CHAPTER 8

Antioxidant Carriers

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8.1 Introduction: The Essential Role of Oxygen in Wound Healing

Wound healing is a restorative response that takes place following any surgical procedures or traumatic injuries. The principles of wound healing, therefore, play a role in surgical practice in all specialties. With wound healing being an ever-present aspect of surgical practice, it makes sense that every surgeon will inevitably take care of patients with problematic wounds. In detail, a problematic wound manifests itself as a compromised wound, and this can represent a convergence of several factors that divert the wound from its normal healing course. Identifying these impediments to wound healing in parallel with the development of an appropriate treatment plan for the situation is the initial step in achieving complete wound healing. One of the main factors limiting healing is hypoxemia, caused by the interruption of vascularity.[1] In fact, the central area of the wound is more hypoxic, with a progressive increase in the oxygen gradient (O_2) the closer you get to the uninjured tissue at the periphery. The blood oxidation index (pO_2), in the case of cutaneous wounds varies from 0 to 10 mm Hg centrally, to 60 mm Hg at the periphery; in the case of arterial blood, on the other hand, we find a pO_2 of about 100 mm Hg.

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The first clinical use of oxygen to improve the healing process was in the 1960s with the administration of systemic hyperbaric oxygen, through a systemic therapy that is implemented through the breathing of pure oxygen inside a hyperbaric chamber brought to a pressure higher than atmospheric. Despite the conditions (*e.g.*, pressure, O_2 concentration, frequency and duration of administration) for systemic hyperbaric oxygen therapy (HBOT) not yet being optimized based on analyses of randomized clinical trials, HBOT stands as a therapeutic modality used for wounds with an excellent success rate, as well as being approved by the Food and Drug Administration (FDA).

The general acceptance of oxygen therapy has been very limited by empirical dependence and the scarce amount of data available that can meet the highest criteria for medicine. Oxygen therapy has therefore not been evaluated as a standard modality in wound care. However, considering this therapy as valid does not only depend on a favorable clinical outcome but also on a stringent mechanical analysis, which must be able to explain the experimental results. In any case, the correction of wound hypoxia is recognized as necessary to ensure a sufficient amount of oxygen in order to support the growth of regenerating tissues.

8.2 Reactive Derivatives of Oxygen: New Horizons for Wound Healing

The search for the mechanisms through which oxygen is able to perform its vital functions in the area of wound healing has taken an important step[2] by making room for a new paradigm.[3] It was recently discovered that not only phagocytes but every type of cell present in the microenvironment of the wound has an enzyme to convert oxygen into reactive oxygen species (ROS), including those that are oxidizing species, such as free radicals and H_2O_2. The role of these ROS is to act as cellular messengers and promote processes that support wound healing. In this type of redox-sensitive processes we also consider the action of cytokines, angiogenesis, cell motility and the formation of the extracellular matrix. This evolution of these systems differs greatly from the classical view, according to which ROS are inherently harmful in nature. In fact, now a more precise view can be formulated, which illustrates that at low concentrations ROS act as a signaling mediator capable of modulating a wide variety of cellular responses.[4–9]

8.2.1 Roles of ROS in Wound Healing

Oxygen plays a fundamental role in wound healing processes, such as oxidative bacterial killing, collagen synthesis, angiogenesis and epithelialization; therefore, the wound healing process is compromised in the case of hypoxia.[10,11] However, the precise role of oxygen in wound healing is still not fully understood. While oxygen is used for energy through oxidative phosphorylation, ROS are produced and cause oxidative injury. Cells in aerobic

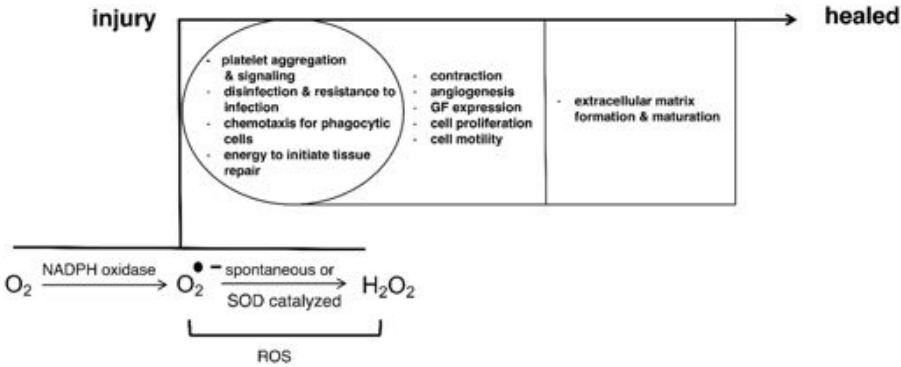

Figure 8.1 Processes associated with wound healing by molecular oxygen and its reactive derivatives. GF, growth factor.

organisms constantly produce ROS during normal metabolic processes, and their production is high under pathological conditions. Although ROS support a variety of physiological processes, they often exert life-threatening damage. In the wound healing process, the physiological role and molecular mechanisms of the reactions involving ROS in individual problems have been investigated.[12–14] In particular, the pathological role of ROS in the inflammatory phase is well understood. The ROS generated directly attack invading pathogens and ultimately kill them to promote phagocytosis. However, excessively produced superoxide damages surrounding tissues. Superoxide is dismantled into hydrogen peroxide (H_2O_2) and molecular oxygen by superoxide dismutase (SOD) or by a spontaneous reaction. H_2O_2 is detoxified by peroxidases, such as catalase, glutathione peroxidase (GPX) and peroxiredoxin (PRDX), to avoid the Fenton reaction that occurs in the presence of transition metal ions, such as iron or copper, and generates hydroxyl radicals, the most harmful ROS (Figure 8.1).

