The metabolic control of collagen deposition and angiogenesis by lactate presents a unifying basis for wound repair. It is remarkable that both forms of ADP-ribosylation reactions that potentially regulate nuclear and posttranslational events of collagen and VEGF production are equally affected by high lactate.

Initiation of collagen synthesis and angiogenesis in wounds can be described as a response to a metabolic demand precipitated in an environment that has little oxygen and/or a high level of lactate. These conditions limit the supply of NAD^+ and, therefore, ADPR and pADPR. In response to this deficit, fibroblasts (and endothelial cells) synthesize and secrete collagen, and macrophages (and endothelial cells) elicit VEGF and keep it in an active form, which stimulates new vessel growth. However, endothelial cells do not respond well to VEGF in hypoxia, despite upregulation of VEGF receptors.[22] Enhanced perfusion caused by new vessel growth subsequently reestablishes normoxia. Endothelial cells now respond actively to VEGF for blood vessel formation. Finally, the endothelial cell response terminates as the macrophage-derived angiogenic signals diminish.

Acknowledgment

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[37] Protocols for Topical and Systemic Oxygen Treatments in Wound Healing

By GAYLE M. GORDILLO, RICHARD SCHLANGER, WILLIAM A. WALLACE, VALERIE BERGDALL, ROBERT BARTLETT, and CHANDAN K. SEN

Wound hypoxia, resulting from disruption of the local vasculature, is a key limiting factor in healing.[1–3] The core of the wound is most hypoxic with a progressive increase in the oxygen gradient toward the uninjured tissue at the periphery. The pO_2 of dermal wounds ranges from 0–10 mm Hg centrally to 60 mm Hg at the periphery.[3–5] This is extremely important

[1] G. M. Gordillo and C. K. Sen, *Am. J. Surg.* **186,** 259–263 (2003).
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[3] T. Hunt, P. Twomey, B. Zederfeldt, and J. Dunphy, *Am. J. Surg.* **114,** 302 (1967).
[4] J. Remensnyder and G. Majno, *Am. J. Pathol.* **52,** 301 (1968).
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when evaluating wounds that will not heal because oxygen plays a critical role in many aspects of the wound healing response. Recent findings support that molecular oxygen as well as its reactive derivatives may support wound healing.[6,7] Oxidants generated at the wound site serve the role of cellular messengers that orchestrate the healing process.[6–12]

Clinical use of O_2 to promote wound healing began in the 1960s with the administration of systemic hyperbaric O_2 to treat wounds. While the conditions (e.g., pressure, O_2 concentration, frequency, and duration of administration) for systemic hyperbaric O_2 therapy (HBOT) have not been optimized on the basis of randomized clinical trials, HBOT is an FDA-approved therapeutic modality used in wound clinics with an encouraging success rate. Reliance on empiricism and a paucity of data that meets the highest criteria for evidence-based medicine have hindered the general acceptance of O_2 therapy as a standard modality in wound care. Embracing the concept of O_2 therapy depends not only on a favorable clinical outcome, but also on detailed mechanistic insight that explains those outcome results. The use of systemic hyperbaric O_2 therapy presents potential advantages as well as widely recognized risks of oxygen toxicity.[13–16] There is evidence to suspect that the use of pressure and systemic pure O_2 may not be essential in wound care. Elimination of these factors by using subpure systemic O_2 under normobaric conditions may significantly minimize the risk of O_2 toxicity. Furthermore, opportunities to treat dermal wounds using topical O_2 therapy warrant further investigation.[17] Given that many growth factors require ROS for their function, it is reasonable to assume that approaches to correct wound pO_2 will serve as an effective adjunct to treat chronic wounds.[8] This article describes some of the currently used protocols for oxygen therapy of wounds.

