

Forum Review

Wounds: An Overview of the Role of Oxygen

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ABSTRACT

We sought to review the role of oxygen in wound healing, with an emphasis on the role tissue oximetry has played in clinical advances in the care of patients with wounds. Oxygen is required for wound healing. Hypoxia sufficient to impair healing is common in wounds, frequently resulting from sympathetically induced vasoconstriction. Correction or prevention of vasoconstriction, as well as provision of increased inspired oxygen in well-perfused patients, has been shown in randomized, controlled clinical trials to improve wound outcomes. Our understanding of the role of oxygen in wound healing has been fueled by tissue oximetry. Advances in technology will lead to further advances in the management of patients with wounds. *Antioxid. Redox Signal.* 9, 1183–1192.

INTRODUCTION

OXYGEN IS REQUIRED for all the major processes of wound healing, including resistance to infection, fibroplasia and collagen deposition, angiogenesis, and epithelialization. The required oxygen reaches the wound *via* the bloodstream, and thus, healing is exquisitely sensitive to perfusion and blood oxygen levels. Wound hypoxia is common, resulting from impaired perfusion due vascular disruption as well as sympathetically induced vasoconstriction. In this article, we review the role of oxygen in wound healing, the causes of wound hypoxia, means of improving tissue oxygen delivery, and the role wound tissue oximetry has played and will continue to play in our understanding of wound healing and clinical wound care.

WOUND REPAIR

Wounds are created by an injury to the skin, which may be caused by surgery or any kind of trauma. The injury disturbs the equilibrium in the local environment, including the microcirculation, and initiates a sequence of events known as healing.

Damage to the local circulation causes platelets to aggregate

and release a variety of substances, including chemoattractants and growth factors. The initial result is coagulation, which prevents exsanguination, but also widens the area that is no longer perfused. Bradykinin, complement, and histamine released by mast cells also perturb the microcirculation. Inflammatory cells (polymorphonuclear leukocytes immediately and macrophages by 24–48 h) migrate to the wound and are activated in response to endothelial integrins, fibrin, lactate, hypoxia, foreign bodies, and growth factors. In turn, macrophages and lymphocytes produce more growth factors [including insulin-like growth factor (IGF-1), leukocyte growth factor, interleukins 1 and 2 (IL-1 and -2), transforming growth factors (TGFs), and vascular endothelial growth factor (VEGF) and lactate 17)]. Oxidants produced by inflammatory cells also play a major role in initiating and directing the healing process (71). This is discussed in detail elsewhere in this volume by Sen and Hunt. The inflammatory phase is characterized by erythema and edema of the wound edges.

Injury also disrupts the normal skin barrier, and thus, wound healing depends on the ability to clear foreign material and resist infection. Neutrophils provide nonspecific immunity and prevent infection. In the absence of infection, they disappear by ~48 h. Nonspecific phagocytosis and intracellular killing are the major pathways activated in wounds (5).

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Bacterial killing by neutrophils depends on a high partial pressure of oxygen. This is because reactive oxygen species are the major component of the bactericidal defense against wound pathogens. After phagocytosis of the pathogen, nicotinamide adenine dinucleotide phosphate (NADPH)-linked oxygenase (also called phagosomal oxidase or primary oxidase), present in the phagocytic membrane, uses oxygen as the substrate to catalyze the formation of superoxide. Superoxide is bactericidal, but more important, the production of superoxide initiates a series of cascades that produces other oxidants that increase bacterial killing capacity (Fig. 1). For example, in the presence of superoxide dismutase, superoxide is reduced to hydrogen peroxide (H_2O_2). H_2O_2 combines with chloride and, in the presence of myeloperoxidase, forms the bactericidal hypochlorous acid (the active ingredient in bleach) (18, 25). Because intraphagosomal oxidant production depends on conversion of oxygen to superoxide, the process is exquisitely sensitive to the partial pressure (not content or saturation) of oxygen in the tissue. The K_m (half-maximal velocity) for the phagosomal oxidase with oxygen as a substrate is 40–80 mm Hg (2). This means that resistance to infection is critically impaired by wound hypoxia and becomes more efficient as pO_2 increases, even to very high levels (500–1,000 mm Hg).

Activated inflammatory cells consume oxygen at a high rate, and, coupled with the impaired microcirculation, this results in hypoxia, especially at the center of the wound (75). Lactate is produced both anaerobically and aerobically, and this results in concentrations of 5–10 mM even in well-oxygenated wounds (64). Lactate is a strong stimulus for collagen secretion and angiogenesis (26, 53). Anti-inflammatory steroids impair healing by suppressing inflammation at this step.

