), topical angiogenesis is favorably influenced, distinguished from the 3% v/v strength available commercially; at this high concentration, severe oxidative damage to wounds is noted, and is thus contraindicated in modern wound management.
- Given that correction of wound hypoxia is beneficial to many aspects of healing, it does not necessarily follow that more is better, and that hyperoxygenation of normally nourished wounds confers a benefit to justify the risks.

INTRODUCTION

Common observations made many decades ago by mountain climbers who noted the inability to clear skin infections at high altitude, and Jacques Cousteau's deep sea divers who noted that their work wounds healed fastest when they were diving, brought general appreciation of the importance of oxygen in healing.[1] Recent years have brought an increased and more detailed scientific appreciation of the diverse roles that oxygen plays in normal physiology and disease states.[2,3] As the individual steps of the wound healing cascade have become elucidated in greater detail, the involvement of oxygen at nearly every stage has become evident. More oxygen is not always better; nature seems to have adapted us to respond constructively to the relative hypoxia that characterizes the healing edge of many wounds.

There remain many gaps in understanding of the biochemical events of healing. Some of the current knowledge regarding oxygen, growth factors, and other mediators is seemingly contradictory, and classification of molecules as promoters or inhibitors of healing (eg, oxygen is good, tumor necrosis factor α [TNF-α] is bad) is simplistic. However, it seems possible to reach a unified understanding of healing that reconciles most of the thousands of basic science investigations into individual steps in the chain, and oxygen is central to this. This article summarizes oxygen physiology in wound biology, and discusses the supporting literature.

Given the central role of oxygen in healing, there is the potential to manipulate the wound

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environment by treatment with supplemental oxygen. Oxygen therapy in various forms has been used to ameliorate many medical conditions for centuries. However, clinical results have been varied, and frequently disappointing. There has been an indiscriminate use of oxygen treatments in the past, and there is still an aura of quackery associated with this area of medicine. However, in the face of deeper scientific understanding of oxygen physiology, and with support from randomized, prospective clinical investigations, the judicious, individualized use of oxygen therapy in wound management may now be considered mainstream. This article reviews the current rationale, regimens, and preclinical and patient data regarding various oxygen treatments that have been used to improve the outcomes of dermal wounds. See **Box 1** for a summary of roles of oxygen in wound healing.

Oxygen Delivery

In normal conditions, oxygen delivery to peripheral tissues is the net result of:

- Cardiac output
- Peripheral vascular resistance
- Oxygen saturation of hemoglobin (usually 90% or greater).

Oxygen in serum

Minimal amounts of oxygen are dissolved in the serum. Release of oxygen is governed by the hemoglobin dissociation curve. Serum P_{O_2} is typically about 100 mm Hg. Once released at the capillary level into normal tissue, oxygen can diffuse up to 64 μm.[4] Given normal capillary density, this diffusion ability is sufficient to nourish and support the viability of the skin. Hunt[1] emphasized the often-neglected but relevant point that oxygen delivery can sometimes be increased significantly by reversing the local vasoconstriction that may result from pain, cold, or other noxious stimuli.

Box 1
Roles of oxygen in healing

- Energy source to fuel biochemical reactions and cellular function
- Nutrient essential to the synthesis and cross-linking of collagen
- Cofactor that is manufactured into signaling molecules such as nitric oxide and hydrogen peroxide
- Substrate for generation of reactive oxygen species (ROS) that combat wound colonization and infection
- Essential component of the redox switch that turns on and off genes that encode proteins critical to the healing cascade
- Deliberate hyperoxygenation recruits endothelial progenitor cells to the wound, increases vascular endothelial growth factor (VEGF), and promotes angiogenesis

Intact skin as barrier to oxygen

The keratin layer of intact epithelium is a barrier to oxygen diffusion; probes designed to exclude air detect only 0 to 10 mm Hg on the skin surface. Warming of the skin makes this layer more permeable and enables P_{O_2} to increase substantially, although it does not reach the ambient level. Stripping the statum corneum with tape enables free diffusion of oxygen into the upper layers of dermis, and the P_{O_2}there closely matches the oxygen tension in the environment. However, within about a day, exudation of serum and accumulation of inflammatory cells lead to formation of a soft eschar that again prevents oxygen diffusion into the skin. Therefore, the oxygen tension in subcutaneous tissue and dermis of intact skin depends on delivery through the underlying circulatory system.

Inadequate Oxygen Delivery is a Causal Factor in Many Chronic Wounds

Each of the most common categories of chronic wounds (arterial, venous, diabetic, pressure) become established or are perpetuated because of factors that limit oxygen delivery to the wound bed. Scheffield[5] noted that chronic wounds have a P_{O_2}in the range of 5 to 20 mm Hg, compared with 35 to 50 mm Hg measured in normal tissue.

In the case of venous leg ulceration, the essential disturbance is abnormal venous hypertension, which is propagated back to the capillary level. The capillary-tissue pressure gradient is increased, causing water to diffuse out of the intravascular space and into the interstitium; large molecules such as fibrinogen, albumin, and α2-macroglobulin are also forced out of the vascular system, and pericapillary cuffs are formed that can be noted histologically.[6] These cuffs and the local edema impair oxygen diffusion and render the cells furthest from the capillary hypoxic.

Lower extremity arterial and diabetic wounds, are prone to suffer macrovascular and/or microvascular occlusive disease, limiting blood flow and therefore oxygen delivery to the lesion. Pressure wounds that are not properly off-loaded become ischemic (and therefore also hypoxic) when capillary closing pressure is exceeded by

the weight of the body part pressing against a support surface.

Reperfusion

In addition to ischemia/hypoxia, another mechanism of injury has been shown to play a major role in a variety of chronic wounds: reperfusion.[6,7] Patients with impaired arterial inflow or venous return have repeated episodes of ischemia and reperfusion related to leg elevation or dependency. Restoring circulation induces endothelial stickiness, which draws white cells into the lesion; the already established proinflammatory environment established by ischemia intensifies as ROS flood the wound and cause further tissue destruction. Repeated episodes of ischemia and reperfusion are more detrimental to wound healing than are prolonged phases of uninterrupted ischemia.[8,9]

Inflammatory cycle

It is a popular current concept that many chronic wounds are stuck in a self-perpetuating inflammatory cycle. Hypoxia may contribute to this pathophysiology in many cases. Under significant hypoxic conditions, mitochondrial adenosine-triphosphate (ATP) production ceases and ATP-dependent transmembrane transport systems such as sodium/potassium ATPase or calcium ATPase fail. Intracellular accumulation of calcium promotes release of proinflammatory cytokines such as TNF-α and interleukin (IL)-1, which attract neutrophils and macrophages. Endothelial adhesion molecules are overexpressed in hypoxia, and enable white blood cells to localize to the wound. The net effect is a self-perpetuating inflammatory vicious cycle in which tissue destruction leads to increased white cell recruitment and release of proinflammatory mediators and ROS, which leads to even more tissue destruction.[7] Bacterial colonization, a nearly universal feature of chronic wounds, adds to the inflammatory burden by attracting and activating leukocytes. Although inflammatory cells are capable of producing ROS at low oxygen tensions, the antidotes to ROS (the most potent of which is nitric oxide) require higher oxygen tension for their synthesis.

Measurement of Wound Oxygen

Accurate, repeatable measurements of wound oxygen are central to many in vivo investigations into the role of oxygen. Although numerous investigators have refined research-grade systems, measurement of oxygen at the tissue/cellular level in routine clinical practice is difficult and imprecise. There is a vast literature on oxygen measurement and a detailed review is beyond the scope of this article. Most methods are indirect and measure oxygenation of periwound skin rather than the wound bed.