Compared with immune cells, ROS are produced by other cells at much lower levels. Low ROS levels play a physiological role, particularly in cell signaling in response to stimuli.[15,16] It has been noted that an excess of ROS signaling, by creating an unbalanced redox homeostasis, would lead to impaired wound healing. Typical causes of delayed wound healing can be diabetes, aging, immunodeficiency and malnutrition. In these specific cases of complicated pathological conditions, redox imbalance occurs and high oxidative damage is observed.[17]

8.2.2 Reactive Derivatives of Oxygen: Oxygen as an Antibiotic

Blood oxygenation levels (pO_2) in wound tissue are an important factor in assessing susceptibility to infections, this concept has been demonstrated in both experimental models and human subjects. In detail, in a guinea pig model, the amount of skin loss that could be observed after the subcutaneous

inoculation of bacteria was inversely proportional to the oxygenation of the wound. Indeed, hypoxic wounds were large, while smaller wounds were observed in animals receiving supplemental oxygen. In practice, the effectiveness of this supplemental oxygen was similar in preventing skin loss to the administration of antibiotics. The combination of supplemental oxygen and antibiotic action resulted in additive beneficial effects.[18] Obviously, all these experimental observations are supported by clinical studies. Furthermore, it has also been observed that the oxygenation of the wound tissue is an extremely sensitive indicator of the risk of infection in surgical patients.[19] In particular, therefore, this study confirmed a clear clinical correlation between oxygenation at the wound site and the development of wound infection.

8.2.3 Reactive Derivatives of Oxygen: Diagnostic, Preventive and Therapeutic

The availability of oxygen in wound tissues depends on several factors: (i) the vascular supply, (ii) the vasomotor tone, (iii) the arterial pO_2 and (iv) the diffusion distance of molecular oxygen. The diffusion distance of oxygen from the wound in particular is increased by edema and necrotic debris, so debridement is an important step to reduce the obstacles to oxygenation of the wound. In addition, peripheral vasoconstriction can also act as an obstacle to oxygenation of the wound, thus leading to little or no improvement in the wound's pO_2 levels, despite any additional oxygen.[20,21]

It is also important to report that the correction of hypoxemia and vasoconstriction are able to increase collagen deposition by up to ten times.[22,23] It goes without saying that in order to obtain suitable and optimal perfusion and oxygenation of the wound, the following conditions must exist: warm patients with adequate intravascular volume and suitable pain and anxiety control. With respect to the estimation of the intravascular volume, to which tissue oxygenation is extremely sensitive, it is an unreliable method in this case. The production of urine is not a reliable parameter of the intravascular volume and even the standard maintenance fluids that are administered after surgery are insufficient.[24] Capillary filling (1.5 seconds on the forehead) and ocular turgor, on the other hand, are more precise and sensitive indicators of the state of the intravascular volume. Results from several clinical studies have also indicated that keeping patients normothermic and administering supplemental oxygen would improve wound oxygenation, thus reducing the rate of wound infection in surgical patients and simultaneously reducing the average length of hospital stay.[25,26] Oxygen is clinically applied to wound healing at different levels: (i) diagnostic, (ii) preventive and (iii) therapeutic. Considering the first level, several surgeons already use wound oxygenation measurements to plan treatment when obtaining transcutaneous oxygen (TcO_2) measurements with non-invasive vascular studies. These TcO_2 measurements are able to provide relevant and reliable prognostic information on what the ability to heal is. Significant examples of this method are those

involving the determination of amputation levels.[27,28] However, it is important to specify that TcO_2 measurements are not able to assess the pO_2 of the wound site, in fact this fact is often overestimated in the perimeter of the intact wound tissue. These measurements are then made under conditions where the skin has to be heated to 42 °C. The reason for this required condition is the fact that heat contributes to the overestimation of pO_2, not least because oxygen therapy on the wound, in most cases, is not supported by heating the wound site. To avoid errors of excessive overestimation, technological progress requires a direct estimate of the pO_2 in the wound core. However, there is a substantial difference between the intact skin of the wound perimeter and the core of the wound itself. In the first case there is vascularization, while in the second case the wound nuclei are generally characterized by interrupted vascularization, which makes it unlikely there will be benefit from the breathed oxygen transported to the tissues by the blood vessels.

There are different methods of application of therapeutic oxygen to wounds, oxygen can be administered to the patient systemically, using pure oxygen (pressurized or not), or it can be administered locally on the wound using a topical device. Hyperbaric oxygen therapy (HBOT), already mentioned, is capable of delivering 100% oxygen at 2 to 3 atmospheres (atm) of pressure and, depending on the diagnosis, patients receive 10 to 30 treatments. These treatments last about 60 to 120 minutes, are performed in specialized rooms with medical supervision and are generally administered 5 days a week. This type of HBOT treatment is capable of increasing arterial pO_2 up to 1200 mm Hg. As previously reported, the administration of oxygen systemically is based on the vascularization to be delivered to the tissues, so although an effective improvement can be observed for the skin in the perimeter of the wound, in the case of areas of the wound not supported by blood vessels it is suggested that the benefit will be minor, if not zero. In the case of HBOT application in a single-seat chamber, the exposed skin wound also receives topical oxygen. This additional path of oxygen delivery to the wound is not always considered, thus leading to the justification of any benefits based only on oxygen administered systemically. Although it is true that topical oxygen is not able to diffuse to the deeper tissues, it still has the advantageous potential of oxygenating the superficial areas of the wound that are not characterized by intact vascularization. So topical oxygen is able to correct the pO_2 of the cells present in the wound center, while also correcting the impairment induced by hypoxia in those cells. A further note to be addressed in the comparison between the effects of systemic and topical oxygen is the risk of systemic toxicity of pure oxygen for vital organs. In the case of HBOT there is no immediate manifestation of clinical abnormalities, as in the case of many other risk factors. However, it is known that exposure of cells and biological tissues to pure oxygen is capable of causing oxidative stress and genotoxicity.[29] It is therefore clear that exposure to pure oxygen should be avoided if it is not strictly necessary. In this regard, we can consider the favorable results of cases in which subpure oxygen is used

Table 8.1 Comparison between systemic hyperbaric oxygenation and topical delivery of oxygen.

