REVIEW ARTICLE

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Oxygen in acute and chronic wound healing

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Summary

Oxygen is a prerequisite for successful wound healing due to the increased demand for reparative processes such as cell proliferation, bacterial defence, angiogenesis and collagen synthesis. Even though the role of oxygen in wound healing is not yet completely understood, many experimental and clinical observations have shown wound healing to be impaired under hypoxia. This article provides an overview on the role of oxygen in wound healing and chronic wound pathogenesis, a brief insight into systemic and topical oxygen treatment, and a discussion of the role of wound tissue oximetry. Thus, the aim is to improve the understanding of the role of oxygen in wound healing and to advance our management of wound patients.

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An injury to the skin may disturb the integrity of the epidermis, the dermis, the connective tissue and the microcirculation, and thus inevitably results in a wound. The disturbed equilibrium of the local environment induces wound healing.[1] Physiological wound healing is a well-regulated stepwise process that ends with wound closure within days or weeks, depending on diameter and depth of the wound.[2,3] One critical parameter for wound healing is oxygen that is required for almost every step of the healing process.[4–7] The oxygenation of wound tissue is dependent on both the oxygen supply to the wound tissue – that is determined by the pulmonic gas exchange, the blood haemoglobin level, the cardiac output, the peripheral perfusion rate, and by the capillary density in the wound tissue and its periphery – and on the oxygen consumption rate of parenchymal, stromal and inflammatory cells of which the wound tissue is composed. Interestingly, in literature dealing with wound tissue oxygenation, the unit mmHg for oxygen partial pressure (pO_2) is still widely used even though it is not the standard SI unit, which is pascal (1 Pa = $7{\cdot}5006 \times 10^{-3}$ mmHg). Therefore, we use mmHg throughout this review to make it easier for the reader to compare data from the different publications cited herein.

In wound healing, biochemical energy supply is a basic requirement. Oxygen is essential for the production of biological energy equivalents (e.g. adenosine triphosphate, ATP) in aerobic glycolysis, the citric acid cycle, and the oxidation of fatty acids.[4,7] Therefore, sufficient oxygenation of tissue is a prerequisite for adequate energy levels, which are essential for proper cellular function.

In healing tissue, sufficient oxygenation is particularly relevant because of the increased energy demand for reparative processes such as cell proliferation, bacterial defence and collagen synthesis. The strictly oxygen-dependent NADPH-linked oxygenase represents a further highly important enzyme in wound healing; it catalyses the production of reactive oxygen species (ROS) such as peroxide anion (HO_2^-), hydroxyl ion (HO^-) and superoxide anion (O^{2-}).[8] ROS play a prominent role in oxidative bacterial killing[9,10] and coregulate prevalent processes in wound healing such as cytokine release, cell proliferation and angiogenesis.[8,11]

Against this background, the crucial role of reduced oxygen supply in chronic wound pathogenesis becomes obvious. Chronic wounds are characterized by an insufficient repair process that precludes the establishment of a sustained anatomical and functional result in an appropriate length of time.[1,2] Chronic wounds represent a frequent interdisciplinary disease affecting about 1% of the European population. According to the United Nations (see http://www.un.org/), the population of Europe was approximately 830 million in 2009, using a definition including the whole of the transcontinental countries of Russia and Turkey. Based on these figures, about 8 million people in Europe suffered from chronic wounds in 2009. Besides the tremendous impact on the quality of life of the affected patients, chronic wounds are

of fundamental economic relevance: nearly 2% of European health budgets are spent on the impaired healing of chronic wounds.[12,13] In Germany, over 2·8 million sick days per year are caused by chronic wounds. In the U.S.A., approximately one-third of the dermatological health budget is spent on the treatment of chronic wounds.[14]

This review summarizes the role of oxygen in the sequential steps of physiological wound healing. The pathogenesis of chronic wounds is explained against the background of impaired wound tissue oxygenation. Moreover, we question the benefits of treatment strategies for improving wound tissue oxygenation and discuss the role of wound tissue oxygen measurement either to classify chronic wounds or to monitor different treatment approaches in clinical routine.

Physiological wound healing

Physiological (syn. acute) wound healing is a dynamic stepwise process consisting of partially overlapping phases that are determined by interacting events on a molecular, cellular and extracellular matrix (ECM) level. This process, which is not yet fully understood, starts with a disturbance of tissue integrity and ends with a *restitutio ad integrum* or a scar formation within an appropriate length of time. Nearly every step of wound healing requires oxygen.[5,7,10,15,16] For didactic reasons, the course of physiological wound healing is schematically divided into three overlapping phases: the inflammatory phase, the proliferative phase (neoangiogenesis, tissue formation, re-epithelialization) and the tissue remodelling phase (Fig. 1).[1,2,17]

