World J. Surg. 28, 294–300, 2004
DOI: 10.1007/s00268-003-7400-2

WORLD
Journal of
SURGERY


# Oxygen in Wound Healing—More than a Nutrient

Andrea A. Tandara, M.D., Thomas A. Mustoe, M.D.

Wound Healing Research Laboratory, Northwestern University, 303 East Chicago Avenue, Tarry Bldg 4-720, Chicago, Illinois 60611, USA

Published Online: February 17, 2004

**Abstract. This article provides an overview of the role of oxygen in wound healing. The understanding of this role has undergone a major evolution from its long-recognized importance as an essential factor for oxidative metabolism, to its recognition as an important cell signal interacting with growth factors and other signals to regulate signal transduction pathways. Our laboratory has been engaged in thestudy of animal models of skin ischemia to explore in vivo the impact of hypoxia as well as the use of oxygen as a therapeutic agent either alone or in combination with other agents such as growth factors. We have demonstrated a synergistic effect of systemic hyperbaric oxygen and growth factors that has been substantiated by Hunt's group. Within the past 10 years research in the field of wound healing has given new insight into the mechanism of action of hypoxia and hyperoxia as modifiers of the normal time-course of wound healing. The article concludes with a discussion of why hypoxia and hyperoxia intercurrently play an important role in wound healing. Hypoxia-inducible factor 1 is crucial in that interplay.**

Oxygen is an essential nutrient for cell metabolism, especially energy production. During wound healing the presence of oxygen takes on additional importance because of the increased demand of reparative processes like cell proliferation and synthesis of collagen [1]. In addition, superoxide generation by polymorphonuclear leukocytes, which is essential for bacterial killing, is critically dependent on oxygen levels. Many clinical observations, strongly supported by experimental evidence in animals, have led to the conclusion that wound healing is delayed under hypoxia. Tissue oxygen tensions from 5 to 20 mmHg were measured transcutanously in nonhealing chronic wounds compared to control tissue values of 30–50 mmHg [2]. Under controlled experimental conditions in vivo, we were able to show in young and aged ischemic rabbit ear ulcers that wound healing is delayed under hypoxia as a result of reduced granulation tissue production and delayed epithelialization [3, 4]. Ischemic ears in this rabbit ear model have tissue oxygen tensions of approximately 24 mmHg versus the level of 53 mmHg measured in non-ischemic ears [5].

*Correspondence to:* Thomas A. Mustoe, M.D., Plastic Surgery, Department of Surgery, Feinberg School of Medicine, Northwestern University, Galter 19-250, 675 North St. Clair Street, Chicago, Illinois 60611-5964, USA

## Role of Oxygen in Wound Healing: Nutrient

### *Role of Oxygen on Subcellular (Enzymatic) Level*

Intracellular processes like biosynthesis, movement, and transport need energy to be functional. This energy is supplied by the coenzyme adenosine-tri-phosphate (ATP). ATP is the most important depot for chemical energy on molecular/enzymatic level and is synthesized in mitochondria by oxidative phosphorylation. This oxidative chain reaction is obligatorily oxygen dependent; it cannot take place without oxygen. Aerobic glycolysis, $\beta$-oxidation of fatty acids, and the citric acid cycle are tightly attached to the energy acquisition by oxidative phosphorylation and are therefore also oxygen dependent. In the case of injury, two additional oxygen-dependent enzymes join this group of crucial subcellular processes during wound healing:

NADPH-linked oxygenase is the responsible enzyme for the respiratory burst that occurs in leukocytes. During the inflammatory phase of wound healing NADPH-linked oxygenase produces high amounts of oxidants by consuming high amounts of oxygen. To work at 50% of its maximum enzymatic speed, the enzyme requires oxygen tensions between 40 and 80 mmHg, and to work at 90%, even tensions above 400 mmHg may be required [6]. Successful wound healing can only take place in the presence of this enzyme, because oxidants are required for prevention of wound infection.

Molecular oxygen is also essential during collagen synthesis [1]. Hydroxylation of proline and lysine in procollagen is a crucial step in collagen maturation. Procollagen molecules cannot form stable triple-helices without hydroxyproline. Hydroxylation requires high amounts of oxygen. The responsible enzyme prolyl hydroxylase needs 20 mmHg to display 50% of its maximum speed and more than 150 mmHg to display 90% of its maximum speed [7].

### *Role of Oxygen on Cellular Level*

Oxygen consumption by cells, a result of the above-mentioned enzymatic activities, is detectable in vitro by measuring changes of oxygen tension in the conditioned media. The levels of oxygen at a monolayer fibroblast cell surface are highly dependent on and inversely correlated with cellular density. By blocking cellular utilization of oxygen with rotenone, the drop in oxygen tension observed

in high-density cultures failed to occur. Indirect correlation between oxygen tension and cellular density is measurable at both high (20% $O_2$ or atmospheric) and low (2% $O_2$) oxygen environments [8].

Fibroblasts need oxygen for their proliferative activity [9]. In vitro it has been shown that human dermal fibroblasts decrease their proliferative activity under chronic hypoxia (six passages at 1% $O_2$) [10]. In vivo oxygen tissue tensions of nearly 5–15 mmHg were measured at the threshold between the epicenter of the wound and granulation tissue where leukocytes (mainly macrophages) were most commonly seen. Areas with active proliferating fibroblasts were only seen at oxygen tensions of more than 15 mmHg [9].

Human dermal fibroblasts also decrease their collagen synthesis rate under chronic hypoxia in vitro [10]. The results from cell cultures matched observations made in vivo. In a clinical study collagen deposition was determined in patient wounds. The amount of deposited collagen was directly proportional to the measured tissue oxygen tensions [11].