The proliferative phase begins ~4 days after injury, concurrent with a waning of the inflammatory phase. It consists of new (granulation) tissue formation and epithelialization. Granulation requires angiogenesis as well as synthesis, modification, and deposition of collagen and connective tissue proteins. Angiogenesis is required to replace the injured microcirculation

and involves the movement of endothelial cells in response to three waves of growth factors. The first wave of growth factors comes with the release by platelets of platelet growth factor (PDGF), TGF, IGF-1, and others during the inflammatory phase. The second wave comes from fibroblast growth factor (FGF) released from normal binding sites on connective tissue molecules. The third and dominant wave comes from VEGF, delivered largely by macrophages stimulated by fibrinopeptides, hypoxia, and lactate (70). Although it is usually present, hypoxia is not required for granulation because of constitutive (aerobic) lactate production by inflammatory cells and fibroblasts. Too little lactate leads to inadequate granulation, whereas levels in excess of 15 mM (usually associated with excess inflammation or infection), delay granulation (6). The capillary endothelial response to angiogenic agents (*i.e.*, migration into the wound, tubule formation, and connecting to sources of blood flow) requires oxygen, so that angiogenesis progresses proportional to blood perfusion and arterial pO_2 (56).

New blood vessels grow into the matrix that is produced by fibroblasts. Although fibroblasts replicate and migrate mainly in response to growth factors and chemoattractants, production of mature collagen requires oxygen (13, 60, 66). Lactate, hypoxia, and some growth factors induce collagen mRNA synthesis and procollagen production. Posttranslational modification by prolyl and lysyl hydroxylases is required to allow collagen peptides to aggregate into triple helices. Collagen can be exported from the cell only when it is in this triple helical structure. The helical configuration is also primarily responsible for tissue strength. The activity of the hydroxylases is critically dependent on vitamin C and tissue oxygen tension (66). Wound strength, which results from collagen deposition, is, therefore, highly vulnerable to wound hypoxia.

Angiogenesis and extracellular matrix (primarily collagen) production are closely linked. Fibroblasts cannot produce mature collagen in the absence of mature blood vessels that deliver oxygen to the site. Conversely, new blood vessels cannot mature without a strong collagen matrix. Mice kept in a hy-

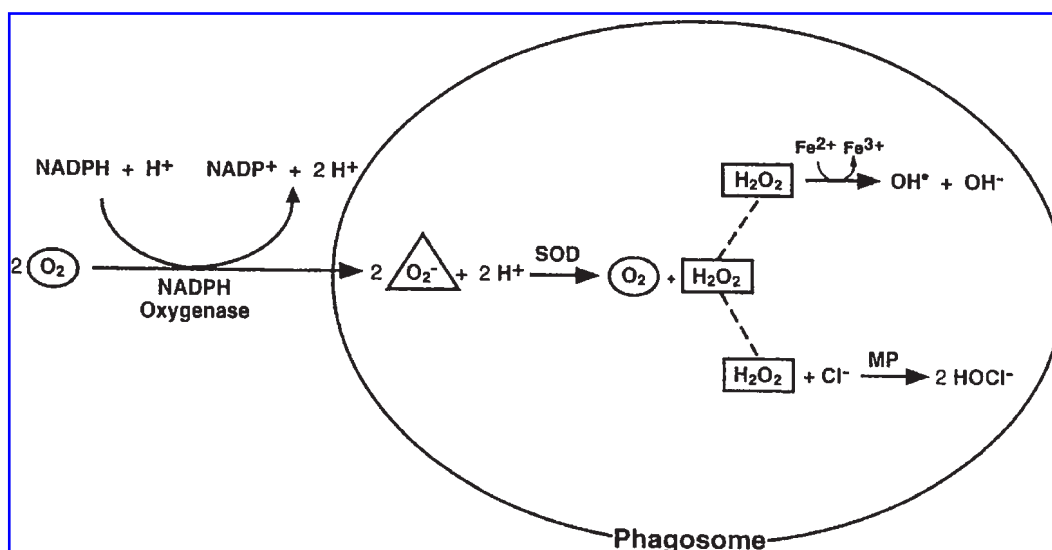


FIG. 1. Schematic of superoxide and other oxidant production within the phagosome. From Hunt and Hopf (47), with permission.

poxic environment (13% inspired oxygen) develop some new blood vessels in a test wound (with VEGF or lactate added), but these vessels are immature, with little surrounding matrix, and demonstrate frequent areas of hemorrhage (4). Mice kept in a hyperoxic environment (50% inspired oxygen), conversely, develop mature vessels with thick surrounding matrix and do so more rapidly than mice kept in either a normoxic (21% inspired oxygen) or a hypoxic environment.

Epithelialization restores the skin barrier. It is characterized by replication and migration of epithelial cells across the skin edges. In acute wounds that are primarily closed, because only a narrow area requires epithelial coverage, it is normally complete within 1–3 days. Like immunity and granulation, epithelialization depends on oxygen and growth factors (58). Silver (76) and Medawar (58) demonstrated that the rate of epithelialization depends on local oxygen concentration *in vivo*. Topical oxygen (applied so that it does not dry out epithelial cells) has been advocated as a method to increase the rate of epithelialization (22). In contrast, O'Toole (65) demonstrated that hypoxia increases epithelial migration *in vitro*. This may be explained, at least in part, by the dependence of epithelialization on the presence of a bed of healthy granulation tissue, which is known to be oxygen dependent. In open wounds, epithelialization occurs only after the wound space is filled with granulation tissue, and, because of the greater area to be covered, is more frequently delayed or impaired.