Systemic hyperbaric oxygenation	Topical delivery of oxygen
Systemically oxygenates blood at 2–3 atmospheres	Topically oxygenates wound tissue at 1 atmosphere
Requires specialized facilities and personnel	Portable devices: available bedside and in the field
Relatively expensive	Inexpensive
Relies on vascular system to deliver O_2 to wound	Can deliver oxygen directly to superficial wounded tissue severed from circulation
Poor vascularity of wound tissue limits O_2 diffusion	Oxygenation not dependent on vascular bed
Risk of multiorgan oxygen toxicity	No risk of multi-organ oxygen toxicity
Relatively well-studied for outcome, limited studies addressing underlying mechanisms	More limited research literature on outcome and mechanism

under normobaric conditions,[25] which lead us to doubt the use of pure oxygen under pressure for wound therapy.

Other encouraging results were obtained from the use of topical oxygen alone,[27] which would justify a more detailed investigation by comparing the systemic and topical modes of oxygen administration, under normobaric and hyperbaric conditions. Optimizing the conditions for oxygen therapy should therefore result in more convenient and efficient treatments, with the aim of reducing as much as possible the various risks associated with the use of pure pressurized oxygen. If optimization proves effective, topical oxygen therapy, compared with systemic therapy, has the further advantage of being applicable to much larger population of potential patients (Table 8.1).

8.2.4 Reactive Derivatives of Oxygen: Synthesis

Although ROS can be produced from reactions catalyzed by different cytoplasmic enzymes (such as xanthine, oxidase, lipoxygenase, cyclo-oxygenase and nitric oxide synthase), most phagocytic ROS derive from the superoxide generated by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. NADPH is a nucleotide generally used in the oxidation reactions of metabolism, *i.e.* in catabolic processes. This type of membrane-bound multimeric electron transfer complex is the main component of ROS formation in phagocytes and changes its intercellular components several times during the activation of leukocytes. It should be noted that this NADPH oxidase complex is not specific to phagocytes, and different types of mammalian cells that develop it in various forms. In fact, tissue-specific NAPDH oxidase reactions differ on the basis of their NADPH oxidase (NOX)/dual oxidase (DUOX) isoform and there are different possible tissue distributions of this. It is therefore fascinating to note how a single cell can have multiple NADPH complexes containing, in turn, different NOX components.[30] The production

of superoxide from NADPH oxidase takes place through a two-step process. When an electron is transferred from NADPH to flavin adenine dinucleotide (FAD) the initiation reaction occurs. At this point the electron is transferred to be able to interact with molecular oxygen to produce superoxide. The reaction can be described as:

$$NADPH + 2O_2 \rightarrow NADP^+ + 2O_2^- + H^+$$

8.2.5 Reactive Derivatives of Oxygen: Measuring ROS Production

The measurement of cellular ROS production, characterized by ROS that induce the excitation or spectroscopic shift of one or more reporter molecules, can be carried out using various techniques. On the basis of this it is easy to understand how this manufacturing process can be quantified by luminometry, flow cytometry or microscopy. In addition, ROS signaling can be customized to study the role of particular oxidizing species in a cellular response following the use of probes with specific interactions. In particular, these probes exploit target oxidants or scavenger molecules such as formate (OH), deferoxamine (Fe^{2+}), diethyldithiocarbamic acid (SOD inhibitor) and salicylhydroxamic acid (myeloperoxidase inhibitor).

8.2.6 Reactive Derivatives of Oxygen: Wound Healing

Wound healing is identified as a complex cellular and biochemical event that requires the union of different cells to synergize and remodel compromised tissues. In the specific field of wound healing there are numerous cell types, and each of these possesses reciprocal signals, in endocrine and paracrine modalities, capable of ensuring a return to normal homeostasis of the damaged site. Ideal wound healing requires a specific coexistence of the constructive and destructive roles of cells and ROS, simultaneously used for tissue remodeling and eradication of infectious organisms and non-autonomous material.

White blood cells, or leukocytes, are characterized by a large number of systems for influencing the healing of a tissue. The most considered are the family of interleukin proteins, which express the main language with which the cells inside the wound communicate during the healing process. The interleukins are capable of various functions: (i) mediating the chemotaxis of leukocytes and (ii) controlling the development and specificity of memory inflammation. Beyond these signaling capabilities, these proteins secreted by leukocytes possess destructive properties. In particular, when these are in the form of enzymes, they are able to degrade or reduce the integrity of the extracellular bacterial matrices (perforin, defensins). As in the case of proteins, ROS are also able to externalize both the signaling and the defense function of the host. ROS, always parallel to proteins, are also capable of creating significant damage to tissues that otherwise would have been healthy,

so it is very important also in this case to formulate an effective balance between the antimicrobial and degenerative effects of the ROS tissues of phagocytes. In this regard, it is precisely this possible tissue damage mediated by ROS that significantly affects aging and could create dependence on ROS themselves during inflammation as aging increases.[31] Chronic wounds are generally characterized by the excessive presence of ROS or the absence of antioxidant ROS scavenger molecules (vitamins E, C and glutathione). It can also be noted that the levels of antioxidants in wounds tend to decrease as we age, and this explains the delayed wound healing responses in the case of elderly patients. It is therefore possible to consider the phenomenon of wound healing being reduced or delayed by the lower concentrations of antioxidants. This leads to the possibility of the ROS reaction of the wound proceeding in an uncontrolled manner, progressively aggravating the tissue damage.[32] Examples of this behavior have been found in tissues such as the brain, demonstrating, in detail, a greater synthesis of ROS in the absence of vitamin C.[33] The use of antioxidants to improve the healing of chronic wounds in combination with hyperbaric oxygen therapy has also been shown to be beneficial.[34] The proliferation, matrix deposition and potential differentiation of fibroblasts play an important role in the sealing operation of a wound through the secretion of collagen and other proteins of the extracellular matrix and are also influenced by ROS.[35]