As mentioned, oxygen is also crucial for the inflammatory phase of wound healing: The bactericidal activity of leukocytes—mainly granulocytes—depends on high amounts of oxidants (see previous paragraph). The rate of production of toxic radicals—and hence the adequacy of oxidative killing—is directly proportional to local oxygen tensions [12].

### *Role of Oxygen on Supracellular Level*

The decline in oxygen tension in skin after wounding is caused not only by interruption of blood flow but also, in large part, by consumption of oxygen by cells that are metabolically more active and in a proliferative state.

There is a steep gradient between capillary and reparative cells in the wound: distances up to 150 $\mu$m may have to be bridged. Within 70 $\mu$m (corresponding to the distance between capillary and outer edge of the dividing fibroblast layer) a major fall in oxygen tension occurs. Oxygen delivery from the capillary to the wound site depends only on diffusion, and therefore on arterial oxygen tension and perfusion [2].

If infection is present, a further decrease in oxygen tension can be observed. This in turn leads to delayed wound healing and reduced wound tensile strength as collagen synthesis is decreased [13]. The decreased oxygen tension results in a lower level of leukocyte killing, which results in higher bacterial levels. Therefore, as reported elsewhere, to prevent wound infection, it is necessary to maintain sufficient tissue oxygenation for a powerful respiratory burst [14–16].

## Impact of Hypoxia on Wound Healing

Shortly after acute tissue injury, the microenvironment of the wound is virtually devoid of oxygen. This is a result not only of the vascular disruption caused by the injury but also of the high oxygen consumption caused by high cell density and cell activity in granulation tissue. In the first days after wounding, granulation tissue consists primarily of leukocytes, which are later replaced by fibroblasts [9, 17].

Despite the hypoxic environment early in the course of wound healing, endothelial cells and fibroblasts still function in terms of migration, protein synthesis, and proliferation. In the course of wound healing (within days) oxygen tensions normalize stepwise through revascularization that takes place in the newly formed granulation tissue that supplants the injured tissue. Thus, it is reasonable to hypothesize that low oxygen tensions could play a role as an early stimulus for tissue repair/angiogenesis. As a result of this observation, there is new interest in the impact of low oxygen tension on growth factor synthesis and gene activation in general.

### *Acute Hypoxia: Stimulus*

Proliferative activity of human dermal fibroblasts in vitro is enhanced under acute hypoxia. After 72 hours exposure to 1% oxygen, the proliferative rate of fibroblasts increases by 71% [10]. Low oxygen tensions have been shown to initiate clonal expansion of fibroblasts [18], a finding that would be surprising if only the previously made statements in this review were taken into account.

### *Hypoxia and TGF-β-1*

Human dermal fibroblasts in vitro secrete up to ninefold more transforming growth factor-beta 1 (TGF-$\beta$1) after being exposed for 72 hours to low oxygen tension (1% to 2% $O_2$). This increase seems to be regulated on a transcriptional level, as TGF-$\beta$1 mRNA levels are as much as eight times higher in hypoxic cells as in their normoxic controls (15% $O_2$) [10, 19]. Low oxygen tension is also a powerful stimulus for the synthesis and transcription of the $\alpha$1(I) procollagen gene [10, 20]. Recently it has been shown that this collagen transcription activation is due to direct activation by TGF-$\beta$1 [21].

The situation seems to be more complex in vivo. If in addition to the normal hypoxic stimulus of wounding (with blood vessel interruption), a baseline ischemia is induced, the overall result is not stimulation of healing, but rather a major delay in wound healing. Rabbit ear ulcers were made additionally ischemic by dividing two of the three arteries in one of the ears ("ischemic rabbit ear ulcers"). These ischemic ulcers demonstrated a continuous increase of TGF-$\beta$1 mRNA up to 12 days after wounding versus the nonischemic control ear of the same animal. In the control ear TGF-$\beta$1 mRNA decreased already after day 7. This delayed decrease of-TGF-$\beta$1 mRNA was consistent with the delayed wound healing in ischemic animals. (TGF-$\beta$1 is increased more in the early phases of wound healing, which are prolonged in ischemic ears). In contrast, rabbit ear ulcers in aged animals failed to show any significant increase in TGF-$\beta$1 mRNA under ischemic and nonischemic conditions until day 12, a finding consistent with the greater delay in wound healing in the aged animals [3]. Further research into the signaling pathway of TGF-$\beta$ showed that aged human dermal fibroblasts have altered TGF-$\beta$ signal transduction because of downregulated receptors. Both TGF-beta receptors (T$\beta$RI and II) were shown to be downregulated on the mRNA level as early as after 20 hours of 1%$O_2$, whereas young cells showed unchanged expression of both receptors. After TGF-$\beta$1 stimulation the activity of p42/p44 mitogen-activated kinase was reduced by 24% in aged fibroblasts under hypoxia compared to normoxic aged cells. In contrast, in young fibroblasts there was a 50% increase of activity under hypoxia [22].

The expression of TGF-beta receptors in human keratinocytes showed the same pattern as fibroblasts [23].

### *Hypoxia and Platelet-derived Growth Factor (PDGF)*

It has been shown that endothelial cells under hypoxic conditions, in the range of 1% to 2% $O_2$, produce increased amounts of PDGF

[24]. In vivo, PDGF-receptor-$\beta$ mRNA increases dramatically in young rabbits 7 days after they received a full-thickness wound on ischemic ears. In the same model, aged animals showed a fourfold to sevenfold lower increase of PDGF-receptor-$\beta$ mRNA than young animals [25].