The final phase of wound repair is maturation, which involves ongoing remodeling of the granulation tissue and increasing wound tensile strength. The movement of fibroblasts pulls the collagen fibers together, promoting contraction of the tissue. This contraction is responsible for shortening scars and shrinking wounds, and it is inhibited by the use of steroids in doses >30 mg/day (15).

WOUND OXIMETRY

The measurement of wound tissue oxygen has led to a detailed and valuable understanding of the role of oxygen in wound healing. Tissue oxygen levels can be measured by a number of means, including subcutaneously implanted silicone tonometers with inserted polarographic or optical electrodes, directly implanted polarographic or optical electrodes, subcutaneously implanted wire-mesh cylinders with inserted oxygen probes, transcutaneous oximetry with polarographic technology (limited in usefulness to lower-extremity wounds), electron spin resonance, fluorine-based MRI, injectable fluorescent dyes that respond to oxygen as does an optode, and near-infrared spectroscopy or other technology that allows monitoring of tissue oxygen "saturation." The technical aspects of oxygen monitoring with various methods are discussed in detail elsewhere in this volume. In this review, we focus largely on the methods that have led to major insights into wound healing and oxygen.

As outlined in the previous section, oxygen plays a central role in wound healing. It is required most importantly for bacterial killing by neutrophils, collagen production by fibroblasts, angiogenesis, and epithelialization. The role of oxygen was largely unexamined until the early 1960s. Hunt was a pioneer in this research and had the insight that defining the role of oxy-

gen in wound healing required accurate wound-tissue oxygen measurements (46).

Hunt used polarographic microelectrodes in his initial studies. Clark (12) and Fatt (21) were among those who improved oxygen-measurement technology to the point that it could be used for such studies. For example, they made a number of modifications to the polarographic (Clark) electrode that addressed critical issues such as the "stirring effect," poisoning of the electrode by proteins and other organic materials, and controlling the area of the probe exposed to the solution in which oxygen was being measured. A major advance was sealing the anode and cathode, along with a small volume of electrolyte solution, behind the same electrically nonconductive oxygen-permeable membrane.

Hunt identified the two major drawbacks to using oxygen microelectrodes in wound-healing research:

One of the barriers to progress in hyperbaric oxygen and its physiology has been the lack of a satisfactory method of determining tissue oxygen tension. Implantable electrodes have been valuable, but they have two major disadvantages: they cannot be calibrated while in tissue, and their relation to acute bleeding caused during insertion is unknown (46).

He also noted that, because of electrode instability, "There has been no way to accurately determine tissue oxygen tension in the same site repeatedly and for hours at a time under varying conditions" (44).

Initially, Hunt used two methods to measure wound oxygen tension, neither one of which was practical in humans, but both of which contributed to the development of the eventual human device. The first method used subcutaneously implanted Hunt-Schilling stainless steel wire-mesh cylinders (Fig. 2). Oxygen was measured in wound fluid aspirated into airtight syringes. Several drawbacks exist to this method: (a) it was easy to contaminate the sample with air bubbles, which rendered the results invalid; (b) it took several days for enough wound fluid to collect to allow sampling, although fluid could be added and allowed to equilibrate; (c) multiple sampling from a single cylinder was not possible, or was possible only on an infrequent (no more than daily) basis; and (d) the wound was a dead-space wound, and thus more hypoxic (pO_2 15–25 mm Hg) than most acute surgical wounds. Oxygen transport in a dead space also differs from that in perfused tissue. Nonetheless, much of value was determined by this technique, including the basic observation that wounds may be very hypoxic (48) and that increasing inspired oxygen tension increases wound oxygen tension and, with it, wound healing (45). The cylinders are still in use today for the study of hypoxic, dead-space wounds, with the refinement that most oxygen measurements are made with stable oxygen electrodes inserted into the wound cylinders for continuous measurement.

The second method was the true beginning of the subcutaneous tonometer used today. Hunt used an implantable tube (tonometer) that was oxygen permeable as a way to sample a larger area of tissue pO_2 than that available to a microelectrode. Wounds have a heterogeneous distribution of oxygen tension, and thus, microelectrodes require measurement at multiple sites with creation of a histogram to determine average tissue pO_2 . The original tonometer was a 100-cm coil of Teflon placed subcutaneously in an animal model. The tonometer was perfused with deoxygenated Ringer's lactate or saline, and pO_2 was mea-