[6] C. K. Sen, S. Khanna, B. Babior, T. Hunt, E. Ellison, and S. Roy, *J. Biol. Chem*. **277,** 33284 (2002).
[7] C. K. Sen, S. Khanna, G. Gordillo, D. Bagchi, M. Bagchi, and S. Roy, *Ann. N. Y. Acad. Sci*. **957,** 239 (2002).
[8] C. K. Sen, *Wound Repair Regen*. **11,** 431–438 (2003).
[9] C. K. Sen and L. Packer, *FASEB J*. **10,** 709 (1996).
[10] C. K. Sen and L. Packer, *Methods Enzymol*. **352,** 580 (2002).
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[17] L. Kalliainen, G. Gordillo, R. Schlanger, and C. Sen, *Pathophysiology* **9,** 81 (2003).

Measuring Responses to Oxygen Interventions

It is important to recognize that simply administering supplemental oxygen does not in itself guarantee increased wound oxygenation. The availability of oxygen to wound tissues depends on vascular supply, vasomotor tone, arterial pO_2, and the diffusion distance for molecular O_2. Edema and necrotic debris both increase the diffusion distance for O_2 to reach the wound. Thus, debridement is an important step to diminish obstruction to wound oxygenation. Peripheral vasoconstriction can also significantly limit wound perfusion and oxygenation so that little to no enhancement of wound pO_2 levels are achieved despite breathing supplemental oxygen.[2,18,19] Therefore, for optimal wound perfusion and oxygenation, patients must be warm, have adequate intravascular volume, and have adequate control of pain and anxiety.

Currently, the only clinically feasible method for measuring tissue oxygenation is transcutaneous oxygen measurements (TcOM). This technique entails placement of an electrode on the surface of the skin. The electrode (modified Clark polargraphic electrode) heats the underlying skin to 42–44° to dilate the dermal vascular bed and obtains a measurement of pO_2 in mm Hg. A minimum of three electrodes are used with one placed in the second intercostal space as a reference lead, one electrode placed at the proximal border of the wound, and one electrode placed at the distal border of the wound. The TcOM at the periphery of a wound indicates the relative blood flow/vascularity at the periphery of the wound site and hence the capacity for O_2 delivery to the wound. At present, it is the only quantitative and noninvasive diagnostic procedure available to assess wounds. TcOMs are measured at room air and in response to an oxygen supplement. If the wound is on an extremity, it is tested in a dependent and an elevated position to determine if there is vascular stenosis limiting blood flow to the wound.

The current protocol for TcOM measurements is based on the guidelines established by the Undersea and Hyperbaric Medicine Society (UHMS). The protocol is as follows.

1. Calibrate electrodes as per manufacturer's instructions (Radiometer America, Westlake, OH).
2. Place patient in a semireclining position with room temperature 24° (75.2°F) and breathing room air.
3. Select a reference site.
   a. Usually anterior chest wall second intercostal space.

[18] F. Gottrup, R. Firmin, J. Rabkin, B. Halliday, and T. Hunt, *Crit. Care Med.* **15,** 1030 (1987).
[19] H. Hopf, J. West, and T. Hunt, *Wound Repair Regen.* **4,** A129 (1996).

b. If necessary shave site, debride stratum corneum with adhesive tape, and wipe down with alcohol.
c. Place electrode fixator ring by pressing down firmly to make sure there are no gaps or debris between ring and skin surface.
d. Fill ring with 2–3 drops of couplant solution (Contact Liquid, Radiometer America).

4. Select two sites around wound—one proximal to wound edge and one distal to the wound edge, if possible—and prepare and apply electrode fixator rings as described in steps 3b–3d.

5. Snap electrodes into fixator rings and obtain readings. This usually takes 10–15 min to allow sufficient time for skin to be heated.

6. If the wound is on an extremity, repeat TcOM with extremity elevated for 5 min prior to taking the measurements.

7. Repeat with patient breathing 100% O_2 using a nonrebreathing mask and obtain measurements at 2, 5, and 10 min.

8. Patients receiving hyperbaric oxygen therapy (HBOT) have in-chamber TcOM evaluation performed with their first treatment to document a positive response to supplemental oxygen therapy.

