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2.1. PHYSIOLOGY OF WOUND HEALING

Introduction
In this modern jet age, the incidence of accidents has steeply risen, which is responsible for the different types of wounds. A wound is disruption of the anatomic structure and its functional continuity of living tissue. Healing is the process of repair that follows injury to the skin and other soft tissues. Wound healing is essentially, a survival mechanism and represents an attempt to maintain the normal structure and function. The capacity of a wound to heal depends partly on its depth, as well as on the overall health and nutritional status of the individual.

Clinically wound may be categorized as acute or chronic based on the timeliness of healing. The acute wound is a breakdown of the integrity of the soft tissue envelope surrounding any portion of the body. Acute wound is defined by its size, depth and involved anatomic structures. However, the exact duration of healing and the distinction between acute and chronic is arbitrary and often based on variables including the site and cause of the wound, age and physical condition of the patient. The time course between an acute versus chronic wound is a continuum between 4 and 6 weeks. It is during this time that if an acute wound has not healed spontaneously, it is likely to become a chronic, “problem wound” that requires further intervention. The acute wound can present as simple or complex, depending on its location, size, involved anatomic structures and bio-burden. The foundation for closure of an acute wound lies in an adequate surgical debridement and a systematic approach to options for closure.

Etiology of acute wound is usually violation of the skin and subcutaneous tissue integrity through multiple mechanisms. These mechanisms include penetrating or blunt trauma as well as various environmental exposures, such as chemical substances, extremes of temperature, prolonged or excessive pressure and radiation. Disruption of the continuity of the skin from any of these mechanisms allows entry of organisms that can lead to local or systemic infection. Irrespective of the nature of the cutaneous injury, acute wounds are expected to heal within a predictable timeframe, although the treatment required to facilitate healing will vary according to the type, site and depth of a wound.
Chronic wound is defined as those wounds that fail to progress through a normal, orderly and timely sequence of repair or wounds that pass through the repair process without restoring anatomic and functional results. Orderly refers to the progression of the wound through the biologic sequences that comprise the phase of repair of acute wounds. Timeliness relates to the progression of phases of repair in a manner that will heal the wound expeditiously. Timeliness is determined by the nature of the wound pathology, medical status of the patient, and environmental factors. Most chronic wounds are associated with a small number of clinical entities, particularly chronic venous stasis, diabetes mellitus and pressure necrosis.

Although some components of the healing process have regenerative aspects, skin is an example of a tissue in which the response to injury is predominantly one of repair. Phases of wound healing are hemostasis, inflammation, proliferation, epithelization and maturation-remodeling. All the phases may occur in orderly and overlapping manner. The primary processes that contribute to the closure of skin wounds are epithelization, wound contraction and collagenous scar formation. Although the relative contribution of each process is different depending on the type of wound, all of these processes are stimulated in response to injury. A partial thickness burn will heal primarily by epithelization, whereas collagenous scar formation is much more important in the healing of an incisional wound. Wound contraction is the primary process involved in the secondary healing of large open wounds such as pressure sores. Epithelization, wound contraction and collagenous scar formation represent terminal aspects of the wound healing response. There are multiple necessary precursors to these terminal events. Wound healing consists of the combined effect of all these preliminary and terminal processes occurring in a carefully regulated manner.

2.1.1. Phases of healing
When human skin tissue is injured, the body supports a complex variety of cellular and molecular reactions in order to return the tissue to homeostasis. These are categorized into 4 phases.
2.1.1.a. Hemostasis/ coagulation
This phase involves a series of complex reactions leading to hemostasis and clot formation. The clot consists of a fibrin mesh with aggregated platelets embedded in it. The mesh traps red cells that become another component of the clot plug. Fibrin is the end product of the coagulation cascades that are stimulated by vascular injuries. There is an intrinsic and an extrinsic coagulation cascade triggered by separate events\textsuperscript{26}. Activation of factor XII initiates the intrinsic coagulation pathway and occurs when blood is exposed to foreign surfaces. An exposure to tissue factor that binds factor VII initiates the extrinsic coagulation pathway. Tissue factor is not found on vascular endothelial cells, but is found in abundance on extravascular cellular surfaces, especially on adventitial fibroblasts. On injuring to the cells the factor is released. Both coagulation pathways result in the production of thrombin catalyzing the conversion of fibrinogen to fibrin. In addition to contributing to hemostasis, fibrin is also the primary component of the provisional matrix that forms in the wound during the early healing period. Fibronectins are a class of glycoproteins that facilitate attachment of migrating cells onto the fibrin latticework and they are an extremely important component of the early matrix as well as mature dermis\textsuperscript{27,28}. Fibronectin is produced by fibroblasts and epithelial cells\textsuperscript{29}. Stimulation of the haemostatic mechanisms is limited to the site of injury in that normal endothelial cells produce prostacyclin that inhibits platelet aggregation. In addition, in uninjured areas, antithrombin III binds thrombin and limits its activity and protein C degrades factors V and VII\textsuperscript{27,28}.

