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**Question One**

**1.0 Cytokine signalling and its role in wound healing**

**1.1 Introduction**

Cytokines are a broad and loose category of small [proteins](https://en.wikipedia.org/wiki/Protein%22%20%5Co%20%22Protein) (~5–20 [kDa](https://en.wikipedia.org/wiki/KDa%22%20%5Co%20%22KDa)) important in [cell signaling](https://en.wikipedia.org/wiki/Cell_signaling%22%20%5Co%20%22Cell%20signaling). Cytokines are [peptides](https://en.wikipedia.org/wiki/Peptide%22%20%5Co%20%22Peptide) and cannot cross the [lipid bilayer](https://en.wikipedia.org/wiki/Lipid_bilayer%22%20%5Co%20%22Lipid%20bilayer) of cells to enter the [cytoplasm](https://en.wikipedia.org/wiki/Cytoplasm%22%20%5Co%20%22Cytoplasm). Cytokines have been shown to be involved in [autocrine](https://en.wikipedia.org/wiki/Autocrine_signaling%22%20%5Co%20%22Autocrine%20signaling), [paracrine](https://en.wikipedia.org/wiki/Paracrine_signaling%22%20%5Co%20%22Paracrine%20signaling) and [endocrine signaling](https://en.wikipedia.org/wiki/Endocrine_signaling%22%20%5Co%20%22Endocrine%20signaling) as [immunomodulating agents](https://en.wikipedia.org/wiki/Immunomodulation%22%20%5Co%20%22Immunomodulation). Their definite distinction from [hormones](https://en.wikipedia.org/wiki/Hormones%22%20%5Co%20%22Hormones) is still part of ongoing research.

Cytokines include [chemokines](https://en.wikipedia.org/wiki/Chemokine%22%20%5Co%20%22Chemokine), [interferons](https://en.wikipedia.org/wiki/Interferon%22%20%5Co%20%22Interferon), [interleukins](https://en.wikipedia.org/wiki/Interleukin%22%20%5Co%20%22Interleukin), [lymphokines](https://en.wikipedia.org/wiki/Lymphokine%22%20%5Co%20%22Lymphokine), and [tumour necrosis factors](https://en.wikipedia.org/wiki/Tumour_necrosis_factor%22%20%5Co%20%22Tumour%20necrosis%20factor), but generally not hormones or [growth factors](https://en.wikipedia.org/wiki/Growth_factor%22%20%5Co%20%22Growth%20factor) (despite some [overlap in the terminology](https://en.wikipedia.org/wiki/Growth_factor%22%20%5Cl%20%22Growth_factors_versus_cytokines%22%20%5Co%20%22Growth%20factor)). Cytokines are produced by a broad range of cells, including immune cells like [macrophages](https://en.wikipedia.org/wiki/Macrophage%22%20%5Co%20%22Macrophage), [B lymphocytes](https://en.wikipedia.org/wiki/B_cell%22%20%5Co%20%22B%20cell), [T lymphocytes](https://en.wikipedia.org/wiki/T_cell%22%20%5Co%20%22T%20cell) and [mast cells](https://en.wikipedia.org/wiki/Mast_cell%22%20%5Co%20%22Mast%20cell), as well as [endothelial cells](https://en.wikipedia.org/wiki/Endothelium%22%20%5Co%20%22Endothelium), [fibroblasts](https://en.wikipedia.org/wiki/Fibroblast%22%20%5Co%20%22Fibroblast), and various [stromal cells](https://en.wikipedia.org/wiki/Stromal_cell%22%20%5Co%20%22Stromal%20cell); a given cytokine may be produced by more than one type of cell (Wolters Kluwer Health *et al.*, 2006).