Interpretation of TcOM results and prognosis for healing can be assessed according to guidelines presented in Table I. When performing TcOM evaluation or troubleshooting the interpretation, keep in mind the following conditions that can affect TcOM measurements.

Systemic

a. Comorbid conditions in the patient that affect their ability to oxygenate blood or the ability of their hemoglobin to bind oxygen.
b. Conditions that impair oxygen delivery, for example, limitations in cardiac output or vascular stenosis limiting blood flow.
c. Infection.

Local

a. Increased skin thickness.
b. Obesity.
c. Edema.
d. Cellulite.

Mechanical

a. Pressure on the coupling ring will diminish measurements; do not attempt to fix the coupling rings in place with adhesive tape.

b. Reference lead reads ≤50 mm Hg; the reference lead should always be >50 mm Hg and, if not, the electrodes should be recalibrated.

Caution: TcOM does not measure oxygenation of the wound itself.[1] It indicates the oxygenation status of the intact skin along the periphery of the wounds. Ideally, one would like to get a three-dimensional spatial imaging of the actual wound. Such an approach would identify pockets of hypoxia and provide information regarding compartments of O_2 tension at the actual wound site. We are currently exploring options to noninvasively image O_2 and the redox status of the wound employing electron paramagnetic resonance (EPR) imaging techniques.[20–25]

TABLE I
INTERPRETATION OF TRANSCUTANEOUS OXYGEN MEASUREMENT RESULTS

Oxygenation status	Room air (mm Hg)	100% O_2 at 1 ATA[a] (mm Hg)	100% O_2 at pressure (mm Hg)
Normally perfused skin	50–90	>300	1000–1500
Wound—marginal hypoxia	30–39	65–75 approximately 75% should heal	
Wound—moderate hypoxia	20–29	35–74 approximately 50% heal	
Wound—severe hypoxia	0–19	<25% needs healing	≥200—consider trial HBO <200—dismal prognosis not HBOT candidate

[a] Absolute atmospheres of pressure.

[20] S. J. Ellis, M. Velayutham, S. S. Velan, E. F. Petersen, J. L. Zweier, P. Kuppusamy, and R. G. Spencer, *Magn. Reson. Med*. **46,** 819 (2001).
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[23] G. Ilangovan, H. Li, J. L. Zweier, M. C. Krishna, J. B. Mitchell, and P. Kuppusamy, *Magn. Reson. Med*. **48,** 723 (2002).
[24] P. Kuppusamy, H. Li, G. Ilangovan, A. J. Cardounel, J. L. Zweier, K. Yamada, M. C. Krishna, and J. B. Mitchell, *Cancer Res*. **62,** 307 (2002).
[25] S. S. Velan, R. G. Spencer, J. L. Zweier, and P. Kuppusamy, *Magn. Reson. Med*. **43,** 804 (2000).

Oxygen Therapy for Wound Infection Prophylaxis

Normobaric systemic oxygen therapy can be administered in the perioperative period to decrease the incidence of surgical wound infection. This has been demonstrated in a double-blinded randomized controlled trial involving 500 patients undergoing colorectal surgery.[26] In this study, patients were randomized to treatment arms consisting of either 30% oxygen + 70% nitrogen ($n = 250$) or 80% oxygen + 20% nitrogen ($n = 250$) administered with the following protocol.

1. Assigned concentrations were given at the start of anesthesia induction until immediately before extubation.
2. Patients were given 100% O_2 from the time of extubation until the anesthesiologist deemed it safe to resume administration of O_2 at the specified concentrations.
3. For the first 2 h of recovery, O_2 was administered to patients at the specified concentrations *via* a nonrebreathing mask.
4. Oxygen levels were monitored by continuous pulse oximetry and arterial blood gas at 1 and 2 h after recovery from anesthesia.
5. Patients were hydrated aggressively during and after surgery: crystalloid basal infusion rate of 15 ml/kg/h during surgery, blood replaced with crystalloid at a 4:1 ratio or with colloid at a 2:1 ratio, and crystalloids administered at 3.5 ml/kg/h for the first 24 h after surgery and at 2 ml/kg/h for the subsequent 24 h.
6. After 48 h, patients in both groups breathed ambient air or received supplemental oxygen as needed to maintain an oxyhemoglobin saturation >92%.

