**ANA 404 ASSIGNMENT ON WOUND HEALING.**

**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.**

***BY***

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* 1. **Cytokine Signaling and its role in wound healing**

**Cytokine Signaling**

 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) (Zhang and An, 2007).

**PROINFLAMMATORY CYTOKINES**

Proinflammatory cytokines, particularly IL‐1 and interleukin‐6, and TNF‐α are up‐regulated during the inflammatory phase of wound healing (Singer and Clark, 1999). IL‐1 is produced by neutrophils, monocytes, macrophages, and keratinocytes. Upon wound healing it is immediately released by keratinocytes. In addition to having a paracrine effect, it also works in an autocrine fashion increasing keratinocyte migration and proliferation (Raja *et al.,* 2007). IL‐1 has been shown to induce the expression of K6 and K16 in migrating keratinocytes. In addition, IL‐1 activates fibroblasts and increases the secretion of FGF‐7 (Tang and Gilchrest, 1996). IL‐6 is produced by neutrophils and monocytes and has been shown to be important in initiating the healing response. Its expression is increased after wounding and tends to persist in older wounds. It has a mitogenic  and proliferative effect on keratinocytes and is chemoattractive to neutrophils (Komine *et al.,* 2000).

Much like IL‐1, TNF‐α can induce the production of FGF‐7, suggesting that it can indirectly promote reepithelialization. Alone, TNF‐α has been shown to inhibit wound reepithelialization.

The effects of exogenous TNF‐α are dependent on concentration and duration of exposure emphasizing the importance of balancing the proinflammatory signals controlling wound healing. TNF‐α, at low levels, can promote wound healing by indirectly stimulating inflammation and increasing macrophage produced growth factors. However, at higher levels, especially for prolonged periods of time, TNF‐α has a detrimental effect on healing. TNF‐α suppresses synthesis of ECM proteins and TIMPs while increasing synthesis of MMPs (MMP‐1, MMP‐2, MMP‐3, MMP‐9, MMP‐13, and MT1‐MMP). In addition, elevated levels of IL‐1β have a similar response to that of TNF‐α. Both TNF‐α and IL‐1β have been shown to perpetuate each others expression and therefore amplify this signal (Gallucci *et al*., 2004).

Levels of TNF‐α and IL‐1β are elevated in chronic wounds. In addition, infection that is common in chronic wounds further contributes to prolonged inflammation. Furthermore, nonhealing wounds also exhibit elevated levels of interstitial collagenases, gelatinases, and stromelysins that have been shown to be induced by TNF‐α and IL‐1β. It has, therefore, been hypothesized that in chronic wounds, chronic inflammation causes inflammatory cells to secrete TNF‐α and IL‐1β that synergistically increase production of MMPs while reducing synthesis of TIMPs. It is increased MMP activity that degrades the ECM inhibiting cell migration and collagen deposition. MMPs also break down growth factors and their target cell receptors (Tarnuzzer and Schultz, 1996).

**CHEMOKINES**

Chemokines are also active participants in the wound healing process because they stimulate the migration of multiple cell types in the wound site particularly inflammatory cells. In addition, the presence of chemokine receptors on resident cells suggests that they also contribute to the regulation of reepithelialization, tissue remodeling, and angiogenesis (Raja *et al*., 2007). The CXC, CC, and C families of ligands act by binding to G protein‐coupled surface receptors, CXC‐receptors and the CC‐receptor.

Macrophage chemo‐attractant protein (MCP‐1 or CCL2) is a CC family chemokine. MCP‐1 is induced in keratinocytes upon wounding. It is a chemoattractant for monocytes/macrophages, T‐cells, and mast cells Sustained expression of this chemokine permits a prolonged presence of neutrophils and macrophages in the chronic wound contributing to a prolonged inflammatory response. However, lack of MCP‐1 in vivo significantly delays wound healing particularly with reepithelialization, angiogenesis, and collagen synthesis as seen in mouse models. This suggests that in the mouse MCP‐1 may be influencing gene expression/protein synthesis of growth factors in murine macrophages (Low *et al*., 2001). However, in humans MCP‐1 does not seem to regulate growth factor production by these cells. Addition of exogenous MCP‐1 to wounds in animals yielded only moderate improvements in wound healing (Brockman *et al*., 2017).

Interferon inducible protein 10 (IP‐10 or CXCL10) is another cytokine part of the CXC family. In acute wounds and chronic inflammatory states, there is increased expression by keratinocytes. IP‐10 has been demonstrated to negatively impact wound healing. Overexpression

of IP‐10 results in a more intense inflammatory response by recruiting lymphocytes to the wound site. In vitro studies show that IP‐10 delays reepithelialization and prolongs the granulation phase (Morand *et* al., 2017).This cytokine inhibits the migration of dermal fibroblasts by blocking their release from the substratum regulated by IP‐10 inhibition of EGF and heparin‐binding EGF‐like growth factor receptor‐mediated calpain activity. In addition, it has been shown that IP‐10 inhibits angiogenesis (Belperio *et al*., 2000). A suggested mechanism can be seen in the related cytokine, PF4. PF4 inhibits endothelial cell migration, proliferation, and angiogenesis in response to bFGF. PF4 inhibits bFGF binding its receptor by forming heterodimeric complexes via heparin binding. It has been suggested that IP‐10 might work in a similar fashion (Tan *et al*., 2018).