Transcutaneous oximetry

Perhaps the most popular of these techniques, transcutaneous oximetry ($TcPo_2$) is subject to high variability related to fluctuations in vasomotor tone at the site of measurement, light penetration of skin, and hemoglobin level.[7] Even perfectly performed $TcPo_2$ typically overestimates wound Po_2 because the skin is warmed to the point of maximal local vasodilatation, which is not representative of the ordinary state of the local vasculature. In addition, there can be significant oxygen consumption along the path from periwound intact skin to the healing tissue edge in the center of the wound.[10] Thus, arterial blood Po_2 is ordinarily about 100 mm Hg; the Po_2 of dermal wounds ranges from 60 mm Hg at the periphery to 0 to 10 mm Hg centrally. There are many reports supporting the usefulness of $TcPo_2$ in determining levels of amputation healing, but many practitioners have found that the method is cumbersome and yields results that have poor repeatability.[3,11] Measurements at 1 point in time and only a limited number of skin sites may not accurately portray the wound microenvironment, because wounds are not uniform and vasomotor tone may change from moment to moment.[12]

Luminescence imaging

Luminescence lifetime imaging has recently shown significant advantages compared with earlier methods of wound oxygen estimation.[7] A phosphorescent indicator is held in place on a plastic matrix; quenching of the phosphorescence is proportional to the amount of oxygen present. The technology provides a noninvasive, painless, reliable, sensitive estimate of wound Po_2.[13] Although offering potential for widespread clinical adoption, the technology is not yet available commercially in a format and at a cost that is compatible with ordinary clinic operations and economics.

Role of Oxygen in the Essential Steps of Dermal Wound Healing

Collagen synthesis

Extracellular matrix deposition is inadequate in many chronic wounds because of poor fibroblast production and inadequate remodeling of collagen, which are both oxygen dependent, and because of excessive degradation of extracorporeal membrane oxygenation (ECM) by matrix metalloproteinases (MMPs). Molecular oxygen is required for the hydroxylation of proline and lysine during collagen synthesis and for the maturation of protocollagen into stable triple-helical collagen. In

the absence of sufficient oxygen, only protocollagen, which does not have the functional abilities of collagen, can be made.[2] Collagen synthesis proceeds in direct relation to P_{O_2} over the range of 25 to 250 mm Hg.[10,12] Prolyl hydroxylase under 20 mm Hg O_2 functions at 20% of maximal speed; the enzyme requires more than 150 mm Hg to reach 90% of maximal speed.[4,14]

Angiogenesis

Many authorities have noted an apparent inconsistency in well-known observations of wound healing, whereby hypoxia is noted to increase VEGF production from fibroblasts and macrophages, but angiogenesis seems to proceed more successfully under normoxic or even hyperoxic conditions.[12,15,16] In one set of instructive experiments, mice underwent subcutaneous injection of a gel alone, gel with VEGF, or with anti-VEGF antibodies. The animals were then maintained in various environments of 13% to 100% oxygen at 1 absolute atmosphere (ATA) to 2.8 ATA to simulate hypoxia, normoxia, and hyperoxia. The explanted gel plugs were then sectioned and graded for the degree of angiogenesis. Angiogenesis was significantly decreased in the hypoxic animals ($P = .001$) and increased in those who were rendered hyperoxic ($P<.05$). Addition of VEGF to the implanted gel did not prevent the deleterious effect of hypoxia. In contrast, the beneficial effect of hyperoxia was blocked by anti-VEGF antibody. These findings suggest that both adequate oxygen and the presence of VEGF are required for angiogenesis.

Sen[12] noted that all developing vascular buds require a sheath of ECM, mainly consisting of collagen and proteoglycans, to guide tube formation and resist the pressures of blood flow. His investigations have confirmed that optimal angiogenesis requires high P_{O_2}; hypoxia, by retarding synthesis of the collagen to support developing vessels, decreases angiogenesis.[12]

Fibroblast growth

Oxygen consumption by cells has been studied by measuring changes in oxygen tension in supernatant media covering cells in tissue culture.[4] Fibroblast cellular proliferation is directly correlated with ambient oxygen level:

- Freshly harvested cells only grow in an environment that includes 15 mm Hg O_2, or more
- Initially, after 72 hours exposure to 1% oxygen, fibroblast proliferation increases by 71%
- During this period, fibroblast secretion of transforming growth factor (TGF)-β1 increases 9-fold, in turn causing upregulation of the procollagen gene.

This adaptation to hypoxia is only transient, and chronic oxygen deprivation severely diminishes fibroblast activity.[4] The surface of a poorly healing wound can be mapped with oxygen tension measurements, and fibroblast growth is progressively diminished until the hypoxic center of the lesion is reached, where proliferation is minimal or nil. By contrast, under hyperoxic conditions, fibroblasts are induced to differentiate into myofibroblasts, which are critical to wound closure through contraction.[17]

Epithelial

Numerous studies have shown that keratinocytes and fibroblasts migrate faster under hypoxic conditions.[18,19] Keratinocytes express more lamellipodia proteins and collagenase, and decrease lamellin-5 (motility brake) under hypoxia. This finding is expected, considering the mechanisms of natural dry wound healing, in which new skin regenerates underneath a stable eschar. The microenvironment through which the healing edge advances is protected from ambient oxygen, and the lack of blood vessels inside the eschar engender local hypoxia. In order for the epithelial edge to migrate the keratinocytes must carve a path through the tissue/eschar interface using collagenase and other enzymes.

Energy generation and use

Fundamental biochemical events such as molecular synthesis and transport cannot occur without a source of energy, and the ubiquitous source of energy in the human body is the coenzyme ATP. ATP is synthesized in mitochondria and stores chemical energy to fuel diverse biochemical processes in the body. The process by which ATP is created is known as oxidative phosphorylation and is critically dependent on the availability of molecular oxygen.[4] About 90% of the oxygen consumed by tissues goes to ATP synthesis.[5] Other energy stores generated through the citric acid cycle and the breakdown of fatty acids are also highly oxygen dependent.[7] The requirement for energy and, hence, the need for oxygen, is accentuated in healing tissue because cellular activities such as collagen synthesis, cell migration, and bacterial defense are heightened.

Influence of age

Another critical dimension to oxygen's role in healing relates to the age of the wounded host. The demographics of chronic nonhealing wounds indicate a strong relationship between incidence and advanced age, with most cases occurring in

patients more than 60 years of age. Aging fibroblasts show: [6,7,20]

- Reduced proliferative ability
- Diminished capacity to respond to growth factor stimulation
- Increased production of destructive enzymes such as MMPs.

Advanced age induces greater sensitivity to the negative effects of hypoxia. The migratory response of fibroblasts to TGF-β1 stimulation is blunted in older patients, compared with younger ones. Mustoe and colleagues[6] compared the effects of hypoxia on young (age 24–33 years) human dermal fibroblasts in tissue culture with fibroblasts from older (age 61–73 years) donors. Under 1% oxygen, there was greater decrease in TGF-β1 receptor expression in the aged cells (decrease of 12% in young fibroblasts vs 43% in old fibroblasts).

Responsiveness to TGF-β1 stimulation was reduced by advanced age:

- Activity of p42/p44 mitogen-activated kinase increased 50% in young cells versus decreasing 24% in the aged cells
- Unstimulated fibroblast migration increased 30% in young cells exposed to hypoxia, whereas the aged cells showed no change
- When TGF-β1 stimulation was added to the migration assay, young cells increased activity by 109% versus only 37% in the aged cells.[2]

Mendez and colleagues[20] studied fibroblasts harvested from the venous ulcer wound beds and used, as controls, cells taken from normal skin of the thigh.

- The 7 patients who were evaluated (mean age 51; range 36–67 years) had suffered wounds for a mean of 12.7 months (range 11–17 months)
- Chronic wound fibroblast growth rates were only one-third of those observed in the control cells ($P = .006$).

β-Galactosidase (β-GAL) activity was used as a sign of cellular senescence, a state of irreversible arrest of proliferation despite maintenance of metabolism.

- Six of 7 controls had no senescence associated (SA)-β-GAL activity
- All samples from the chronic wounds had measurable activity (mean 6.3%, median 2%)
- Level of SA-β-Gal correlated inversely with cellular growth rate ($R^2 = 0.77$).

The issue of cellular senescence may be more complex than mere chronologic age of the wounded patient. It may be the number of cell divisions that fibroblasts have undergone, rather than the age of the host, that defines the age of a cell. Many cell lines, including fibroblasts, are capable of only a finite number of cell divisions. Chronic wounds in young individuals may contain fibroblasts that have already reached the limit of their proliferative ability and are prematurely senescent.[7] Thus, although the lessons learned about the roles of oxygen in wound physiology are generally applicable, the degree to which patients may react to hypoxia and hyperoxia with the predicted responses may vary according to the physiologic age of the host cells.