They act through [cell surface receptors](https://en.wikipedia.org/wiki/Cell_surface_receptor%22%20%5Co%20%22Cell%20surface%20receptor) and are especially important in the [immune system](https://en.wikipedia.org/wiki/Immune_system%22%20%5Co%20%22Immune%20system); cytokines modulate the balance between [humoral](https://en.wikipedia.org/wiki/Humoral_immunity%22%20%5Co%20%22Humoral%20immunity) and [cell-based](https://en.wikipedia.org/wiki/Cell-mediated_immunity%22%20%5Co%20%22Cell-mediated%20immunity) immune responses, and they regulate the maturation, growth, and responsiveness of particular cell populations. Some cytokines enhance or inhibit the action of other cytokines in complex ways. They are different from hormones, which are also important cell signaling molecules. Hormones circulate in higher concentrations, and tend to be made by specific kinds of cells. Cytokines are important in health and disease, specifically in host immune responses to infection, [inflammation](https://en.wikipedia.org/wiki/Inflammation%22%20%5Co%20%22Inflammation), trauma, [sepsis](https://en.wikipedia.org/wiki/Sepsis%22%20%5Co%20%22Sepsis), cancer, and reproduction.

**1.2 Structural**

Structural homogeneity has been able to partially distinguish between cytokines that do not demonstrate a considerable degree of redundancy so that they can be classified into four types:

The four-[α-helix bundle](https://en.wikipedia.org/wiki/Helix_bundle%22%20%5Co%20%22Helix%20bundle) family: member cytokines have three-dimensional structures with a bundle of four [α-helices](https://en.wikipedia.org/wiki/Alpha_helix%22%20%5Co%20%22Alpha%20helix). This family, in turn, is divided into three sub-families:

The [IL-2](https://en.wikipedia.org/wiki/Interleukin_2%22%20%5Co%20%22Interleukin%202) subfamily. This is the largest family. It contains several non-immunological cytokines including [erythropoietin](https://en.wikipedia.org/wiki/Erythropoietin%22%20%5Co%20%22Erythropoietin) (EPO) and [thrombopoietin](https://en.wikipedia.org/wiki/Thrombopoietin%22%20%5Co%20%22Thrombopoietin) (TPO).Some members share a gamma-chain in their receptors.

1. the [interferon (IFN)](https://en.wikipedia.org/wiki/Interferon%22%20%5Co%20%22Interferon) subfamily.
2. the [IL-10](https://en.wikipedia.org/wiki/Interleukin_10%22%20%5Co%20%22Interleukin%2010) subfamily.

Furthermore, four-α-helix bundle cytokines can be grouped into *long-chain* and *short-chain* cytokines by topology.

1. The [IL-1](https://en.wikipedia.org/wiki/Interleukin_1%22%20%5Co%20%22Interleukin%201) family, which primarily includes IL-1 and [IL-18](https://en.wikipedia.org/wiki/Interleukin%22%20%5Co%20%22Interleukin).

2. The [IL-17](https://en.wikipedia.org/wiki/Interleukin_17%22%20%5Co%20%22Interleukin%2017) family, which has yet to be completely characterized, though member cytokines have a specific effect in promoting proliferation of T-cells that cause cytotoxic effects.

**1.3 Functions**

A classification that proves more useful in clinical and experimental practice outside of [structural biology](https://en.wikipedia.org/wiki/Structural_biology%22%20%5Co%20%22Structural%20biology) divides immunological cytokines into those that enhance [cellular immune responses](https://en.wikipedia.org/wiki/Cellular_immune_response%22%20%5Co%20%22Cellular%20immune%20response), type 1 (TNFα, IFN-γ, etc.), and type 2 (TGF-β, [IL-4](https://en.wikipedia.org/wiki/Interleukin_4%22%20%5Co%20%22Interleukin%204), IL-10, [IL-13](https://en.wikipedia.org/wiki/Interleukin_13%22%20%5Co%20%22Interleukin%2013), etc.), which favor [antibody](https://en.wikipedia.org/wiki/Antibody%22%20%5Co%20%22Antibody) responses. A key focus of interest has been that cytokines in one of these two sub-sets tend to inhibit the effects of those in the other. Dysregulation of this tendency is under intensive study for its possible role in the [pathogenesis](https://en.wikipedia.org/wiki/Pathogenesis%22%20%5Co%20%22Pathogenesis) of [autoimmune disorders](https://en.wikipedia.org/wiki/Autoimmune_disorder%22%20%5Co%20%22Autoimmune%20disorder). Several [inflammatory cytokines](https://en.wikipedia.org/wiki/Inflammatory_cytokine%22%20%5Co%20%22Inflammatory%20cytokine) are induced by [oxidative stress](https://en.wikipedia.org/wiki/Oxidative_stress%22%20%5Co%20%22Oxidative%20stress) (Vlahopoulos *et al.*, 1999).