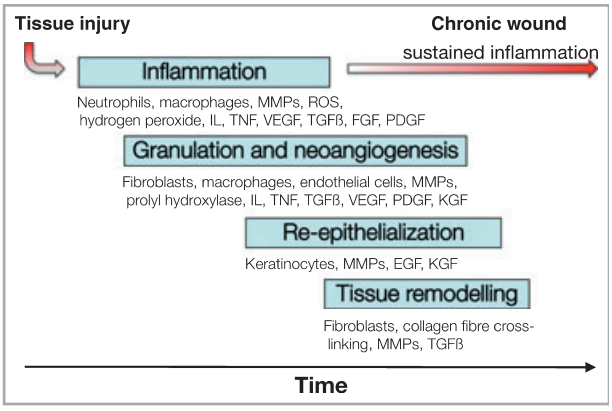

Fig 1. Wound healing phases. The inflammatory phase starts after tissue injury. At this stage, cytokines, chemokines and reactive oxygen species are released and cells are recruited to the wound site. In the subsequent proliferative phase (neoangiogenesis, tissue formation, re-epithelialization) new tissue is formed by endothelial cells, fibroblasts and keratinocytes. After these initial steps, tissue remodelling starts. EGF, epidermal growth factor; FGF, fibroblast growth factor; IL, interleukin; KGF, keratinocyte growth factor; MMPs, matrix metalloproteinases; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; TGF, transforming growth factor; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

Inflammatory phase

Physiologically, the inflammatory phase lasts between 4 and 6 days and starts immediately after wounding. Blood vessels constrict after traumatization, and platelets aggregate along the activated endothelium. Vascular disruption and vasoconstriction cause a hypoxic microenvironment that is intensified by increased oxygen consumption due to metabolically active cells contributing to wound healing. Hypoxia actuates the initial steps of wound healing by boosting ROS activity, by activating platelets and endothelium, and by inducing cytokines released from platelets, monocytes and parenchymal cells [e.g. vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-β, tumour necrosis factor (TNF)].[9,18] However, even if acute hypoxia initiates wound healing, the recovery of wound tissue oxygenation is of major importance for physiological healing as chronic hypoxia impairs all processes necessary for healing. The aggregated platelets initiate the coagulation cascade leading to a blood clot, which prevents the leakage of blood and forms a provisional ECM. The provisional ECM, which is composed of fibronectin, fibrinogen, fibrin, thrombospondin and vitronectin, fills the tissue defect and enables migration of the different cytokines and cells required for the healing process.[19] Besides these structural contributions, the activated platelets direct the healing process through the secretion of several mediators of wound healing such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), TGF-β1 and TGF-β2.[2] ROS stimulate cytokine and chemokine release as well as their functions. The primary effect of these mediators is the recruitment and activation of neutrophils and macrophages to the wound site and the activation of fibroblasts. However, these platelet-derived processes are not the only ones that initiate healing. The injury activates epithelial and nonepithelial cells in the wound area. Consecutively, cytokines and chemokines are secreted, which initiate stress pathways and activate the complement cascade, both oxygen-dependent processes. In consequence, a set of secreted factors [TGF-α, TGF-β1, keratinocyte growth factor (KGF), EGF, PDFG and insulin-like growth factor (IGF)] is released, which attracts and stimulates relevant players of wound healing such as inflammatory leucocytes and fibroblasts. Just recently, it has been shown that hydrogen peroxide (H_2O_2) is an important mediator in wound–leucocyte interaction.[20] A tail-fin model of a zebrafish with a genetically encoded H_2O_2 sensor showed that H_2O_2 at the wound margins peaks as early as a few minutes after injury, and that leucocytes are recruited to the wound site by a tissue-scale H_2O_2 gradient. Whether H_2O_2 is produced by damaged cells or by their neighbours, and how neutrophils sense H_2O_2 gradients yet remains to be answered.[21]

Even very superficial traumas without the destruction of blood vessels and activation of platelets initiate wound healing.[1] Injured parenchymal cells secrete prostaglandins, histamine, bradykinin and serotonin, which induce vasodilatation and increase capillary permeability. Subsequently, diapedesis of cells is accelerated and oxygen supply to the wound site is

increased. Clinical correlates of these processes are erythema and oedema, which become apparent at the wound edges during the inflammation phase. The infiltration of leucocytes, monocytes, and – 24–48 h later – macrophages is the key event in initial wound healing; their functions, such as degradation of cell detritus, counteraction of tissue infection and phagocytosis of microorganisms, are indispensable for wound healing. The amount and proportion of these inflammatory cells as well as the duration of the inflammatory phase depend on the wound extent, the degree of tissue infection, and the extent of debris that needs to be removed.[1] Metabolically, inflammatory cells are extremely active and therefore depend on high amounts of oxygen. The high consumption of oxygen may lead to areas of hypoxia, even in well-oxygenated wounds.[5] During the inflammatory phase, one central product of neutrophils, macrophages, monocytes, endothelial cells and fibroblasts is ROS. Thrombin, PDGF and TNF stimulate the release of ROS from endothelial cells, whereas interleukin (IL)-1 and platelet-activating factor stimulate ROS release from fibroblasts.[22] ROS are the main force against microorganisms and thus wound infection. As mentioned above, NADPH-linked oxygenase is responsible for the production of ROS, a highly oxygen-dependent process: the K_m (half maximal velocity) for NADPH-linked oxygenase with oxygen as a substrate is a pO_2 value of 40–80 mmHg.[8,23] Neutrophils were shown *in vitro* to lose their bacterial killing capacity at a pO_2 level below 40 mmHg.[24,25] This loss may explain the significant bacterial colonization apparent in hypoxic chronic wounds. Besides their role in the oxidative killing of bacteria, ROS are able to augment neutrophil chemotaxis.[9,18] Activated inflammatory cells themselves produce cytokines and growth factors such as IGF, leucocyte growth factor, IL-1, IL-2, TNF, TGF-α, TGF-β, VEGF, PDGF and lactate.[26] VEGF and PDGF are both potent chemoattractants and mitogens for fibroblasts and angiogenic growth factors; their release initiates the formation of granulation tissue and thus the proliferative phase at day 4–5 after wounding.