8.3 Enzymatic and Non-enzymatic Antioxidants

Antioxidants can be divided into two groups: enzymatic (endogenous) and non-enzymatic (exogenous). The first group is composed of antioxidants produced by the body for defense against free radicals, the second group instead involves antioxidants introduced into the body from the outside. The first group of physiological antioxidants include the ROS-detoxifying enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX) and peroxiredoxins (PRDX).[36] Generally low molecular weight antioxidants, such as glutathione, vitamins E and C and phenolic compounds, are non-enzymatic defenders for the control of ROS. The operating mechanism of this group of low molecular weight compounds is to sacrifice themselves by being oxidized and thus becoming radicals, less reactive, and therefore less harmful, than the radicals that are eliminated.[37] The antioxidants then work together, in a continuous chain reaction mechanism, to regenerate the low molecular weight antioxidants that have now become free radicals. In this way it is possible to always guarantee new reduced forms for cellular defense.[38] Very low levels of antioxidants have indeed been associated with slow and poor wound healing.[36] Antioxidants, therefore, appear to be the main host defense produced in response to the production of reactive oxygen species. The following sections will initially address the functions in the wound healing process of enzymatic antioxidants and then the work will focus on non-enzymatic antioxidants.

8.4 The Role of Enzymatic Antioxidants in Wound Healing

The most important of the enzyme group are SOD, GPX, PRDX and catalase. Only the first three will be analyzed, as catalase does not appear to be a particularly necessary antioxidant compound in the healing process. In fact, this enzyme, which is found uniquely in peroxisomes, has the main function of catalyzing the dismutation of H_2O_2 into an oxygen and a water molecule.

8.4.1 Enzymes With Antioxidant Activity: Superoxide Dismutase (SOD)

The primary ROS generated by molecular oxygen are the superoxide anions (Figure 8.2).[39] If these superoxide anions are found in the vicinity of nitric oxide (NO), produced by nitric oxide synthase, they react with it, causing the generation of peroxynitrite.

This compound is a strong and toxic oxidizing agent but is also used for the oxidative killing of bacteria for the protection of wounds. The superoxide anion produced in excess, to avoid harmful reactions, is rapidly dismantled into H_2O_2 by SOD. SOD is a family consisting of: (i) SOD1, which is found in the cytoplasm and intermembrane space of mitochondria, (ii) SOD2, which is found in the matrix of mitochondria, and (iii) SOD3 which is found in the extracellular space. The latter constitutes the primary defense system against oxidative stress in the corresponding subcellular positions (Figure 8.3).

As the skin is continually exposed to oxygen toxicity, SOD has aroused much interest from a wound healing perspective,[40–42] and, therefore, the question arises as to whether SOD activity is necessary for the healing of wounds. The results presented in the literature,[42–44] provide us with information on the role that SOD1 plays not only in wound care but also in the construction of new skin layers. Furthermore, the results of other studies[45–47] indicate that SOD is effectively useful in improving the wound healing rate.

8.4.2 Enzymes With Antioxidant Activity: Glutathione Peroxidase (GPX)

GPX is classified as a member of a family of proteins capable of reducing H_2O_2 and a series of organic peroxides in a glutathione-dependent manner. There are eight different genetic products in the GPX family (GPX1–GPX8) in humans. The first four (GPX1–4) are selenoproteins containing a selenocysteine residue (SeCys) in the catalytic center, while GPX6 is a selenoprotein expressed only in humans.[48] Since GPX requires the presence of glutathione as an electron donor, it is likely that a decrease in glutathione levels in a wound lesion suppresses GPX activity.[49] Furthermore, while the alkylation or nitric oxide-dependent oxidation of SeCys reduces GPX activity,[50] a major oxidative stress, such as an excess of H_2O_2 or a deficiency of SOD1, causes the

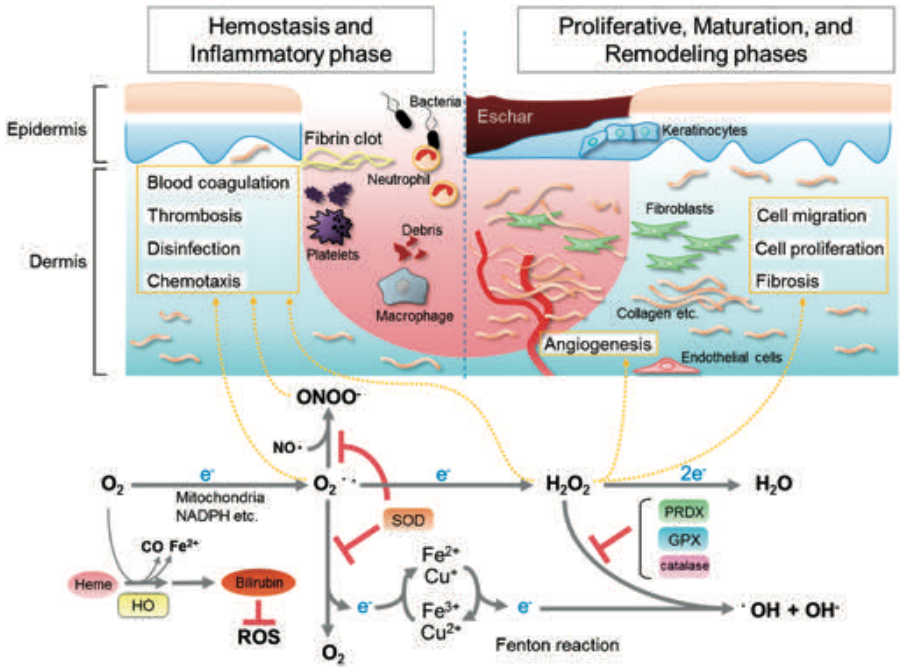

Figure 8.2 Superoxide is dismutated into hydrogen peroxide (H_2O_2) and an oxygen molecule by superoxide dismutase (SOD), in this way the generation of highly deleterious ROS is avoided. Reproduced from ref. 39, https://doi.org/10.3390/jdb3020057, under the terms of the CC BY 4.0 license, http://creativecommons.org/licenses/by/4.0/.