The fact that cytokines themselves trigger the release of other cytokines (Carpenter LR *et al*., 2002) and also lead to increased oxidative stress makes them important in chronic [inflammation](https://en.wikipedia.org/wiki/Inflammation%22%20%5Co%20%22Inflammation), as well as other immunoresponses, such as fever and acute phase proteins of the liver (IL-1,6,12, IFN-a). Cytokines also play a role in anti-inflammatory pathways and are a possible therapeutic treatment for pathological pain from inflammation or peripheral nerve injury (Zhang JM *et al*., 2007). There are both pro-inflammatory and anti-inflammatory cytokines that regulate this pathway.

**1.4 Receptors**

the cytokine receptors have come to demand the attention of more investigators than cytokines themselves, partly because of their remarkable characteristics and partly because a deficiency of cytokine receptors has now been directly linked to certain debilitating immunodeficiency states. In this regard, and also because the redundancy and pleomorphism of cytokines are, in fact, a consequence of their homologous receptors, many authorities think that a classification of cytokine receptors would be more clinically and experimentally useful.

A classification of cytokine receptors based on their three-dimensional structure has, therefore, been attempted. Such a classification, though seemingly cumbersome, provides several unique perspectives for attractive pharmacotherapeutic targets.

1. [Immunoglobulin (Ig) superfamily](https://en.wikipedia.org/wiki/Immunoglobulin_superfamily%22%20%5Co%20%22Immunoglobulin%20superfamily), which are ubiquitously present throughout several cells and tissues of the vertebrate body, and share [structural homology](https://en.wikipedia.org/wiki/Structural_homology%22%20%5Co%20%22Structural%20homology) with immunoglobulins ([antibodies](https://en.wikipedia.org/wiki/Antibody%22%20%5Co%20%22Antibody)), [cell adhesion molecules](https://en.wikipedia.org/wiki/Cell_adhesion_molecule%22%20%5Co%20%22Cell%20adhesion%20molecule), and even some cytokines. Examples: IL-1 receptor types.
2. Hemopoietic Growth Factor (type 1) family, whose members have certain conserved motifs in their extracellular [amino-acid](https://en.wikipedia.org/wiki/Amino-acid%22%20%5Co%20%22Amino-acid) domain. The IL-2 receptor belongs to this chain, whose γ-chain (common to several other cytokines) deficiency is directly responsible for the x-linked form of [Severe Combined Immunodeficiency](https://en.wikipedia.org/wiki/Severe_Combined_Immunodeficiency%22%20%5Co%20%22Severe%20Combined%20Immunodeficiency) ([X-SCID](https://en.wikipedia.org/wiki/X-SCID%22%20%5Co%20%22X-SCID)).
3. Interferon (type 2) family, whose members are receptors for IFN β and γ.
4. [Tumor necrosis factors](https://en.wikipedia.org/wiki/Tumor_necrosis_factor%22%20%5Co%20%22Tumor%20necrosis%20factor) (TNF) (type 3) family, whose members share a [cysteine](https://en.wikipedia.org/wiki/Cysteine%22%20%5Co%20%22Cysteine)-rich common extracellular binding domain, and includes several other non-cytokine [ligands](https://en.wikipedia.org/wiki/Ligands%22%20%5Co%20%22Ligands) like [CD40](https://en.wikipedia.org/wiki/CD40%22%20%5Co%20%22CD40), [CD27](https://en.wikipedia.org/wiki/CD27%22%20%5Co%20%22CD27) and [CD30](https://en.wikipedia.org/wiki/CD30%22%20%5Co%20%22CD30), besides the ligands on which the family is named.
5. [Seven transmembrane helix](https://en.wikipedia.org/wiki/7TM_Receptor%22%20%5Co%20%227TM%20Receptor) family, the ubiquitous receptor type of the animal kingdom. All [G protein-coupled receptors](https://en.wikipedia.org/wiki/G_protein-coupled_receptors%22%20%5Co%20%22G%20protein-coupled%20receptors) (for hormones and neurotransmitters) belong to this family. Chemokine receptors, two of which act as binding proteins for [HIV](https://en.wikipedia.org/wiki/HIV%22%20%5Co%20%22HIV) ([CD4](https://en.wikipedia.org/wiki/CD4%22%20%5Co%20%22CD4) and [CCR5](https://en.wikipedia.org/wiki/CCR5%22%20%5Co%20%22CCR5)), also belong to this family.
6. [Interleukin-17 receptor](https://en.wikipedia.org/wiki/Interleukin-17_receptor%22%20%5Co%20%22Interleukin-17%20receptor) (IL-17R) family, which shows little homology with any other cytokine receptor family. Structural motifs conserved between members of this family include: an extracellular fibronectin III-like domain, a transmembrane domain and a cytoplasmic SERIF domain. The known members of this family are as follows: IL-17RA, IL-17RB, IL-17RC, IL17RD and IL-17RE

