**DEPARTMENT OF HUMAN ANATOMY**

**AFE BABALOLA UNIVERSITY, ADO EKITI STATE**

**COURSE TITLE:** INTRODUCTION TO HISTOPATHOLOGY

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An Assignment On

Wound Healing.

BY

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**Question One**

**Write on cytokine signaling and its role in wound healing**

Cytokine biology is now recognized as a fundamental component of immunology and the actions of cytokines are understood to be essential mechanisms underlying host defense, immunoregulation, and autoimmunity. Moreover, cytokines themselves and cytokine antagonists have become some of the most successful new drugs. On a more basic level, the biochemistry of cytokine action has become a paradigm for understanding rapid, evolutionarily conserved membrane-to-nucleus signal transduction, offering remarkable opportunities for understanding how extracellular cues are sensed and translated into the control of gene expression.  (John *et al*., 2011). Cytokines represent a diverse group of molecules that transmit intercellular signals. These signals may either be autocrine (where the same cell both produces the cytokine and responds to it) or paracrine (where the cytokine is made by one cell and acts on another). Both these situations can occur simultaneously. Cytokines use multiple signaling pathways (Warren *et al*., 2000).

Some cytokine-signaling pathways involve hormones such as growth hormones and leptin, the hormone that controls fat storage.

The immune system depends on cytokine signaling to keep the human body healthy. Macrophages and dendritic cells engulf foreign particles and send a cytokine signal to nearby dormant lymphocytes. The receptors on the lymphocytes recognize the signal and activate. Those cells are specialized to recognize certain antigens. The combination of the macrophages and activation of lymphocytes through cytokine signaling help keep the body in homeostasis — or the proper internal equilibrium.

Some cytokine signals are not local but rather travel a long distance throughout the body. These cytokines are sometimes classified as hormones. This classification is changing, however, because cytokines are not secreted from glands. Instead, they are secreted from glial cells of the nervous system. These growth hormones are essential for embryonic development. Cytokines bind to receptors on target cells and activate a cascade of intercellular signals. The most common of these pathways is the protein kinase transduction cascade. After the cytokine binds to the receptor embedded in the membrane of the cell, inactive protein kinases are activated by a process known as phosphorylation.

The coordination of normal wound healing occurs through communicating molecules, known as cytokines and growth factors, which provide many of the required molecular and cellular signals. Cytokines, expressed by numerous cell types, are part of a family of molecules that have autocrine, paracrine, and endocrine effects (Rumalla and Borah, 2001). Historically, mediators produced during the healing process have been frequently classified by the terms “cytokines” and “growth factors.” These terms have no real biological significance but rather represent how these mediators were initially described or defined. Cytokines were initially described as protein mediators produced by inflammatory cells that coordinate communication between leukocytes and parenchymal tissues. In contrast, growth factors were considered to be peptide mediators involved in cell proliferation, cell cycling, and apoptosis. Clearly, many cytokines have growth factor properties (interleukin [IL]-2 as a T-cell growth factor, IL-6 as a B-cell growth factor), whereas all growth factors can also be considered as cytokines involved in intercellular communication (Steed, 1997). Cytokines can be further subclassified based on some of their properties. For example, chemokines, such as IL-8, are traditionally known for their ability to regulate the recruitment and movement of leukocyte populations, mostly during lymphoid organogenesis and in response to inflammatory challenges. There has been a great deal of research regarding both the

CC and CXC families of chemokines, which appear responsible for the recruitment and activation predominantly of mononuclear cell and granulocyte cell populations, respectively. Evaluation of wound fluid has demonstrated that different cytokines are expressed in the wound at different time periods during the healing process (Dvonch *et al*., 1992). Common to many processes in the body directed by cytokines, the timing and pattern of the cytokine response may be more important than the magnitude of cytokine expression. Because cytokines and growth factors play an important role in tissue repair.

**Question 2**

**When is wound healing referred to as impaired and why?**

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, et al., 2008; 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 et al., 2004).

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 et al., 2008, Rodriguez et al., 2008). 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).

**Question 3**

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

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 (Wlaschek *et al*., 2015). ROS are required for the defense against invading pathogens, and low levels of ROS are also essential mediators of intracellular signaling (D’Autreaux and Toledano, 2007). For example, a recent study revealed that low levels of hydrogen peroxide are important for efficient wound angiogenesis (Roy *et al*., 2006). These positive roles of ROS in the wound repair process have recently been reviewed (Sen and Roy, 2008). However, excessive amounts of ROS are deleterious due to their high reactivity. In this review, we will first summarize the evidence for the presence of oxidative stress in skin wounds, in particular in chronic non-healing wounds. Subsequently, we will report on the presence of low molecular weight antioxidants in the wound tissue and their function in the repair process. Finally, we will summarize recent results on the expression and function of ROS-detoxifying enzymes in the wound healing process.