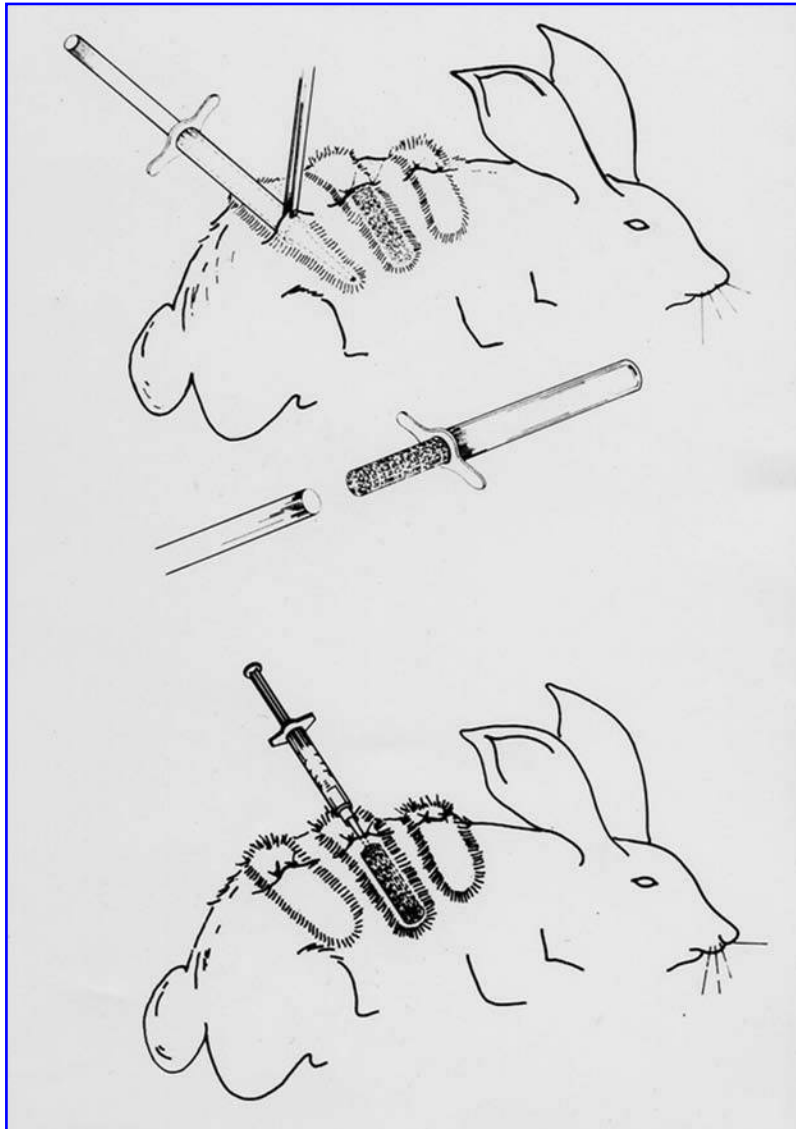


FIG. 2. Method for insertion of wire-mesh cylinders and sampling of fluid. Incisions are made in the dorsal skin. A rod is used to insert the cylinder, and the incision is closed. After a few days, a syringe is used to sample wound fluid percutaneously. Reprinted with permission from Forrester *et al.*, 1968 (24).

sured in the effluent (46). The flow rate for the device was 0.1–0.2 ml/min to allow adequate equilibration between the tubing and the surrounding tissue. The 100 cm of Teflon required for equilibration was bulky and unwieldy, and obviously not suitable for use in humans.

In 1972 (63), Niinikoski and Hunt replaced the Teflon tubing with silicone tubing (Silastic; Dow Corning, Midland, MI). Silicone is significantly more oxygen permeable, and thus, only 10 cm of subcutaneous tubing was required at perfusate flow rates of 0.1–0.2 ml/min. This was the major innovation that made measurement in humans possible (61), and the oxygen tonometer used today is made from silicone tubing. Subcutaneous tissue oxygen measurements in human volunteers were first reported in 1972 (61, 62). The first data in trauma patients were reported in 1973 (33, 73) and confirmed the vulnerability of wounds to hypoxia.

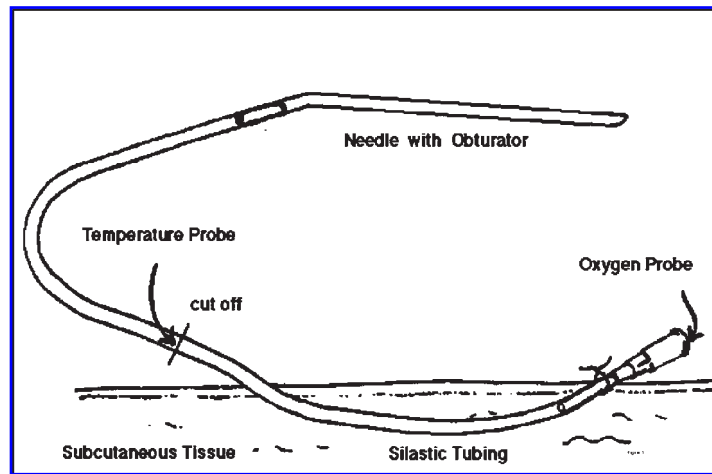
The use of the continuous-perfusion technique remained a limitation, however, that was addressed by placing an oxygen electrode directly in the tonometer. This was more efficient and

accurate, and allowed continuous recording (27) but required that the tonometer be modified slightly. A 20-gauge angiocatheter was added to create a Luer hub at one end where the electrode could be secured in place. A three-way stopcock assembly was also added to allow the tonometer to be flushed with saline to remove air bubbles (Fig. 3).

