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**An assignment submitted to the**

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**1. Write on cytokine signalling and its role in wound healing.**

**2. When is wound healing referred to as 'impaired'? And why?**

**3. Examine the role of oxidative stress in the development and progression of impaired wound healing.**

Answers

1) Cytokines are small secreted proteins released by cells have a specific effect on the interactions and communications between cells. Cytokine is a general name; other names include lymphokine (cytokines made by lymphocytes), monokine (cytokines made by monocytes), chemokine (cytokines with chemotactic activities), and interleukin (cytokines made by one leukocyte and acting on other leukocytes). Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells (endocrine action) (Zhang and An, 2007).

Growth factors and cytokines are signaling molecules that control cell activities in an autocrine, paracrine or endocrine manner. They exert their biological functions by binding to specific receptors and activating associated downstream signaling pathways which in turn, regulate gene transcription in the nucleus and ultimately stimulate a biological response (Nicola, 1994).

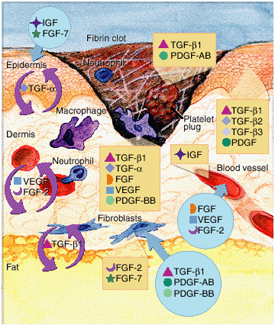
Wound healing is a complex and dynamic biological process that involves the coordinated efforts of multiple cell types and is executed and regulated by numerous growth factors and cytokines (Barrientos et al., 2015). The response to injury is a phylogenetically primitive, yet essential innate host immune response for restoration of tissue integrity. Tissue disruption in higher vertebrates, unlike lower vertebrates, results not in tissue regeneration, but in a rapid repair process leading to a fibrotic scar. Wound healing, whether initiated by trauma, microbes or foreign materials, proceeds via an overlapping pattern of events including coagulation, inflammation, epithelialization, formation of granulation tissue, matrix and tissue remodeling. The process of repair is mediated in large part by interacting molecular signals, primarily cytokines, that motivate and orchestrate the manifold cellular activities which underscore inflammation and healing.

Response to injury is frequently modeled in the skin (Singer and Clark, 1999), but parallel coordinated and temporally regulated patterns of mediators and cellular events occur in most tissues subsequent to injury. The initial injury triggers coagulation and an acute local inflammatory response followed by mesenchymal cell recruitment, proliferation and matrix synthesis. Failure to resolve the inflammation can lead to chronic nonhealing wounds, whereas uncontrolled matrix accumulation, often involving aberrant cytokine pathways, leads to excess scarring and fibrotic sequelae. Continuing progress in deciphering the essential and complex role of cytokines in wound healing provides opportunities to explore pathways to inhibit/enhance appropriate cytokines to control or modulate pathologic healing.

1. Platelet activation and cytokine release

Most types of injury damage blood vessels, and coagulation is a rapid-fire response to initiate hemostasis and protect the host from excessive blood loss. With the adhesion, aggregation and degranulation of circulating platelets within the forming fibrin clot, a plethora of mediators and cytokines are released, including transforming growth factor beta (TGF-beta), platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), that influence tissue edema and initiate inflammation. VEGF, a vascular permeability factor, influences the extravasation of plasma proteins to create a temporary support structure upon which not only activated endothelial cells, but also leukocytes and epithelial cells subsequently migrate. Angiopoietin-1 (Ang-1), the ligand for Tie-2 receptors, is a natural antagonist for VEGF’s effects on permeability, a key regulatory checkpoint to avoid excessive plasma leakage (Liekens et al., 2001).