Interleukin‐8 (IL‐8 or CXCL8) is a member of the CXC family. Its expression is increased in acute wounds and it has been shown to play a role in reepithelialization by increasing keratinocyte migration and proliferation. It also induces the expression of MMPs in leukocytes, stimulating tissue remodeling (Patruno *et al*., 2018). It is, however, a strong chemoattractant for neutrophils, thus participating in the inflammatory response. High levels of this chemokine accumulate in nonhealing wounds. Furthermore, addition of IL‐8 in high levels decreases keratinocyte proliferation and collagen lattice contraction by fibroblasts. It has been shown that there are relatively low levels of IL‐8 in the fetus. This finding may be responsible for the lack of inflammation during the fetal wound healing and contribute to scarless wounds (Wang *et al*., 2017).

* 1. **When is wound healing referref to as “impaired” and why?**

Wound healing, as a normal biological process in the human body, is achieved through four precisely and highly programmed phases: hemostasis, inflammation, proliferation, and remodeling (Rocchetti and Braga, 2012). For a wound to heal successfully, all four phases must occur in the proper sequence and time frame. 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 (Guo and DiPietro, 2010).

 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 (Menke *et al*., 2007). Multiple factors can lead to impaired wound healing. In general terms, the factors that influence repair can be categorized into local and systemic. Local factors are those that directly influence the characteristics of the wound itself, while systemic factors are the overall health or disease state of the individual that affect his or her ability to heal (Guo and DiPietro, 2010). An example of a local factor that affects wound healing is oxygenation.

 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)).

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/%22%20%5Cl%20%22bibr95-0022034509359125)).

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)). 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 ([Rodriguez et al., 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903966/#bibr81-0022034509359125)). 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)).

An example of a systemic factor is stress (Guo and DiPietro, 2010). Stress has a great impact on human health and social behavior. Many diseases such as cardiovascular disease, cancer, compromised wound healing, and diabetes; are associated with stress. Studies in both humans and animals have demonstrated that psychological stress causes a substantial delay in wound healing. Caregivers of persons with Alzheimer’s and students undergoing academic stress

during examinations demonstrated delayed wound healing (Marucha et al, 1998). . Stress up-regulates glucocorticoids (GCs) and reduces the levels of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α at the wound site. Stress also reduces the expression of IL-1α and IL-8 at wound sites both chemoattractants that are necessary for the initial inflammatory phase of wound healing (Boyapati and Wang, 2007). Stressors can lead to negative emotional states, such as anxiety and depression, which may in turn have an impact on physiologic processes and/or behavioral patterns that influence health outcomes. In addition to the direct influences of anxiety and depression on endocrine and immune function, stressed individuals are more likely to have unhealthy habits, which include poor sleep patterns, inadequate nutrition, less exercise, and a greater propensity for abuse of alcohol, cigarettes, and other drugs. All of these factors may come into play in negatively modulating the healing response (Guo and DiPietro, 2010).



Figure 1: Effects of stress on wound healing (Guo and DiPietro, 2010).

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

Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism and environmental factors, such as air pollutants or cigarette smoke. ROS are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions. The shift in the balance between oxidants and antioxidants in favor of oxidants is termed "oxidative stress." (Birben et al., 2012).

**Role of ROS in wound repair**

The wound healing process is regulated by a large variety of different growth factors, cytokines and hormones. In addition, a series of recent studies revealed that nitric oxide as well ROS are crucial regulators of this process. ROS are required for the defense against invading pathogens

and low levels of ROS are also essential mediators of intracellular signaling. For example, a recent study revealed that low levels of hydrogen peroxide are important for efficient wound angiogenesis. These positive roles of ROS in the wound repair process have recently been reviewed. However, excessive amounts of ROS are deleterious due to their high reactivity. A series of recent studies have highlighted the important role of ROS in the wound healing process. On the one hand, they are required for efficient defense against invading pathogens. Even in the absence of infection, low levels of ROS are required for cellular signalling, in particular for angiogenesis. However, excessive production of ROS or impaired detoxification of these aggressive molecules causes oxidative stress, and this has been identified as an

important feature in the pathogenesis of chronic, non-healing wounds. Therefore, a tight regulation of ROS production and detoxification is crucial for the normal repair process. The identification of proteins involved in these processes and their functional in vivo analysis have already identified some key regulators of the redox balance in healing wounds. However, the in vivo functions of many additional proteins need to be explored and the interaction of individual ROS-producing and -detoxifying enzymes will have to be characterized in the future. This knowledge will likely result in the identification of novel targets for the treatment of wound healing disorders.

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