The importance of temperature measurement not just for determining pO_2 , but also as a reflection of perfusion and thus a data point of interest in itself, was increasingly recognized (26, 68). To allow continuous subcutaneous temperature monitoring, Hunt began to place a second tonometer 1 cm lateral to the first to house a temperature probe in the same tissue as the oxygen probe (68). Current probes incorporate thermistors within the probe, which allow both temperature correction and independent monitoring of subcutaneous temperature.

Currently, at least four companies market oxygen probes suitable for use in the tonometer. GMS (Licox pO_2 computer, Kiel, Germany), Integra Neurosciences (Plainsboro, NJ), which is licensed by GMS, and Diametrics (Paratrend 7) make polaro-

FIG. 3. Schematic drawing of the silicone subcutaneous oxygen tonometer. The oxygen probe is inserted through the catheter hub. The temperature probe is inserted into the protruding end of the tonometer. Reprinted with permission from Hopf *et al.*, 2003 (41).



graphic electrodes, whereas Oxford Optronix (Oxylite, Oxford, U.K.) uses optode technology. All provide stable, accurate measurements, either within the tonometer or when directly inserted into tissue.

Over the past 35 years, wound oximetry has allowed research into the role of oxygen in wounds. In turn, these measurements have led to clinical advances that have directly resulted in improved wound healing and resistance to infection in human patients. These advances are summarized in the next section.

WOUND PERFUSION AND OXYGENATION

Complications of wounds include failure to heal, infection, and excessive scarring or contracture. Rapid repair has the least potential for infection and excess scarring. The goals of healing, therefore, are to avoid contamination, ensure rapid tissue synthesis, and optimize the immune response. Unless adequate wound-tissue oxygen levels are attained, none of this can happen.

All surgical procedures lead to some degree of contamination that must be controlled by local host defenses. The initial hours after contamination represent a decisive period during which inadequate local defenses may allow an infection to become established.

It is well established that wounds on the extremities and trunk heal more slowly than those on the face. The major difference in these wounds is the degree of tissue perfusion and thus the wound-tissue oxygen tension. As a rule, repair proceeds most rapidly and immunity is strongest when wound oxygen levels are high, and this is achieved only by maintaining perfusion of injured tissue (28). Ischemic or hypoxic tissue, conversely, is easily infectable and heals poorly if at all. Wound oxygen delivery depends on vascular anatomy, the degree of vasoconstriction, and arterial pO_2 (Fig. 4). Wound-tissue oxygenation is complex and depends on the interaction of blood perfusion, arterial oxygen tension, hemoglobin (Hb) dissociation conditions, carrying capacity, mass transfer resistances, and local oxygen consumption.

The standard teaching that oxygen delivery depends more on hemoglobin-bound oxygen (oxygen content) than on arterial

pO_2 may be true of tissues with high metabolic rates and capillary density, like working muscle, but it is not true of wound healing. In subcutaneous tissue, intercapillary distances are large, and oxygen consumption is relatively low (19). In wounds, where the microvasculature is damaged, diffusion distances increase by an order of magnitude. Peripheral vasoconstriction further increases diffusion distance (75). The driving force of diffusion is partial pressure. Hence, a high pO_2 is needed to allow the oxygen to reach injured and healing tissues, particularly in subcutaneous tissue, fascia, tendon, and bone, the tissues most at risk for poor healing.

Although wounds consume oxygen at a relatively low rate [0.6–0.7 vol % (19)], the supply of oxygen at a high concentration to a wound is necessary for normal healing. The processes involved in wound healing have such a high rate constant for oxygen, they do not proceed at a sufficient rate when the pO_2 is below ~ 40 mm Hg. Inflammatory cells use little oxygen for respiration, producing energy largely *via* the hexose-monophosphate shunt (2). Most of the oxygen consumed in wounds is used for oxidant production (bacterial killing), with a significant contribution as well for collagen synthesis, angiogenesis, and epithelialization. The rate constants (K_m) for oxygen for these components of repair all fall within the range of 25–100 mm Hg (2, 13, 18, 49, 58, 60).

Because of the high rate constants for the oxygen substrate for the components of repair, the rate at which repair proceeds varies according to tissue pO_2 from zero to at least 250 mm Hg. Thus, bacterial killing, for example, can be increased well outside the physiologic range by increasing arterial oxygen tension. *In vitro* fibroblast replication is optimal at a pO_2 of ~ 40 –60 mm Hg. Neutrophils lose their ability to kill bacteria *in vitro* below a pO_2 of ~ 40 mm Hg (34, 54). These *in vitro* observations are clinically relevant. “Normal” subcutaneous pO_2 , measured in test wounds in uninjured, euthermic, euvolemic volunteers breathing room air, is 65 ± 7 mm Hg (51). Thus, any reduction in wound pO_2 may impair immunity and repair. In surgical patients, the rate of wound infections is inversely proportional (42), whereas collagen deposition is directly proportional (55), to postoperative subcutaneous wound-tissue oxygen tension.