Latent TGF-beta1, released in large quantities by degranulating platelets, is activated from its latent complex by proteolytic and non-proteolytic mechanism (Khalil, 1999) to influence wound healing from the initial insult and clot formation to the final phase of matrix deposition and remodelling (Wahl, 1999). Active TGF-beta1 elicits the rapid chemotaxis of neutrophils and monocytes to the wound site (Wahl et al., 1987) in a dose-dependent manner through cell surface TGF-beta serine/threonine type I and II receptors and engagement of a Smad3-dependent signal (Ashcroft et al., 1999). Autocrine expression of TGF- beta 1 by leukocytes and fibroblasts, in turn, induces these cells to generate additional cytokines including tumor necrosis factor alpha (TNF-a), interleukin 1 beta (IL-1 beta) and PDGF, as well as chemokines, as components of a cytokine cascade (McCartney-Francis and Wahl, 2001). Such factors act to perpetuate the inflammatory cell response, mediating recruitment and activation of neutrophils and monocytes. In response to TGF- beta and other cytokines, which engage their respective cell surface receptors, intracellular signaling pathways are mobilized to drive phenotypic and functional responses in target cell populations (Heldin et al., 2001). Among the upstream signaling cascades engaged in acute tissue injury are NF-?B, Egr-1, Smads, and MAP kinases, which result in activation of many cognate target genes, including adhesion molecules, coagulation factors, cytokines and growth factors (Heldin et al., 2001; Braddock, 2001).



**Fig 1:** Wound healing is a complex process encompassing a number of overlapping phases, including inflammation, epithelialization, angiogenesis and matrix deposition. During inflammation, the formation of a blood clot re-establishes hemostasis and provides a provisional matrix for cell migration. Cytokines play an important role in the evolution of granulation tissue through recruitment of inflammatory leukocytes and stimulation of fibroblasts and epithelial cells (Singer and Clarke, 1999).

1. Inflammation

Of the myriad of cytokines that have been investigated in terms of wound healing, TGF- beta 1 has undoubtedly the broadest effects. Despite the vast number of reports documenting the actions of TGF-beta in this context, both in vitro and in vivo, controversy remains as to its endogenous role. The paradoxical actions of TGF-beta are best appreciated in inflammation, where dependent upon the state of differentiation of the cell and the context of action, TGF-beta acts in a bi-directional manner (Wahl, 1994). Moreover, this understanding of the nature of TGF-beta has led to the hypothesis that it may act as a therapeutic tool in some circumstances, but also a target for therapeutic intervention in others (Wahl, 1994; Chen and Wahl, 1999). Recent studies, in particular those utilizing genetically manipulated animal models, have highlighted the impact of TGF-beta on various aspects of wound healing, and surprisingly, not all of its effects are conducive to optimal healing. Intriguingly, mutations within the TGF-beta1 gene, or in the cell signaling intermediate Smad3, lead to normal or even accelerated cutaneous wound healing responses (Ashcroft et al., 1999). The rate of healing of full-thickness wounds in Smad3 null mice was significantly greater than in their wild-type counterparts, associated with enhanced epithelialization and keratinocyte proliferation, and a markedly diminished inflammatory response. These observations have broad implications for understanding the role of TGF-beta in the endogenous wound healing response, in that an excess of TGF-beta may be a normal constituent of the response for rapid and optimal protection of the host. In the absence of infection, however, reduction of this overexuberant recruitment, inflammation and keratinocyte suppression may result in a more cosmetically acceptable scar. This knowledge may allow us to optimize the response by modulating selective cell pathways and to tailor therapy to specific cellular defects in pathological conditions such as chronic ulcers and fibrotic processes.

With the initial barrage of mediators, including TGF-beta, a chain reaction is set in motion, with recruitment, proliferation and activation of the cellular participants. Among the first cells to respond are the vascular endothelial cells, which not only respond to cytokines, but release them as well. Cytokine-induced enhancement of adhesion molecules (VCAM-1, ELAM-1, ICAM-1) on the endothelium provides the platform upon which circulating leukocytes expressing counter-adhesion molecules (integrins, selectins, Ig superfamily members) tether, slowing them down to sense the microenvironment and respond to chemotactic signals at the site of tissue injury (Sundy and Haynes, 2000). Adhesion molecule interactions between blood leukocytes and endothelium enables transmigration from inside to outside the vessel wall in response to multiple chemotactic signals. In addition to the powerful chemotactic activity of TGF-beta1 for neutrophils and monocytes (Wahl et al., 1987; Wahl, 1994), multiple chemokines are released to entice leukocytes into the site of tissue injury. Chemokines are represented by several families of related molecules based on the spatial location of the cysteine residues. Deletion of genes for chemokines leads to specific alterations in wound healing, underlying their role in this process.