### *Hypoxia and Vascular Endothelial Growth Factor (VEGF)*

VEGF mRNA levels increased within hours of exposing different cell cultures to hypoxia and returned to background when normoxia was re-established [26]. In vivo, VEGF mRNA increases threefold to fivefold in normal tissue when exposed to hypoxia in our ischemic rabbit ear model. However, the stimulus of wounding alone (with local transient ischemia due to vessel interruption) caused a sixfold to sevenfold increase in VEGF mRNA. Adding the ischemic insult in our rabbit ear wound model did not result in any further increase over the levels observed in nonischemic rabbit ear wounds [27]. This indicates that the ischemic stimulus induced by wounding is maximal, and that making the ears ischemic did not increase the stimulus for VEGF production.

### *Epithelialization*

Hypoxic conditions enhance cell migration of skin-derived cells: Keratinocytes move faster under hypoxia [23, 28]. Also, human dermal fibroblasts have increased motility under short-term hypoxia [22].

### *Glycolysis/Lactate*

It has been shown that a number of glycolytic enzymes are synthesized in greater amounts in cell cultures when exposed to hypoxia [29].

Lactate has been thought to be a stimulus for VEGF-secretion [30]. This could be the explanation for the earlier observations that angiogenesis and collagen deposition enhanced when increased lactate levels were present [31]. Interestingly, lactate is also present in well-oxygenated wounds because of accumulation of leukocytes, fibroblasts, and endothelial cells. Lacking mitochondria, all these cells rely on anaerobic glycolysis for energy recruitment. As a consequence, lactate levels increase in wounds under normoxic conditions as well.

### *Impact of Aging on Hypoxic Stimulus*

Most chronic wounds occur in the elderly, so in the aged the response to hypoxia is particularly relevant clinically. The aged have an altered response to hypoxia, and wound healing is markedly delayed. The ischemic rabbit ear model evidences such delay in aged animals, and the depression was shown to be evident even in middle-aged rabbits [3]. By maintaining chronic ischemia in the rabbit ear model, a chronic wound model can beproduced in which there is minimal healing, even after 26 days [32].

Aged human keratinocytes show decreased motility in response to hypoxiakeratinocytes in comparison to young adult [23]. Aged human fibroblasts show a decrease in migration when stimulated by TGF-$\beta$. TGF-$\beta$1 mRNA expression, when compared to collagen 1 mRNA synthesis, TGF-$\beta$1 auto-induction by TGF-$\beta$1, and PDGF-R$\beta$, all show a marked depression in the ischemic rabbit ear model when compared to young rabbit controls [3, 25].

### *Chronic Hypoxia Decreases All Processes in Wound Healing*

Most chronic wounds, such as pressure sores, venous ulcers, diabetic ulcers, or are a result of local tissue ischemia or ischemia-reperfusion injury. As mentioned above, chronic wounds generally occur in people over age 60 years old [33]. Acute wounds in the elderly usually heal without complications, but it is the presence of chronic hypoxia or repeated ischemia-reperfusion injury that results in chronic wounds.

In vitro, there is substantial experimental evidence showing the difference in cellular response to chronic hypoxia versus acute transient hypoxia (analogous to acute versus chronic wounds clinically). In contrast to the findings in acute hypoxia, the proliferation rate of human dermal fibroblasts decreases under chronic hypoxic conditions in vitro (six passages at 1% $O_2$). Also $\alpha$1(I) procollagen is significantly downregulated on the protein level as well as on the mRNA level under chronic hypoxia. In addition to these findings, TGF-$\beta$1 mRNA levels are 3.1-fold lower in fibroblasts under conditions of chronic hypoxia [10].

Comparable observations have been made clinically. It was recently demonstrated that TGF-$\beta$-signaling in fibroblasts from chronic wounds is altered. In chronic wounds the TGF-beta type II receptor is downregulated. As a logical consequence, decreased TGF-beta type II receptor expression is accompanied by the failure of ulcer fibroblasts to phosphorylate Smad 2, Smad 3, and p42/p44 mitogen-activated kinase (MAPK). These resident cells from chronic wounds display signs of senescence in addition to unresponsiveness to the stimulatory action of TGF-$\beta$1 [34]. One important observation in relation to chronic wounds is that aged cells are more sensitive to stress-induced senescence [35, 36]. Most chronic wounds occur in the aged and are subjected to the chronic stress of inflammation, hypoxia, and ischemia-reperfusion.

### *Effectiveness of Recombinant Growth Factors on Wound Healing under Hypoxia*

*External Application of Growth Factors on Ischemic Rabbit Ear Ulcers.* Recombinant TGF-$\beta$1 enhances wound healing by increasing granulation tissue formation, epithelialization, and breaking strength under ischemia in the young, but it fails to enhance healing in ear ulcers of aged rabbits [3, 37]. This matches the previously described observations of reduced responsiveness to TGF-$\beta$1 in chronic wounds and aged cells, and it strongly suggests altered TGF-$\beta$ signal transduction in the aged under hypoxic conditions.

Recombinant PDGF-BB improves wound healing under ischemia through enhancing granulation tissue formation and by increasing angiogenesis as well as epithelialization [37, 38]. Application of collagen-embedded PDGF plasmid DNA promotes wound healing the same way as recombinant PDGF-BB, by enhancing granulation tissue formation and epithelialization, with higher wound closure rates as consequence [39].

Recombinant vascular endothelial growth factor (VEGF) increases granulation tissue formation by 100% to 150% in ischemic wounds versus 50% to 79% in nonischemic wounds; however, it has no effect on epithelialization [27].

Recombinant basic fibroblast growth factor (bFGF–FGF-2) en-

hances wound healing in nonischemic wounds but fails to show an effect in ischemic wounds under a wide dose range, indicating altered FGF signal transduction in response to hypoxia. The addition of hyberbaric oxygen restores the wound-enhancing effects of bFGF [37, 38].

Recombinant KGF enhances epithelialization and granulation tissue formation in ischemic wounds [40]. Recombinant KGF-2 also enhanced epithelialization as well as granulation tissue formation in both young and aged ischemic rabbit ear ulcers, [41].