The processes of blood coagulation and platelet aggregation terminate when the stimuli for clot initiation dissipate. Clot breakdown begins as soon as the clots form. Plasminogen activator mediates the clot lysis and converts plasminogen to plasmin, an extremely potent enzyme that can degrade a wide variety of extracellular matrix proteins\textsuperscript{28}.

2.1.1.b. Inflammation
Following an injury, inflammatory response is an essential part of the wound-healing process. A reaction of vascularized living tissues after injury is inflammation. The physiologic process underlying this inflammation begins immediately upon tissue
injury, but reaches completion usually after neutralization of the injurious influence. The inflammatory response triggers events that have implications for the entire healing process\textsuperscript{30}.

The physical signs of inflammation include erythema, edema, pain and heat. These signs are largely a result of changes that occur in the microcirculation and particularly in microvenules of 15 to 20 µm in diameter. Areas of injury cause vasodilatation that generate erythema and heat. Vasodilatation is mediated by histamine, kinins, prostaglandins and possibly additional factors such as leukotrienes and endothelial cell product. In addition to vasodilatation, the capillaries develop gaps between the endothelial cells lining them. Gap formation and increased permeability are also partially mediated by histamine and prostaglandins, although neutrophil factors contribute as well. These gaps allow plasma to leak from the intravascular space to the extravascular compartment\textsuperscript{31}. The prostaglandins PGE$_1$ and PGE$_2$ stimulate vasodilatation as well as capillary permeability. Prostaglandins affect vasodilatation through activation of adenyl cyclase and production of cAMP\textsuperscript{32}. Prostaglandins accumulate in injured tissue, probably from activation of phospholipases on injured cell membranes. The migration of cells and fluid into the injured area generates edema.

\textit{Role of white blood cells in inflammation}

Neutrophils are the first of the leukocytes found in wounded tissue in large numbers. Neutrophils function as defensive units that engulf foreign material and digest it through the action of hydrolytic enzymes and oxygen radicals. After phagocytosing damaged tissue or bacteria, neutrophils are themselves phagocytosed by macrophages and destroyed. Alterations in pH resulting from breakdown products of tissue and bacteria, along with swelling and decreased tissue oxygenation resulting from damage to the blood supply, produce the pain noted in areas of injury\textsuperscript{33}.

As monocytes migrate from the capillaries into the extra vascular space, they transform into macrophages in a process mediated by serum factors and fibronectin\textsuperscript{34}. Specific factors that stimulate macrophage migration include collagen fragments, fibronectin fragments\textsuperscript{35} and elastin\textsuperscript{36} derived from damaged matrix, as well as complement.
components. Macrophages are tremendously important in normal wound healing. Macrophages phagocytose bacteria and dead tissue and also secrete collagenase and elastases that break down damaged matrix\textsuperscript{37,38}.

Lymphocytes produce factors essential for normal healing. In addition to functioning as immunoreactants, it is involved in cellular immunity and antibody production. IL-2 and other factors have been demonstrated to be chemotactic for lymphocytes\textsuperscript{39,40}.

Eosinophils are only present in limited quantities in the peripheral circulation under normal circumstances. They can also migrate into the extravascular tissues in response to injury\textsuperscript{41}.

In normal healing, changes that occur in tissue over time are extremely reproducible. After hemostasis is accomplished, inflammatory cells migrate into the wound with neutrophils initially predominating. At 48 to 72 hours, macrophages begin to outnumber neutrophils and large number of macrophages remains in the wound for several days. This is critical in that macrophages\textsuperscript{42,43} unlike neutrophils\textsuperscript{44} are essential for normal healing. After 5 to 7 days, few inflammatory cells remain in normally healing wounds and fibroblasts become the predominant cell type.