Migrating through the provisional matrix (scaffolding) provided by the fibrin-enriched clot, leukocytes release proteases and engage in essential functions including phagocytosis of debris, microbes and degraded matrix components. Proteolytic activity is not constitutive, but transcriptionally driven by the cytokines, TGF-beta, IL-1beta and TNF-&alhpa;, released from multiple cellular sources (Fig 1). Neutrophil recruitment typically peaks around 24-48 hours post wounding, followed by an increasing representation of monocytes which are essential for optimal wound healing (Leibovich and Ross, 1975; Clarke 1996). Activation of these cells in the context of the wound microenvironment results in enhanced release of chemokines, recruitment of reinforcements, and amplification of the response, with the further release of cytokines, TNF-a, IL-1 and IL-6, that act as paracrine, autocrine and potentially, endocrine mediators of host defense. Antigen stimulation drives lymphocytic recruitment and activation, but at a delayed pace compared to the rapid acute response essential to maintain tissue integrity. Beyond the neutrophil, monocyte/macrophage and lymphocyte participants, mast cells have become increasingly recognized as active participants with increased numbers noted at sites of cutaneous injury (Huttunen et al., 2000). Mast cells respond to monocyte chemotactic protein (MCP-1) and TGF-beta1, -beta2 and -beta3, and within the lesion, release mediators (histamine, proteoglycans, proteases, platelet activating factor, arachidonate metabolites) and cytokines, including TGF-beta and IL-4 (Fig 1). Once the inflammatory cells are activated, they become susceptible to TGF-beta1 mediated suppression to reverse the inflammatory process (McCartney-Francis and Wahl, 2001; Wahl, 1994). Moreover, IL-4 may also dampen the inflammatory response as the inciting agent/trauma is dealt with and promote collagen synthesis during the repair phase.

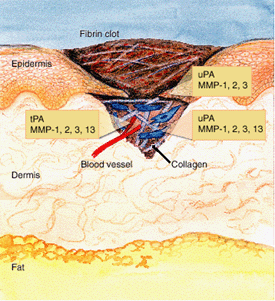
1. Re-epithelialization

Clearance of debris, foreign agents, and/or infectious organisms promotes resolution of inflammation, apoptosis, and the ensuing repair response that encompasses overlapping events involved in granulation tissue, angiogenesis, and re-epithelialization. Within hours, epithelial cells begin to proliferate, migrate and cover the exposed area to restore the functional integrity of the tissue. Re-epithelialization is critical to optimal wound healing not only because of reformation of a cutaneous barrier, but because of its role in wound contraction. Immature keratinocytes produce matrix metalloproteases (MMPs) and plasmin to dissociate from the basement membrane and facilitate their migration across the open wound bed in response to chemoattractants. The migration of epithelial cells occurs independently of proliferation, and depends upon a number of possible processes including growth factors, loss of contact with adjacent cells, and guidance by active contact. TGF-beta1 stimulates migration of keratinocytes *in vitro* (Ashcroft et al., 1999; Hebda, 1998), possibly by integrin regulation and/or provisional matrix deposition (Wikner et al., 1998). Behind the motile epidermal cells, basal cell keratinocyte proliferation is mediated by the local release of growth factors, with a parallel up-regulation of growth factor receptors including TNF-a, heparin-binding epidermal growth factor (EGF) and keratinocyte growth factor (KGF or FGF-7) (Barrandon and Green, 1987; Higashiyama et al., 1991; Werner et al., 1994). Such growth factors are released not only by keratinocytes themselves, acting in an autocrine fashion, but also by mesenchymal cells and macrophages (Fig 1), as paracrine mediators (Coffey et al., 1987; Werner et al., 1992). Numerous animal models in which cytokine genes have been deleted or over-expressed have provided further evidence that such factors are involved in the process of epithelialisation (Werner et al., 1994). TGF-beta1, and -beta2 are potent inhibitors of keratinocyte proliferation, with the Smad3 pathway implicated as the negative modulator (Ashcroft et al., 1999). Since epithelialization is significantly accelerated in mice null for the Smad3 gene, with unchecked keratinocyte proliferation, but impaired migration in response to TGF-beta1, the implication is that the early proliferative event is critical to normal epithelialisation (Ashcroft et al., 1999). Once contact is established with opposing keratinocytes, mitosis and migration stop, and in the skin, the cells differentiate into a stratified squamous epithelium above a newly generated basement membrane. Other factors secreted by keratinocytes may exert paracrine effects on dermal fibroblasts and macrophages. One such factor is a keratinocyte-derived non-glycosylated protein termed secretory leukocyte protease inhibitor (SLPI), which inhibits elastase, mast cell chymase, NF-?B and TGF-beta1 activation. In rodents, SLPI is a macrophage-derived cytokine with autocrine and paracrine activities (Ashcroft et al., 2000; Song et al., 1999), but production by human macrophages has not yet been demonstrated. In mice, an absence of this mediator of innate host defense (SLPI null) is associated with aberrant healing.26