Based on existing knowledge, we interpret these positive responses of recombinant growth factor treatment under ischemia to be the result of enhanced activation of the hypoxia-inducible protein-1 pathway (HIF-1). It has been shown that TGF$\beta$, PDGF-$\beta$, and VEGF stimulate HIF-1$\alpha$ stabilization and hence its pathway (see last chapter).

## Impact of Hyperoxia on Wound Healing

As a logical consequence of the observation that the lack of oxygen decreases wound healing, the application of oxygen either topically or systemically has a long history. For many years, hyperbaric oxygen chambers, originally constructed to treat diving decompression sickness, were used empirically to treat a wide variety of conditions, including chronic wounds. The absence of a clear mechanism of action of oxygen in healing, and the intermittent nature of the treatments resulted in much skepticism within the medical community. However, research in the last few years, has both given insights into possible mechanisms and produced more compelling evidence of oxygen's efficacy, although the clinical data are still limited. We will review the current rationales for oxygen therapy based on experimental evidence, including the recent renewal of enthusiasm for topical oxygen therapy.

Experimentally there is a benefit to hyperbaric oxygen therapy (HBO) in a clinically relevant ischemic rabbit ear ulcer model [42], particularly when given for a duration equivalent to clinical protocols [32]. In a clinical study periwound-tissue oxygen tension measured during HBO correlated directly with the improvement in wound healing of chronic wounds: during HBO treatment of patients with chronic leg wounds, the oxygen tensions of their wounds were measured and further compared to the wound healing rate. Only if the peri-wound tissue oxygen tension increased under systemic application of 100% at $O_2$ 1 atm and showed further increase at 100% $O_2$ 2.4 atm did the wounds respond with enhanced healing rates [43]. Continuous 100% topical oxygen delivery by a battery-powered catalytic converter has been shown to be beneficial in wound healing by enhancing epithelialization by 104% in ischemic rabbit ear ulcers [44].

### *Physiology of Oxygen Delivery*

Tissue oxygenation of the skin ($Ptco_2$) depends only on local blood supply, and arterial oxygen tension as delivery of oxygen to the wound occurs solely by diffusion [2, 9]. The oxygen-carrying capacity of hemoglobin is not rate limiting for adequate tissue oxygenation until hemoglobin levels are very significantly depressed. In humans—healthy volunteers—tissue oxygen tension levels did not decrease (they stayed at the baseline) despite hemoglobin values of 5 g/dL, if adequate circulation was maintained [45].

The diffusion distance for effective tissue oxygen delivery increases from 64 $\mu$m at $PO_2$ 100 mmHg to 246 $\mu$m at $PO_2$ 2000 mmHg oxygen tension [2]. Therefore, even under conditions of decreased blood flow, hyperbaric oxygen therapy[1] at a standard 2.0 atm can transiently elevate tissue oxygen levels significantly. Under nonischemic conditions, in our rabbit ear model, $ptco_2$ rose from a baseline value of 53 mmHg $PO_2$, to 850 mmHg $PO_2$, whereas in our ischemic rabbit ear model we observed an increase of $ptco_2$ from the baseline at 24 mmHg to 312 mmHg $O_2$ after the first HBO-treatment. After 14 daily HBO treatments of 90 minutes each, $ptco_2$ increased up to 507 mmHg in ischemic ears immediately after the last treatment, indicating an increased responsiveness to oxygen, most consistent with increased blood flow. Consistent with this observation, the oxygen washout (which should be related to blood flow) decreased after 7 daily HBO treatments within one hour, in contrast to a four-hour decrease at the beginning of the treatment. Also, the baseline levels of tissue oxygen tensions increased from 24 mmHg to 32 mmHg after 14 days of HBO treatment versus an unchanged 53 mmHg in the nonischemic ear. These changes are most likely a result of enhanced neovascularization under HBO treatment [5]. In the same model, oxygen delivery at 1 atm pressure (100% $O_2$ or NBO) had no therapeutic effect.

In vitro, the fibroblast proliferation rate is enhanced by HBO dose-dependently. Maximal proliferation rates were reached at oxygen tensions of 1,875 mmHg $Po_2$, which corresponds to 2.4 ata hyperbaric oxygen treatment [46]. Under experimental in vivo conditions, in the ischemic rabbit ear ulcer, wound healing is increased by an amount equivalent to growth factor stimulation [32].

As previously described, oxygenation of the wound by HBO also has a positive effect on prevention of wound infection (see previous section). Soft tissue infections like necrotizing fasciitis or gas gangrene are among the widely accepted indications for $HBO_2$ treatment.

### *HBO and Growth Factors*

Oxygen therapy in wound healing results in increased growth factor production, particularly VEGF, but also PDGF-receptor when combined with PDGF treatment.

Normal angiogenesis occurs in response to an oxygen gradient that usually occurs after wounding [9, 17]. The transiently occurring hypoxia in the wound results in production of angiogenic factors including VEGF [27], PDGF, and TGF-$\beta$.

The additional angiogenic effect of HBO is likely due in part to an enhancement of the normal VEGF response to wounding. After exposure to HBO for 7 days the increase of VEGF is even more accentuated [47]. It has been shown that free $H_2O_2$ increases VEGF levels [30]. The mechanism of this enhancement seems to be due to reactive oxygen species (ROS), and is at least partially independent of the HIF signal-transduction pathway [48, 49].

Combined treatment of ischemic rabbit ear ulcers with HBO and recombinant PDGF-BB increased PDGF-$\beta$–receptor (PDGF-R$\beta$) content of the treated tissue [50]. This positive effect seems to be due to the effect of oxygen as signal transducer via ROS (reactive oxygen species). In vitro, HBO treatment results in an increase in p42/p44 MAPK activity, consistent with the hypothesis that HBO is modulating growth factor dependent signal transduction pathways. The MAP kinases p42/p44 are known to modulate growth factor–dependent cellular responses as proliferation, migration, and gene expression. HBO-treated human dermal fibroblasts had a threefold increase in p42/p44 MAPK activity after the addition of PDGF-BB [51].