This protocol is significant because it demonstrates the efficacy of O_2 therapy in preventing a specific wound healing complication, that is, infection. This is also one of the few O_2 treatment protocols that has been validated by a double-blinded, randomized control trial. It also adds a preventive dimension to the concept of O_2 therapy in addition to its therapeutic uses for refractory wounds. The use of aggressive hydration is a key component of this protocol. The ability to prevent infection with oxygen administration is contingent upon optimal perfusion and oxygention, which fail to occur in postoperative patients that are underresuscitated.[2]

Systemic Oxygen Therapy

HBOT has been used since the 1960s to treat refractory wounds and acute conditions related to pressure (e.g., the bends/air or gas embolism)

[26] R. Grief, O. Akca, E.-P. Horn, A. Kurz, and D. Sessler, *N. Engl. J. Med.* **342,** 161 (2000).

and oxygenation (e.g., carbon monoxide poisoning). This modality entails administration of 100% oxygen usually at a pressure of 2–3 atmospheres and is the most commonly used method for the clinical application of oxygen therapy. Patients receive HBOT in either a multiplace or a monoplace chamber. In the multiplace chamber, the patient sits inside a large room and is administered pressurized oxygen through a face mask. In a multiplace chamber, the amount of inhaled oxygen delivered to the wound is contingent upon the vascular supply to the wounded area. This is a critical concept to appreciate in designing studies and interpreting the literature. In this case, the only way that oxygen is delivered to the wound is *via* saturated hemoglobin and dissolved oxygen in the blood. However, the vast majority of patients receiving HBOT are treated in monoplace chambers. Note that under such circumstances, the wound receives oxygen through the systemic as well as topical routes. The inhaled oxygen is carried by the patients' vasculature to the wound and pressurized oxygen comes into direct contact with the surface of the wound. Thus, HBOT protocols using a monoplace chamber incorporate several variables: pressure, systemic oxygen effects, and topical oxygen effects, all of which may influence wound responses.

Selecting patients for HBOT is based on several criteria. The only absolute contraindication to HBOT is untreated pneumothorax. TcOM evaluation must be obtained when initiating HBOT to make sure the patient has the capacity to respond to oxygen therapy. Federal guidelines from the Health Care Finance Administration (HCFA) indicate the approved conditions for HBOT treatment. In addition, the UHMS has a list of approved conditions and developed the standard protocols for HBOT administration. The indications for HBOT and the treatment protocols that are used are those recommended by UHMS and are summarized in Table II. A physician trained in HBOT must supervise these treatments. Obvious complications that can occur during HBOT administration include the following.

1. Middle ear barotrauma. Perforated tympanic membrane more likely with ventilator-dependent patients and young children.
2. Seizures. HBOT lowers the seizure threshold and blood glucose. Diabetic patients should have blood glucose >200 prior to entering the chamber. Patients on seizure medication must have serum drug levels within the therapeutic range.
3. Pulmonary complications. Untreated or occult pneumothorax can be converted to a tension pneumothorax. Apnea/loss of respiratory drive will occur in patients with significant chronic obstructive pulmonary disease defined as room air $pCO2 \geq 55$ on arterial blood gas. Patients with heart failure, defined as an ejection fraction <30%, can develop pulmonary edema.

