New Insights Into Oxygen Therapy for Wound Healing

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Abstract: Oxygen is a powerful substrate and signal. The ability to understand and control this vital substance will open new avenues of therapy for multiple diseases. New insights into cutaneous oxygenation suggest that there may be a sound basis for topical oxygen therapy. The indications for systemic oxygen therapy are expanding as well, as research delves into the role of oxygen in pathophysiologic conditions. Previous therapeutic approaches simply relied on providing oxygen as a critical substrate for fundamental metabolic processes. New therapies that also utilize oxygen as a needed signal will further expand the indications for oxygen therapy.

Throughout history, native healers have recognized that wounds heal faster if a patient is transported from thin mountain air to a richer atmosphere (e.g., a low-lying valley). In modernity, oxygen has been recognized as the element most essential to healing. Clinicians are now able to diagnose oxygen deficiency and administer oxygen therapy with increasingly advanced mechanisms and devices. The following review will examine the background as well as newer developments in this area.

The Wound Healing Process

Wound healing consists of a series of physiologic events that occur in response to tissue damage. Many of the vital processes of wound healing are oxygen dependent. Wounds vary greatly, and the healing of a wound may proceed with different tempo and quality due to local and systemic factors and due to variation among individuals. Variable healing is present within a specific wound and within different anatomic locations in the same patient. However, there are common themes to the healing of all wounds. The burn literature has classically described three zones of injury: necrosis, stasis, and hyperemia. This construct serves well in considering any wound. Tissue in the zone of necrosis is already lost, while tissue in the zone of stasis may be saved with good local and systemic care; otherwise, it is lost to infection, trauma, or dehydration. Tissue in the zone of hyperemia may become dam-
aged or lost if problems arise in the zone of stasis. Recognizing that the key determinant of tissue survival is oxygenation, one can rephrase the burn zone paradigm as follows: zone of anoxia, zone of hypoxia, and zone of normoxia. In this oxygen-based paradigm, the wound healer must focus on treating tissue in the zone of hypoxia in order to preserve the maximum amount of viable tissue and encourage the healing process.

Oxygen levels in wounded tissue result from a balance of supply and demand. Supply may be low due to problems such as vascular disease, radiation (impaired delivery), or edema (increased diffusion distance). Demand may be high as a result of metabolic needs of specific cells at various points within the wound healing sequence. White blood cells consume oxygen during the respiratory burst necessary for killing ingested bacteria. Fibroblasts require a critical level of oxygen in order to secrete collagen and other extracellular matrix molecules. This allows angiogenesis and granulation tissue formation to fill the wound. Hypoxic wounds deposit collagen poorly and become infected easily. Epithelialization is a parallel process of resurfacing the wound that also proceeds optimally at high oxygen levels. Although the processes described may proceed optimally at elevated pO₂ levels, they may still require some hypoxia as an intermittent signal.

Oxygen as Substrate and Signal

Until recently, oxygen was simply viewed as “fuel for the fire,” a vital metabolic substrate for a multitude of important cellular functions. It was presumed that the effect of a burst treatment of supplemental oxygen was to allow those necessary reactions to proceed at higher rates, and that the effect would subside upon the cessation of therapy. What we have learned recently is that the oxygen also provides an important signal that affects multiple cellular behaviors long after the oxygen level returns to its pretreatment value. In the context of the important biological process of cutaneous wound healing, oxygen functions as both a substrate and a signal (Table 1).

Clinical Relevance of Oxygen Therapy

It has been shown that vital cellular functions such as angiogenesis, fibroplasia, epithelialization, and bacterial killing all proceed at a more rapid pace in response to higher oxygen levels. In wound healing, bacterial burden must be removed and a preliminary matrix must be formed in order to allow fibrous tissue formation and epithelial coverage. Oxygen hastens removal of bacterial bioburden, which allows resolution of inflammation and facilitates matrix production, cell division, and ultimate wound closure. This is especially relevant in conditions where healing is impaired. Common impediments include diabetes, peripheral arterial or venous vascular disease, radiation and other situations where reduced oxygenation and/or large bacterial bioburden may be present.

Of particular note, chronic venous leg ulcers are often colonized with anaerobic bacteria which impede the healing process. In this setting oxygen therapy can change the wound microenvironment to a more aerobic milieu, which will inhibit anaerobe proliferation. It has been demonstrated that hyperbaric oxygen therapy improved the healing of chronic venous ulcers in a controlled randomized clinical trial. As more is learned about bacterial resistance to antimicrobial therapy, alternate methods of antibacterial control such as oxygen therapy become more relevant. Bacteria do not develop resistance to oxygen therapy in the same manner as they do to antibiotic therapy. Oxygen may offer a novel therapeutic strategy against biofilm forming bacteria, where such microbes can greatly increase their resistance to antibiotics.