*HBO and Recombinant Growth Factors*

HBO has been shown to enhance the effect caused by growth factor treatment. Recombinant growth factors (r-TGF-$\beta$1, r-PDGF-BB) increase wound healing in ischemic rabbit ear ulcers by 200%, but still only 40% of normal at 7 days. If growth factors are applied together with HBO, the negative effect of ischemia on wound healing is completely reversed [42]. However, under conditions of 20 treatments with HBO (rather than five [42]), the effects of HBO are much greater, and no additional benefit is seen from the addition of growth factor (TGF-$\beta$3) [32].

**Oxygen: Signaling Molecule**

Recent studies have shown that signal transduction of growth factors also happens through reactive oxygen species (ROS). PDGF, EGF, TNF-$\alpha$, and IL-1$\beta$ increase intracellular ROS synthesis in fibroblasts via Rac1, a guanosine-triphosphate-binding protein [52].

Interestingly both hypoxia and hyperoxia enhance ROS production: In hypoxia through the so-called X-to-O shift and in hyperoxia through activated inflammatory cells [53]. High levels of ROS are toxic, but low levels are stimulants. The levels of ROS on the cellular level are exceedingly hard to determine, but their levels are critical to explain the complex effects of oxygen levels on cell signaling.

*Hydrogen Peroxide ($H_2O_2$) as a Signaling Molecule*

In the case of PDGF, signal transduction was inhibited when the growth-factor–stimulated rise in $H_2O_2$ was blocked [54]. The activation of the JAK-STAT-pathway, an important pathway downstream of several growth factors and cytokines such as PDGF, responds to intracellular ROS as a second messenger ($H_2O_2$): The transcription factors STAT1 and STAT3 are activated in the presence of $H_2O_2$ – the presence of antioxidants blocks PDGF-mediated activation of STAT1 and STAT3 by JAK2 and TYK2 [55]. The HIF-1 pathway is another pathway that can be activated by ROS [56].

*Stimulation of Growth Factor Secretion by ROS*

In addition to activating signal transduction pathways directly, ROS seem to activate secretion of growth factors. For example, macrophages and keratinocytes increase their VEGF mRNA and secrete more VEGF after treatment with $H_2O_2$. It has been demonstrated that $H_2O_2$ stimulates the VEGF promotor directly via a HIF-1 independent pathway [48, 57]. Over-expression of Rac1 downstream of different cytokine receptors, increases VEGF expression. It is known that activation of Rac1 generates reactive oxygen species [52, 58].

*Role of Hypoxia-inducible Factor-1 (HIF-1) Mediated Responses in Wound Healing*

Several genes involved in cellular differentiation are directly or indirectly regulated by hypoxia. HIF-1 was known to interact with *cis*-acting sequences within the erythropoietin gene 10 years ago [59]. Since then it has become more and more clear that the primary molecular mechanism of gene activation during hypoxia occurs via HIF-1, which binds as a transcription factor to hypoxia response elements (HRE) within the promotor region of different genes, and thereby starting their transcription. HIF-1 dependent are besides erythropoiesis controlling genes (EPO, ceruloplasmin, transferrin, and transferrin receptor) also genes that control metabolism (exp. 11 glycolytic enzymes), proliferation/cell survival (among those are TGF-b3, VEGF, p21, NOS2, IGF2, IGFBPs), andvascular biology (exp. $\alpha$1B adrenergic receptor, NOS-2, PAI-1, VEGF-receptor FLT-1, VEGF [60, 61]. In vitro, increased cellular motility and cellular activity are the acute responses to hypoxia, which is most likely mediated through HIF-1.

Under normoxic conditions HIF-1$\alpha$ is inactivated through ubiquitinization after hydroxylation of proline residues. Hydroxylation of proline as it also happens during collagen maturation is oxygen dependent. In the case of HIF-1$\alpha$, however, it requires a different enzyme [61]. Though in vitro experiments show that HIF-1 is regulated totally by controlling the stability of the HIF-1$\alpha$ protein, there is solid evidence in vivo that regulation of HIF-1$\alpha$ at the mRNA level also plays an important role [62].

*HIF-1 Activation*

To start transcriptional activity of HIF-1 its sub-fraction HIF-1$\alpha$ must be protected from degradation. Phoshorylation of HIF-1$\alpha$ allows its binding to HIF-1$\beta$ and hence the formation of the HIF-1-dimer. Through this binding, HIF-1$\alpha$ escapes proline hydroxylation, ubiquitinization, and ultimately degradation. The HIF-1-heterodimer binds to HRE and displays its transcriptional activity [56, 61].

Phosphorylation of HIF-1$\alpha$ happens through the ERK-pathway – p42/p44 mitogen-activated protein kinases phosphorylate HIF-1$\alpha$ [63]. The ERK-pathway is activated by many different stimulants: ROS, $Ca^{2+}$, VEGF through the VEGF-R and other growth factors (PDGF, TGF-beta) through their receptors [56]. Activation of p42/p44 MAPK alone is able to promote the transcriptional activity of HIF-1. Furthermore, HIF-1-stabilization under hypoxia seems also to be dependent on the PI-3K/Akt pathway, but this stabilization is growth factor independent [56]. Therefore cooperation between hypoxic and growth factor signals may ultimately lead to the increase in HIF-mediated gene expression.