Proliferative phase

The proliferative phase lasts – depending on the extent of the wound – for a few weeks and comprises elementary processes such as neovascularization, formation of granulation tissue and ECM, and re-epithelialization. Endothelial cells and fibroblasts simultaneously invade the initially built haemostatic clot. Macrophages lead the way by degrading the clot and by releasing cytokines and chemokines that attract fibroblasts and stimulate angiogenesis.[27] Particularly macrophages and their metabolites play a pivotal role in granulation tissue formation as depletion of macrophages was shown to lead to impaired wound healing in an *in vivo* porcine model.[27] Fibroblasts and keratinocytes also secrete growth factors. Hereby, the cytokines of the TGF-β superfamily seem to play the most prominent role in granulation tissue formation.[28] Interestingly, ECM molecules such as fibrinogen, fibronectin, fibrin and vitronectin are interactive with cytokines and also regulate the proliferation, differentiation and migration of fibroblasts.[29] Important stimulators of angiogenesis are hypoxia and ROS. Both stimulate macrophages, fibroblasts, endothelial cells and keratinocytes to synthesize VEGF.[30–32] Again, acute hypoxia is the initiator of this process, whereas chronic hypoxia impairs neovascularization.[30,33] Hypoxia activates the transcription factor hypoxia-inducible factor (HIF)-1α. HIF-1α binds to the hypoxia response element in the gene promoter region of the *VEGF* gene, which in turn upregulates VEGF. VEGF, as the major angiogenic growth factor, stimulates endothelial cells to migrate, proliferate and form countless new capillaries.[32] Rossiter *et al.*[34] showed in a murine model system that keratinocyte-specific deletion of *VEGF* resulted in delayed wound healing due to impaired neoangiogenesis. Complementarily, Hong *et al.*[35] showed enhanced wound healing in transgenic mice with overexpression of VEGF in the skin. The new capillaries branch out and invade the provisional wound matrix, which is replaced piecemeal by a new ECM produced and deposited by fibroblasts. The emerging ECM, in which fibroblasts, myofibroblasts, leucocytes and macrophages are embedded, consists of immature collagen (type III), proteoglycans, glycosaminglycans, fibrin, fibronectin and hyaluronic acid.[36] In this context, the production and deposition of collagen represents a fundamental process as it reconstitutes skin alignment and integrity. The production and deposition of collagen is proportional to oxygen tension: fibroblasts need a pO_2 of 30–40 mmHg for collagen synthesis.[37] A central oxygen-dependent step in the synthesis of collagen is the hydroxylation of proline and lysine residues. In addition, hydroxylase activity is critically dependent on cofactors such as iron and vitamin C. Lysyl hydroxylase and lysyl oxidase, both oxygen-dependent enzymes, catalyse collagen cross-linking, a step that aims at wound stability. Again, in hypoxia, acute hypoxic conditions must be distinguished from chronic hypoxia. Acute hypoxia may stimulate fibroblast proliferation, collagen synthesis and expression of TGF-β1, whereas chronic hypoxia decreases these processes as shown *in vitro* by Siddiqui *et al.*[38] in human dermal fibroblasts. Angiogenesis and ECM synthesis are interdependent processes as new blood vessels need new ECM as a three-dimensional scaffold for their ingrowth while the cell metabolism of, for example, fibroblasts needs new blood vessels that deliver oxygen and other nutrients. The fact that the same cytokines stimulate each process interconnects these steps of wound healing. Parallel to the formation of granulation tissue, re-epithelialization is initiated.

Re-epithelialization aims at covering the wound surface by a layer of epithelium and is based on the differentiation, proliferation and migration of epidermal keratinocytes. The stress pathways activated by injury lead to the oxygen-dependent release of certain cytokines and chemokines (TNF, TGF-α, TGF-β1, KGF, EGF, PDFG and IGF) by parenchymal cells such as keratinocytes. These cytokines, foremost TNF, seem to stimulate epidermal cells at wound edges and hair follicles in an autocrine manner to restructure their cytoskeleton, a process that is oxygen dependent and starts within a few hours after injury.[39,40] The cells retract their intracellular tonofilaments,