Another problem with chronic wounds is that there is poor quality of healing after a prolonged period of inflammation. For example, burns that are not closed within a 3-week period have a 70% chance of developing excessive fibrotic hypertrophic scars. Similarly, chronic leg ulcers often heal with unstable, brittle fibrotic skin that is prone to recurrent ulceration. If better wound oxygenation yields a faster exit from the inflammatory state, then perhaps an increase of healing by regeneration and less by fibrosis will result in higher quality and more durably healed tissue.

Tissue oxygen levels have also been measured as predictors of clinical outcome. For example, limb amputations at the below knee level have been shown to heal in only 11% of patients with an oxygen partial pressure of < 20 mmHg at the amputation site, heal in 46% at 20 mmHg–30 mmHg, and to heal at 97% at ≥ 30 mmHg.

Table 1. Functions of oxygen.

<table>
<thead>
<tr>
<th>Oxygen as substrate</th>
<th>Oxygen as signal</th>
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<tr>
<td>ATP production</td>
<td>Macrophage VEGF secretion</td>
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<tr>
<td>Respiratory burst</td>
<td>PDGF receptor increase</td>
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<td>Protein synthesis</td>
<td>Cell proliferation increase</td>
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Physiology of Oxygen Delivery

How does the body normally deliver oxygen to those tissues that vitally need it? First, oxygen in the atmosphere enters the lungs during respiration. There, the gaseous oxygen must cross from the air spaces (alveoli) of the lung into the fluid spaces (capillaries). Oxygen must become dissolved in the fluid state in order to do so. In other words, gaseous oxygen may not enter the tissues until it first becomes dissolved oxygen. This dissolved oxygen then moves across the alveolar membrane into the lung capillaries where it then enters the bloodstream. Oxygen movement from alveolar air space into the capillary liquid space is dependent on surface area. The human lungs are optimally designed for this purpose. They provide a total surface area of 70 square meters for gas exchange across the very thin alveolar membrane (thickness < 1 µm).14 Note that even with this huge surface area available for gas exchange, only about 50% of the inspired gaseous oxygen actually traverses the alveolar membrane. Oxygen moves by diffusion, from areas of high concentration to low concentration. The size of the diffusion gradient (the difference between high and low concentration) determines the amount of diffused oxygen that may move into the low concentration area. As shown below, the amount of oxygen is reduced as it diffuses out of the bloodstream and reaches the peripheral tissues.

Once in the peripheral tissues, movement of oxygen is driven by a gradient, where it travels from regions of higher concentration to lower concentration (Figure 2). Upon entering the bloodstream, oxygen is carried in its dissolved form, either bound to hemoglobin within the red blood cell or free in an unbound form within the plasma. When the richly oxygenated blood approaches the peripheral tissue capillaries, the dissolved oxygen begins to move again by the diffusion gradient, away from the oxygen-rich capillary to the oxygen-poor tissues. The first oxygen to leave the capillaries is the free or unbound oxygen within the plasma. When the plasma oxygen concentration lowers, hemoglobin begins to unload its bound oxygen into the plasma and this oxygen also diffuses into the tissues. Ultimately, the dissolved oxygen is taken up by the peripheral tissue cells and is consumed. Tissue factors such as edema may limit cellular oxygen uptake due to high diffusion distance from the capillary to the cell. Normal gradients cannot drive oxygen very far into the tissues if there is significant edema. The oxygen-depleted plasma returns via the venous system to reload with oxygen in the lungs for the next cycle.

Delivering Oxygen From the “Inside Out”: \( pO_2 \) vs. \( O_2 \) Content

Oxygenation of the skin may occur by two routes: delivery from the “inside out” via the circulation or delivery from the “outside in” via the atmosphere. Once blood is oxygenated and travels through the body, dissolved plasma oxygen diffuses into peripheral tissues such as the skin. The oxygen moves by gradient from the capillaries into the interstitial space and ultimately to the skin cells. Note the amount of dissolved oxygen in the blood is described by its partial pressure or \( pO_2 \) (expressed in mmHg). Note also that the total \( O_2 \) content includes the dissolved as well as the hemoglobin-bound oxygen. As Hunt speculated, \( pO_2 \) may be more important than \( O_2 \) content in terms of delivery of oxygen to the skin and wound.15 Hopf16 provided further support for this view and found normal levels of subcutaneous tissue \( pO_2 \) at a hemoglobin level of 5 g/dL in normal healthy volunteers who allowed phlebotomy with crystalloid substitution.10 Note that the oxygen content in a person with a hemoglobin level of 5 g/dL is 9 g/100 mL \( O_2 \) content, compared to a hemoglobin level of 14 g/dL (normal) with 20 g/100 mL \( O_2 \) content. This indicates that the dissolved oxygen segment may be more important in driving diffusion into the tissues than the hemoglobin-bound segment, and that \( O_2 \) is carried in the blood greatly in excess...
of the skin and subcutaneous tissue needs. However, one must be cautious in treating sick patients with these low hemoglobin levels, as there are tissues that may have greater oxygen demand (muscle, nerve) or be in a condition of increased oxygen demand (sepsis, shock). Under normal conditions pO₂ is 55 mmHg–70 mmHg in the subcutaneous chest tissue of a healthy subject breathing room air, but this level varies in different regions of the body.¹⁶