\textbf{2.1.1.c. Proliferative Phase}

This phase begins approximately 2-3 days after formation of wound. During the proliferative phase, the repair processes are angiogenesis, fibroplasia and epithelisation. This stage is characterized by the formation of granulation tissue, consisting of a capillary bed, fibroblasts, macrophages and a loose arrangement of collagen, fibronectin, hyaluronic acid and bacteria. The desired outcome of proliferative phase is to fill the wound defect with connective tissue and cover it with epithelium. These sub phases do not happen in discrete timeframe but constitute an overall and ongoing process.

\textbf{2.1.1.c.i. Epithelization:} Occurs early in wound repair. Following injury, renewal of the epithelial barrier is essential to re-establish the barrier functions of skin. Epithelial cells
cover all surfaces of the body including internal surfaces such as the gastrointestinal, respiratory and genito-urinary tract. Injury results in discontinuity of the epithelium. The epithelial cells migrate and initiate the process of epithelization\textsuperscript{45}. This process is important to complete healing quickly, as it reforms the body’s barrier with the outside by minimizing chance of infection and water loss at the wound site. If the basement membrane remains intact, the epithelial cells migrate upwards in the normal pattern. This is equivalent to a first-degree burn of the skin. The epithelial progenitor cells remain intact below the wound and the normal layers of epidermis are restored in two to three days. If the basement membrane is destroyed, similar to a second degree or third degree burn, then the wound is reepithelialized from the normal cells in the periphery and from the skin appendages (hair follicles and sweat glands). Epithelization in an incisional wound involves cellular migration over a distance of less than a millimeter from one wound edge to the adjacent one and is a minor contributor to the healing process. Incisional wounds are generally completely reepithelized in 24 to 48 hours. Epidermal growth factor and platelet-derived growth factor are postulated to stimulate epithelial migration\textsuperscript{46,47}. Transforming growth factor-\(\alpha\) (TGF-\(\alpha\)) is also a potent stimulant of epithelial cell migration and proliferation\textsuperscript{48}.

\textbf{2.1.1 .c. ii. Fibroplasia:} This process predominates in 2 to 4 days after formation of wound and mediated by cytokines. Fibroblasts are the primary mesenchymal cells in dermis and they are the most important mesenchymal cells involved in wound healing. Injury damages smooth muscle cells and other cell types and are involved in the healing response. Fibroblasts from the surrounding undamaged tissue migrate into wound matrix under the influence of chemotactic cytokines derived from inflammatory cells and other factors, some of which may be bound to the matrix. The fibroblasts themselves may contribute chemotactic cytokines that further stimulate their migration\textsuperscript{43}. Undifferentiated mesenchymal cells in the neighbourhood of the wound may differentiate into fibroblasts when stimulated by macrophage products and migrate into the wound as well\textsuperscript{49}. Platelet derived growth factor (PDGF) has been demonstrated to be chemotactic for both fibroblasts\textsuperscript{50} and smooth muscle cells\textsuperscript{51} and has been demonstrated to be present at the site of wound\textsuperscript{51,52}. 
2.1.1.c.iii. Angiogenesis: Reconstructs the vasculature in areas damaged by wounding. The capillary sprouts grow through the proliferation of endothelial cells at the sprout bases. The cells within the sprout develop a curvature, which results in a lumen. The capillary sprouts continue to grow until they contact other sprouts growing from other directions. The sprouts then interconnect forming a vascular loop, and the sprouting process begins anew. After a portion of the wound becomes revascularized with new capillaries, the vascular system subsequently matures possibly through the aggregation of capillaries, resulting in fewer larger vessels in the healed wound53.

Cytokines, many of which are macrophage derived, directly and indirectly stimulate the endothelial cell migration and proliferation required for angiogenesis. The lactic acid, biogenic amines and low oxygen tension in the injured tissue stimulated the release of cytokines54.

2.1.1.c. iv. Granulation tissue formation: This new tissue fills the wound space and revascularizes the injured site. The concurrent infiltration into the wound site of macrophages, fibroblasts and blood vessels allows granulation tissue formation to occur. Granulation tissue consists of a combination of cellular elements including fibroblasts and inflammatory cells along with new capillaries embedded in a loose extracellular matrix of collagen, fibronectin and hyaluronic acid. As early as 24 hours after injury, fibroblasts and vascular endothelial cells begin proliferating to form granulation tissue. It is specialized and its formation is the hallmark of healing25.