dissolve the desmosomal or hemidesmosomal connections, but establish adhesion structures for gripping to the ECM and develop cytoplasmic actin filaments for cell migration.[41–43] Stimulated by EGF, TGF-α, KGF, TGF-β1, hepatocyte growth factor (HGF) and IGF-1, cell migration toward the wound's central point, called shuffling, takes place.[44] Hereby, TGF-β1 is a key cytokine as it controls the expression of integrins in keratinocytes. Integrins are cell surface receptors that interact with ECM, particularly with fibrin and fibronectin.[45] Migration through the wound matrix, which is composed of necrotic material, bacteria, a haemostatic clot of platelets and fibrin, and later on of granulation tissue, is further supported by the activation of plasmin. This process is caused by a plasminogen activator produced by both epidermal cells[46] and matrix metalloproteinases (MMPs). MMPs (MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-13) are released mainly by macrophages, keratinocytes, endothelial cells and fibroblasts[47] and degrade certain constituents of provisional wound tissue such as collagen I, III, IV and VII. It is of outstanding importance that MMP inhibitors such as α_1-antiproteinase, secretory leucocyte protease inhibitor (SLPI), α_2-macroglobulin and tissue inhibitors of MMPs (TIMPs) are sufficiently present, once provisional wound tissue has been removed. To achieve complete closure of larger wound areas, cell migration has to be accompanied by oxygen-dependent cell proliferation. For this, cytokines and chemokines (EGF, TGF-α, KGF, HGF, nerve growth factor, IGF-1, IL-1 and IL-6), most possibly released from keratinocyte stem cells, stimulate the proliferation of keratinocytes in a process called 'proliferative burst'.[48] As processes with a high metabolic activity the different steps of epithelialization are oxygen and ROS dependent. Taking all these considerations into account, the topical administration of pure oxygen on wounds could increase the rate of epithelialization.[49] However, O'Toole *et al.*[50] demonstrated in an *in vitro* study that hypoxic keratinocytes showed a decreased secretion of laminin-5, a laminin isoform known to inhibit keratinocyte motility, but an increased expression and redistribution of the lamellipodia-associated proteins, cytoskeletal proteins which are involved in cell migration. However, the *in vitro* experiments discount the countless interactions of keratinocytes with, for example, inflammatory cells, bacterial colonization, granulation tissue etc. The same research group showed in another study that a very low concentration of H_2O_2 inhibits keratinocyte migration and proliferation.[51] The exact processes are not yet fully understood, but tools like chemiluminescent nanoparticle sensors for H_2O_2 enable us to study H_2O_2 functions *in vivo* in more detail.[52]

Tissue remodelling phase

The tissue remodelling phase starts as early as a few days after injury and lasts up to 2 years thereafter. In the beginning, wound contraction contributes to wound closure. This process is enabled due to the differentiation of a subgroup of fibroblasts to contractile myofibroblasts triggered by oxygen[53] and mediated by TGF-β1, TGF-β2 and PDGF.[54–56] After the main steps of the proliferative phase are fulfilled, unknown stop signals induce a redifferentiation of fibroblasts, keratinocytes and endothelial cells so that the accelerated proliferation and migration normalizes. Gradually, the provisional collagen (type III) is replaced by the more stable collagen type I that is produced strictly oxygen dependently by fibroblasts and is deposited in a physiological alignment. Thus, the healing wound gains increased wound tensile strength. The collagen fibres contract so that the wound tissue shrinks.[55,56] Prominent mediators of collagen anabolism and catabolism are MMPs, which are released oxygen dependently by macrophages, keratinocytes, endothelial cells and fibroblasts. Of great importance are the TIMPs, which contribute to a concerted maturation process that leads to a *restitutio ad integrum* or a scar formation depending on the MMP/TIMP ratio and activity.

Chronic wound healing

Chronic wounds are defined as wounds that do not follow the well-defined stepwise process of physiological healing but are trapped in an uncoordinated and self-sustaining phase of inflammation (Fig. 1).[3] This impairs the constitution of anatomical and functional integrity in a physiologically appropriate length of time. The aetiology of chronic wounds is diverse, but more than 80% are associated with venous insufficiency, high blood pressure or diabetes mellitus.[3,57] Despite the different underlying aetiology, most chronic wounds show a similar behaviour and progress. This uniformity is due to consistent components of the multifactorial pathogenesis of most chronic wounds: local tissue hypoxia, bacterial colonization, repeated ischaemia-reperfusion injury and cellular as well as systemic changes of ageing (Fig. 2).[1,4,58,59]

Common causes of local wound tissue hypoxia are pathological alterations of the vascular bed (arteriosclerosis, micro- or macroangiopathy, venous hypertension), periwound fibrosis and a subsequent local reduction of tissue perfusion, or oedema, which increases the distance between capillaries (Fig. 2). Local tissue hypoxia has been widely accepted to impair wound healing profoundly. Mathematical models showing the importance of oxygen for physiological wound healing and ischaemic wounds have recently been published.[60,61] Sheffield measured a pO_2 of 5–20 mmHg in chronic wound tissue as compared with 30–50 mmHg in control tissue.[62] Ahn and Mustoe[63] showed a wound healing deceleration of 80% in an ischaemic rabbit ear model evoked by a pO_2 decrease from 40–45 mmHg to 28–30 mmHg. The initial implication of tissue hypoxia on the molecular level is the impairment of mitochondrial oxidative phosphorylation with a subsequently reduced ATP production. As a consequence, ATP-dependent membrane transport proteins such as Na^+/K^+-ATPase or Ca^{++}-ATPase drop out, which leads to a loss of the transmembrane potential with subsequent cell swelling. Particularly intracellular accumulation of calcium ions activates a signal transduction pathway that ends up in cell membrane disruption,[64] which results in a promotion of inflammatory cascades via various signal pathways. Proinflammatory cytokines and chemokines such as TNF and IL-1 are