TABLE II
PROTOCOLS FOR HBOT ADMINISTRATION[a]

Indication	Pressure (ATA)	Duration	# Treatments (tx)	Reevaluate	Comment
Air or gas embolism	2.8	Symptom specific	1–14	After 10–14 tx	Follow US Navy tx tables 6 and 6A
Carbon monoxide (CO)	2.4–3.0	90–120 min	1–10	After 5 tx	qid–bid based on neuro function
CO/cyanide complications	2.5–3.0	90 min	1–10	After 5 tx	Same as CO
Gas gangrene	3.0	90 min	5–10	After 10 tx	tid day 1, bid day 2, then qd
Crush injury	2.0–2.5	90 min	3–12	After 6 tx	tid × 2 days, bid × 2 days, qd × 2 days
Decompression illness	2.8	Symptom specific	1–14	No further response	Follow U.S. Navy tx tables 6 or 7
Select nonhealing wounds	2.0–2.5	90–120 min	10–40	After 30 tx	qd and/or bid
Exceptional blood loss[b]	2.0–3.0	90–120 min	Not specific	Hematocrit = 22.9%	
Necrotizing soft tissue infection	2.0–2.5	90–120 min	5–30	>30 tx	bid initially then qd
Chronic osteomyelitis	2.0–2.5	90–120 min	20–40	After 40 tx	qd and/or bid
Radiation tissue damage	2.0–2.5	90–120 min	20–60	After 60 tx	qd and/or bid
Compromised graft or flap	2.0–2.5	90–120 min	6–40	After 20 tx	bid initially then qd
Thermal burn[b]	2.0–2.4	90 min adult 45 min peds	5–45	No specified limit	tid first 24 h then bid
Intracranial abscess	2.0–2.5	60–90 min	5–20	No specified limit	bid initially then qd
Diabetic lower extremity wound	2.0	90 min	30–60	After 30 tx	qd

[a] tx, treatment; qd, once a day; bid, twice a day; tid, three times a day; qid, four times a day.
[b] Not HCFA/CMS-approved condition.

Additional factors must be taken into consideration before any patient can reap the benefits of HBOT. They must be able to (i) fit into the chamber (obesity is an issue), (ii) come to the HBOT facility to receive daily treatments, and (iii) tolerate the treatments. Claustrophobic patients may become uncomfortable or anxious when spending 2 h at pressure in the monoplace chambers.

Topical Oxygen Therapy

The use of topical oxygen to treat refractory wounds was first described in 1969.[27] Widespread application of this modality has not been feasible until recently when commercial products became available that were designed specifically for this purpose.[17] The concept of topical oxygen therapy is appealing because it can deliver oxygen directly to a wound site without the risks and potentially at a significantly less cost than HBOT. Because a physician does not need to be present and special chambers are not required, topical oxygen therapy can be administered practically anywhere and most patients are treated in their own homes. Given the current geopolitical conditions, it also could be applied under conditions of field combat use.

While the concept of topical oxygen therapy is intriguing, there are little data to support the case for its clinical efficacy. We performed a retrospective analysis of our results for patients treated during the first 9 months with this modality. There were no specific inclusion or exclusion criteria for this study. There were no standardized wound care regimens, but all patients received topical oxygen treatments using the following protocol.

1. Remove dressing from wound and apply topical oxygen device (GWR Medical, Chadds Ford, PA). These are single-use disposable devices that come as either sacral bags (like a large colostomy bag) or boots. They have an adhesive strip for fixation of the device to the patient. Wounds should be debrided/free of necrotic debris. Do not use petroleum-based dressings as any residual will prevent oxygen penetration into the wound.
2. Connect device to oxygen gas cylinder.
3. Initiate oxygen flow at 7–8 liters per minute. The bag should be fully insufflated without any wrinkles. Each device has a release valve to prevent excessive pressure buildup within the bag.
4. Treatments last 90 min and are administered for 4 consecutive days followed by 3 days without treatment.

[27] B. Fischer, *Lancet* **2,** 405 (1969).

Using this protocol in 32 patients with 58 wounds, complete healing was achieved in 42/58 wounds. If the two patients with two wounds who were lost to follow-up are not included, then the overall healing rate for our series was 75%.[17] We were pleased with these results when comparing them to overall results at our wound care center, which reports successful healing in 79% of its patients subjected to specialized wound care. We did not have any complications related to the use of this modality, and all patients with smaller wound dimensions responded positively over time, even if they did not heal completely.