2.1.1.c.v. Collagenation: Collagen synthesized primarily by fibroblasts, is a major component of normal skin, granulation tissue and mature scar. Collagen makes up 25% of all body proteins and more than 50% of the protein found in scar tissue55. Fibroblasts produce large quantities of collagen, a family of triple chain glycoproteins, which form the main constituent of the extracellular wound matrix, which are ultimately responsible for imparting tensile strength to the scar. Detection of collagen in the wound occurs around the third day of post-injury, thereafter the levels increase rapidly for approximately 3 weeks. It then continues to accumulate at a more gradual pace for up to 3 months. Initial deposition of collagen is in a haphazard fashion. Subsequently, these
individual collagen fibrils are reorganized by cross-linking into regularly aligned bundles. Fibroblasts have migrated into the wound, laying down new collagen of the subtypes III and I. Early in normal wound healing type III collagen predominates but is later replaced by type I collagen.

Tropocollagen is the precursor of all collagen types that transforms within the cells rough endoplasmic reticulum, where proline and lysine are hydroxylated. Disulfide bonds are established allowing 3 tropocollagen strands to form a left-handed triple helix, termed procollagen. As the procollagen is secreted into the extracellular space peptidases in the cell wall cleave terminal peptide chains, creating true collagen fibrils. The critical component of collagen synthesis is the hydroxylation of lysine and proline moieties within the polypeptide chains, and this occurs in the endoplasmic reticulum. Hydroxylysine is required for covalent cross-link formation. Hydroxyproline is found almost exclusively in collagen and serves as a marker of the quantity of collagen in tissue. This hydroxylation process requires specific enzymes for lysine and proline in addition to oxygen, vitamin C, alpha-ketoglutarate, and ferrous iron functioning as cofactors. Deficiencies in vitamin C, oxygen, or suppression of enzymatic activity by corticosteroids may lead to under hydroxylated collagen, which is incapable of generating strong cross-links and which is early broken down early.25

Age56, mechanical pressure57 and tension58 affect the rate of collagen synthesis. Transforming growth factor-β (TGF-β) is the most potent stimulant of collagen synthesis59. The TGF-β derives from both inflammatory cells and the fibroblasts themselves. Specific antibodies to TGF-β can limit collagen accumulation in wounds.60 Fibroblast growth factor61,62 and epidermal growth factor63 have also been shown to stimulate collagen synthesis. Glucocorticoids inhibit it64.

2.1.1.c.vi. Wound Contraction: The process by which the wound edges pull together to reduce the defect is wound contraction. It plays a considerable role in reducing the volume of tissue for repair. It is the result of the contraction of myofibroblasts in the granulation tissue. These are attached to each other and to the adjacent matrix
components so that granulation tissue as a whole contracts and draws together the surrounding tissues.\textsuperscript{65}

Wound contraction, like collagen synthesis, begins approximately 4 to 5 days after wounding. Wound contraction represents the centripetal movement of the wound edge towards the center of the wound. Maximal wound contraction continues for 2 to 15 days, though it can continue for longer periods if the wound remains open. The wound edges move towards each other at an average rate of 0.6 to 0.75 mm/day. The rate of contraction is dependent on tissue laxity, and there is significant variability among different tissues. A wound in the buttock where the tissue is loose will contract much more than a wound on the scalp or pretibial area where the skin is tighter. Wound shape can also affect contraction. Wounds with square edges contract more rapidly than circular wounds. Forces of contraction in a circular wound cancel each other to some degree, preventing effective centripetal movement of the wound edge.\textsuperscript{25}

Radiation and cytolytic drugs delay contraction adding further evidence that cellular activity is required. TGF-\(\beta\) can stimulate collagen lattice contraction and appears to be a mediator of wound contraction.\textsuperscript{66} Splints can temporarily slow wound contraction, though wound contraction will proceed at an accelerated rate after splint removal. Topical dressings may also delay wound contraction.\textsuperscript{67}

2.1.1.d. Scar formation: Scar formation is the hallmark of the final product of healing process. This relatively avascular and cellular mass of collagen serves to restore tissue continuity, strength and function.