Due to the short half-life of ROS, their concentrations in vivo are difficult to determine. Nevertheless, H2O2 levels could recently be determined in wound fluid from acute murine excisional wounds using a real-time electrochemical H2O2 measurement (Roy et al., 2006). These studies revealed that low concentrations (100–250 M) of H2O2 are present at the wound site. Higher levels were found during the early inflammatory phase (day 2 after injury) compared to the later phase, when new tissue formation occurs (day 5 after injury). In addition to H2O2, the presence of superoxide at the wound edge was detected by staining of frozen sections with the redox-sensitive dye dihydroethidium (Sen and Roy, 2008). The same group recently confirmed these results using an electron paramagnetic resonance spectroscopy-based approach, where the metabolism of topically applied nitroxide 15N-perdeuterated tempone was measured noninvasively. These studies revealed that superoxide levels peak at around day 2 after injury in full-thickness excisional mouse wounds (Ojha, 2008). Superoxide production was impaired in mice lacking Rac2, one of the essential subunits of NADPH oxidase, and this correlated with impaired wound healing in these mice (Ojha, 2008). These results suggest that the low levels of ROS that are produced in normal wounds are important for the repair process. It will be interesting in the future to use these technologies for the analysis of ROS levels in chronic, non-healing wounds. In most studies, ROS levels at the wound site have been determined indirectly through analysis of oxidation products of lipids, proteins or DNA (Urso and Clarkson, 2003). A major product of lipid peroxidation is 4-hydroxy-2-nonenal (4-HNE), which could be detected by M. Schäfer, S. Werner / Pharmacological Research 58 (2008). Different ROS that are produced in inflamed tissues are shown together with the enzymes that generate or detoxify ROS as well as oxidized macromolecules. GSH: glutathione. immunohistochemistry at the edge of murine excisional wounds. Interestingly, co-immunostaining revealed that 4-HNE mainly colocalizes with neutrophils, suggesting that the respiratory burst of these inflammatory cells results in the production of superoxide, which in turn causes lipid peroxidation (Ojha, 2008). Another lipid peroxidation product is malondialdehyde (MDA), which was found at significantly higher levels in wounds of hydrocortisone-treated, healing-impaired rats compared to control animals (Gupta *et al*., 2002). Surprisingly, however, no difference in MDA levels were found between wound fluid from acute and chronic human wounds (Moseley *et al*., 2001). Peroxidation of essential fatty acids (primarily arachidonic acid) results in the formation of isoprostanes, which are prostaglandin-like molecules. A strong increase in the concentration of 8-isoprostanes was found in fluid from chronic venous ulcers in comparison to fluid from acute human wounds (Yeoh and Stacey, 2003). These findings provide evidence for oxidative stress in chronic ulcers, which likely results from the persistence of a strong inflammatory infiltrate (Wlaschek *et al*., 2015). This hypothesis is further supported by the significant elevation of the allantoin to uric acid ratio in wound fluid from chronic leg ulcers compared to wound fluid from acute surgical wounds (James *et al*., 2003). This ratio represents a marker for oxidative stress. An alternative readout for oxidative stress in vivo is the detection of oxidized proteins. Using oxyblot analysis to determine the levels of oxidized proteins, which are characterized by the presence of carbonyl groups, a strong increase in the levels of oxidized proteins was seen in wounded compared to intact mouse skin (Fig. 2A). Interestingly, wounds from male mice had higher levels of oxidized proteins (Kumin *et al*., 2007). This correlates with the enhanced inflammation and reduced wound healing rates seen in male compared to female animals (Ashcroft and Mills, 2002). Surprisingly, however, wound fluid from chronic human wounds showed a lower protein carbonyl content than fluid from acute wounds (Moseley *et al*., 2001). It will be interesting to determine if this can be confirmed for tissue lysates. Another possibility to detect protein oxidation is the immunohistochemical detection of nitrotyrosine. In combination with nitric oxide, ROS contribute to the generation of peroxynitrite. This aggressive molecule can react with tyrosine residues of proteins, resulting in tyrosine nitration. Immunostaining with an antibody against nitrotyrosine revealed the presence of proteins with nitrotyrosine residues in the granulation tissue of wild-type mice. The number of nitrotyrosine-positive cells was strongly increased in mice deficient for the ROS-detoxifying enzyme peroxiredoxin 6.

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