Nitric oxide generation

Nitric oxide synthetase metabolizes the amino acid L-arginine into nitric oxide using oxygen as a substrate. Although nitric oxide is well known for its diverse, generally beneficial, effects on inflammation, angiogenesis, and cell proliferation, a full discussion of the putative benefits of enhanced nitric oxide is beyond the scope of this article.[21]

Role of Oxygen in Infection Control

During the initial phase of wound healing, activated leukocytes enter the wound and engulf bacteria. In the presence of adequate oxygen, an oxidative burst ensues, in which oxygen consumption increases as much as 50-fold compared with baseline conditions, and persists for hours, creating ROS that destroy the invaders.[22] ROS include:

- Peroxide anion (HO_2^-)
- Hydroxyl ion (OH^-)
- Superoxide anion (O_2^-)
- Hydrogen peroxide (H_2O_2).

About 98% of oxygen consumption by leukocytes is related to this respiratory burst, which is facilitated by phagocyte (neutrophil, eosinophil, monocyte, and macrophage) cell membrane–bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase[23]:

$$NADPH + 2O_2 \rightarrow NADP + 2O_2^- + H^+$$

Glucose provides the energy to drive the reaction, generating substantial amounts of lactate in the process.[1]

ROS, and especially superoxide, are toxic and kill bacteria, then are rapidly degraded to H_2O_2 and other by-products.[10,24] The kinetics are such that the killing process works at 50% of maximal

speed in the presence of oxygen at 40 to 80 mm Hg, and as much as 400 mm Hg are required to increase the velocity to 90%.[4] Neutrophils therefore lose most of their ability to kill bacteria at less than 40 mm Hg.[7] Patients afflicted with chronic granulomatous disease, which is characterized by defects in the genes that encode NADPH oxidase, have increased susceptibility to infection, and also show impaired wound healing.[10]

The results of many investigations suggest that, in a reduced P_{O_2} wound environment, the ability of leukocytes to generate ROS is substantially impaired, and therefore the ability to ward off colonization/infection is lowered. In vitro studies of leukocytes obtained from venous blood show that neutrophil oxygen consumption increases as ambient oxygen concentration increases, and suggest that increasing ambient oxygen to more than physiologic levels may induce even greater ROS synthesis. However, there is the theoretic potential that excess ROS may be destructive and counterproductive to wound healing. The body has a robust system for removal of ROS by superoxide dismutase, catalase, and reduced glutathione, and the wound concentration of ROS is the net result of synthesis and destruction. ROS clearance requires an adequate circulatory supply.[25]

The potential to increase local wound oxygenation to supraphysiologic levels and cure or prevent infection was evaluated in 30 patients with chronic diabetic wounds. Patients were randomized to receive standard care (wound dressings; antibiotics guided by culture results) alone or in combination with 4 hyperbaric oxygen therapy (HBOT) treatments given during a 2-week period. In the control group, cultures grew significant colony counts of 16 different isolates at baseline, and 12 at the end of the observation period; in the patients receiving HBOT, isolates were reduced from 19 before treatment to only 3 afterward ($P<.05$). HBOT seemed particularly effective in controlling *Pseudomonas* and *Escherichia coli*. Seven patients in the control group required major lower extremity amputation (5 for spreading infection) versus 2 in the HBOT-treated patients ($P<.05$).[26]

Redox Signaling

The traditional view of ROS has been that they are destructive to bacteria and host cells, necessary for wound hygiene in the early phase of healing, but otherwise counterproductive to the normal healing cascade.[12] This view has prompted numerous clinical trials testing the role of various antioxidants in ameliorating different disease conditions, and these trials typically have shown disappointing results.[27–29]

The late 1990s brought a growing appreciation that, at very low levels, ROS (particularly H_2O_2) serve as signaling messengers, a role independent of bacterial killing.[10] NADPH oxidase exists not only in neutrophils but also in nonphagocytic wound cell lines, and there is a continuous low-level production of ROS that is unrelated to leukocytes, oxidative burst, or response to wound colonization or debris.[30] ROS bind to proteins and can alter their conformation, leading to increases or decreases in their functional abilities.[31] H_2O_2 functions primarily by oxidizing cysteine moieties. The molecule has a potent effect in recruiting leukocytes to the wound site, and the concentration of endogenous hydrogen peroxide increases significantly at the wound margins within the first few minutes of injury. The functions of important growth factors such as VEGF, platelet-derived growth factor (PDGF), keratinocyte growth factor (KGF), and TGF-α are inhibited in the absence of such signals.[32] Thus, overexpression of catalase, which removes H_2O_2 from the wound, is associated with impaired angiogenesis and delayed wound closure.[33] Cellular functions like migration and leukocyte recruitment are also inhibited in the absence of H_2O_2.[12] Micromolar levels of peroxide can be measured in normal wound fluid.[34] Research in oncology indicates that low-level ROS foster angiogenesis, and overexpression of extracellular super oxide dismutase inhibits tumor vascularization in mice.

Note

The low physiologic concentration of H_2O_2 must be distinguished from the 3% v/v strength that is available commercially and often used to clean and disinfect wounds; at such high concentrations, severe oxidative damage to wounds is noted, and this is contraindicated in modern wound management. However, at 0.15% H_2O_2, topical angiogenesis is favorably influenced.[31]

The state of overabundance or underabundance of ROS is reflected in the redox potential of the wound microenvironment. The ratio of NADH to NAD^+ has been used as a redox index, and transcription of various genes important in wound repair seems to be responsive to this ratio. Every phase of the wound healing cascade (hemostasis, inflammation, proliferation, epithelialization) has been shown to have key steps that require NADPH oxidase action.[24] Individuals with mutations in either $p47^{phox}$ or Rac2, which are each associated with action of this enzyme, show impaired wound healing.

Hypoxia

Many of our conclusions about the role of oxygen in wound repair come from observations of the

defects in healing that are associated with hypoxia. Absolute hypoxia is usually defined as an oxygen level less than 30 mm Hg.[2] However, hypoxia is more typically a relative term, indicating insufficient oxygen for the tissue and physiologic situation under consideration.[12] For example, with infection or an open wound, oxygen demand increases and levels of delivery that might be adequate for intact, uninfected dermis may be deficient. Cells challenged with hypoxia must reduce activity and rely on anaerobic metabolism, or die. Acute and mild/moderate hypoxia usually leads to cellular adaptation and survival; prolonged and more extreme hypoxia may result in cellular death.

Hypoxia is a more frequent issue in wound repair than is commonly appreciated. Wound bed oxygenation depends on:

- Pulmonary uptake
- Hemoglobin level
- Cardiac output
- Vascular patency
- Capillary density
- Factors that deplete oxygen such as parenchymal consumption and inflammatory cell activity.

Decreased wound oxygen tension occurs not only from macrocirculatory issues but also because oxygen is consumed by metabolically active and proliferative cells located along the path from capillaries to the healing edge of tissue **Box 2**. As much as a 150-μm distance from the nearest capillary to the healing edge may need to be nourished by oxygen diffusion.[4] This effect is magnified in the presence of heavy bacterial colonization or infection, further consuming oxygen and decreasing the available oxygen to the healing wound. A vicious cycle may ensue in which bacterial oxygen consumption reduces the ability of leukocytes to synthesize ROS, enabling further bacterial proliferation, and so on. The centers of even seemingly well-oxygenated wounds may be hypoxic because of high oxygen extraction along the path of diffusion from capillaries.[7] Although circulating blood may contain a P_{O_2} of 100 mm Hg, the periphery of a dermal wound may be 60 mm Hg and, at the center of the wound, readings can be as low as 0 to 10 mm Hg.[1,35] Many chronic wounds suffer local hypoxia; in one study, normal, nonwounded tissue showed oxygen tensions of 30 to 50 mm Hg, whereas measurements of P_{O_2} in nonhealing chronic wounds in the same patients were in the range of 5 to 20 mm Hg.[4]

Box 2
Clinical pearl: wound hypoxia

Clinical pearl: wound hypoxia is under-recognized and may occur in even normally perfused lesions because of high oxygen extraction along the diffusion path from the feeding capillary to the edge of healing tissue.