At approximately 21 days following injury, net accumulation of wound collagen becomes stable.\textsuperscript{68} During this period the wound becomes less cellular as apoptosis occurs. Endothelial cells appear to be the first cell type to undergo apoptosis followed by myofibroblasts, which disappear on completion of wound contraction approximately 21 days after wounding. The process of scar remodeling dramatically increases wound bursting strength. The greatest rate of increase occurs between 3 and 6 weeks after
wounding. By 6 weeks after wounding, the wound has reached 80% to 90% of its eventual strength\textsuperscript{69}.

During this remodeling phase, a continual turnover of collagen molecules occurs as old collagen is broken down and a dense synthesis of new collagen occurs in a more organized fashion along lines of stress\textsuperscript{70}. During this period of scar remodeling, the number of intra and inter-molecular cross-linkages between collagen fibers increases dramatically. This increase in cross-linking is a major contributor to the increase in wound breaking strength noted during this phase of healing. As collagen matures during scar remodeling, the quantity of type III collagen decreases, it is replaced by type I collagen. The quantity of water and glycosaminoglycans in matrix decreases as well. Wound remodeling is visible as a change in the texture, thickness and color of a healing wound. This process continues for 6 to 12 months after wounding\textsuperscript{25}.

2.1.2 Wound healing types

2.1.2. a. Primary healing or healing by first intention
This occurs on closing a wound within a few hours of its creation. Approximation of a wound edges is done surgically or mechanically and collagen metabolism provides long-term strength. This surgical incision results in the mortality of a minimal number of cellular constituents.

2.1.2.b. Secondary healing or healing by second intention
This involves no formal wound closure. It occurs on allowing an open full thickness wound to close by wound contraction and epithelization. Secondary healing results in an inflammatory response that is more intense than seen in primary wound healing. Fibroblastic differentiation into myofibroblasts, which resemble contractile smooth muscle, contributes to wound contraction. These myofibroblasts are maximally present in the wound from 10-20 days.

2.1.2. c. Delayed primary closure or healing by third intention
If the wound edges are not re-approximated immediately, delayed primary wound healing transpires. Contaminated wounds desite this type of healing, Macrophages,
metamorphose into epitheloid cells, encircled by mononuclear leukocytes forming granulomas, wall off the foreign materials. Now surgical closure of the wound is usually done and if the ‘cleaning’ of the wound is incomplete, chronic inflammation can ensue resulting in prominent scarring\textsuperscript{65}.

2.1.3 Complications of wound healing
Abnormalities, in any of the basic healing process can result in complications.

2.1.3.a. Deficient scar formation: Inadequate formation of granulation tissue or inability to form a suitable extracellular matrix, can lead to deficient scar formation and thereby wound dehiscence.

2.1.3.b. Ulceration: Ulceration of a wound may be due to inadequate blood supply and vascularization.

2.1.3. c. Excessive scar formation: This may be due to excessive deposition of extracellular matrix at the wound site. It results in a hypertropic scar or keloid. The rate of collagen synthesis and the number of reducible cross-links remain high leading to a situation that indicates ‘maturation arrest’ or block in the healing process\textsuperscript{54}.

2.1.4. Factors affecting wound healing
Healing does not always occur in a straightforward, undisturbed fashion. Classification of several factors interfering with healing is as follows

2.1.4.a. General  i) Local factors ii) Systemic factors
2.1.4. b. Specific  i) Growth factors and Cytokines  ii) Endocrine hormones
2.1.4.c. Drugs  i) Corticosteroids  ii) NSAIDs  iii) Anticancer drugs  iv. Drug resistance

2.1.4. a. General
2.1.4. a. i. Local factors
Most commonly used terms in delayed wound healing are wound contamination, wound colonization, critical colonization and wound infection. Wound contamination is the presence of bacteria within a wound without any host reaction. Wound colonization
refers to the presence of bacteria within the wound that multiply or initiate a host reaction. Critical colonization means multiplication of bacteria causing a delay in wound healing, usually associated with an exacerbation of pain not previously reported but still with no overt host reaction. Wound infection is the deposition and multiplication of bacteria in tissue with an associated host reaction. This is the most common reason for delay in poor wound healing. Wound infection is determined by host immune competence and the size of the bacterial inoculum. With normal host defenses and adequate debridement, a wound may bear a level of 1, 00,000 microorganisms per gram of tissue and still heal successfully. Beyond this number, a wound may become infected. The microorganisms contaminating the wound are listed in Table 2.1. Different types of wound infection and frequently colonizing organisms are dealt with later in 2.1.5 section of this chapter.