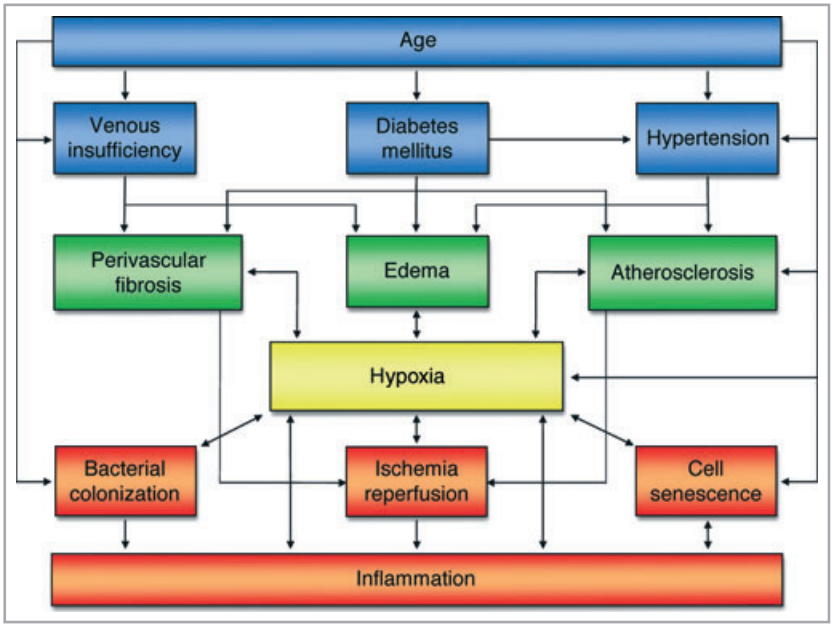

Fig 2. Chronic wound pathogenesis. Schematic representation of the elementary aetiological factors (blue), the resulting morphological correlates (green) and the consequent pathophysiology (red). The reciprocal interference of the pathophysiological factors is shown. These changes perpetuate inflammation in chronic wound pathogenesis.

released, which attracts and activates neutrophils and macrophages.[65] In addition, hypoxia induces a pronounced expression of endothelial adhesion molecules such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and the corresponding ligands leucocyte function-associated antigen-1 and very late antigen-4 that enhance the extravasation and invasion of neutrophils and macrophages into the wound site with a subsequent autocrine synthesis of proinflammatory cytokines such as IL-1α, IL-1β, IL-6 and TNF.[66] Growth factors and cytokines are released in a self-perpetuating manner, macrophages are attracted, and tissue degenerating enzymes, e.g. serine proteases (neutrophil-derived elastases, cathepsin) and MMPs, particularly MMP-8, are generated.[67] Wound fluid of chronic wounds has been demonstrated to show elevated levels of MMPs released from neutrophils (MMP-8) and fibroblasts (MMP-1, MMP-2, MMP-3, MMP-9, MMP-13).[68–70] In physiological wound healing, proteases are inhibited by α_1-antiproteinase, SLPI, α_2-macroglobulin and TIMPs, once all necrotic tissue and debris have been removed. In chronic wound healing, certain proteases (e.g. MMP-8 and MMP-9) exceed their inhibitors, leading to an excessive degradation of growth factors and ECM components such as collagen, fibronectin and vitronectin.[71] The breakdown products further promote inflammatory reaction,[72] changing the inflammation phase from a self-limiting to a self-sustaining process. Accordingly, the activity of neutrophils is long lasting in chronic wounds but is limited to the first 72 h in acute wound healing.[73,74]

Neutrophils and macrophages produce ROS like HO_2^-, HO^- and O^{2-}. As mentioned above, ROS in low concentrations provide signalling and defence against microorganisms and thus play an important role in acute wound healing. A prerequisite for this process is a delicate balance between the amount of oxidants and antioxidants, as high amounts of ROS impair wound healing due to oxidative damage. High amounts of ROS not only damage extracellular structure proteins, lipids and DNA, but also stimulate complex signal transduction pathways, leading to an enhanced expression of MMPs, serine proteases and inflammatory cytokines. The toxic effects of high amounts of ROS were shown by the severe endothelial damage in wounds of mice which lack the ROS-detoxifying enzyme peroxiredoxin-6.[75] The most effective antioxidant is nitric oxide (NO) that is produced by NO synthase in a strictly oxygen-dependent manner.[8] NO not only detoxifies ROS but also switches off nuclear factor-κB, an important transcriptional activator of inflammatory proteins.[76] Lymphocytes that invade wound tissue also activate the oxygen-dependent oxidoreductase thioredoxin as a protective mechanism against oxidative stress.[77] Under oxidative stress, macrophages express the oxygen-dependent haem oxygenase and cysteine transporter to protect themselves against ROS.[78] Thus, nearly all detoxification mechanisms are strictly oxygen dependent. Under hypoxic conditions, as prevalent in chronic wounds, the detoxification process is hindered, leading to a persistent and uncontrolled production of ROS and to a further potentiation of the inflammatory state. The resulting perpetual degradation of wound tissue impairs the maturation of wounds. However, hypoxia promotes wound healing not only by means of enhancing the inflammatory state but also through the impairment of countless other metabolic processes on the molecular, cellular and supracellular level. Siddiqui *et al.*[38] demonstrated *in vitro* that the collagen synthesis rate of human fibroblasts is decelerated under chronic hypoxia. α_1-procollagen was significantly downregulated at the mRNA and at the protein level. Accordingly, Hunt *et al.*[79] showed *in vivo* that fibroblasts were actively proliferating only in wound tissue with a pO_2 level of at least 15 mmHg. Jonsson *et al.*[80] demonstrated this causality in a clinical investigation, in which the amount of deposited collagen was proportional to the pO_2 value present in the respective wound. In another ischaemic rabbit ear model, Wu *et al.*[81] demonstrated significantly

impaired epithelial ingrowth and granulation tissue deposition under ischaemia.