conversion of SeCys to dehydroalanine, which results in irreversible inactivation and degradation.[51] Therefore, the SeCys biosynthesis processes, using selenium, and the translational incorporation of SeCys are essential for the production of some GPX products.[52] In detail, the availability of selenium limits the presence of the GPX1 protein. In other words, therefore, selenium can function independently of the formation of GPX proteins and their activity and could be applicable for therapeutic purposes in the treatment of wound healing.[53,54]

8.4.3 Enzymes With Antioxidant Activity: Peroxiredoxins (PRDX)

PRDX are a family of enzymes that catalyze the reduction of H_2O_2, different types of organic peroxides and peroxynitrite using thioredoxin as an electron donor.[55] Six components of the PRDX family of proteins can be considered, which show differential localization in tissues and cells and are able to accelerate wound healing and it is possible to uniquely assign individual functions to each skin component.[56] To date, PRDX are considered the

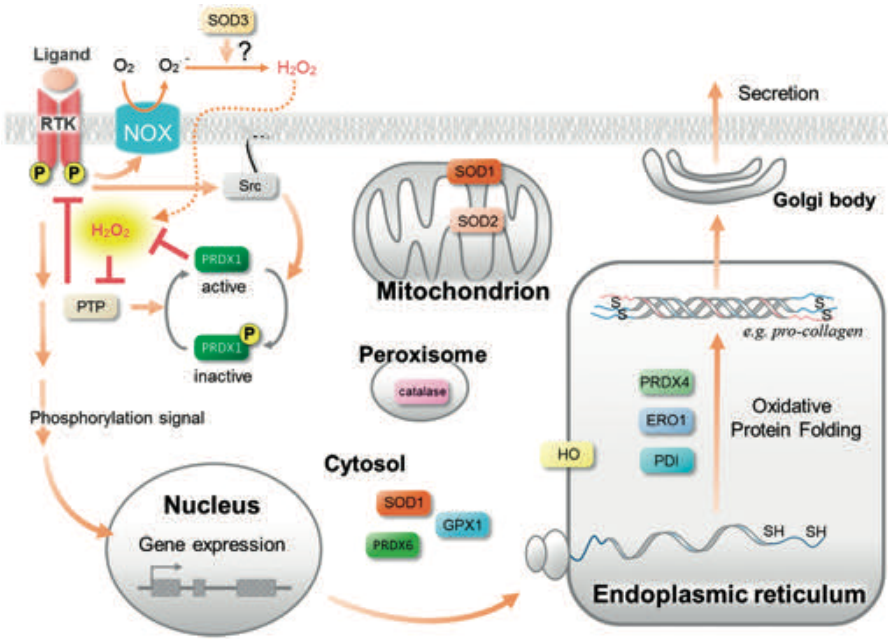

Figure 8.3 Antioxidative enzymes together with their isoforms constitute a cellular defense system against oxidative stress at the corresponding subcellular positions. They also permit, simultaneously, the low concentrations of ROS to equilibrate, serving as signaling pathways to regulate the wound healing processes. Abbreviations: RTK, receptor tyrosine kinase; PTP, protein tyrosine phosphatase; Src, Rous sarcoma virus oncogene product homolog; ERO1, endoplasmic reticulum oxidase 1; PDI, protein disulfide isomerase. Reproduced from ref. 39, https://doi.org/10.3390/jdb3020057, under the terms of the CC BY 4.0 license, http://creativecommons.org/licenses/by/4.0/.

predominant enzymes for the reduction of peroxides, more effective than GPX and catalase.[57,58] Considering the various compounds we can identify: (i) PRDX1, mainly localized in the cytosol and nucleus, (ii) PRDX4, unique in possessing a hydrophobic N-terminal signal peptide that leads to its secretion from the cells and to its predominant localization in the endoplasmic reticulum (ER) (Figure 8.3), (iii) PRDX6, a monomeric enzyme, belonging to the atypical class 1-Cys PRDX, showing a more divergent structure.

8.5 The Role of Non-enzymatic Antioxidants in Wound Healing

Since the purpose of this chapter is to cover, as far as possible, the entire spectrum of non-enzymatic antioxidant compounds for wound healing, studies evaluating the effects or mechanisms of antioxidants in the healing process were also considered.[59] These studies considered will be divided according

Table 8.2 Antioxidant compounds.

Compound	Type	Chemical properties	Biological properties	Origin
Curcumin	Polyphenolic	Non-water-soluble	Anti-inflammatory, antibacterial and antioxidant	Vegetal, *Curcuma longa* rhizome
Chitosan	Polysaccharide	Water-soluble	Highly biocompatible hemostatic, antibacterial and antioxidant	Animal, exoskeleton from crustaceans
N-acetyl cysteine (NAC)	Sulfhydryl	Water-soluble	Precursor in the formation of glutathione, antioxidant	Modified form of the amino acid L-cysteine
Gallic acid	Polyphenol	Soluble in alcohol, ether, acetone	Antioxidant, anti-inflammatory, analgesic	Vegetables, fruits, leaves and wildflowers
Edaravone	3-Methyl-1-phenyl-2-pyrazolin-5-one	Soluble in hot water and hot alcohol	Free radical scavenger, antioxidant	Chemical synthesis
Crocin and safranal	Carotenoid	Lipophilic	Antioxidant, anti-inflammatory, antitumoral	Vegetal from saffron crocus
Quercetin	Flavonoid	Non-water-soluble	Antioxidant, anti-inflammatory	Vegetables and fruits

to the type of compound with antioxidant activity considered. The following compounds with antioxidant activity were identified: (1) Vitamin E, (2) Vitamin C, (3) *N*-acetylcysteine (NAC), (4) curcumin, (5) chitosan, (6) gallic acid, (7) edaravone, (8) crocin, (9) safranal and (10) quercetin. They were mainly chosen for their antioxidant activity and are shown listed in Table 8.2 and some of their structures are shown in Figure 8.4.