**1.5 Its role in wound healing**

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,1 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.

### **1.6 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.

Latent TGF-beta1, released in large quantities by degranulating platelets, is activated from its latent complex by proteolytic and non-proteolytic mechanisms to influence wound healing from the initial insult and clot formation to the final phase of matrix deposition and remodeling. Active TGF-beta1 elicits the rapid chemotaxis of neutrophils and monocytes to the wound site in a dose-dependent manner through cell surface TGF-beta serine/threonine type I and II receptors and engagement of a Smad3-dependent signal. 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. 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.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.



### **Figure 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

### **1.7 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.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. 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. 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.

### **1.8 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 possibly by integrin regulation and/or provisional matrix deposition.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).Such growth factors are released not only by keratinocytes themselves, acting in an autocrine fashion, but also by mesenchymal cells and macrophages as paracrine mediators.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 epithelialization. TGF-beta1, and -beta2 are potent inhibitors of keratinocyte proliferation, with the Smad3 pathway implicated as the negative modulator.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 epithelialization. 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 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.

### **1.9 Granulation Tissue and Angiogenesis**

Granulation tissue forms below the epithelium and is composed of inflammatory cells, fibroblasts and newly formed and forming vessels. 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 and by the chemokines, MCP-1 and macrophage inflammatory protein (MIP-1a). VEGF, released from wound epithelium and from the extracellular matrix by endothelial-derived proteases, stimulates endothelial cell proliferation and increases vascular permeability.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.Mice heterozygous for targeted inactivation of VEGF or homozygous for inactivation of its receptors are embryonically lethal, confirming the essentiality of VEGF in angiogenesis.Besides VEGF, FGFs transduce signals via four protein tyrosine kinase receptors 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.

### **1.10 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.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.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.36 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,whereas BMP-6 over-expression inhibits epithelial proliferation.

### **1.11 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) reduce scarring, as does local administration of exogenous TGF-beta3 or systemic delivery of TGF-beta1.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.IL-10 may be considered anti-fibrotic via its anti-inflammatory activities,as are inhibitors of TNF-a.

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.

**Question Two**

**2.0 When is wound healing referred to as ‘impaired’ and why**

**2.1 Introduction**

The wound-healing process consists of four highly integrated and overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling or resolution ([Gosain and DiPietro, 2004](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr36-0022034509359125)).These phases and their biophysiological functions must occur in the proper sequence, at a specific time, and continue for a specific duration at an optimal intensity ([Mathieu](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr62-0022034509359125)*[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr62-0022034509359125)*[., 2006](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr62-0022034509359125)). There are many factors that can affect wound healing which interfere with one or more phases in this process, thus causing improper or impaired tissue repair.