<table>
<thead>
<tr>
<th>Table 2.1: Examples of potential wound infecting organisms</th>
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<tr>
<td>Gram-positive cocci</td>
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<tr>
<td>• Beta Haemolytic Streptococci (<em>Streptococcus pyogenes</em>)</td>
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<tr>
<td>• Enterococci (<em>Enterococcus faecalis</em>)</td>
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<tr>
<td>• Staphylococci (<em>Staphylococcus aureus/MRSA</em>)</td>
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<tr>
<td>Gram-negative aerobic rods</td>
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<tr>
<td>• <em>Pseudomonas aeruginosa</em></td>
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<tr>
<td>Gram-negative facultative rods</td>
</tr>
<tr>
<td>• Enterobacter species</td>
</tr>
<tr>
<td>• <em>Escherichia coli</em></td>
</tr>
<tr>
<td>• Klebsiella species</td>
</tr>
<tr>
<td>• Proteus species</td>
</tr>
<tr>
<td>Anaerobes</td>
</tr>
<tr>
<td>• Bacteroides</td>
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<tr>
<td>• Clostridium</td>
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<tr>
<td>Fungi</td>
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<tr>
<td>• Yeasts (<em>Candida</em>)</td>
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<tr>
<td>• Aspergillus</td>
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**Wound Colonization**

Microbial colonization is usually described as a process that occurs when exposed subcutaneous tissue provides a favorable substratum for a wide variety of microorganisms to contaminate and colonize. If the involved tissue is devitalized (e.g., ischemic, hypoxic, or necrotic) and the host immune response is compromised, the
conditions become optimal for microbial growth. Wound contaminants are likely to originate from either exogenous or endogenous sources. Exogenous contamination is either from environment or from the surrounding skin (involving members of the normal skin microflora such as Staphylococcus epidermidis, micrococci, skin diphtheroids, and propionibacteria). Endogenous contamination may occur from mucous membranes (primarily the gastrointestinal, oropharyngeal, and genitourinary mucosa). The normal microfloras of the gut, the oral cavity, and the vagina are both diverse and abundant, and these sources (particularly the oral and gastrointestinal mucosa) supply the vast majority of microorganisms that colonize wounds. A minor healing wound may allow sufficient time for only relatively small number of skin contaminants to take residence, but the continued exposure of devitalized tissue associated with a slowly healing chronic wound is likely to facilitate the colonization and establishment of a wide variety of endogenous microorganisms.

Many researchers opine that aerobic or facultative pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa, and beta-hemolytic streptococci are the primary causes of delayed healing and infection in both acute and chronic wounds. Such opinion has been formed on the basis of referenced comments and studies performed largely during the last two decades that have investigated the role of microorganisms in wound healing\(^72-81\). The role of anaerobic organisms in causing wound infection has been underestimated. The failure to recognize the prevalence of anaerobic bacteria in wounds may be due to several reasons. Firstly, anaerobes, were not regarded as being detrimental to normal wound healing\(^82-84\), secondly compared with aerobic and facultative microorganisms, the culture, isolation, and identification of anaerobic bacteria is more time-consuming, labor-intensive and expensive, it is deemed to be too demanding for many diagnostic microbiology laboratories. Lastly, anaerobes perceived to die rapidly in air, the method of specimen collection and transportation to the laboratory is critical for maintaining viability and for effective culture\(^2\).
**Pathogenic effects of virulent micro-organisms:** The following are the ways by which microorganisms cause the pathogenic effects.

- Some species of micro-organisms such as Staphylococcus and Streptococcus produce superantigens.
- Superantigen released into the blood stream, initiates an uncontrolled proliferation of T lymphocytes allowing the release of cytokines that initiates cell and tissue damage.
- Biofilm, usually attached to a wound surface is a microbial colony encased in an adhesive polysaccharide matrix. Cells in biofilms exhibit a decreased sensitivity to host immunological defense mechanisms, decreased susceptibility to antimicrobial agents and increased virulence. They have also been implicated in persistent infections\(^71\).