8.5.1 Compounds With Antioxidant Activity: Vitamin E

Two subfamilies of vitamin E can be considered, tocopherols and tocotrienols, with the latter being classified as more powerful antioxidants. Several studies have evaluated the role of these two vitamin E families in wound healing, considering mainly the properties of tocotrienols.[60] Vitamin E has also been studied in a topical application for the treatment of surgical incision wounds in children.[61] The main comparison was made between treatment with vitamin E and treatment with petroleum jelly, considered as a control. The study result was promising, with reduced scar formation during wound healing. However, it is necessary to specify that this study of this type of treatment did not have adequate controls, since the topical formulation

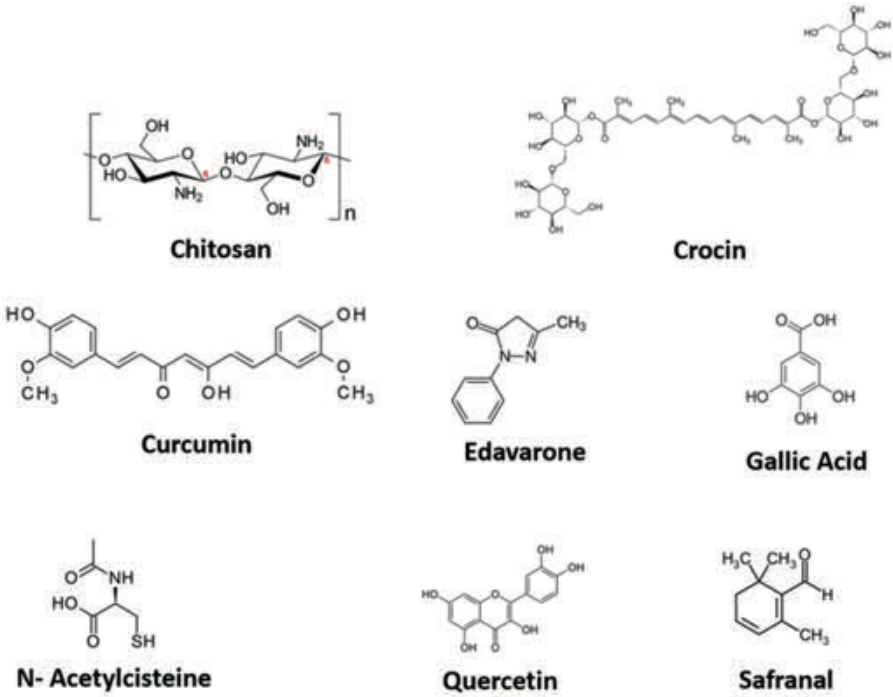

Figure 8.4 Chemical structures of different compounds with antioxidant activity.

of vitamin E also in this case contained castor oil and palmitate to accelerate the wound healing process. Despite vitamin E being a promising option, recent results still fail to be convincing that topical vitamin E is useful for treating wounds.

8.5.2 Compounds With Antioxidant Activity: Vitamin C (Ascorbic Acid)

The choice to consider vitamin C stems from its role in collagen formation. The notoriety of this vitamin stems from its role in scorbutus (scurvy), where a dietary deficiency leads to a lack of structural integrity of the blood vessels and consequent bleeding. At the molecular level, vitamin C is then a fundamental part of the hydroxylation of the two amino acids, proline and lysine. These two products are the ones that will contribute to the stabilization of the triple helix structure of collagen. Despite the correlation between vitamin C deficiency and poor wound healing, the beneficial role of vitamin C in the treatment and healing of wounds has not been demonstrated. Two studies that examined the integration of vitamin C in humans can be considered. In the first case a controlled group of hospitalized surgical patients with pressure sores was studied,[62] in the second this was repeated in a randomized multi-center study.[63] It was not possible to detect a difference, evaluated as a

percentage change in the wound surface area and as a healing rate in centimeters per week, in wound healing between the two groups. The fact of having this difference in the studies considered makes it difficult to formulate a recommendation on the evidence of the effects of vitamin C supplementation on wound healing. It remains to be demonstrated that supplementation, at a low or high dose, can improve healing.

8.5.3 Compounds With Antioxidant Activity: *N*-acetylcysteine (NAC)

N-acetylcysteine (NAC) is a sulfhydryl compound that acts as a precursor for the formation of glutathione, which has a relevant antioxidant activity. The fundamental role of NAC is that of regulating the redox state in tissues, reducing oxidative stress through the transformation of ROS produced by macrophages, endothelial cells and fibroblasts.[64,65] The first study that deserves a mention in considering *N*-acetyl cysteine is one in which the topical and systemic effects of the antioxidant component were evaluated by applying it to damaged wounds.[64] The application of NAC caused a reduction in oxidative stress parameters thanks to its antioxidant properties, but additional studies are needed to fully understand the mechanisms of NAC in healing. In another study,[66] NAC was used to treat burn wounds so that the mechanisms of action could be studied in greater depth. Wounds treated with NAC were found to exhibit better re-epithelialization characteristics. These results more precisely demonstrate that NAC has the potential to promote wound healing activity and thus stands as a promising drug for accelerating burn wound healing. To summarize, positive effects of NAC on wound healing have been shown in several studies; but it is important to specify that further studies are still needed to evaluate the actual clinical benefits in order to actually consider the use of NAC as a standard treatment in wound healing.