Wounds that exhibit impaired healing, including delayed acute wounds and chronic wounds, generally have failed to progress through the normal stages of healing. Such wounds frequently enter a state of pathologic inflammation due to a postponed, incomplete, or uncoordinated healing process. Most chronic wounds are ulcers that are associated with ischemia, diabetes mellitus, venous stasis disease, or pressure. Non-healing wounds affect about 3 to 6 million people in the United States, with persons 65 years and older accounting for 85% of these events ([Mathieu](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr62-0022034509359125)*[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr62-0022034509359125)*[., 2006](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr62-0022034509359125); [Menke](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr65-0022034509359125)*[et al.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr65-0022034509359125)*[, 2007](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr65-0022034509359125)).

Laboratory investigations and clinical studies have yielded a wealth of information about both normal and impaired wound healing. More recently, a great deal of research has been directed at understanding the critical factors that influence poorly healing wounds. While much remains to be learned, these studies may lead to therapeutics that will promote proper tissue repair and improve impaired wound healing. This review will discuss the many different factors that affect cutaneous wound healing and the potential cellular and molecular mechanisms involved.

Wound healing is a dynamic process consisting of four continuous, overlapping, and precisely programmed phases. The events of each phase must happen in a precise and regulated manner. Interruptions, aberrancies, or prolongation in the process can lead to delayed wound healing or a non-healing chronic wound.

**2.2 The wound healing process**

In adult humans, optimal wound healing involves the following the events: (1) rapid hemostasis; (2) appropriate inflammation; (3) mesenchymal cell differentiation, proliferation, and migration to the wound site; (4) suitable angiogenesis; (5) prompt re-epithelialization (re-growth of epithelial tissue over the wound surface); and (6) proper synthesis, cross-linking, and alignment of collagen to provide strength to the healing tissue ([Gosain and DiPietro, 2004](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr36-0022034509359125); [Mathieu](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr62-0022034509359125)*[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr62-0022034509359125)*[., 2006](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr62-0022034509359125)). The first phase of hemostasis begins immediately after wounding, with vascular constriction and fibrin clot formation. The clot and surrounding wound tissue release pro-inflammatory cytokines and growth factors such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). Once bleeding is controlled, inflammatory cells migrate into the wound (chemotaxis) and promote the inflammatory phase, which is characterized by the sequential infiltration of neutrophils, macrophages, and lymphocytes ([Gosain and DiPietro, 2004](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr36-0022034509359125); [Broughton](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr9-0022034509359125)*[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr9-0022034509359125)*[., 2006](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr9-0022034509359125); [Campos](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr12-0022034509359125)*[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr12-0022034509359125)*[., 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr12-0022034509359125)). A critical function of neutrophils is the clearance of invading microbes and cellular debris in the wound area, although these cells also produce substances such as proteases and reactive oxygen species (ROS), which cause some additional bystander damage.

Macrophages play multiple roles in wound healing. In the early wound, macrophages release cytokines that promote the inflammatory response by recruiting and activating additional leukocytes. Macrophages are also responsible for inducing and clearing apoptotic cells (including neutrophils), thus paving the way for the resolution of inflammation. As macrophages clear these apoptotic cells, they undergo a phenotypic transition to a reparative state that stimulates keratinocytes, fibroblasts, and angiogenesis to promote tissue regeneration ([Meszaros](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr66-0022034509359125)*[et al.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr66-0022034509359125)*[, 2000](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr66-0022034509359125); [Mosser and Edwards, 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr70-0022034509359125)). In this way, macrophages promote the transition to the proliferative phase of healing.