High oxygen tensions (>100 mm Hg) can be reached in wounds, but only if perfusion is rapid and arterial pO_2 is high

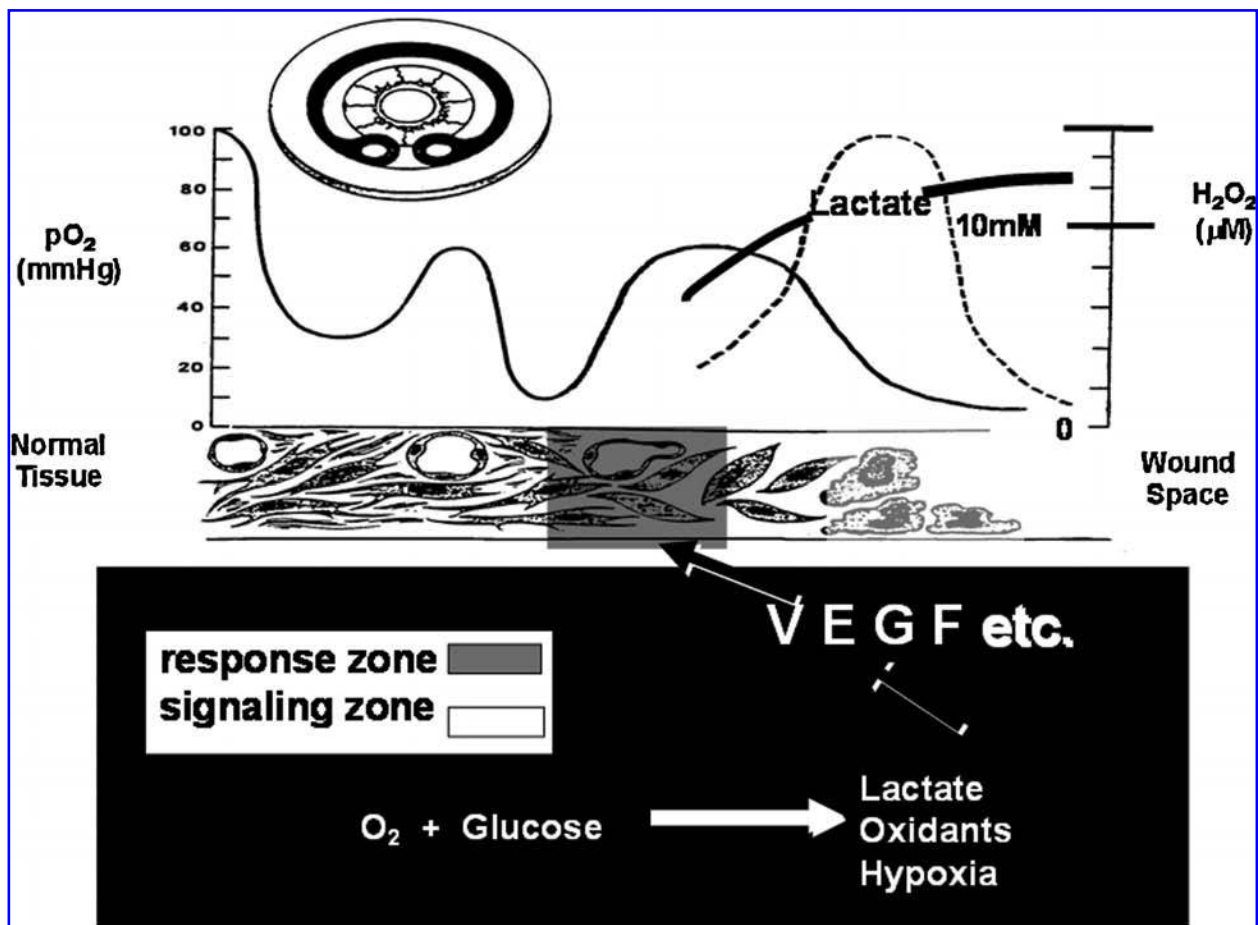


FIG. 4. The wound module. Cross section of the wound module in a rabbit ear chamber (diagram of chamber is in left upper corner of figure). Note that pO_2 , depicted graphically above the cross section, is highest next to the vessels, with a gradient down to zero at the wound edge. Note also the lactate gradient, high in the dead space and lower (but still above plasma) toward the vasculature. Hydrogen peroxide is present at fairly high concentrations and is also a major stimulus to wound repair (71). Modified version reprinted with permission from Silver, 1980 (74).

(28, 72). This is because (a) subcutaneous tissue serves a reservoir and a thermoregulatory function, so normally flow occurs in excess of nutritional needs; and (b) wound cells consume relatively little oxygen, ~ 0.7 ml/100 ml of blood flow at a normal perfusion rate (19, 36). At high levels of pO_2 , this small volume can be carried by plasma alone. Contrary to conventional wisdom, therefore, oxygen-carrying capacity (*i.e.*, hemoglobin concentration) is not particularly important to wound healing, provided that perfusion is normal (35). Wound pO_2 and collagen synthesis remain normal in individuals who have hematocrit levels as low as 15–18%, provided they can appropriately increase cardiac output and vasoconstriction is prevented (38, 52).