8.5.4 Compounds With Antioxidant Activity: Curcumin

Curcumin occurs as a natural polyphenolic molecule that is extracted from the rhizome of *Curcuma longa*. This compound has important functions, including anti-inflammatory, antibacterial and antioxidant properties. Curcumin affects several states during the healing process: the formation of granulation tissue, the deposition of collagen, the remodeling of the tissues and the contraction of wounds.[67] On the other hand, however, curcumin has a low solubility in water and this represents an important barrier to therapeutic use, since it limits bioavailability. Therefore, the development of suitable vectors is required for this compound to release the molecule in a manner compatible with therapeutic levels, thus improving its bioavailability. Examples of carriers are hydrogels, nanoparticles, micelles and hyaluronic or oleic acid.[68,69]

8.5.5 Compounds With Antioxidant Activity: Chitosan

D-Glucosamine and *N*-acetyl-D-glucosamine derived from chitin (present in the exoskeleton of crustaceans), make up the linear polysaccharide called chitosan. Chitosan has significant properties including biocompatibility and biodegradability.[70,71] In particular, this compound acts as a hemostatic agent, thanks to the bond it creates with the surface of the platelets, as an antibacterial agent and as a bioadhesive material, which could make it a promising option in the field of wound dressings.[72–74]

8.5.6 Compounds With Antioxidant Activity: Gallic Acid

Gallic acid is one of the natural polyphenolic compounds found in almost all plants. Attention has turned to this compound thanks to its important biological effects, such as its antioxidant, anti-inflammatory and analgesic action.[75,76]

8.5.7 Compounds With Antioxidant Activity: Edaravone

Edaravone, 3-methyl-1-phenyl-2-pyrazolin-5-one, is a compound with the function of a free radical scavenger capable of suppressing the effects of oxidative stress. In particular, its antioxidant function is used to treat cases of acute cerebral infarction since this molecule positively influences the cerebral blood flow, thus suppressing delayed neuronal death and thus significantly improving the main neurological deficits. However, it should be noted that its low stability and solubility limit its topical applications.[77,78]

8.5.8 Compounds With Antioxidant Activity: Crocin and Safranal

Crocin and safranal are carotenoid compounds extracted from *Crocus sativus* L. (saffron crocus). Both are characterized by significant antioxidant and free radical scavenging activity. Furthermore, anti-inflammatory and anticancer properties have been demonstrated for both compounds.[79,80]

8.5.9 Compounds With Antioxidant Activity: Quercetin

Quercetin is a flavonoid compound generally found in fruits and vegetables. It is characterized by important antioxidant and anti-inflammatory properties that make it suitable as a possible compound to be applied during wound healing. In fact, quercetin is able to inhibit both acute and chronic phases of inflammation.[81] Finally, this could regulate oxidative stress and inflammation, which are the two main factors in delaying the healing process.[82–84]

8.6 Effects on the Healing Process of Non-enzymatic Antioxidant Compounds

For some of the antioxidant compounds the effects on the healing process and their effectiveness can be listed. In particular the following can be considered: (i) studies on curcumin alone or together with chitosan or NAC; (ii) studies on NAC alone or with curcumin; (iii) studies on chitosan alone or with curcumin, NAC or gallic acid. In addition, studies on gallic acid, edaravone, crocin and safranal and studies on quercetin can be considered.

8.6.1 Effects on the Healing Process of Antioxidant Compounds: Curcumin

For this type of compound, five studies can be considered that evaluated the effect of curcumin by various methods. *In vitro* trials were carried out to test the feasibility of controlled release of curcumin for wound healing applications. This study demonstrated the possibility of this compound being used to improve wound closure *in vivo*.[85] In another study it was shown that curcumin is able to reduce the size of wounds, is able to promote cell migration and is able to improve overall wound healing. In confirmation of what has been verified in this study, a supplementary study[86] also revealed that animals treated with curcumin showed more significant wound closure, higher degrees of re-epithelialization, well-organized granulation tissue and significant fibroblastic deposition compared with those that did not receive curcumin-based treatment. It is also useful to cite a comparative study conducted to study the potential of curcumin in healing temporal wounds. Curcumin had the effect of increasing the contraction of the wound, of showing a better granulation of the tissue, dominated by a remarkable proliferation of fibroblasts and collagen, and of promoting the covering of the wounds by thick regenerated epithelial layers.[87]

8.6.2 Effects on the Healing Process of Antioxidant Compounds: Curcumin and Chitosan

For the case of curcumin plus chitosan it is also possible to evaluate the effects on healing through various studies. In the first case[88] curcumin used with chitosan in a new medical formulation was able to facilitate the healing of skin wounds. It was also noted that wounds treated with this combination of antioxidants showed lower levels of superoxide dismutase and lipid peroxidation. The accelerated healing process can therefore be attributed to the action of the antioxidant and anti-inflammatory effects of the chitosan–curcumin dressing. Another study worth commenting on is one in which the synergistic potential of curcumin alone and cross-linked with chitosan was tested.[89] The most significant results were the increase in free radical scavenging properties as curcumin increased, which would help in speeding

wound recovery, and the fact that with the presence of chitosan it increased the same scavenging activities. In view of the results, it can be concluded that the chitosan–curcumin combination improves the wound healing process while at the same time showing excellent free radical scavenging capabilities.