*Possible Explanation for the Response under Chronic Hypoxia*

We believe that the altered growth factor response to ischemia in old age plays an important role in the pathogenesis of chronic wounds. The signal transduction of TGF-$\beta$1 under ischemic conditions is substantially reduced in the aged rabbit as previously described. TGF-$\beta$ and procollagen-$\alpha$(I) mRNA are downregulated [3].

A possible explanation for this reduced responsiveness under chronic hypoxia and in old age may be found in the HIF-1 pathway. It has been observed that the HIF-1$\alpha$ synthesis rate is decreased in aged cells. In vitro, smooth muscle cells from aged rabbits have lower VEGF mRNA levels under hypoxia (0.1% $O_2$). This decrease is due to reduced HIF-1$\alpha$ protein levels and reduced DNA binding activity in spite of unchanged mRNA levels in aged cells when compared to young cell lines [64].

As a logical consequence it must be concluded that HIF-1–

dependent cell signaling in general is reduced in aged or chronic hypoxic cells, as is the case of TGF-$\beta$.

Chronically decreased HIF levels due to senescence in chronic hypoxic wounds may change when ″shocked″ by high oxygen levels temporarily as occurs in hyperbaric oxygen treatment. HBO could reset the HIF sensing on the protein or mRNA level and hence reset the responsiveness to hypoxia (in behavioral terms a loss of habituation by resetting the oxygen gradient as a new stimulus). HIF-1 is very unstable in post-hypoxic cells; degradation of the protein takes place within 5 minutes, as does the decrease of HIF-1 mRNA [65].

## Conclusions

Efforts to explore the mechanisms for the altered response to ischemia in old age will undoubtedly produce new therapeutic strategies. Combining oxygen as a therapeutic agent with other agents that affect other signal transduction pathways offers considerable promise.

We have utilized transfection with a plasmid containing a hypoxia response element linked to a marker gene in our rabbit ear model to demonstrate substantial regional variations in local tissue perfusion related to the severity of the ischemia, and the proximity of the feeding vessel [66]. Future linkage of hypoxia response elements to other relevant genes will offer new therapeutic potential.

**Résumé. Cet article constitue un aperçu rôle de l'oxygène dans la cicatrisation. La compréhension de ce rôle a évolué considérablement depuis qu'on avaitreconnu que l'oxygène était un facteur essentiel dans le métabolisme oxydatif, jusqu' le reconnaître comme déclencheur au niveau cellulaire interagissant avec les des facteurs de croissance et d'autres signaux pour régler les voies de transduction. Notre laboratoire s'est lancé dans l'étude de tests modèles sur animaux en vue de l'ischémie cutanée afin explorer l'impact in vivo aussi bien de l'hypoxie que de l'utilisation oxygène comme agent thérapeutique, seul ou en combinaison avec d'autres facteurs tels que les facteurs de croissance. Nous avons démontré les effets synergéstiques de l'oxygène hyperbare systémique et de facteurs de croissance ce qui a été étayé par le groupe de recherche de l'équipe de Hunt. La recherche ddes dix derniéres années dans le domaine de la cicatrisation a apporté une nouvelle perception du mécanisme de l'action de l'hypoxie et de l'hyperoxie comme éléments modificateurs du délai normal de la cicatrisation.L'article aborde finalement la question pourquoil'hyperoxie et de l'hypoxiejouent tant l'un que l'autre un rôle important pour la cicatrisation.Le facteur de l' induction de l'hypoxie est essentiel dans cette interaction.**

**Resumen. Este artículo suministra el sumario sobre el rol del oxígeno en la cicatrización. Habia mucho cambio en el reconocimiento de este rol. Aparte del reconocimiento radicional como un factor esencial en el metabolismo oxidativo se ha aceptado como importante señal celular que esta interactúa con factores de crecimiento y otros señales en la regulación de las vías celulares de transducción. Nuestro laboratorio haestablecido modeles de animales de isquemia cutánea parainvestigar in vivo el impacto de la hipoxia, así como del oxígeno como agente terapéutico solo o en combinación con otros factores como los factores de crecimiento. Hemos demostrado un efecto sinergístico de la terapia de oxigeno hiperbárico y los factores de crecimiento, lo cual ha sido confirmado por el quipo de Hunt. En los últimos años la investigación en el area de la cicatrizaciónha aportado una nueva perspectiva sobre el mecanismo de acción de la hipoxia y la hiperoxia como modificadores del curso natural de la cicatrización. Se concluye el articulo con la discussion sobre el papel importante de la hipoxia concominante hiperoxia en la cicatrización. El factor 1 inducible de hipoxia es crucial en esta interrelación.**