At present, it is not easy to reconcile all the scientific knowledge about effects of hypoxia and hyperoxia on wound tissues and to synthesize all the evidence into a coherent story.[19] Hypoxia has been traditionally regarded as an important stimulus to fibroblast growth and angiogenesis. Hypoxia encourages angiogenesis by increasing levels of hypoxia-inducible factor 1 (HIF-1), which in turn binds to the promoter segment of the VEGF gene and activates transcription, leading to higher synthesis of VEGF, the principal angiogenetic growth factor in human physiology.[2,7] Paradoxically, multiple studies have shown that hyperoxic conditions induce greater angiogenesis, perhaps by increasing local ROS.[32,36]

Acute hypoxia induces temporary increases in cellular replication (3-fold increase under 5 mm Hg compared with 150 mm Hg). This has been associated with a 6.3-fold increase in the expression of TGF-β1 and enhanced procollagen synthesis.[7] However, these increases in proliferation and metabolic activity are short lived and, when these conditions are maintained for more than a week, cellular growth and synthetic activity decrease to significantly less than baseline physiologic levels.[16] The situation is reversible; restoration of normal oxygen levels restores typical proliferation rates, and a second bout of hypoxia induces a second temporary burst of fibroblast activity. In chronic hypoxia, the production and secretion of the most important cytokines and chemokines central to healing (including TNF-α, TGF-α, TGF-β1, KGF, EGF, PDFG, and insulin growth factor) require oxygen and are reduced or absent.

There seem to be at least 2 ways to reconcile the older concept that hypoxia is beneficial with modern understanding that healing proceeds more quickly with increased oxygen delivery.

1. The true primary stimulus to VEGF secretion, angiogenesis and collagen deposition may be lactate, more than hypoxia.[4,5] Even in well-perfused and properly oxygenated wounds, lactate may accumulate because leukocytes, fibroblasts, and endothelial cells lack mitochondria and rely on anaerobic glycolysis for energy production; a principal by-product of this glycolysis is lactate.[1] Thus, lactate levels may still be increased in hyperoxia, albeit less so compared with hypoxia.

2. It is possible that the P_{O_2} gradient between the capillary and the most distant cell may drive angiogenesis more than the absolute level of P_{O_2}. The immediate effect of hyperoxygenation of the blood may be to increase this gradient, and increased oxygen diffusion to areas of low P_{O_2} may follow.

Therapeutic Oxygen Supplementation

Because of the high incidence of wound hypoxia and the knowledge of the deleterious effects of inadequate oxygen on healing, it is natural to consider the potential of oxygen supplementation to improve wound repair **Box 3**. In some instances, the most effective way to improve wound oxygenation may involve measures to enhance blood flow rather than changing the oxygen content of the blood. Such maneuvers may include hydration and relief of vasoconstriction using local warmth and analgesics. Because hemoglobin is nearly saturated in most individuals, enhancing inspired oxygen may only modestly increase peripheral delivery. Other techniques for hyperoxygenation of wounds include breathing oxygen under supranormal pressures (hyperbaric oxygen [HBO]) or bathing the wound topically with an enhanced oxygen environment (topical oxygen therapy [TOT). The remainder of this article focuses on various attempts to supplement oxygen for the benefit of healing acute and chronic wounds.

Enriched inhaled oxygen to prevent surgical site infections

Under normal circumstances, hemoglobin is almost completely saturated while breathing room air under normobaric conditions, so the opportunity for increased oxygen delivery with enhanced forced inspiratory oxygen (FiO_2) is limited. However, by increasing the serum P_{O_2}, the tissue diffusion gradient is increased. For example, in the rabbit ischemic ear model, breathing 100% O_2 at 1 ATA increases blood P_{O_2} from 90 to 450 mm Hg. At less than 20% O_2, most of the oxygen is consumed within 70 μm of the nourishing capillary, but, after breathing 100% O_2 for 45 minutes, there is measureable increase in P_{O_2} even 150 μm away.[5]

Knighton and colleagues[37] in 1984 showed that higher inspired concentrations of oxygen lowered the extent of infection induced by intradermal injection of bacteria. Guinea pigs were given intradermal injections containing 10^8 *E coli*, then were treated with either 12%, 21%, or 45% inhaled normobaric oxygen. The end point, the size of the resulting lesion, was substantially reduced with increased oxygen ($P<.005$ for either 21% or 45% vs 12%; $P<.01$ for 45% vs 21%). The therapeutic effect was attributed to improved leukocyte killing with higher oxygen tension.

Conversely, it has been noted that the rate of surgical site infection (SSI) in surgical patients increases as systemic subcutaneous oxygen tension decreases.[38]

- Upper arm subcutaneous P_{O_2} measurements were conducted in 130 patients who underwent major general surgery (mostly abdominal) on the day of surgery and during postoperative days 1 and 2
- A total of 24 patients developed infection at the surgical site
- There was a strong correlation between decreased P_{O_2} and the development of infection ($r = 0.91$; $P<.001$).

The potential for supplemental inspired oxygen to lower the rate of SSI has been studied extensively. Grief and colleagues[39] randomized 500 adult patients undergoing colorectal resection to breathe 30% or 80% oxygen during, and for 2 hours after, the procedure. After surgery, patients were evaluated daily until hospital discharge, and then in the clinic 2 weeks later. Although the anesthesiologists knew the treatment to which each patient was randomized, the surgeon conducting the operation and the postoperative evaluation was blinded to this assignment. Wounds were considered infected if there were clinical signs and symptoms of infection, and if fluid or pus expressed from the wound cultured positive.

- Thirteen patients in the 80% oxygen group developed wound infection versus 28 patients in the 30% group (5.2% vs 11.2%; $P = .01$)
- Six patients in the 30% group had signs of infection but cultured negative, compared with 4 in the 80% group.

The investigators concluded that supplemental inspired oxygen was effective in lowering the perioperative wound infection rate.

Box 3
Clinical pearl: wound open to breathe

Clinical pearl: leaving a wound open to breathe

I wish I had a penny for every time I have explained to patients and families that wounds do not breathe! Under normobaric room air conditions, all the oxygen delivery to a wound is internal, and wounds can (and generally should) be occluded to maintain a moist wound environment and exclude dirt.

A similar prospective, randomized trial of 30% versus 80% inspired oxygen was conducted by Belda and colleagues.[40]

- Three hundred patients were randomized to yield 291 who were evaluable
- The rate of SSI was unusually high (57 patients [39.3%]) and there was no clear explanation for this finding
- The incidence of SSI was reduced significantly in response to higher inspired oxygen: 24% versus 15% infected for patients treated with 30% and 80% oxygen, respectively ($P = .04$).

Chura and colleagues[41] reviewed the literature and conducted a meta-analysis on the value of supplemental oxygen in preventing SSI in colorectal surgery. Among thousands of articles that emerged from a keyword search on "surgical site infection" and "perioperative oxygen" only 4 studies met criteria for sufficient scientific rigor to warrant analysis. Although there was significant heterogeneity among these studies, the investigators concluded that there was sufficient evidence to support the assertion that perioperative oxygen supplementation reduces SSI. The aggregate number of patients in these studies was 943 (477 who received supplemental O_2 and 466 who were controls). The pooled relative risk for SSI with perioperative O_2 supplementation was 0.68 (95% confidence interval, 0.49 to 0.94).

Only 1 major study failed to show reduction of SSI with supplemental oxygen. Meyhoff and colleagues[42] studied 1400 Danish patients undergoing laparotomy at 14 hospitals. Patients and observers were blinded to the random assignment to 30% or 80% O_2. The study end point was superficial or deep wound infection, or intra-abdominal infection, using the Centers for Disease Control and Prevention definitions. The low-oxygen group had an SSI rate of 20.1% versus 19.1% for 80% O_2 (not significant [NS]). Most patients received perioperative antibiotic prophylaxis with cefuroxime and metronidazole or benzylpenicillin and gentamycin; perhaps this accounts for the apparently discrepancy between this study and the others cited earlier.