1. Granulation tissue and angiogenesis

Granulation tissue forms below the epithelium and is composed of inflammatory cells, fibroblasts and newly formed and forming vessels (Figure 2). This initial restructuring of the damaged tissue serves as a temporary barrier against the hostile external environment. Within granulation tissue, angiogenesis (i.e. the generation of new capillary blood vessels from pre-existing vasculature to provide nutrients and oxygen) is potentiated by hypoxia, nitric oxide (NO), VEGF and fibroblast growth factor 2 (FGF-2) and by the chemokines, MCP-1 and macrophage inflammatory protein (MIP-1a) (Belperio et al., 2000). VEGF, released from wound epithelium and from the extracellular matrix by endothelial-derived proteases, stimulates endothelial cell proliferation and increases vascular permeability (Liekens et al., 200; Brown et al., 1992; Ferrara, 1999). VEGF may be transcriptionally up-regulated in response to NO, which also influences vasodilatation, an early step in angiogenesis. In a cyclic fashion, VEGF also drives nitric oxide synthase (NOS) in endothelial cells. Endothelial cells express high affinity receptors for VEGF, VEGF R1 (Flt-1) and VEGF R2 (Flk-1), and represent a primary target of this angiogenic and vascular permeability factor (Ferrara, 1999). Mice heterozygous for targeted inactivation of VEGF or homozygous for inactivation of its receptors are embryonically lethal, confirming the essentiality of VEGF in angiogenesis (Ferrara et al.,1996; Shibuya, 2001). Besides VEGF, FGFs transduce signals via four protein tyrosine kinase receptors (Ornitz et al., 1996) to mediate key events involved in angiogenesis. FGFs recruit endothelial cells, and also direct their proliferation, differentiation and plasminogen activator synthesis. Clearly a multifactorial process, the cellular events underlying neovascularization are also impacted by TGF-beta1, EGF, TGF-a, endothelin 1, leptin, and indirectly, TNF-a and IL-1beta.

Of necessity, angiogenesis is a tightly controlled process. It is characterized not only by the presence of endogenous inducers, but also inhibitors which mediate a decline in the process as the granulation tissue, named for the granular appearance of the blood vessels in the wound, matures and scar remodeling continues. Among the identified endogenous inhibitors of re-vascularization are thrombospondin (TSP-1), IFN-?, IP-10, IL-12, IL-4 and the tissue inhibitors of MMPs (TIMPs), in addition to the recently recognized activities of angiostatin and endostatin. Since loss of angiogenic control may have negative consequences as evident in tumors, rheumatoid arthritis, and endometriosis, identification of potential endogenous and therapeutic modulators continues.



**Fig 2:** The remodeling phase (i.e. re-epithelialization and neovascularization) of wound healing is also cytokine-mediated. Degradation of fibrillar collagen and other matrix proteins is driven by serine proteases and MMPs under the control of the cytokine network. Granulation tissue forms below the epithelium and is composed of inflammatory cells, fibroblasts and newly formed and forming vessels (Singer and Clarke, 1999).