## References

1. Hunt TK, Zederfeldt B, Goldstick TK. Oxygen and healing. Am. J. Surg. 1969;118:521–525
2. Sheffield PJ. Tissue oxygen measurements. In Davis JC, Hunt TK, editors, Problem Wounds: The Role of Oxygen, New York, Elsevier, 1988:17–52
3. Wu L, Xia YP, Roth SI, et al. Transforming growth factor-beta1 fails to stimulate wound healing and impairs its signal transduction in an aged ischemic ulcer model: importance of oxygen and age. Am. J. Pathol. 1999;154:301–309
4. Ahn ST, Mustoe TA. Effects of ischemia on ulcer wound healing: a new model in the rabbit ear. Ann. Plast. Surg. 1990;24:17–23
5. Siddiqui A, Davidson JD, Mustoe TA. Ischemic tissue oxygen capacitance after hyperbaric oxygen therapy: a new physiologic concept. Plast. Reconstr. Surg. 1997;99:148–155
6. Allen DB, Maguire JJ, Mahdavian M, et al. Wound hypoxia and acidosis limit neutrophil bacterial killing mechanisms. Arch. Surg. 1997;132: 991–996
7. Hopf HW, Humphrey LM, Puzziferri N, et al. Adjuncts to preparing wounds for closure: hyperbaric oxygen, growth factors, skin substitutes, negative pressure wound therapy (vacuum-assisted closure). Foot Ankle Clin. 2001;6:661–682
8. Tokuda Y, Crane S, Yamaguchi Y, et al. The levels and kinetics of oxygen tension detectable at the surface of human dermal fibroblast cultures. J. Cell. Physiol. 2000;182:414–420
9. Hunt TK, Niinikoski J, Zederfeldt B. Role of oxygen in repair processes. Acta Chir. Scand. 1972;138:109–110
10. Siddiqui A, Galiano RD, Connors D, et al. Differential effects of oxygen on human dermal fibroblasts: acute versus chronic hypoxia. Wound Repair Regen. 1996;4:211–218
11. Jonsson K, Jensen JA, Goodson WH, et al. Tissue oxygenation, anemia, and perfusion in relation to wound healing in surgical patients. Ann. Surg. 1991;214:605–613
12. Rabkin JM, Hunt TK. Infection and oxygen. In Davis JC, Hunt TK, editors, Problem Wounds: The Role of Oxygen, New York, Elsevier, 1988:1–16
13. Niinikoski J, Grislis G, Hunt TK. Respiratory gas tensions and collagen in infected wounds. Ann. Surg. 1972;175:588–593
14. Robson MC. Wound infection. A failure of wound healing caused by an imbalance of bacteria. Surg. Clin. North Am. 1997;77:637–650
15. Hopf HW, Hunt TK, West JM, et al. Wound tissue oxygen tension predicts the risk of wound infection in surgical patients. Arch. Surg. 1997; 132:997–1004
16. Hunt TK, Hopf HW. Wound healing and wound infection. What surgeons and anesthesiologists can do. Surg. Clin. North Am. 1997;77:587–606
17. Niinikoski J, Hunt TK, Dunphy JE. Oxygen supply in healing tissue. Am. J. Surg. 1972;123:247–252
18. Falanga V, Kirsner RS. Low oxygen stimulates proliferation of fibroblasts seeded as single cells. J. Cell. Physiol. 1993;154:506–510
19. Falanga V, Qian SW, Danielpour D, et al. Hypoxia upregulates the synthesis of TGF-beta 1 by human dermal fibroblasts. J. Invest. Dermatol. 1991;97:634–637
20. Falanga V, Martin TA, Takagi H, et al. Low oxygen tension increases mRNA levels of alpha 1 (I) procollagen in human dermal fibroblasts. J. Cell. Physiol. 1993;157:408–412
21. Falanga V, Zhou L, Yufit T. Low oxygen tension stimulates collagen synthesis and COL1A1 transcription through the action of TGF-beta1. J. Cell. Physiol. 2002;191:42–50
22. Mogford JE, Tawil N, Chen A, et al. Effect of age and hypoxia on TGFbeta1 receptor expression and signal transduction in human dermal fibroblasts: impact on cell migration. J. Cell. Physiol. 2002;190:259–265
23. Xia YP, Zhao Y, Tyrone JW, et al. Differential activation of migration by hypoxia in keratinocytes isolated from donors of increasing age: implication for chronic wounds in the elderly. J. Invest. Dermatol. 2001; 116:50–56
24. Kourembanas S, Hannan RL, Faller DV. Oxygen tension regulates the expression of the platelet-derived growth factor-B chain gene in human endothelial cells. J. Clin. Invest. 1990;86:670–674
25. Brucker M, Gruskin E, Farrell C, et al. Differential expression of platelet-derived growth factor receptor-beta in an aging model of wound repair. Wound Repair Regen. 1996;4:219–223
26. Shweiki D, Itin A, Soffer D, et al. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 1992;359:843–845
27. Corral CJ, Siddiqui A, Wu L, et al. Vascular endothelial growth factor