On balance, it seems that increasing the concentration of inhaled oxygen is likely of benefit. In 2008, the UK National Institute for Health and Clinical Excellence concluded that, "The mechanism for improved blood oxygen carriage due to increased FiO_2 is physiologically not clear. However, this simple, cheap intervention deserves further investigation."[43]

Systemic HBOT

HBOT is conducted in single-person or multiple-person chambers that completely envelop patients in an environment of 2 to 2.5 atmospheres (atm) and 100% oxygen.[44] Sessions are typically delivered for 90 to 120 minutes daily (sometimes twice daily), and therapeutic courses lasting 2 to 8 weeks are typical. Because most patients under room air conditions show hemoglobin oxygen saturations of 90% or greater, the incremental binding of oxygen to hemoglobin induced by HBOT is marginal. However, independently of the normal mechanism of oxygen transport to tissues via hemoglobin binding, HBOT dissolves substantial amounts of oxygen directly into serum, much as carbon dioxide is dissolved under pressure into carbonated beverages.

- Serum P_{O_2} can reach 1200 to 2000 mm Hg during treatment sessions.[35]
- Total blood oxygen content, which is ordinarily in the range of 20 volume %, increases to about 27 volume % during 100% oxygen breathing at 3 ATA pressure.
- Because oxygen delivery from capillaries into tissue is driven by diffusion along a gradient, large increases of capillary P_{O_2} result in substantial increases in oxygenation at the cellular level. When the P_{O_2} is increased to 2000 mm Hg, oxygen may diffuse as far as 246 μm.
- As shown by the classic report of Boerema and colleagues[45] in 1960, sufficient oxygen can be dissolved in the bloodstream to maintain life and vital organ function even in the absence of hemoglobin.
- Following a session of HBOT, skin oxygen tension remains increased for a period of 30 minutes to 4 hours.

The details of nearly 400 years of hyperbaric medicine have been described by Kindwall.[46] The history of HBOT is checkered. On a naïve level, hyperoxygenation of tissue seems to be potentially beneficial in a wide variety of pathologic conditions. Given the sensitivity of peripheral and central nervous tissue to hypoxia, for example, HBOT has been attempted in a range of neurologic diseases and conditions. In past decades, hyperbaric operating rooms were used to conduct procedures such as carotid endarterectomy, in which temporary brain ischemia was anticipated. In general, results of HBOT for these and many other conditions were disappointing.

The combination of overly optimistic expectations and disappointing outcomes led to the perception that HBOT was not useful in any

medical condition, and the therapy was relegated to the realm of quackery in the opinion of many practitioners since the 1970s. Nevertheless, an increase in interest and effort to understand the physiology of wound healing in the past 20 years has generated a large body of evidence both in favor of the results of HBOT and to explain the mechanisms by which HBOT is beneficial. Thus, opinion has returned to a middle ground in which HBOT is understood to be a valuable adjunctive wound healing therapy but not a panacea. Nevertheless there are still skeptics of HBOT who think that, for most patients, the therapy is unnecessary and that oxygenation of most wounds can be achieved in other ways, such as by improving local perfusion.[1]

HBOT: growing scientific basis

Medical societies, government agencies, and health insurers now agree that there is a legitimate role for HBOT in a variety of conditions, including many aspects of chronic open wounds. The American Diabetes Association endorsed HBOT for treating recalcitrant diabetic foot ulcers in 1999; 3 years later, the Centers for Medicare and Medicaid Services announced its concurrence and its policy to reimburse for such treatments in patients who had failed to heal with a month of standard care.[14] Professional societies such as the Undersea and Hyperbaric Medical Society and the Wound Healing Society have included adjunctive HBOT in suggested algorithms of care for diabetic foot ulceration.

There is a large and growing body of scientific information to indicate the potential physiologic benefits of HBOT in wound healing. Typical HBOT increases P_{O_2} to 1200 mm Hg and increases O_2 diffusion distance from 60 to 250 μm.[47] Fibroblast proliferation is improved by this treatment. Hehenberger and colleagues[22] described a series of experiments in which fibroblasts harvested from normal patients undergoing reduction mammoplasty were compared with cells derived from specimens of diabetic foot ulcer beds. Cells were plated in tissue culture medium and placed inside a monoplace hyperbaric oxygen (HBO) chamber, then subjected to air (as control, at 795 and 1875 mm Hg) and 100% oxygen at various pressures from 795 to 2250 mm Hg. Total cell count was determined by measuring DNA content in the specimens immediately before treatment and after 24 hours. Although pressurized air did not induce fibroblast growth, fibroblasts proliferated more as P_{O_2} was increased, and maximal proliferation rate was observed at 1875 mm Hg (2.4 ATA).[22] HBOT was able to restore fibroblast growth in diabetic ulcer cells to the level seen in the normal control cells. Other investigators have noted that HBOT induces fibroblasts to differentiate into myofibroblasts, which are responsible for wound contraction.[48]

See **Box 4** for a summary of HBOT wound healing indications.

HBOT treatment and wound vascularity

A common observation among hyperbaric physicians is the large increase in wound vascularity after patients are treated for several weeks. HBOT has been shown to increase VEGF mRNA levels in endothelial cells and macrophages, and increased VEGF is noted in wound fluid of patients receiving this treatment.[7] Much of this effect may be mediated by increased nitric oxide.[49,50] HBOT induces endothelial progenitor cells (EPCs) to migrate out of bone marrow, circulate, and settle in the peripheral wound, forming vascular buds.[51,52] Circulating EPCs in the peripheral blood are diminished in diabetes mellitus; HBOT reverses this. The HBOT-induced steep oxygen gradient between capillary and hypoxic wound bed prompts macrophage migration and release of angiogenetic growth factors. There is a distinction between angiogenesis (proliferation and migration of resident endothelial cells supported by fibroblasts) and vasculogenesis (a de novo process whereby EPCs enter a wound, differentiate into endothelial cells, and create a new vascular network). HBOT facilitates both these regenerative processes by increasing wound VEGF, basic fibroblast growth factor, and TGF-β1. Patients undergoing 20 HBO treatments in preparation for dental procedures related to osteoradionecrosis of the mandible were found to have a 5-fold increase in EPCs. Under inhibition of nitric oxide synthetase, this effect is blocked. Maximal stimulation of peripheral nitric oxide synthetase requires up to 2.8 ATA.[12]

HBOT and hypoxia/hyperoxia

We have noted that both hypoxia and hyperoxia have certain salutary effects on healing. Perhaps

Box 4
Generally accepted wound-related indications for HBOT

- Necrotizing soft tissue infections
- Radiation damage to soft tissue and/or bone
- Moderate and deep diabetic foot and leg wounds
- Crush injury
- Gas gangrene

by fortune more than design, the current regimen of HBOT makes use of both stimuli: during HBO treatment, and for about 4 hours afterward, the supplemental oxygen facilitates fibroblast growth and collagen deposition and maturation, whereas during the remaining 20 hours before the next treatment, relative hypoxia stimulates angiogenesis.

HBOT and antibiotics

HBOT also increases the susceptibility of various bacteria to antibiotics, but is this strain specific and the effect cannot be generalized to all pathogens, whether aerobic or anaerobic.[5,7]

HBOT and MMPs

Sander and colleagues[36] used a model of impaired healing created by depleting mice of macrophages to study the effects of HBOT. Healing was restored to normal pace in HBOT-treated animals, even though the wounds were not ischemic or hypoxic. The author proposed that the benefit of HBOT was mediated through contradictory effects on MMPs. The activity of tissue inhibitor of metalloproteinase-1 (TIMP-1) was increased by the treatment. However, HBOT increased TNF-α in the wound bed, which typically increases the supply of MMPs available for selective tissue breakdown necessary for neovascularization and epithelial migration.[36]

HBOT and granulation tissue

In the rabbit ischemic ear model, HBOT increased granulation tissue and epithelial regrowth; the response was accentuated when growth factors were added to the HBOT.[53] Two of the 3 arteries supplying the external ear were ligated to create tissue ischemia, then 6-mm, full-thickness dermal wounds were created (n = 42). Wounds were treated with PDGF-BB, TGF-β1, or buffered saline; animals underwent HBO for 90 min/d at 1, 2, or 3 ATA 100% O_2 for up to a week. Treatment at 1 ATA did not alter epithelial advance or granulation tissue. Hyperoxygenation at 2 and 3 ATA increased granulation and increased wound P_{O_2} to as high as 300 mm Hg, increasing tissue volume by 100% (P = .03) but not influencing epithelial advance.[54] In combination with topical growth factors, granulation tissue increased by 200% (P = .0001) and epithelial advance increased significantly.