### Matrix Production and Scar Formation

With the generation of new vasculature, matrix-generating cells move into the granulation tissue. These fibroblasts degrade the provisional matrix via MMPs and respond to cytokine/growth factors by proliferating and synthesizing new extracellular matrix (ECM) to replace the injured tissue with a connective tissue scar. Although the stage is being set for tissue repair from the beginning (provisional matrix, platelet release of PDGF and TGF-beta, cytokine reservoir), fibroblasts migrate into the wound and matrix synthesis begins in earnest within a couple of days, continuing for several weeks to months. TGF-beta contributes to the fibrotic process by recruiting fibroblasts and stimulating their synthesis of collagens I, III, and V, proteoglycans, fibronectin and other ECM components (Liekens et al.., 2001; Branton and Kopp, 1999). TGF-beta concurrently inhibits proteases while enhancing protease inhibitors, favoring matrix accumulation. In vivo studies have confirmed that exogenous TGF-beta1 increases granulation tissue, collagen formation, and wound tensile strength when applied locally or given systemically in animal models. Increased levels of TGF-beta are routinely associated with both normal reparative processes, as well as fibropathology. In Smad3 null mouse wounds, matrix deposition (fibronectin) could be restored by exogenous TGF-beta, implying a Smad3-independent pathway, whereas collagen deposition was not restored, suggesting a dichotomous Smad3-dependent regulation (Ashcroft et al., 1999). The progressive increase in TGF-beta3 over time and its association with scarless fetal healing have implicated this member of the TGF-beta family in the cessation of matrix deposition (Niesler and Ferguson, 2001). Other members of the TGF-beta superfamily may also contribute to the wound healing response. Activin A when over-expressed in basal keratinocytes stimulates mesenchymal matrix deposition (Munz et al., 1999), whereas BMP-6 over-expression inhibits epithelial proliferation (Blessing et al., 1996).

PDGF, released at the outset by degranulating platelets, represents a family of cytokines consisting of two polypeptide chains (A and B) which form the dimers PDGF-AA, AB and B (Ross et al., 1990). In addition to platelets, PDGF is released by activated macrophages, endothelial cells, fibroblasts and smooth muscle cells (Fig 1) and is a major player in regulating fibroblast and smooth muscle cell recruitment and proliferation through PDGF specific receptor-ligand interactions (Claesson-Welsh, 1996). Beyond its role in fibroblast migration and matrix deposition, PDGF-A and -B also up-regulate protease production, in contrast to the anti-protease activity of TGF-beta (Circolo et al., 1991; Laiho et al., 1987). PDGF represents the only FDA approved cytokine/growth factor for the clinical enhancement of delayed wound healing. Also central to repair are the FGFs, which signal mitogenesis and chemotaxis (Onitz et al., 1996), underlying granulation tissue formation, and the production of MMPs (Powers et al., 2000). FGF-1 (acidic FGF) and FGF-2 (basic FGF) have been the most intensely studied, but the additional members of this family may also support tissue repair and/or have clinical application (Payne et al., 2001). The role of FGF-2 has been confirmed in the FGF-2 null mouse which shows not only retarded epithelialization but also reduced collagen production (Ortega et al., 1998).

With many overlapping functional properties with FGFs, epidermal growth factor (EGF) orchestrates recruitment and growth of fibroblasts and epithelial cells in the evolution of granulation tissue. EGF and TGF-a, which share sequence homology, enhance epidermal regeneration and tensile strength in experimental models of chronic wounds (Andresen and Ehlers, 1998). TNF-a and IL-1beta, key mediators of the inflammatory process, also contribute to the reparative phase either directly by influencing endothelial and fibroblast functions or indirectly, by inducing additional cytokines and growth factors. IL-6 has also been shown to be crucial to epithelialization and influences granulation tissue formation, as shown in the wound healing studies of mice null for the IL-6 gene (Gallucci et al, 2000). As repair progresses, fibroblasts display increased expression levels of adhesion molecules and assume a myofibroblast phenotype, mediated in part by TGF-beta and PDGF-A and -B, to facilitate wound contraction (Grinnell, 1994).