Patient selection criteria are far les rigorous for topical oxygen than for HBO. Contraindications for using the topical oxygen devices include (i) fistulous tract that the end cannot be contacted with a probe and (ii) patient refuses to refrain from smoking while administering the oxygen treatment. There are no known risks for this method of oxygen therapy and the duration of treatment is determined by the prescribing physician. We have not observed any complications related to the use of this modality at our institution. Favorable outcomes in the clinical setting implied that the benefits of oxygen therapy could be achieved with this method of oxygen therapy. There was enough merit in these findings to commit the resources to pursue more mechanistic studies of topical oxygen.

An experimental pig model has been developed for this purpose (Fig. 1). The protocol for implementation is as follows.

1. Premedicate a female Yorkshire pig (80–100 pound) with an intramuscular injection of Telazol (500 mg) and acepromazine (5 mg).
2. Place pig in prone position on operating table and administer general anesthesia via a nose cone using isofluorane and a standard respirator (Harvard Apparatus, NP 72-3001) for the duration of the surgery.
3. Shave the back and sterilely prep and drape the area.
4. Create 2.5 $\times$ 2.5 cm full-thickness skin wound defects using a scalpel. Place four wounds on the back in the thoracic area and four wounds in the lumbar area. Arrange the wounds in a 2 $\times$ 2 pattern separated by 2.5 cm. Hemostasis is achieved by packing the wounds with gaze until bleeding stops.
5. Treat four wounds in one anatomic location with topical oxygen; the other four wounds serve as untreated controls.
6. Attach the topical oxygen device to the pig and cover the untreated wounds with an occlusive dressing (e.g., Op-site, Tegaderm) and Elasticon tape. Transfer the pig back into its cage.
7. Attach the topical oxygen device to the oxygen cylinder and set the flow at 2 liters/minute. Administer topical oxygen is administered every

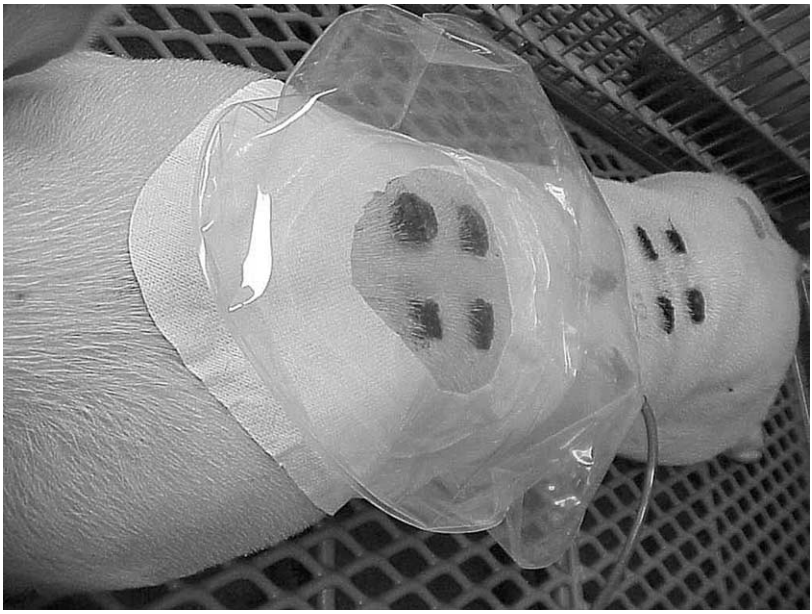

FIG. 1. Pig under general anesthesia receiving topical oxygen treatment. Full-thickness skin wounds are created on the dorsum. Half of the wounds are treated with topical oxygen using the sacral bag and half the wounds are untreated and remain outside the device.

other day for 3 h starting the day of wounding. At the end of treatments the wounds are dressed in the same manner as the control wounds.

This protocol deviates from the human application because we could not place the pig under general anesthesia to receive the topical oxygen treatments as frequently as humans receive treatment. However, it is important to note that none of the protocols used for topical oxygen or HBOT have ever been optimized by a randomized clinical trial to determine optimal concentration, pressure, frequency, or duration of oxygen exposure.

Acknowledgment

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