Bacterial colonization, obligatory in all chronic wounds,[82] attracts leucocytes, which results in high levels of proinflammatory cytokines and proteases and therefore directly initiates and maintains the inflammatory cascade. Different authors investigated wound fluids from acute and chronic wounds and showed an increased level of proinflammatory cytokines and proteases as well as a decreased level of growth factors in chronic wounds.[65,83,84] Correspondingly, in healing wounds, a reduction in bacterial counts and markers of inflammation was reported.[85,86] A direct correlation between bacterial colonization and the hypoxic state of the wound was shown in numerous studies. Knighton *et al.*[87,88] compared the wound extent after subcutaneous inoculation of bacteria in hypoxic wounds with wounds in animals treated with oxygen and found an inversely proportional correlation between the wound extent and the wound oxygenation status. Grief *et al.*[89] performed a prospective study on 500 patients with colorectal resection. They compared the wound infection rates in two patient groups who had received 80% vs. 30% oxygen perioperatively and 2 h postoperatively. Wound infection rates were 5·2% (80% oxygen) and 11·2% (30% oxygen), respectively. Other studies showed that even a moderate decrease of the tissue oxygen level significantly increases the risk of infection.[90,91] Correspondingly, in an *in vitro* experiment, neutrophils were shown to lose their bacterial killing capacity at a pO_2 level below 40 mmHg.[24,25]

In the past years, different authors postulated ischaemia-reperfusion injury as an important aetiological factor of chronic wounds.[58,92,93] Patients with impaired circulation due to venous insufficiency, arteriosclerosis, diabetes mellitus etc. suffer cyclic intervals of ischaemia and reperfusion in their lower legs when changing posture (leg elevation or leg dependency). As the leg position is changed repetitively, injury occurs in a self-potentiating cycle.[58,92] Ischaemia in combination with subsequent hypoxia induces a proinflammatory state (see above). With reperfusion, oedema is increased. Moreover, additional neutrophils flood into the wound tissue and transmigrate to the activated endothelium, which further contributes to the inflammatory vicious circle leading to cell death and tissue damage.[94] Besides, reperfusion accounts for partial reoxygenation with a subsequently enhanced production of ROS. In turn, ROS have a deleterious effect on vascular and cellular processes.[95] The impact of repetitive ischaemia-reperfusion injury on wound healing was demonstrated in animal models in which the tissue damage correlated with the number of ischaemia-reperfusion cycles.[93,96] Remarkably, repeated ischaemia-reperfusion cycles seem to be more deleterious for wound healing than prolonged phases of single ischaemia.[93,96]

The fact that ageing cells show reduced cell viability and proliferative capacity, altered patterns of gene expression and decreased response to growth factors is of great importance for our understanding of abnormal healing, as most chronic wounds occur in the elderly (average age over 60 years).[58,92,97,98] Senescent fibroblasts showed an increased generation of MMPs and a decreased release of MMP inhibitors,[99] which could explain the well-documented fact that MMP inhibitor (TIMP-1) and serine protease inhibitor (α_1-antiproteinase, antileucoproteinase SLPI, α_2-macroglobulin) activity is reduced in chronic wound fluids. The healing response in an aged organism is basically and essentially delayed[7] and additional pathogenetic factors such as local tissue hypoxia soon overpower response. Here, tissue hypoxia as a mainstay of chronic wound pathogenesis plays a crucial role because aged cells are significantly more susceptible to hypoxia than young adult cells. In an ischaemic rabbit ear model, aged human fibroblasts showed decelerated migration under stimulation with TGF-β, depression of PDGF receptor β, and decreased TGF-β1 mRNA expression compared with young controls.[81,100,101] Under hypoxic conditions, aged human keratinocytes showed a decelerated motility[101] and a decreased proliferation rate[102] *in vitro* compared with younger cells. Tandara *et al.*[103] demonstrated increased cell death of aged human fibroblasts compared with young adult cells if exposed to oxidant stress plus ischaemia, conditions that are analogous to chronic wounds.

Interestingly, cells of chronic wounds show signs of senescence even independently of a patient's age. Compared with fibroblasts taken from the healthy leg, fibroblasts harvested from the margin and bed of chronic wounds exhibited characteristics of premature senescence.[97,104] Agren *et al.*[105] showed in an *in vitro* setting a significantly decreased proliferative activity of fibroblasts of chronic wounds in comparison with fibroblasts isolated from acute wounds. These observations might be partially explained by the exposure of cells in chronic wounds to stress factors such as chronic inflammation and the respective proinflammatory cytokines (TNF, TGF-β), the presence of ROS,[106] and the aggressive proteolytic milieu caused by bacterial infection and toxins – factors that may accelerate cell senescence.[97] An additional explanation might be that fibroblasts are driven through countless cell divisions to induce wound healing. Due to ongoing stimulation, cells seem to lose their proliferative capacity. This causality could explain the success of wound debridement as this procedure removes senescent cells from the ulcer surface. These data demonstrate that stress factors apparent in chronic wounds, specifically hypoxia, and the resulting premature senescence create a vicious circle that is difficult to break, particularly in elderly patients.

From the clinical point of view, the main surface area of a common nonhealing wound shows extensive fibrin deposition and necrosis due to the prevalent inflammatory state. However, a typical hallmark of chronic wounds is the persistent occurrence of islands of granulation tissue or epithelialization within the inflammatory battlefield. Hunt *et al.*[79] showed that wound areas with actively proliferating fibroblasts were seen only at pO_2 above 15 mmHg. Only rarely are these islands the origin of a structured healing process as, in most cases, they are overwhelmed by inflammation. The conditions in chronic wounds are changing repeatedly, not only temporally but also spatially. Thus, a chronic wound represents an extremely heterogeneous structure.