is more important than basic fibroblastic growth factor during ischemic wound healing. Arch. Surg. 1999;134:200–205
28. O'Toole EA, Marinkovich MP, Peavey CL, et al. Hypoxia increases human keratinocyte motility on connective tissue. J. Clin. Invest. 1997; 100:2881–2891
29. Webster KA, Gunning P, Hardeman E, et al. Coordinate reciprocal trends in glycolytic and mitochondrial transcript accumulations during the in vitro differentiation of human myoblasts. J. Cell. Physiol. 1990; 142:566–573
30. Constant JS, Feng JJ, Zabel DD, et al. Lactate elicits vascular endothelial growth factor from macrophages: a possible alternative to hypoxia. Wound Repair Regen. 2000;8:353–360
31. Hunt TK, Conolly WB, Aronson SB, et al. Anaerobic metabolism and wound healing: an hypothesis for the initiation and cessation of collagen synthesis in wounds. Am. J. Surg. 1978;135:328–332
32. Bonomo SR, Davidson JD, Tyrone JW, et al. Enhancement of wound healing by hyperbaric oxygen and transforming growth factor beta3 in a new chronic wound model in aged rabbits. Arch. Surg. 2000;135:1148–1153
33. Mustoe TA. Understanding chronic wounds: a unifying hypothesis on their pathogenesis, and implications for therapy. Am. J. Surg. 2003;186
34. Kim BC, Kim HT, Park SH, et al. Fibroblasts from chronic wounds show altered TGF-beta-signaling and decreased TGF-beta type II receptor expression. J. Cell. Physiol. 2003;195:331–336
35. Toussaint O, Dumont P, Remacle J, et al. Stress-induced premature senescence or stress-induced senescence-like phenotype: one in vivo reality, two possible definitions? Sci. World J. 2002;2:230–247
36. Holbrook NJ, Ikeyama S. Age-related decline in cellular response to oxidative stress: links to growth factor signaling pathways with common defects. Biochem. Pharmacol. 2002;64:999–1005
37. Wu L, Mustoe TA. Effect of ischemia on growth factor enhancement of incisional wound healing. Surgery 1995;117:570–576
38. Mustoe TA, Ahn ST, Tarpley JE, et al. Role of hypoxia in growth factor responses: differential effects of basic fibroblast growth factor and platelet-derived growth factor in an ischemic wound model. Wound Repair Regen. 1994;2:277–283
39. Tyrone JW, Mogford JE, Chandler LA, et al. Collagen-embedded platelet-derived growth factor DNA plasmid promotes wound healing in a dermal ulcer model. J. Surg. Res. 2000;93:230–236
40. Wu L, Pierce GF, Galiano RD, et al. Keratinocyte growth factor induces granulation tissue in ischemic dermal wounds. Importance of epithelial-mesenchymal cell interactions. Arch. Surg. 1996;131:660–666
41. Xia YP, Zhao Y, Marcus J, et al. Effects of keratinocyte growth factor-2 (KGF-2) on wound healing in an ischaemia-impaired rabbit ear model and on scar formation. J. Pathol. 1999;188:431–438
42. Zhao LL, Davidson JD, Wee SC, et al. Effect of hyperbaric oxygen and growth factors on rabbit ear ischemic ulcers. Arch. Surg. 1994;129: 1043–1049
43. Smith BM, Desvigne LD, Slade JB, et al. Transcutaneous oxygen measurements predict healing of leg wounds with hyperbaric therapy. Wound Repair Regen. 1996;4:224–229
44. Said HK, Hijjawi JB, Burk M, et al. Topical oxygen improves wound healing in a rabbit model. J. Am. Coll. Surg. 2002;195:S51–S52
45. Hopf HW, Viele M, Watson JJ, et al. Subcutaneous perfusion and oxygen during acute severe isovolemic hemodilution in healthy volunteers. Arch. Surg. 2000;135:1443–1449
46. Hehenberger K, Brismar K, Lind F, et al. Dose-dependent hyperbaric oxygen stimulation of human fibroblast proliferation. Wound Repair Regen. 1997;5:147–150
47. Sheikh AY, Gibson JJ, Rollins MD, et al. Effect of hyperoxia on vascular endothelial growth factor levels in a wound model. Arch. Surg. 2000; 135:1293–1297
48. Sen CK, Khanna S, Babior BM, et al. Oxidant-induced vascular endothelial growth factor expression in human keratinocytes and cutaneous wound healing. J. Biol. Chem. 2002;277:33284–33290
49. Sen CK, Khanna S, Gordillo G, et al. Oxygen, oxidants, and antioxidants in wound healing: an emerging paradigm. Ann. N. Y. Acad. Sci. 2002;957:239–249
50. Bonomo SR, Davidson JD, Yu Y, et al. Hyperbaric oxygen as a signal transducer: upregulation of platelet derived growth factor-beta receptor in the presence of $HBO_2$ and PDGF. Undersea Hyperb. Med. 1998; 25:211–216
51. Saulis AS, Davidson JD, Mustoe TA, et al. Oxygen growth environment-dependent activation of human dermal fibroblasts P42/P44 MAPK by hyperbaric oxygen ($HBO_2$). Abstract, unpublished data, 2000
52. Sundaresan M, Yu ZX, Ferrans VJ, et al. Regulation of reactive-oxygen-species generation in fibroblasts by Rac1. Biochem. J. 1996;318: 379–382
53. Hunt TK, Hussain MZ, Sen CK. Give me ROS or give me death. Pressure 2001;30:10–11
54. Sundaresan M, Yu ZX, Ferrans VJ, et al. Requirement for generation of H2O2 for platelet-derived growth factor signal transduction. Science 1995;270:296–299
55. Simon AR, Rai U, Fanburg BL, et al. Activation of the JAK-STAT pathway by reactive oxygen species. Am. J. Physiol. 1998;275:C1640–C1652
56. Minet E, Michel G, Mottet D, et al. Transduction pathways involved in hypoxia-inducible factor-1 phosphorylation and activation. Free Radic. Biol. Med. 2001;31:847–855
57. Cho M, Hunt TK, Hussain MZ. Hydrogen peroxide stimulates macrophage vascular endothelial growth factor release. Am. J. Physiol. Heart Circ. Physiol. 2001;280:H2357–H2363
58. Kheradmand F, Werner E, Tremble P, et al. Role of Rac1 and oxygen radicals in collagenase-1 expression induced by cell shape change. Science 1998;280:898–902
59. Wang GL, Semenza GL. General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. Proc. Natl. Acad. Sci. U. S. A. 1993;90:4304–4308
60. Semenza GL. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J. Appl. Physiol. 2000;88:1474–1480
61. Semenza GL. HIF-1, O(2), and the 3 PHDs: how animal cells signal hypoxia to the nucleus. Cell 2001;107:1–3
62. Lee SH, Wolf PL, Escudero R, et al. Early expression of angiogenesis factors in acute myocardial ischemia and infarction. N. Engl. J. Med. 2000;342:626–633
63. Richard DE, Berra E, Gothie E, et al. p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1alpha (HIF-1alpha) and enhance the transcriptional activity of HIF-1. J. Biol. Chem. 1999;274:32631–32637
64. Rivard A, Berthou-Soulie L, Principe N, et al. Age-dependent defect in vascular endothelial growth factor expression is associated with reduced hypoxia-inducible factor 1 activity. J. Biol. Chem. 2000;275: 29643–29647
65. Wang GL, Jiang BH, Rue EA, et al. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc. Natl. Acad. Sci. U. S. A. 1995;92:5510–5514
66. Mogford JE, Roy NK, Cross KJ, et al. Use of hypoxia-inducible factor signal transduction pathway to measure $O_2$-levels and modulate growth factor pathways. Wound Repair Regen. 2003;in press