### Remodeling Phase

The remodeling phase, during which collagen is synthesized, degraded and dramatically reorganized (as it is stabilized via molecular crosslinking into a scar), is also cytokine-mediated. Although repaired tissue seldom achieves its original strength, it provides an acceptable alternative. Degradation of fibrillar collagen and other matrix proteins is driven by serine proteases and MMPs under the control of the cytokine network. MMPs not only degrade matrix components, but also function as regulatory molecules by driving enzyme cascades and processing cytokines, matrix and adhesion molecules to generate biologically active fragments. TIMPs provide a natural counterbalance to the MMPs and disruption of this orderly balance can lead to excess or insufficient matrix degradation and ensuing tissue pathology (Birkedal-Hansen, 1995). Similarly, there exists a naturally occurring inhibitor of elastase and other serine proteases (i.e. SLPI) (Ashcroft et al., 2000; Song et al., 1999). The coordinated regulation of enzymes and their inhibitors ensures tight control of local proteolytic activity. In physiologic circumstances, these molecular brakes limit tissue degradation and facilitate accumulation of matrix and repair.

### Aberrant Healing

Rapid clearance of the inciting agent and resolution of inflammation during healing minimizes scar formation, whereas persistence of the primary insult results in continued inflammation and chronic attempts at healing. Prolonged inflammation and proteolytic activity prevent healing as evident in ulcerative lesions. On the other hand, continued fibrosis in the skin leads to scarring and potentially, disfigurement, whereas progressive deposition of matrix in internal organs such as lungs, liver, kidney or brain compromises not only their structure, but also function, causing disease and death. Inhibitors of TGF-beta (e.g. antibodies, decorin, Smad 7, antisense oligonucleotides) (Wahl et al., 1993; Border and Noble, 1998), reduce scarring, as does local administration of exogenous TGF-beta3 (Niesler and Ferguson, 2001) or systemic delivery of TGF-beta1 (Song et al., 1999). IFN-? is a natural antagonist of fibrogenesis through its ability to inhibit fibroblast proliferation and matrix production and has been shown to have clinical efficacy (Ghosh et al., 2001; Duncan et al., 1985). IL-10 may be considered anti-fibrotic via its anti-inflammatory activities (Akdis, 2001), as are inhibitors of TNF-a (Wahl et al., 2001).

Wound healing is a complex process encompassing a number of overlapping phases, including inflammation, epithelialization, angiogenesis and matrix deposition. Ultimately these processes are resolved or dampened leading to a mature wound and macroscopic scar formation. Although inflammation and repair mostly occur along a proscribed course, the sensitivity of the process is underscored by the consequences of disruption of the balance of regulatory cytokines. Consequently, cytokines, which are central to this constellation of events, have become targets for therapeutic intervention to modulate the wound healing process. Depending on the cytokine and its role, it may be appropriate to either enhance (recombinant cytokine, gene transfer) or inhibit (cytokine or receptor antibodies, soluble receptors, signal transduction inhibitors, antisense) the cytokine to achieve the desired outcome.

2) In wounds where oxygenation is not restored, healing is impaired.

Oxygen is important for cell metabolism, especially energy production by means of ATP, and is critical for nearly all wound-healing processes. It prevents wounds from infection, induces angiogenesis, increases keratinocyte differentiation, migration, and re-epithelialization, enhances fibroblast proliferation and collagen synthesis, and promotes wound contraction ([Bishop, 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/#bibr5-0022034509359125); Rodriguez et al., 2008). In addition, the level of superoxide production (a key factor for oxidative killing pathogens) by polymorphonuclear leukocytes is critically dependent on oxygen levels.

Due to vascular disruption and high oxygen consumption by metabolically active cells, the microenvironment of the early wound is depleted of oxygen and is quite hypoxic. Several systemic conditions, including advancing age and diabetes, can create impaired vascular flow, thus setting the stage for poor tissue oxygenation. In the context of healing, this overlay of poor perfusion creates a hypoxic wound. Chronic wounds are notably hypoxic; tissue oxygen tensions have been measured transcutaneously in chronic wounds from 5 to 20 mm Hg, in contrast to control tissue values of 30 to 50 mm Hg ([Tandara and Mustoe, 2004](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/#bibr95-0022034509359125)).

In wounds where oxygenation is not restored, healing is impaired. Temporary hypoxia after injury triggers wound healing, but prolonged or chronic hypoxia delays wound healing ([Bishop, 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/#bibr5-0022034509359125); [Rodriguez et al., 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/#bibr81-0022034509359125)). In acute wounds, hypoxia serves as a signal that stimulates many aspects of the wound-healing process. Hypoxia can induce cytokine and growth factor production from macrophages, keratinocytes, and fibroblasts. Cytokines that are produced in response to hypoxia include PDGF, TGF-β, VEGF, tumor necrosis factor-α (TNF-α), and endothelin-1, and are crucial promoters of cell proliferation, migration and chemotaxis, and angiogenesis in wound healing ([Rodriguez et al., 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/#bibr81-0022034509359125)).