Obstacles to defining benefits of HBOT

A major obstacle to precise definition of the benefits of HBOT is the paucity of scientifically rigorous clinical studies. In 2005, Roeckl-Wiedmann and colleagues[55] performed an extensive search through some 24 electronic databases as well as a manual search through texts to gather 78 articles that purportedly offered clinical evidence on the role of HBOT in wound management. Of these, only 21 were deemed to be suitable human clinical trials, and 15 were excluded because they were not randomized, not clearly focused on 1 wound cause, used TOT instead of HBOT, or reported data that had already been published elsewhere. Six publications of at least moderate scientific merit were left: 5 on diabetic foot ulcers and 1 on venous leg ulcers.

Kessler and colleagues' study on diabetic foot ulcers

Kessler and colleagues[56] noted that HBOT doubled the rate of wound healing (P = .037) compared with controls during 2 weeks of treatment, but, once the twice-daily HBOT treatments were discontinued, the rate of healing in the control and HBOT groups equalized.[56] Twenty-eight patients were randomized, 15 to HBOT and 13 to control therapy, including off-loading. Patients were excluded if they suffered significant arterial occlusive disease or serious contraindications to HBOT (emphysema, claustrophobia, proliferating retinopathy). The groups were similar in terms of demographic factors and indicators of diabetic complications (renal, ocular, vascular), and all suffered with neuropathy. Lesions ranged from Wagner grade I to III. HBOT was delivered as 100% oxygen at 2.5 ATA pressure, giving two 5-minute air breaks during each treatment session. Only 1 patient had barotrauma to the ear and had to discontinue participation. Peri-wound $TcPo_2$ increased from a mean of 22 to 454 mm Hg with first treatment, and 26 to 550 mm Hg after 20 treatments (P<.001). At day 15 (completion of HBOT), the surface area of ulcers treated with HBOT was reduced substantially more than the healing that was observed in control subjects (41.8%$\pm$25.5% surface area reduction with HBOT vs 21.7% $\pm$ 27.3% in controls; P = .037). By day 30, the control group had caught up: respective surface area reductions were 48.1% ($\pm$30.3%) and 41.7% ($\pm$27.3%; NS).

Faglia and colleagues' study on ischemic diabetic wounds

HBOT reduced lower amputation rates significantly in patients with ischemic diabetic wounds.[57] Seventy diabetic patients who were hospitalized for severe foot wounds were considered appropriate candidates for HBOT. They consented to randomization to standard therapy with or without HBOT. Two patients dropped out (1 died of a stroke shortly after hospital admission; the other withdrew consent for treatment). Of the remaining patients, 35

underwent HBOT and 33 did not. There were no differences in the treatment groups in terms of demographic features, depth of foot ulceration, other diabetic complications, circulatory status, or duration of hospitalization. In the treatment groups, Wagner grade III and IV lesions predominated (25.7% and 62.8% in the HBOT group, and 24.2% and 60.6% in the control groups, respectively). All patients underwent an initial aggressive excisional debridement shortly after admission to the hospital, then twice-daily dressing changes and antibiotics guided by frequent wound cultures. Patients receiving HBOT received an average of 38 treatments; daily in the beginning and then 5 days per week later on (2.5 ATA 100% O_2, 90 minutes). The end point of the study was the incidence of major amputation. Three patients receiving HBOT required below-knee or above-knee amputations, compared with 11 controls (8.6% vs 33.3%, respectively; $P = .016$). $TcPo_2$ measurements were included in the study: at baseline the 2 patient groups had similar readings (23.2±10.7 mm Hg vs 21.3±10.7 mm Hg, respectively; NS). However, at hospital discharge, the values had increased to 37.3 (±16.1) mm Hg and 26.3 (±13.5) mm Hg, respectively. The greater improvement in the HBOT group was highly statistically significant ($P = .0002$). The investigators concluded that the enhanced increase in $TcPo_2$ was related to improved angiogenesis in and around the wound, and they proposed that HBOT is effective in lowering the incidence of major amputation in diabetic wounds.

Baroni and colleagues' study on diabetic foot ulcers

Baroni and colleagues[58] conducted a nonrandomized comparison of the outcomes of 18 hospitalized patients with diabetic foot ulcers treated with standard of care plus HBOT with another group of 10 patients treated identically but without HBOT. All patients had advanced diabetes, and most suffered retinopathy, neuropathy, and vascular occlusive disease. In the HBOT group, 16 patients healed versus 1 in the comparator group ($P = .001$). Two patients receiving HBOT and 4 comparator patients underwent amputation. The investigators concluded that there is major benefit in HBOT for amputation avoidance.

Abidia and colleagues' study on ischemic lower extremity ulcers

Abidia and colleagues[59] conducted a small double-blind study in which patients with ischemic lower extremity ulcers were randomized to hyperbaric air or hyperbaric oxygen treatment. By 6 weeks, 5 of 8 wounds healed in the oxygen group versus 1 of 8 of the controls (NS because of small numbers). At 1 year, all the HBOT wounds remained healed but the wound that had healed in the control group reopened, and the difference in 1-year closure was significantly better in the treated group ($P = .026$). The investigators analyzed overall direct patient treatment costs (fees for dressings, clinic visits, and hospitalizations) and found that HBOT was also able to show cost-effectiveness (based on 100 UK pounds per HBO treatment session). At 6 weeks, median wound surface area closure was 100% in the HBOT group and 52% in controls ($P = .027$). Although the study included few patients, the randomization was strict and the methodology was rigorous.

Kranke and colleagues' study on diabetic foot ulcers

Kranke and colleagues[60] found only 5 publications that met the standards for inclusion in a Cochrane Review of the evidence in favor of HBOT for chronic wounds. The investigators concluded that HBOT significantly reduces the chance of amputation and may increase the chance of healing at 1 year for diabetic foot ulcers. However, they concluded that there is a need for further substantiation because the number of patients on which these conclusions were based is small, and the trial methodologies imperfect. They could not justify the use of HBOT in the management of other wound causes based on current evidence. The report estimated that 4 diabetic ulcers needed to be treated with HBOT to prevent 1 amputation.

Lin and colleagues' study on diabetic foot lesions

Lin and colleagues[61] randomized 29 patients with Wagner 0, 1, and 2 foot lesions to standard care with and without HBOT. Although patients started the study with similar values, after 30 HBO treatments, the patients receiving HBOT had significantly reduced HgA1c (6.6±1.7 vs 9.3±4.3) $TcPo_2$ (57.5±20.7 vs 35.8±21.2), and laser Doppler perfusion scan flux (35.6±7.0 vs 25.8±4.1; all 3 comparisons were statistically significant at the $P<.05$ level).

Predictive ability in HBOT

A frequent objection to HBOT is the inability to predict accurately which patients will receive benefit. Because there are certain indications that sometimes have good responses, patients are usually treated empirically for several weeks, assessed for response, and then continued or terminated according to clinical parameters. Perhaps because of well-intentioned optimism, many patients are treated for weeks or even

months, at great expense ($1000 per treatment at our center) but with minimal tangible improvement. Fife and colleagues[34] found only moderate predictive ability of $TcPo_2$ to distinguish patients who would benefit from HBOT, whether measured under room air, breathing 100% oxygen at sea level, or in the chamber. Barnes[47] noted that, for diabetic lesions, the extent of the wound according to the Wagner scale correlated well with response to HBOT, with Wagner 3, 4, and 5 lesions responding well 77%, 64%, and 30% of the time, respectively. In the same report, patients who achieve a periwound $TcPo_2$ of 200 while breathing 100% oxygen at 2.5 ATA were likely to heal.