Therapeutic wound oxygenation

The ability of systemic oxygen therapy as well as topical oxygen therapy (TOT) to improve wound healing and prevent infection is documented in animal models and clinical trials.[107–110] Hyperbaric oxygen therapy (HBOT) delivers 100% O_2 at 2–3 atmospheres of pressure over 60–120 min 5 days a week in a specialized patient chamber. Usually, 10–30 treatments are performed. HBOT has turned out to be an effective tool to increase pO_2 values in wound tissue, and the effects of HBOT on chronic wound healing have been described by a mathematical model.[108,109,111] Sheikh *et al.*[112] demonstrated increased VEGF expression in rats treated with HBOT, and Hopf *et al.*[30] showed a stimulation of neovascularization in hypoxic tissue after HBOT. Moreover, oxygen administration has been shown to increase VEGF mRNA levels in endothelial cells and macrophages and VEGF protein expression in wound fluids *in vivo*.[112–114] Knighton *et al.*[16] reported accelerated vessel growth following supplemental oxygen administration. The transcutaneous pO_2 in wound-surrounding tissue measured during HBOT correlated directly with the improvement in wound healing of chronic wounds.[108,109] In a randomized controlled trial in diabetic patients ($n = 68$) with ulcers of the lower legs, Faglia *et al.*[115] ascertained that treatment with HBOT and standard care vs. standard care alone resulted in a significant lower amputation rate in the HBOT group. Kranke *et al.*[116] assessed the benefits and harms of HBOT for treating chronic ulcers of the lower legs and found that HBOT both significantly reduced the risk of major amputation and improved the chance of healing. Abidia *et al.*[117] evaluated in a double-blind study the role of HBOT in the management of ischaemic lower-extremity ulcers. Patients were given 30 treatments of 100% oxygen vs. air. Healing was achieved in five of eight ulcers in the treatment group compared with one of eight ulcers in the control group. Despite the expense of HBOT, the authors stated reduced total treatment costs for every patient during the study. Another controlled study demonstrated that HBOT did not reduce hospital days of wound patients but the amount of bacterial colonization.[118] Bonomo *et al.*[107,119] showed that HBOT stimulates the release and activity of growth factors and their receptors. Zhao *et al.*[110] measured the amount of epithelial regrowth and granulation tissue production following HBOT alone and in combination with PDGF or TGF-β1 in an ischaemic rabbit ear ulcer model. They demonstrated that HBOT alone increased the production of new granulation tissue. However, the addition of growth factors to HBOT synergistically led to increased healing rates. This supports the clinical experience that, in the majority of cases, the different treatment strategies for chronic wounds are successful only if combined. *In vitro* experiments of Roy *et al.*[53] demonstrated that oxygen triggers the differentiation of fibroblasts to myofibroblasts – a possible explanation for the accelerated healing. It has also been shown *in vitro* that HBOT may increase the susceptibility of certain bacteria to antimicrobial agents.[120] For example, Kenward *et al.*[120] reported that under HBOT the zones of inhibition were reduced in Gram-negative bacterial cultures, whereas for Gram-positive bacteria a mixture of effects was found. It seems that these effects are quite strain specific and may not easily be generalized to all aerobic or anaerobic bacteria. Grief *et al.*[89] reported significantly fewer postoperative infections in patients who had received 80% oxygen compared with patients who had received 30% oxygen during surgery and 2 h afterwards. However, costs of therapy, the risk of systemic oxygen toxicity, and the lack of large evidence-based studies with standardized treatment protocols impede the general acceptance of oxygen therapy as a standard treatment option in wound care.

TOT is characterized by the administration of pure oxygen to the wound area using a portable inflatable device. A major advantage of TOT is its independence of the wound's microcirculation. Other advantages are lower costs, the lower risk of oxygen toxicity, and the possibility of home treatment. Fries *et al.*[33] studied the efficacy of TOT in excisional dermal wounds in pigs. They showed that exposure of open dermal wounds to TOT increases wound tissue pO_2 and, if repetitively applied, accelerates wound closure. Kalliainen *et al.*[121] conducted a retrospective uncontrolled study on 58 wounds in 32 patients given TOT. They documented a complete healing of 38 wounds in 15 patients during TOT and concluded that TOT had no detrimental effects on wounds and showed beneficial indications in promoting wound healing. However, the data currently available have a restricted informative value because of small sample sizes, the inclusion of different wound types and patient ages, additionally applied wound care regimens, nonstandardized treatment protocols, or a poor evaluation of comorbidities. Therefore, additional evidence-based studies with standardized treatment protocols are required to evaluate the efficacy of oxygen therapy.

Oxygen monitoring in wounds

Different methods allow the evaluation of wound tissue oxygenation. Direct and indirect as well as invasive and noninvasive methods must be distinguished, whereas the noninvasive methods are preferable in routine clinical settings. Indirect methods estimate tissue oxygenation only by calculating the relation of oxygenated to nonoxygenated haemoglobin. This calculation is possible as haemoglobin changes its spectroscopic as well as its magnetic properties with its degree of oxygenation. Respective methods (for instance, near-infrared spectroscopy, tissue reflectance photometry, magnetic resonance chemistry, magnetic resonance saturation, blood oxygen-dependent magnetic resonance imaging) are rather imprecise: first, they do not allow the measurement of absolute values; second, the exact penetration depth of light is unknown;[122–124] and third, they are falsified by certain disturbance variables such as other tissue chromophores or global perfusion (for further details see reviews[122,123]). Direct methods allow direct measurements of oxygen or pO_2 values. Tissue oxygen-dependent magnetic resonance imaging is based on the paramagnetic properties of oxygen in tissue. However, this technique is not applicable at the skin surface as the skin–air interface evokes a severe artifact.[124] The polarographic electrode technique allows the measurement of pO_2 and is still