Peripheral vasoconstriction, which results from central sympathetic control of subcutaneous vascular tone, is probably the most frequent and clinically the most important impediment to wound oxygenation. Subcutaneous tissue is both a reservoir to maintain central volume and a major site of thermoregulation. Little local regulation of blood flow occurs, except by local heating (68, 72). Therefore, subcutaneous tissue is particularly vulnerable to vasoconstriction. Sympathetically induced peripheral vasoconstriction is stimulated by cold, pain, fear, and

blood-volume deficit (14, 30) and by various medications including smoking (nicotine) β -adrenergic antagonists, and α -1 agonists, all commonly present patients with wounds, whether surgical or traumatic. These factors are clearly the source of a great amount of impaired wound healing. Conversely, the importance of vasoconstriction leads to the ability to improve wound healing by modulating the sympathetic nervous system.

Prevention or correction of hypothermia (57) and blood-volume deficits (31) has been shown to decrease wound infections and increase collagen deposition in patients undergoing major abdominal surgery. Preoperative systemic (forced air warmer) or local (warming bandage) warming have also been shown to decrease wound infections, even in clean, low-risk surgeries such as breast surgery and inguinal hernia repair (59). Subcutaneous tissue oxygen tension is significantly higher in patients with good pain control than in those with poor pain control after arthroscopic knee surgery (1). Stress also causes wound hypoxia and significantly impairs wound healing and resistance to infection (43, 69). These effects are clearly mediated, in large part, by changes in the partial pressure of oxygen in the injured tissue resulting from vasoconstriction or the prevention of vasoconstriction.

Greif *et al.* (29) demonstrated in a randomized, controlled, double-blind trial that, in warm, well-hydrated patients with good pain control, that is, in well-perfused patients ($N = 500$) undergoing major colon surgery, administration of 80% versus 30% oxygen intraoperatively (via endotracheal tube) and for the first 2 postoperative hours (via non-rebreather face mask) significantly reduced the wound-infection rate by 50%. Belda *et al.* (7) recently replicated these results (significant 40% reduction in surgical-site infection) in a randomized, controlled, double-blind trial in 300 colon surgery patients randomized to 80% versus 30% oxygen intraoperatively and during the first 6 postoperative hours. Surgical and anesthetic management were standardized and intended to support optimal perfusion. A smaller ($N = 165$) randomized, controlled study by Pryor (67) demonstrated a doubling of surgical-site infection in patients undergoing major abdominal surgery randomized to 80% versus 35% oxygen intraoperatively. A number of methodologic flaws were found in the study, including lack of standardized patient management, particularly with respect to maintaining peripheral perfusion, and retrospective collection of wound-infection data from the surgical record rather than daily evaluation by a blinded researcher. In addition, the two groups of patients were not equivalent (more obesity, baseline pulmonary disease, blood loss, and postoperative need for mechanical ventilation in the 80% group), which likely explained the increase in infections seen in the 80% oxygen group. Thus, substantial evidence exists that use of 80% inspired oxygen intraoperatively and providing supplemental oxygen postoperatively in well-perfused patients will reduce the risk of wound infection.

Measurement and control of wound oxygen, supported by technological advances in tissue oximetry, have led to increased understanding of the fundamental physiology and pathophysiology of wound healing, as well as to major improvements in wound healing in patients with both surgical and traumatic wounds. Nonetheless, more remains to be done. Tissue oximetry remains a largely experimental method, despite the promise it holds for improved patient monitoring. Tissue oxygen monitoring (whether in subcutaneous tissue or in other tissues such as gut, brain, heart, or liver) has not become a standard monitor because (a) most techniques are invasive (at least minimally so) and thus less attractive to clinicians and patients; (b) the techniques are time and effort intensive and require excess troubleshooting; (c) interventions to increase tissue oxygen have only begun to be developed and evaluated; and (d) interventions to increase tissue oxygen/perfusion (warmth, volume, increased inspired oxygen) seem to be effective even if wound oxygen tension is not measured.

Wound oximetry would appear to have a use for monitoring patients at high risk for wound infections. Some might argue that just providing normothermia, fluids, pain control, and high inspired oxygen concentrations is sufficient, without the need to monitor actual wound oxygen tension. However, this approach does not eliminate all wound complications. Moreover, these interventions may have side effects (volume overload, excess sedation), and thus, tissue oximetry might help to guide management, particularly in high-risk patients. Finally, other potential means of increasing wound oxygen tension cannot be evaluated without a direct measure of wound pO_2 .

Tissue oxygen monitoring has shown some promise in critically ill patients, but again the techniques must be made less

complicated to perform and interpret, and means of addressing confounders must be developed. Measurement of small-bowel mucosal pO_2 seems particularly interesting, but current techniques only work well in small animal models.

The work discussed here has largely involved studies of acute wound healing, but it is clear that the principles demonstrated in acute surgical wounds apply to chronic wounds as well. For several reasons, the principles are not as well developed for chronic wounds. First, the tonometer is invasive, although minimally so, and is thus not ideal for use in lower-extremity, non-healing wounds. Second, acute wounds provide a model that can be standardized and where conditions can be carefully controlled, whereas the size, duration, location, and etiology of chronic wounds are far more difficult to control. Finally, wound infections occur, by definition, within 30 days of surgery, and thus, the primary outcome of acute wound studies can be measured in a short period. Chronic wounds, conversely, generally take at least several months to heal, and thus, data collection is much more protracted.