Diabetes mellitus is a classic example where wound healing is slow because of interruption of the inflammatory phase as well as the proliferative phase. Neutrophils and macrophages cannot adequately keep the bacterial load of the wound in check, as their glycosylation is inhibitory to phagocytic function. Infection thus prolongs\(^85\) the inflammatory phase.\(^85\)

**Tissue Perfusion:** A good blood supply is a basic factor in the success of wound repair. It is essential for the supply of oxygen, other nutrients required in the cellular, and biochemical process of repair and it is necessary for the removal of wound metabolites. Arterial diseases that limit blood flow and venous abnormalities retarding drainage are, well documented to cause impairment to the healing of wounds\(^86\).

**Radiation:** Radiation affects wound healing specially on skin wounds. Irradiation of the skin has early and late effects, and it is the late effects, which are most relevant to wound healing. Use of Vitamin A reverses the healing impairment caused by radiation therapy\(^87\).

**Local wound environment:** Moist wounds heal faster than dry ones. Local supply of oxygen in chemically dissolved form in aqueous solution enhances healing\(^88\).
2.1.4.a. ii. Systemic factors

Age: Proteolysis is an essential component of wound healing, but if uncontrolled, it may lead to degradation of the neo-matrix and a delay in wound repair. Despite numerous reports of impaired wound healing associated with increasing age, the control of proteolysis is completely unknown. Ashcroft GS et al have reported that tissue inhibitor of matrix metalloproteinases (TIMP)-1 and -2 inhibit the activity of matrix metalloproteinases and the pattern of regulation of these molecules determines in part the spatial and temporal regulation of proteolytic activity\textsuperscript{89}.

Antioxidant defense system: Free radicals and other oxygen-derived species are constantly generated in the body during metabolism. Oxidant induced protein denaturation with subsequent breakdown is a well-recognized process after wound injury. A marked increase in oxidant release and a decrease in antioxidant defense is well defined after post wound. The reactivity of some free radicals can cause severe damage to biological molecules especially to DNA, lipids and proteins. Antioxidant defense systems like reduced glutathione, glutathione peroxidase and superoxide dismutases are produced by the body to protect the cellular constituents from the damages caused by reactive oxygen species\textsuperscript{90,91}. The leaves of plant Adhatoda vesica Nees (Acanthaceae) has shown to exert antioxidant activity by scavenging the lipid peroxidation\textsuperscript{92}. The increased lipid peroxide formation in the tissues of carbon tetrachloride (CCl\textsubscript{4}) treated rats have shown significant inhibition by Glycyrrhiza glabra Linn\textsuperscript{93}.

Tissue oxygen: This is the most important factor-regulating wound healing. Oxygen influences angiogenesis, epithelization and resistance to infection. Reduced wound oxygen tension can delay wound healing by slowing the production of collagen. Collagen fibril cross-linking begins to fail as tissue oxygen pressure falls below 40 mm Hg because oxygen is required for the hydroxylation of proline and lysine to synthesize mature collagen. Wound hypoxia also predisposes to bacterial infection as the leukocyte’s oxidative phosphorylation, bactericidal activities are severely impeded without normal tissue oxygen levels. Oxygen enhanced environments have been shown to be bactericidal for most clostridial species and inhibit alpha toxin release\textsuperscript{94}. Thus, cell
death and tissue necrosis caused by tissue hypoxia or anoxia creates ideal growth conditions for members of the wound microflora, including fastidious anaerobe that proliferate as residual oxygen is consumed by facultative bacteria. Oxygen is a critical component of the respiratory burst activity in polymorphonuclear leukocytes (PMNs), resulting in the intracellular production of highly potent antimicrobial metabolites. A significant reduction in the killing capacity of PMNs at a \( pO_2 \) of 30 mm Hg has been reported\(^9\). This signifies, poorly perfused wound tissue is more susceptible to infection than are wounds involving well-perfused tissue\(^6\). Although many endogenous anaerobes survive prolonged periods of exposure to air and tolerate oxygen tensions up to 60 mm Hg (8% oxygen)\(^7\), the redox (oxidation-reduction) potential (Eh) of tissue is also important for their survival. Generally, a low Eh (measured in millivolts) favors the growth of anaerobic bacteria. Hypoxic or anoxic wound environment that has a low oxygen tension and a low redox potential will facilitate the development of polymicrobial aerobic-anaerobic populations\(^2\).