Complications in HBOT

HBOT generally is safe and comfortable; nevertheless, there are well-known complications that may occur. One set of risks relates to maintaining the patient under supranormal external pressure. About 60% to 70% of patients experience measureable, but reversible, myopia; patients with presbyopia may experience temporary improvement in visual acuity during the course of therapy. The incidence of otic barotrauma has been quoted between about 2% and 20%.[35,47,55] In my experience, the incidence is even higher, and fully one-quarter of my patients require temporary tympanostomy tubes to equalize pressures in the middle ear and prevent permanent damage. The tubes are easily removed (or sometimes fall out spontaneously) and the tympanic membranes typically heal within days afterward. Other barotrauma is less frequent; the incidence of pneumothorax has been cited at 1 in 1 million treatments. HBOT imposes increased afterload on the left ventricle and patients with New York Heart Association class 3 and 4 congestive heart failure, with ejection fraction less than 35%, should not be treated, because their cardiac situation may worsen.

The other set of potential complications relates to the effects of oxygen as a medicine. Perhaps the most feared complication is oxygen toxicity seizure, the risk of which is about 0.01% to 0.03%. These seizures, which are usually over within the brief time it takes to switch the breathing mixture from 100% to air and to decompress the HBO chamber, do not cause permanent brain damage and are random events that do not necessarily preclude further HBOT. Regimens dictating a 90-minute treatment and limiting pressure to about 2.5 ATA are based on known tolerance of patients to high levels of oxygen for given periods of time. The risk of seizure may be lowered by giving a 5-minute period of air breathing, rather than 100% oxygen, every half hour while in the chamber. There are other theoretic risks of excessive oxygen, such as pulmonary toxicity and of enhancing tumor growth, but these have not been borne out in everyday practice.

Indications for HBOT

At present, the generally approved wound-related indications for HBOT include moderate and deep diabetic foot/leg lesions, necrotizing soft tissue infections, radiation damage to soft tissue and/or bone (osteoradionecrosis), selected problem wounds (**Figs. 1** and **2**), compromised skin grafts and flaps, crush injury, clostridial myositis, gas gangrene, and refractory osteomyelitis. Perioperative treatments have been particularly useful in patients with osteoradionecrosis of the mandible who are undergoing dental extractions or implants; typical regimens call for 10 preoperative treatments and then postprocedure treatment until full healing is observed. Similarly, in anticipation of skin grafts or flaps that may be compromised because of tension or because the tissue to be closed has previously been irradiated, a brief period of preoperative treatment followed by resumption of HBOT as soon as practical after surgery optimizes graft/flap take.

The American Diabetes Association has since 1999 endorsed the used of HBOT as an adjunctive therapy for severe diabetic wounds that fail to respond to standard therapy.[14] The Centers for Medicare and Medicaid Services in 2002 approved the therapy for treatment Wagner III to IV ulcers. Professional organizations such as the Undersea and Hyperbaric Medical Society and Wound Healing Society have included HBOT in their suggested algorithms of care. More than 90% of wounds closed with HBOT remain healed 4 years later. Although health economic aspects of treatment are beyond the scope of this article, a case can be made that the expense of HBOT is justifiable with cost-effectiveness data.[47]

TOT

TOT involves enclosing a body part (usually an extremity) in a portable chamber that circulates pure oxygen over the wound surface. Potential advantages to this approach, compared with HBOT, include lower cost, greater convenience, therapy not limited to 2 hours per day, and success not dependent on the macrocirculation and microcirculation to the wound bed.[7] Potential complications of HBOT, such as oxygen toxicity to tissues/organs, may be avoided by using TOT.[35]

TOT Penetration of Wound Surface

Perhaps the most controversial issue is the ability of TOT to achieve meaningful penetration of the

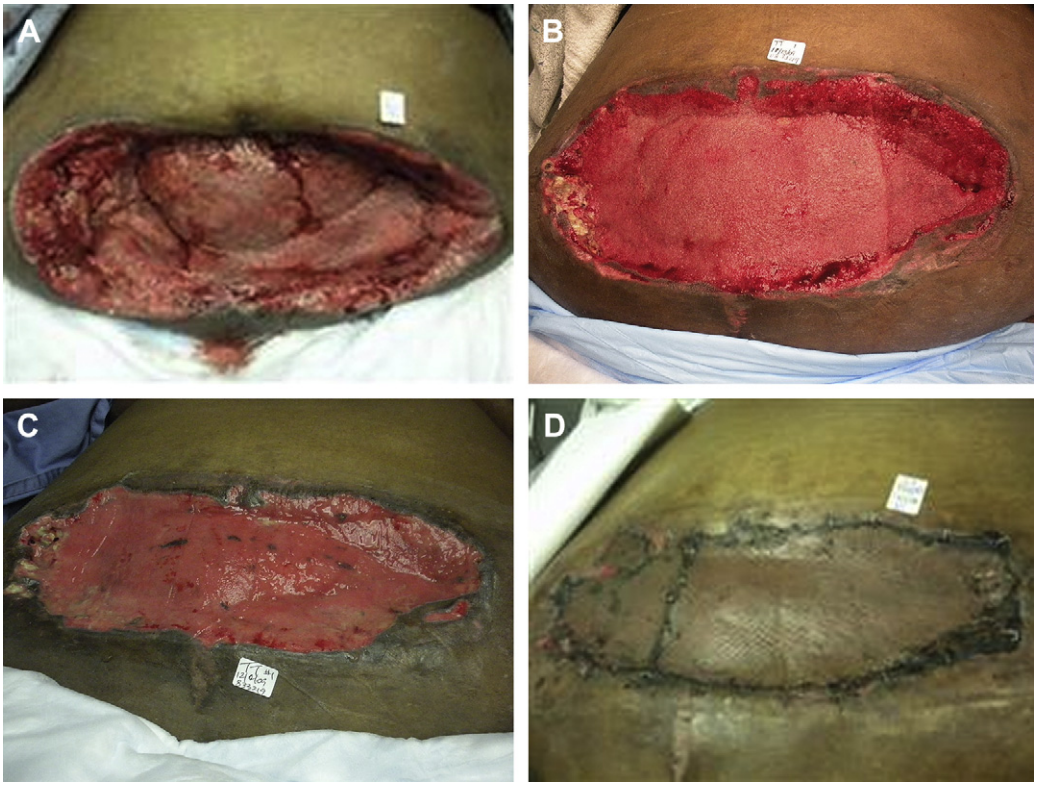

Fig. 1. Multiple-recurrent ventral hernia had recently been repaired with fascial flaps and mesh, but the wound became infected and dehisced shortly after surgery. The patient was returned to surgery and the mesh was removed. (*A*) Exposed abdominal organs thinly covered with collagen mesh. (*B*) After 20 HBO treatments, there is substantial in-growth of vessels and soft tissue. (*C*) After nearly 40 HBO treatments, the wound has partially contracted and the soft tissue coverage has thickened. (*D*) Perioperative HBOT was used to support the take of partial-thickness skin grafts, achieving definitive closure of the wound.

wound surface and reach the cells/tissues that would benefit from hyperoxygenation. This issue has been addressed both with direct measurement of tissue oxygen levels and, indirectly, by showing the biochemical and biologic effects of TOT. Fries and colleagues[35] showed that, at a depth of 2 mm into the wound bed, Po_2 increased from its baseline of 5 to 7 mm Hg to 40 mm Hg

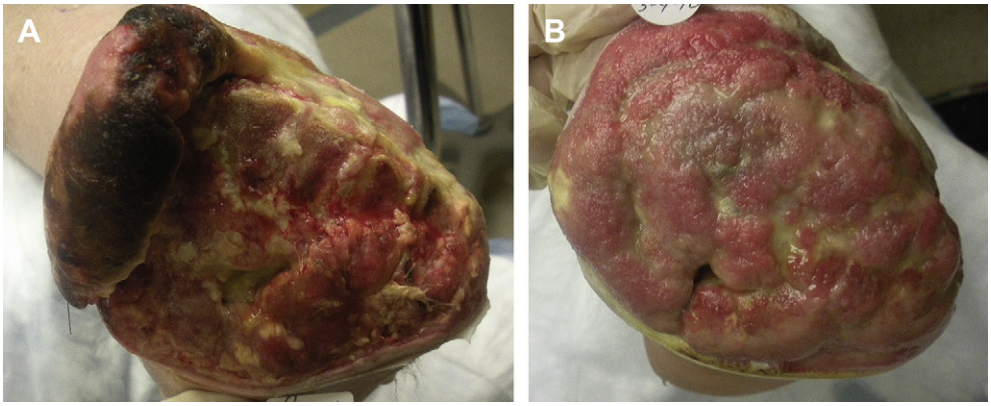

Fig. 2. Open transmetatarsal amputation. (*A*) Early postoperative period. Cut ends of metatarsals #2 to 5 are exposed. (*B*) After approximately 40 HBO treatments. Bone ends are covered. Healthy granulation tissue fills the wound.

after 4 minutes of TOT. Standardized full-thickness wounds on the backs of pigs were treated with either topical air or topical pure oxygen. By the second day of treatment, the surface area of the oxygen wounds was significantly ($P<.05$) reduced compared with the controls, and the acceleration in healing was maintained to 3 weeks ($P<.005$). Healed wounds were subjected to 3-mm punch biopsy and histologic evaluation; TOT wounds were more mature than the controls, in terms of epithelial architecture, expression of keratin-14, and expression of VEGF and α-smooth muscle actin. Under resting conditions, wound site tissue P_{O_2} levels were 4 times higher in the TOT versus the control wounds (42 vs 11 mm Hg; $P<.05$).