T-lymphocytes migrate into wounds following the inflammatory cells and macrophages, and peak during the late-proliferative/early-remodeling phase. The role of T-lymphocytes is not completely understood and is a current area of intensive investigation. Several studies suggest that delayed T-cell infiltration along with decreased T-cell concentration in the wound site is associated with impaired wound healing, while others have reported that CD 4+ cells (T-helper cells) have a positive role in wound healing and CD8+ cells (T-suppressor-cytotoxic cells) play an inhibitory role in wound healing ([Swift](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr93-0022034509359125)*[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr93-0022034509359125)*[., 2001](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr93-0022034509359125); [Park and Barbul, 2004](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr72-0022034509359125)). Interestingly, recent studies in mice deficient in both T- and B-cells have shown that scar formation is diminished in the absence of lymphocytes ([Gawronska-Kozak](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr29-0022034509359125)*[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr29-0022034509359125)*[., 2006](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr29-0022034509359125)). In addition, skin gamma-delta T-cells regulate many aspects of wound healing, including maintaining tissue integrity, defending against pathogens, and regulating inflammation. These cells are also called dendritic epidermal T-cells (DETC), due to their unique dendritic morphology. DETC are activated by stressed, damaged, or transformed keratinocytes and produce fibroblast growth factor 7 (FGF-7), keratinocyte growth factors, and insulin-like growth factor-1, to support keratinocyte proliferation and cell survival. DETC also generate chemokines and cytokines that contribute to the initiation and regulation of the inflammatory response during wound healing. While cross-talk between skin gamma-delta T-cells and keratinocytes contributes to the maintenance of normal skin and wound healing, mice lacking or defective in skin gamma-delta T-cells show a delay in wound closure and a decrease in the proliferation of keratinocytes at the wound site ([Jameson and Havran, 2007](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr44-0022034509359125); [Mills](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr67-0022034509359125)*[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr67-0022034509359125)*[., 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr67-0022034509359125)).

The proliferative phase generally follows and overlaps with the inflammatory phase, and is characterized by epithelial proliferation and migration over the provisional matrix within the wound (re-epithelialization). In the reparative dermis, fibroblasts and endothelial cells are the most prominent cell types present and support capillary growth, collagen formation, and the formation of granulation tissue at the site of injury. Within the wound bed, fibroblasts produce collagen as well as glycosaminoglycans and proteoglycans, which are major components of the extracellular matrix (ECM). Following robust proliferation and ECM synthesis, wound healing enters the final remodeling phase, which can last for years. In this phase, regression of many of the newly formed capillaries occurs, so that vascular density of the wound returns to normal. One critical feature of the remodeling phase is ECM remodeling to an architecture that approaches that of the normal tissue. The wound also undergoes physical contraction throughout the entire wound-healing process, which is believed to be mediated by contractile fibroblasts (myofibroblasts) that appear in the wound ([Gosain and DiPietro, 2004](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr36-0022034509359125); [Campos](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr12-0022034509359125)*[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr12-0022034509359125)*[., 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr12-0022034509359125)).

The role of stem cells (SC) in cutaneous wound healing and tissue regeneration is a topic of increasing research attention, with a focus on the role of adult stem cells such as epidermal stem cells and bone-marrow (BM)-derived cells (BMDCs). Epidermal stem cells reside in the bulge area of hair follicles and in the basal layer of the epidermis and give rise to the keratinocytes that migrate andre-epithelialize wounds. Normal skin is also a target organ for BMDCs. Two main stem cell populations are present in the bone marrow: hematopoietic SC (HSC) and mesenchymal SC (MSC). BM-MSCs are able to differentiate into a variety of cell types, including adipocytes, osteoblasts, chondrocytes, fibroblasts, and keratinocytes ([Cha and Falanga, 2007](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr13-0022034509359125); [Rea](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr79-0022034509359125)*[et al.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr79-0022034509359125)*[, 2009](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr79-0022034509359125)). Endothelial progenitor cells (EPCs) derived from the HSC lineage are key cells that contribute to neovascularization. Both BM-MSCs and EPCs are involved in the cutaneous wound-healing process. Wound-induced hypoxia triggers the mobilization of bone marrow EPCs to the circulation, playing a significant role in the process of neovascularization ([Wu](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr104-0022034509359125)*[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr104-0022034509359125)*[., 2007](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr104-0022034509359125); [Liu and Velazquez, 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr58-0022034509359125); [Rea](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr79-0022034509359125)*[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr79-0022034509359125)*[., 2009](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/%22%20%5Cl%20%22bibr79-0022034509359125)).