Despite these limitations, major advances have been made in studying wound oxygen in lower-extremity ulcers. These include identifying mechanisms for increasing oxygen in hypoxic wounds, such as hyperbaric oxygen (20), sympathetic blockade (37, 39), and increased fluid intake (78). This has largely been accomplished by using transcutaneous oximetry ($PtcO_2$). Transcutaneous oximetry certainly has limitations, including the need to warm a small area of skin to 42–44°C to allow oxygen to diffuse through the stratum corneum to the electrode and the inability to measure within the wound directly, but the information gained from these studies has changed the management of patients with lower-extremity ulcers.

A number of studies have demonstrated that periwound $PtcO_2$ below a cut-off of ~ 40 mm Hg is associated with impaired healing due to inadequate oxygen supply. Clearly, arterial insufficiency results in reduced oxygen supply and thus decreased $PtcO_2$. A large number of studies have demonstrated the value of $PtcO_2$ in predicting healing of an amputation site (3, 8, 10, 11, 16, 32, 50, 77, 81). The studies were performed in patients scheduled for amputation, and thus, presumably, options for revascularization were exhausted. These studies specifically assess the relation between $PtcO_2$ and healing when no effort is made to change $PtcO_2$ (e.g., revascularization). Revascularization would be expected to increase $PtcO_2$ and thus change the predicted outcome.

Hyperbaric oxygen (HBO) therapy is an effective means for increasing wound oxygen levels in hypoxic wounds, given perfusion adequate (although often impaired) to carry the oxygen there. It is often used in patients for whom revascularization has failed or whose wound fails to heal despite successful revascularization. Tissue oximetry has been a valuable tool in defining the role of HBO in patients with hypoxic wounds. Faglia *et al.* (20) demonstrated a significant reduction in overall and major (below-knee or above-knee) amputation rates in a randomized, controlled trial in 68 diabetic patients with ischemic lower-extremity ulcers treated with adjunctive HBO and standard care (8.6% versus standard care alone (33%; $p < 0.02$). As expected for ischemic wounds, the mean $PtcO_2$ in enrolled patients in both groups was hypoxic. In the HBO group, $PtcO_2$ increased by 14 mm Hg after HBO treatment, significantly greater than the control group ($p = 0.0002$). Thus, the study

demonstrated not only the benefit of HBO in ischemic ulcers, but also, by using tissue oximetry, a potential mechanism for the improvement seen in healing.

Although HBO was traditionally thought to exert its beneficial effects on healing only through correction of tissue hypoxia, its effects are now known to be more complex than that. HBO increases wound oxygen well above physiologic levels (77), and therefore, its effects result from a pharmacologic, rather than physiologic, dose of oxygen. HBO is known to (a) increase tissue oxygen in ischemic tissue (given some degree of arterial inflow) (23, 77); (b) increase angiogenesis in hypoxic or injured tissue, probably *via* oxidant-mediated mechanisms (39); (c) increase signal transduction (possibly *via* nitric oxide) and thereby growth factors and receptors (9, 79); and (d) decrease inappropriate inflammation by inhibitory effects on neutrophil adhesion (80).

FUTURE DIRECTIONS

Over the past 40 years, huge advances have been made in the technology available for monitoring oxygen in the wound. These advances have led to the ability to perform the research to define the role of oxygen in wound healing. These, in turn, have led to major improvements in patient care and wound outcomes. Nonetheless, substantial work remains to be done, both in understanding oxygen delivery in wounds and in advancing management of wounds. Much of this work will require further advances in technology. The ideal wound oximeter would have the following attributes:

- Noninvasive
- Accurate and precise
- Simple to use
- Single point calibration in room air
- Stable for ≥ 24 h *in vivo*
- Can be calibrated *in vivo*
- Oxygen and temperature can be measured simultaneously in the same site
- Accuracy in the range from 0 to >300 mm Hg
- Motion, pH, and CO_2 do not induce artifacts
- Continuous, long-term measurement possible
- Repeatable
- Produces number that is relevant, absolute, and easy to interpret
- No pitfalls or troubleshooting

SUMMARY

Tissue oximetry has played an instrumental role in research into the fundamental mechanisms of wound healing. This research has demonstrated that

- Oxygen is required for wound healing
- Wound hypoxia is common and impairs wound healing and resistance to infection.
- Wound hypoxia can be corrected, and doing so improves wound healing and patient outcomes

The development of improved and novel methods of measuring oxygen will enable us to improve our understanding of oxygen delivery and metabolism in the wound, as well as to improve monitoring and care in patients with wounds.

ABBREVIATIONS

FGF, fibroblast growth factor; H_2O_2 , hydrogen peroxide; Hb, hemoglobin; HBO, hyperbaric oxygen; IGF-1, insulin-like growth factor 1; IL-1 and -2, interleukins 1 and 2; NADPH, nicotinamide adenine dinucleotide phosphate; PDGF, platelet-derived growth factor; Ptc O_2 , transcutaneous oximetry; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

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