Delivering Oxygen From the “Outside In”: The Role of Skin Permeability

Oxygen can diffuse through any gas permeable surface, and can therefore enter the skin. However, the skin is a limited surface for oxygen exchange as the total surface area of the human skin is relatively small (slightly greater than 1 m² compared to the lungs 70 m²) and its thickness is a significant barrier to oxygen diffusion (epidermis up to 1.5 mm thick and dermis up to 3 mm thick, compared to the lungs < 1 µm). It has been long known that the skin is capable of gas exchange. In 1851, when Gerlach¹⁷ secured a varnished horse bladder to his skin for 24 hours and measured the gas content before and after, he observed a decrease in O₂ and an increase in CO₂. The skin can exchange 80 mL–100 mL O₂/m², or provide about 1%–2% of the total O₂ exchange surface needed by the body.¹⁸ Though the skin’s gas exchange is not systemically significant, it is locally important. Recent advances in the technology of cutaneous oxygen measurement have changed quite a few long held beliefs about how the skin receives its oxygen supply. It had been observed that oxygen will pass through the stratum corneum in vitro as well as 0.30 mm down into the superficial dermis,¹⁹,²⁰ but advances in oxygen measurement technology have produced in-vitro data as well. Two investigators have demonstrated that the atmospheric ambient O₂ almost exclusively supplies the outer 0.25 mm–0.40 mm of the skin.¹⁵,²¹ A nadir of pO₂ occurs at 0.1 mm below the skin surface (Figure 3).²²

It was previously believed that poorly diffusing oxygen was further limited in its penetration of the skin by stratified epithelium, and that all O₂ delivered to the skin occurred via the capillary beds. However, oxygen levels in the superficial dermis and epithelium are well maintained even in the setting of cuff occlusion of limb arterial inflow.²² The oxygen is therefore concluded to have been directly absorbed from the ambient atmospheric O₂, rather than from the capillary beds by diffusion. Interestingly, when an experimental wound surface was deprived of atmospheric oxygen by substituting an atmosphere of pure nitrogen, wound surface pO₂ dropped to 4 mmHg–5 mmHg, indicating that was the level achievable via diffusion from the underlying circulation alone.²³ Another recent study using oxygenated water (dissolved oxygen) on porcine skin noted penetration of the skin by oxygen that was enhanced when tape stripping of the epidermis was performed.²⁴

Hyperbaric Oxygen Therapy and its Efficacy

Hyperbaric oxygen therapy (HBOT) has been in use for many years and its clinical indications continue to increase. It is known from experimental and clinical evidence that HBOT can accelerate healing through increased angiogenesis.²⁵ Hyperbaric oxygen will substantially increase the amount of dissolved oxygen in the blood, since at a pO₂ of 100 mmHg, hemoglobin is nearly fully saturated. These changes can have a significant impact on healing tissues through a combination of substrate and signaling roles. Hyperbaric oxygen therapy creates a large (pO₂ > 2000 mmHg) driving gradients from the high systemic O₂ concentration in the bloodstream, which allows better tissue penetration as long as the vasculature can deliver the oxygenated blood to the target tissue.

The effectiveness of HBOT has been studied extensively in many different clinical settings. Although HBOT treatments are brief, subcutaneous oxygen tensions are elevated for several hours.²⁶,²⁷ One effect has been to
improve angiogenesis and tissue transcutaneous oxygen tension in a diabetic foot wound and irradiated tissue. 28-29 The success of HBOT has been seen in a prospective study of chronic leg wounds. 10 Patients receiving HBOT treatment had a significant reduction in wound size (35.7%) at 6 weeks compared to control patients (2.7%). Similarly, an extensive retrospective analysis of patients with intractable wounds evidenced a 70%-90% success rate. 30-31 It should also be mentioned that perioperative normobaric supplemental oxygen breathing (FIo₂ of 80%) reduced the incidence of surgical wound infections. 32

Topical Oxygen Therapy and its Efficacy: Gaseous vs. Dissolved

Many wound patients cannot tolerate systemic HBOT side effects, afford HBOT, or gain access to a hyperbaric chamber. Sometimes the patient’s cardiovascular system is inadequate for carrying the oxygenated blood to the wounded tissue, or the tissue is so edematous that the oxygen cannot reach the wound well. Topical oxygen therapies are designed to allow oxygen to enter the wound or skin via the external surface of the body rather than from capillaries within. The oxygen is therefore delivered directly to the wound and the systemic side effects are eliminated.