Several different cell types are involved in the wound-healing process, and, as described above, the cellular activities of any particular cell type may also vary during different stages of repair. The complexity and coordination of the healing process are major hurdles to therapeutic approaches, since any therapeutic must effectively be sequenced to the appropriate stage.

**2.3 Conclusion**

Wound healing is a complex biological process that consists of hemostasis, inflammation, proliferation, and remodeling. Large numbers of cell types—including neutrophils, macrophages, lymphocytes, keratinocytes, fibroblasts, and endothelial cells—are involved in this process. Multiple factors can cause impaired wound healing by affecting one or more phases of the process and are categorized into local and systemic factors. The influences of these factors are not mutually exclusive. Single or multiple factors may play a role in any one or more individual phases, contributing to the overall outcome of the healing process.

**Question Three**

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

**3.1 Oxidative stress in the development of impaired wound healing**

A large percentage of the population suffers from wound healing abnormalities, in particular aged individuals, patients with diabetes, and those treated with immunosuppressive drugs, chemo- or radiotherapy. The mechanisms underlying the impaired healing response are still poorly understood. Recent studies provided strong evidence for a role of oxidative stress in the pathogenesis of non-healing ulcers. Therefore, it is of major importance to identify and functionally characterize the factors involved in the generation and detoxification of reactive oxygen species (ROS). This will provide the basis for the development of new strategies for therapeutic intervention. The current information about the roles of low molecular weight antioxidants and ROS-detoxifying enzymes in normal and impaired wound repair, and we report on the consequences of their modulation at the wound site.

**3.2 Progression of impaired wound healing**

At times difficult to appreciate, the wound healing process (WHP) is a highly structured and well-organized biological process (Heughan C *et al*., 1975). Wound healing can be divided into 4 phases:

Hemostasis

Inflammation

Proliferation

Tissue remodeling

When a wound forms, whether due to trauma or surgery, immediate vasoconstriction occurs via the action of thromboxane A2 and prostaglandin . Parallel to this process, the initiation of the clotting cascade takes place. Platelets arrive first to provide hemostasis and release cytokines and growth factors. These chemoattractants, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF), as well as cytokines, promote the migration of inflammatory cells to the wound. After approximately 24 to 48 hours, vasodilation occurs, allowing for inflammatory cells such as neutrophils, monocytes, macrophages, and lymphocytes to arrive at the injured tissue and perform a host of different functions. Neutrophils are the first of the inflammatory cells to arrive, peaking at 24 hours. They phagocytize bacteria, clear microbial and other cellular debris. Also, polymorphonuclear leukocytes (PMNs) release reactive oxygen species that potentiate this killing process  (Rodrigues M *et al*., 2019).

The next major step in wound healing involves the accumulation of macrophages, usually around 48 to 72 hours. Macrophages help initiate the proliferation phase of the WHP. These cells also perform a variety of diverse functions, including promoting the inflammatory healing process through the release of cytokines, clearance of cellular debris, and attracting blast cells to the area of the wounding (Rodrigues M *et al*., 2019).

 T-lymphocytes also play a critical but still poorly understood role, as their absence in the wound or delayed arrival has been associated with WHP impairment. As the proliferation phase gives way to remodeling, fibroblasts lay down the extracellular matrix (ECM) and allow for re-epithelialization of the wound. Fibroblasts produce components of the ECM, including collagen-glycosaminoglycan scaffolds and proteoglycans. Furthermore, endothelial cells promote angiogenesis and formation of a new capillary bed to allow for continued remodeling. Myofibroblasts promote wound contracture via actin filaments. Over time, the wound will regain up to 70% to 80% of its original tensile strength (Rodrigues M *et al*., 2019).

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