8.6.3 Effects on the Healing Process of Antioxidant Compounds: Curcumin and *N*-acetyl Cysteine

The first scientific evidence to be considered concerns a new antioxidant dressing based on curcumin and *N*-acetyl cysteine for moist wound care. In particular, the study used an antioxidant hydration solution composed of the two compounds considered capable of ensuring adequate hydration to the wound bed. This solution, in addition to its antioxidant power, was able to exert a marked protective effect, reducing ROS levels and at the same time regulating inflammation. The dressing components were also non-cytotoxic and demonstrated good biocompatibility. So, the antioxidant dressing under consideration not only modulates the inflammatory phase of wound healing, thus also controlling excessive cell activation, but allows for a more orderly transition between the inflammatory, proliferative and remodeling phases of wound healing. A further case study for this combination of antioxidants was acute and chronic wounds. The analysis of the findings leads to the conclusion that dressing done in the presence of curcumin and NAC works well for both types of wounds and can be used on wounds regardless of their level of recurrence or severity. The antioxidant dressing therefore represents a new and advanced alternative in the wound dressing landscape.

8.6.4 Effects on the Healing Process of Antioxidant Compounds: Chitosan

In the first study involving chitosan as an antioxidant compound for wound care, it was shown that the wound healing process can be accelerated thanks to a chitosan-based dressing, which has adequate antioxidant and mechanical properties and good ability to scavenge free radicals. All of these dressing optimization features represent great potential in the application of chitosan to wound healing.[90] Furthermore, through a study that exploited a new type of hydrogel loaded with chitosan,[91] the ability of the antioxidant to accelerate wound healing by promoting the formation and deposition of collagen with the consequent formation of the epidermis was demonstrated. Chitosan, therefore, stands as a promising means for healing wounds, providing a new cutting-edge method for the exploitation of antioxidant compounds. The results of a further study of chitosan with a hydrogel dressing[92] indicated that it allowed promotion of the potential of antioxidants in the wound healing process. In detail, chitosan demonstrated excellent blood coagulation capacity and favored the synthesis of the extracellular matrix, together with the deposition of collagen and the development of the thickness of the

granulation tissue. Antioxidants therefore rank as suitable candidates for bioactive dressings for healing skin wounds. A final study on chitosan that is important to mention is one conducted as a prospective randomized comparative clinical study with the aim of evaluating the safety and efficacy of a chitosan dressing in the field of chronic wounds.[93] The study demonstrated the safety of the new chitosan dressing for various clinical applications. This type of dressing is therefore safe and can be used effectively in the management of chronic wounds.

8.6.5 Effects on the Healing Process of Antioxidant Compounds: Chitosan and Gallic Acid

The effect of chitosan and gallic acid on healing was tested by considering a study performed with an injectable gelatin hydrogel.[76] In this case the hydrogels, given their anti-ROS properties, avoided any oxidative damage to the cells and accelerated the wound healing process, with effectively healed skin as a final result. Better re-epithelialization and better wound remodeling were observed for the case of hydrogels treated with gallic acid and chitosan. It can be concluded that this type of anti-ROS injectable hydrogel may indeed be the future of wound treatment and the future of tissue regeneration. In a second study[94] conducted *in vitro* on the effect of gallic acid on wound healing, it was shown that gallic acid has important antioxidant properties, is capable of stimulating cell migration on fibroblasts and can activate healing factors, such as focal adhesion kinase or N-terminal kinase. It can be concluded that gallic acid together with chitosan could be a potential pair of wound healing agents.

8.6.6 Effects on the Healing Process of Antioxidant Compounds: Edaravone

The first study considered for the antioxidant compound edaravone is one that examined the key factors for the correct application of the drug in wounds. In particular, for low doses of edaravone, an acceleration of wound healing was noted, while for a high dose of this antioxidant, an obstacle to healing was observed. In addition, the study demonstrated edaravone's ability to ensure a prolonged release.[77] A second study addressed the issue of a possible accelerated wound closure in the edaravone group. Histologically, it was interesting to observe that in the case of a wound treated with edaravone the blood vessels in the wound were more abundant than those in the control wound sites.

8.6.7 Effects on the Healing Process of Antioxidant Compounds: Crocin and Safranal

A study was conducted in which the performance of a hydrogel enriched with the antioxidant compounds of crocin and saffron was tested.[95] The results indicated improved biocompatibility and faster cell growth than in controls.

In particular, safranal has been proven to be able to stimulate the expansion of fibroblasts. Obviously, it should be specified that the concentration of the two compounds was high enough to exert an antioxidant activity and, therefore, capable of protecting against the effects of reactive oxygen species. The real advantage of this loaded hydrogel lies in its ease of administration, in fact it is an easy, economical and enriched system with applications in the treatment of difficult wounds or in wound healing without the need for complicated guidelines. In other studies, safranal was considered as a therapeutic option in the management of burn wounds thanks to its biological effects and its benefits in tissue regeneration.[96,97] Safranal significantly improved the vascularization of tissues and the proliferation and migration of fibroblasts at the end of the experiment. There was also a radical decrease in inflammation and an increase in the wound closure rate, including re-epithelialization and wound contraction compared with other wounds treated. Finally, thanks to this treatment with antioxidants, the count of neutrophils, free radicals and ROS in the burn environment was reduced. In any case, further studies are needed to clarify the exact mechanism of action of safranal combined with crocin in wound healing.

8.6.8 Effects on the Healing Process of Antioxidant Compounds: Quercetin

Turning to the effect of quercetin on wound healing, two studies in particular can be considered. In the first, a dressing composed of nanofiber and quercetin was used.[98,99] Important features highlighted with this nanofiber were the inhibition of the bacterial load and the excessive activity of free radicals in the wound, as well as the promotion of the vitality of the fibroblasts through protection from oxidative damage. Overall, the results indicated that quercetin with this nanofiber effectively reduced any possible infection and promoted collagen synthesis by preventing oxidative damage to fibroblasts. The second study focused on the detailed assessment of the healing potential of quercetin at different concentrations in wounds.[100] A 0.3% concentration of quercetin promoted better healing than in controls and also compared with other doses. In particular, this particular concentration accelerated healing through a rapid contraction of the wound. Further merits of quercetin were related to the control of the modulation of inflammatory and anti-inflammatory cytokines, the improvement of neovascularization and the antioxidant status at the wound site.

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