Topical oxygen therapy may be delivered to the external surfaces of the body in a gaseous or dissolved form. However, the administered oxygen must be transformed from the gaseous to the dissolved form to become biologically available to the target cells being treated.

Topical gaseous “oxygen boot” systems have been proposed since 1932 with refinements since. Treatment methods that deliver gaseous oxygen include enclosures around a limb or wound site that are flushed with pure gaseous oxygen, or machines that generate gaseous oxygen at the wound surface. 33 A recent review discusses the evidence based recommendations for topical oxygen therapy. 34

For oxygen to be transferred from a gas bubble to an individual cell, several independent partial resistances must be overcome including 35:

- resistance within the gas film to the phase boundary
- penetration of the phase boundary between gas bubble and liquid
- transfer from the phase boundary to the liquid
- movement within the nutrient solution
- transfer to the surface of the cell.

These intrinsic issues limit the efficacy of gaseous topical oxygen systems; yet, topical low-pressure oxygen therapy has shown some encouraging results in preliminary studies. One hundred percent oxygen is administered at atmospheric or slightly greater pressure without a hyperbaric chamber via an enclosure that surrounds the affected area. It is inexpensive and can be provided in the home environment. It has been shown to promote wound angiogenesis and healing in animal studies as well as in human clinical studies. 36-38 A topical device that administers a stream of 100% oxygen bubbles to the wound surface has been shown to improve epithelial healing. 39

Methods that deliver topical dissolved oxygen include those which catalytically produce dissolved oxygen at the wound surface, those which contain diffusible dissolved oxygen bound to a carrier such as a fluorocarbon, or those which allow a reservoir of gaseous oxygen to diffuse through the vehicle. There have been difficulties reported in creating stable fluorocarbon emulsions, however some promising early results have been reported.

Recent experimental data on a set of devices that deliver unbound dissolved oxygen have demonstrated significant oxygen penetration through viable human skin samples. 40 Levels of transcutaneous oxygen 4-6 times normal subcutaneous oxygen (250 mmHg) were observed after topical dissolved oxygen treatment of skin samples with or without a stratum corneum epidermal layer present. This level of penetration achieved was twice the depth of that noted in previous studies of skin exposed to hyperbaric gaseous oxygen. 41 Another recent study demonstrated penetration of topical dissolved oxygen through both intact (with epidermis) and tape stripped (epidermis removed) viable porcine skin samples.

There appears to be a significant advantage to delivering oxygen topically in its dissolved form, as it is biologically available immediately upon administration. The fundamental challenge to topical oxygenation methods is to create a large enough driving oxygen gradient to allow oxygen delivery into zones of tissue hypoxia. If this can be achieved clinically, topical tissue oxygenation procedures will become complementary to systemic methods of oxygenation and will allow the treating physician greater therapeutic versatility in treating wounds.

The ability to control duration and depth of topical oxygen delivery into human tissue will allow new strategies in individualizing patient therapy. A deeper understanding into the pharmacokinetics of topical oxygen administration will allow manipulation of specific healing
processing (Table 2). Targeted oxygen therapies will be based on pharmacokinetics that will control oxygen tissue penetration depth, with degree and duration of oxygenation. New insights into oxygen’s ability to penetrate intact skin may prove therapeutic in other conditions besides wounds, where inflammatory or degenerative conditions of the skin require repair and rejuvenation.

Conclusion
Oxygen is a powerful and multifunctional substrate and signal. The ability to understand and control this vital substance may open new avenues of treatment in multiple disease states. Oxygen-based treatment strategies for wounded soft tissues can be designed on an understanding of the zones of anoxia, hypoxia, and normoxia. Recent insights into cutaneous oxygenation suggest that there may be a sound basis for topical tissue oxygenation procedures. Recent technological developments now allow delivery of biologically available dissolved oxygen directly to the wound and skin. The ability to drive oxygen deep into zones of tissue hypoxia will lead to better metabolic support of cellular function, more rapid clearance of bacteria and resolution of inflammation, and ultimately faster and better tissue preservation and healing.

References

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Table 2. Theoretical examples of targeted oxygen therapy.