In another report, db/db diabetic obese mice were subjected to standardized full-thickness wounds and then randomized to TOT or control dressings.[62] TOT was delivered as pure oxygen at low flow (3 mL/h) over the wounds. The surface area of the TOT wounds was significantly smaller at 6 and 10 days ($P = .022$ and $P = .008$, respectively). Histologic sections of the wounds showed more extensive collagen deposition in the TOT-treated lesions.

TOT and Increased VEGF

Gordillo and colleagues[63] showed that TOT increased VEGF up to 20-fold in diabetic foot ulcers. A large clinic population was screened to enroll 57 patients who suffered a variety of chronic wounds. The criteria by which these patients were selected were not described in detail. These patients were assessed for HBOT but patients who had contraindications were offered TOT; this yielded a population of 32 HBO-treated and 25 TOT-treated patients. Patients who consented to serial biopsies underwent wound edge biopsy just before initiation of treatment and again at 7 and 14 weeks into therapy. Tissue levels of mRNA expressed by the genes for VEGF, TGF-β1, and COLIA1 were measured. In patients who experienced complete wound healing, VEGF expression was substantially increased by TOT but not by HBOT; expression of the other 2 genes did not show a significant difference with either treatment. The issues with study design limit the interpretation of these results, but demonstration of increased VEGF expression implied that TOT succeeded in penetrating the wound with oxygen to a meaningful extent.

TOT Versus Silver Alginate

Blackman and colleagues[64] conducted a prospective, but not randomized, comparison of patients treated with TOT versus a silver alginate dressing. TOT treatments were conducted for 60 minutes, 5 times each week. Fourteen of 17 patients (82.4%) healed in the TOT group, compared with 5 of 11 (45.5%) in the alginate group ($P = .04$). Median time to closure was also improved by TOT (56 vs 93 days, respectively). The results were particularly impressive considering that the wounds in the TOT group were significantly larger (mean 4.1 vs 1.4 cm^2; $P = .02$) and of greater duration (6.1 vs 3.2 months, P = NS). Although subject to all the flaws inherent in a nonrandomized and nonblinded trial, these results are provocative.

TOT Versus Standard Wound Care

Heng and colleagues[25] reported a prospective study comparing TOT and standard care (wet-to-dry or hydrocolloid dressings) in the care of wounds of various causes, including 39 diabetic foot ulcers and 40 wounds of other types (mostly pressure ulcers). Many patients suffered significant comorbidities. The TOT was delivered at 1.03 atm, 4 h/d, 4 d/wk up to 4 weeks. Complete wound healing was noted in 90% of the TOT group versus 22% of controls. Four of 7 stage IV wounds healed with TOT versus none of 5 in long-term follow-up. The methodology used clouds the results: many patients were not randomized and most patients had more than 1 study wound, making the statistical analysis and interpretation of the data difficult. Wound biopsies were conducted and showed substantial increase in the density of blood vessels and collagen in the TOT wounds. Although acknowledging the limitations of this report, the results argue in favor of TOT compared with standard wound care alone.

Tawfick and Sultan[65] published a nonrandomized comparison of venous leg ulcers treated with TOT and others who were managed using standard therapy (n = 83). At 12 weeks, 80% of patients receiving TOT were healed (median 45 days) compared with 35% of controls (median 182 days; $P<.0001$ for both incidence of healing and time to healing). Patients were allowed to choose whether to be treated with TOT or standard of care. TOT was given in an inpatient setting, with 3-hour treatments twice daily, O_2 at 10 L/min with continuous humidification. Patients were treated until full healing or 12 weeks, whichever came first. Standard of care consisted of wound cleaning, debridement, and Profore (Smith and Nephew, London, United Kingdom) compression dressings 1 to 3 times weekly. Initial patient and wound characteristics were not significantly different between the 2 groups. All wounds were at least 2 years old, and most were larger than 10 cm^2. The methodology raised the question of whether the

therapeutic effect came more from mandatory leg elevation 6 hours per day, or from TOT. In 32 of 46 TOT-treated ulcers, granulation tissue developed first in the center of the wound, and then extended peripherally to cover the surface of the lesion.

Clinical Evidence for TOT

Although there is sufficient penetration of TOT to achieve meaningful physiologic effects within the wound bed, the current clinical evidence in favor of this modality needs to be supplemented with more rigorous investigations. In choosing between HBOT and TOT, the benefits for TOT of lower cost and avoidance of potential complications are outweighed by the less potent clinical effect achieved with this approach. Particularly in the case of diabetic foot and leg ulceration, in which there is substantial risk of amputation, the preponderance of data seems to support HBOT in preference to TOT.

HOW MUCH OXYGEN IS OPTIMAL?

Given that correction of wound hypoxia is beneficial to many aspects of healing, it does not necessarily follow that more is better, or that hyperoxygenation of normally nourished wounds confers enough benefit to justify the risks. It is possible that, for some individuals, imposing oxidative stress and excessive ROS may be more harmful than helpful.[2,12] The rate of production of toxic radicals is directly proportional to local oxygen tension.[4] Heng and colleagues[25] make important points about potential negative influences of excess oxygen on wound repair. Unphysiologically high levels of oxygen can react with NADP and be metabolized to ROS in the cytoplasm without the usual catalysis by the mitochondrial cytochrome system, which ordinarily also controls the release of ROS. In this event, the ROS created may be injurious to the host cell (fibroblast, endothelium) rather than serving a useful role in killing foreign organisms. This may be one mechanism by which oxygen exerts brain toxicity, occasionally causing seizures in patients under HBOT.[66] In addition, bypassing the cytochrome system sacrifices production of ATP, so the extra oxygen is wasted. There is the potential for cell cycle arrest and genotoxicity from exposure of cells to pure oxygen.[10,67] Cellular senescence is accelerated in hyperoxic conditions.[12]

Cellular Adaptation to Ambient Oxygen Level

Cells seem to accustom themselves to chronic hypoxia or hyperoxia. For example, the PHD family of proteins, which are known to suppress cellular proliferation, modulate the cell cycle and encourage apoptosis, is expressed when hypoxia is sensed.[68] Cells cultured under 20% oxygen and then subjected to a 5% oxygen environment synthesize higher levels of PHDs; over longer periods of time, PHDs return to baseline levels. Similarly, cells accustomed to culture under 30% oxygen overexpress PHDs when suddenly put under 20% oxygen.[12] Thus there is a hypoxia set point that is tunable. In theory, the optimal oxygen treatment strategy may be to restore hypoxic areas of the wound bed to normal oxygen tension without overtreating and risking oxidative stress.

Individualized Oxygen Dosing

In common practice, nearly all patients receiving HBOT are dosed similarly: 90-minute sessions, once daily at 2.0 to 2.5 ATA. It is possible that this one-size-fits-all approach to dosing might not be appropriate for all patients; some wounds are more hypoxic than others, therefore some may be overdosed and others underdosed. Everyone has endogenous genes that encode antioxidant molecules to protect them against oxidative stress; however, individuals may vary in the levels of expression of these genes and therefore some patients may be less capable than others of dealing with the oxygen load. Real-time wound Po_2 mapping assist understand of the degree of hypoxia in each wound, and could be the basis for more individualized therapy.

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