In normally healing wounds, ROS such as hydrogen peroxide (H2O2) and superoxide (O2) are thought to act as cellular messengers to stimulate key processes associated with wound healing, including cell motility, cytokine action (including PDGF signal transduction), and angiogenesis. Both hypoxia and hyperoxia increase ROS production, but an increased level of ROS transcends the beneficial effect and causes additional tissue damage ([Rodriguez et al., 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/#bibr81-0022034509359125)).

In summary, the proper oxygen level is crucial for optimum wound healing. Hypoxia stimulates wound healing such as the release of growth factors and angiogenesis, while oxygen is needed to sustain the healing process ([Bishop, 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/#bibr5-0022034509359125)). One therapeutic option that can sometimes overcome the influence of tissue hypoxia is hyperbaric oxygen therapy (HBOT; [Rodriguez et al., 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/#bibr81-0022034509359125)). While HBOT can be an effective treatment for hypoxic wounds, its availability is limited.

#### Example: Smoking strongly impairs wound healing

Smoking demonstrates detrimental effects on physiologic wound healing. Amongst over 4000 substances detected in tobacco smoke, several ones negatively impact healing processes (Pizzino et al., 2017). To this end, nicotine strongly promotes vasoconstriction leading to disturbed microcirculation that negatively impacts wound healing (Ahn et al., 2008). Further, smoking attenuates the inflammation phase by impairing white blood cell migration, reducing neutrophil bactericidal activity, and depressing IL-1 production (Sørensen et al., 2009). The proliferative phase is impaired by the reduced fibroblast migration and proliferation in addition to the downregulated collagen synthesis and deposition in smokers (Sørensen et al., 2010). Additionally, smoking disrupts epithelial regeneration and normal angiogenesis and decreases ECM production (Chan et al., 2006). Overall, smokers show delayed wound healing, increased frequency of wound healing complications and wound dehiscence compared to non-smokers (Manassa et al., 2003).

3) Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products. ROS can play, and in fact they do it, several physiological roles (i.e., cell signaling), and they are normally generated as by-products of oxygen metabolism; despite this, environmental stressors (i.e., UV, ionizing radiations, pollutants, and heavy metals) and xenobiotics (i.e., antiblastic drugs) contribute to greatly increase ROS production, therefore causing the imbalance that leads to cell and tissue damage (oxidative stress) (Pizzino et al., 2017).

i) Oxidative stress was a condition which was the imbalance of prooxidant and antioxidants, abnormally high levels of free radicals and/or the decline of antioxidant defense mechanisms. Excessive oxidative stress could lead to damage of tissue, which played an important role in the development of many kinds of diseases ((Yang et al., 2007).).

ii) Free radical relatively increased during oxidative stress. Normally free radical was necessary for defense of organism and there was a balance between its produce and scavenge (Yang et al., 2007).

iii) Oxidative stress was closely associated with reactive oxygen species. Reactive oxygen species could play an important role in physiology in some extent, also it led to damage of tissue or cells when organism could not defend excessive reactive oxygen species (Yang et al., 2007).

iv) Excessive reactive oxygen species and its degradation product generated during the healing of cutaneous wound. Oxidation increased in acute and chronic wound. After wound oxidative stress generates, antioxidation increased in chronic wound, which indirectly reflected the increasing of oxidative stress and compensation and defense of tissue to oxidative stress (Yang et al., 2007).

v) The generation of oxidative stress in wound maybe closely relate to inflammatory reaction. In the inflammatory stage of wound healing, oxidative stress induced the damage of tissue because of the imbalance of prooxidant and antioxidant (Yang et al., 2007).

Conclusion: Oxidative stress should be considered in the inflammatory processes of wound healing and treatment of chronic wound. The treatment of antioxidation is a good strategy. If it is used in wound healing in time, it can be good to wound healing (Yang et al., 2007).

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