the gold standard for assessing tissue oxygenation. This technique is usually applied using a planar electrode for surface measurements or a needle electrode for measurements within the tissue.[124,125] An alternative application method for measurements in wound tissue is to place the polarographic electrode in a subcutaneously implanted tonometer or to implant the polarographic electrode directly into subcutaneous tissue.[80,90,126] However, placing an electrode into tissue (i) is invasive and painful, (ii) causes tissue injury that alters microcirculation and thus pO_2 levels, and (iii) may lead to irritation at the electrode membrane. A further fundamental limitation of the polarographic technique is the oxygen consumption during measurement, which makes long-term measurements impossible and overestimates pO_2 values as the electrode 'sucks' oxygen through the tissue.[127] Besides, this technique provides only scattered single measurements, making multiple measurements necessary. The required calibration before and after each measurement and the lacking spatial resolution further limit the application of the polarographic electrode technique.[124,128] Therefore, the available methods suffer from disadvantages when measuring pO_2 levels in tissue. Because of these disadvantages, only a few studies exist on tissue oxygenation in acute wounds and hardly any study on tissue oxygenation in chronic wounds.

The use of luminescence lifetime imaging (LLI) overcomes these limitations.[127–130] This method for two-dimensional pO_2 measurements is based on the oxygen-dependent quenching of phosphorescence of the indicator platinum(II)-octaethyl-porphyrin. Hereby, the indicator is immobilized in a polystyrene matrix as a transparent planar sensor. This method was validated *in vitro* and *in vivo*, as well as in clinical settings.[127–129,131] This body of work has characterized this method as particularly suitable for surface measurements. First, sensors are transparent, allowing a simultaneous visualization of the underlying wound tissue. Second, sensor sensitivity remains stable during measurement because alterations due to ageing, moisture, toxic cell products, local enzymes or photobleaching as a result of exposure to light could be excluded in calibration sequences over 8 days both *in vivo* and *in vitro*.[128,130] Third, pO_2 levels can be visualized in two dimensions with a high resolution (approximately 25 μm) over large areas,[128,131] thus allowing the simultaneous visualization of pO_2 gradients in different skin conditions. This fact is of fundamental relevance in heterogeneously oxygenated tissues such as chronic wounds as the heterogeneity can be registered simultaneously in a single measurement. Single point measurements as provided by the Clark electrode are unable to provide oxygen gradients. Fourth, *in vitro* experiments documented a high sensitivity over a broad pO_2 range (±0·2 mmHg at 0 mmHg; ±1·5 mmHg at 160 mmHg pO_2).[128] The Clark electrode provides a sensitivity of at least ±10%. Fifth, in clinical investigations, accurate and reproducible pO_2 values were provided under changing microcirculatory conditions. The lack of oxygen consumption during measurement allowed both a more realistic estimation of pO_2 values compared with the gold standard and the permanent use in regions with critical oxygen supply.[127] Sixth, this noninvasive and rapid technique is simple to perform and prevents patient discomfort. Zhang *et al.*[132] recently published a dual-emissive material for luminescence imaging of pO_2 in tumours, which may also be modified for use in two-dimensional wound oximetry.

Because of the great interest in new technologies that advance research on the role of oxygen in wound healing, Hopf and Rollins listed attributes of an ideal wound oximeter: noninvasive; repeatable; simple to use; stable for at least 24 h *in vivo*; not disturbed by motion, pH and CO_2; provides accurate, precise and easily interpretable results in the range from 0 to ≥300 mmHg. Furthermore, such an oximeter should enable continuous long-term measurements, require just a single point calibration at room conditions *in vivo*, and simultaneously measure oxygen and temperature at the same site.[5] All these requirements are fulfilled by LLI.

Conclusions

Oxygen is well known to be required for wound healing. The K_m for enzymes involved in bacterial killing, collagen synthesis, angiogenesis and epithelialization requires pO_2 levels in wound tissue ranging from 25 to 100 mmHg.[9,23,37] Therefore, restricted oxygenation, as common in chronic wounds, impairs the healing process. Several studies have demonstrated that enhancing wound tissue oxygenation improves wound healing and reduces bacterial colonization. Further research should establish LLI as a new, promising tool for wound oximetry. This will probably enhance our understanding of the pathophysiology of chronic wound healing and of oxygen delivery and metabolism in the different healing zones of chronic wounds. Of special interest is monitoring the wound oxygenation under different therapeutic regimens to enable the well-founded selection of a suitable and efficient therapeutic modality for the individual patient. This will contribute to cost-effectiveness and hopefully will improve the quality of life of the affected patients.

What's already known about this topic?

- Oxygen is a prerequisite for wound healing due to the increased demand for reparative processes such as cell proliferation, bacterial defence, angiogenesis and collagen synthesis.
- Wound healing is impaired under hypoxia and topical oxygen therapy is known to affect healing.

What does this study add?

- We present an up-to-date overview on the role of oxygen in wound healing and chronic wound pathogenesis, a brief insight into the impact of systemic and topical oxygen treatment, and a discussion of the role of wound tissue oximetry.

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