Nutrition: Nutrition is a crucial aspect of a holistic approach to the healing of wounds. Poor nutritional status can delay the wound healing process or cause inadequate healing when nutritional deficiencies are not corrected\(^9\). Synthesis of collagen appears gets inhibited in protein deficient animals. A light protein diet hastens the acquisition rate of tensile strength\(^9\). Malnutrition causes a decreased rate of fibroblastic proliferation and neovascularization and impairs both cellular and humoral immunity\(^10\).

Vitamin deficiency:

**Vitamin C:** Participates in the process of wound healing by promoting the synthesis of collagen. Hence, its deficiency contributes to fragile granulation tissue\(^10\). It affects collagenation and causes impaired wound healing. Supplementation with Vitamin C twice a day accelerates ulcer healing\(^10\).

**Vitamin A:** Rats fed with vitamin A supplemented diet showed enhanced wound healing compared to those fed a standard diet. The beneficial effect of vitamin A on wound healing may be due to an increase in collagen synthesis. Vitamin A has a stabilizing effect on lysosomal membrane. Thus, excessive doses of vitamin A being reported to
increase inflammatory reactions\textsuperscript{103}. It is known to reverse the inhibitory effect of cyclophosphamide\textsuperscript{104} and corticosteroids on wound healing\textsuperscript{105}.

**Trace elements:** Copper is a cofactor required for the enzyme lysyl oxidase, which plays a role in cross-linking and strengthening of connective tissue. A copper supplement as a part of a comprehensive nutritional program has been recommended to promote wound healing\textsuperscript{106}. Copper complexes have antineoplastic activity\textsuperscript{107}. Copper complexes of Tolmetin (Tol-Cu) found to have prohealing action\textsuperscript{108}.

Zinc is a component of many enzymes that is necessary for repair wounds. Even a mild deficiency of zinc can interfere with optimal recovery from everyday tissue damage as well as from more serious trauma\textsuperscript{109,110}. It is found that zinc reverses the healing suppressant effect of corticosteroids\textsuperscript{111,112}. Zinc salts also reverse the healing suppressant effects of non-steroidal anti-inflammatory agents\textsuperscript{113,114}.

21.4.b. Specific

2.1.4.b. i. Growth Factors and Cytokines

Growth factors and cytokines are two distinct categories of signaling proteins that modulate wound healing at a molecular and cellular level. Growth factors are constitutively present, released by a few selected subsets of cells and have primarily trophic effects on cells. However, they may have an indirect inflammatory influence. A summary of the profiles of a few major growth factors is in Table 2.2. Cytokines are small molecular weight mediators, which primarily have variable effect on inflammatory process by their influence on the cells of the immune system. All nucleated cells release this expressing transient, local and elicit varying responses in different cells\textsuperscript{115}. A summary of the profiles of the most common cytokines are given in Table 2.2.
Table 2.2: Profile of selected growth factors of importance in wound healing (Senthil K et al. 2004)

<table>
<thead>
<tr>
<th>Growth factor</th>
<th>Physiological effects</th>
<th>Clinical and experimental correlates</th>
</tr>
</thead>
</table>
| TGFβ Transforming growth factor    | - Chemotactic to fibroblasts, monocytes, macrophages, lymphocytes  
- Proliferation of epithelial cells, macrophages, lymphocytes, fibroblasts  
- Stimulates keratinocyte migration  
- Induces expression of Pro-MMP-9 in keratinocytes  
- Induces fibroblasts to secrete TIMPs  
- Augments cell adhesion to matrix proteins by modulating integrin receptor  
- Stimulates fibroblasts to contract collagen matrix | Chronic wound fluid contains lower TGFβ when compared to acute wounds  
While higher concentrations of TGFβ1 and TGFβ2 are associated with hyperfibrotic disorders  
TGFβ3 has been found to reduce scarring  
Scarless healing in the embryo has been attributed to an absence of TGFβ |
| PDGF Platelet derived growth factor | - Neutrophil, monocyte, lymphocyte chemotaxis  
- Monocyte maturation  
- Potentiates VEGF production  
- Stimulates MMP production by fibroblasts  
- Stimulates myofibroblasts to contract matrix collagen | First growth factor to be licensed for topical therapy |
| KGF Keratinocyte growth factor     | - Potent mediator of keratinocyte proliferation which under the influence of KGF-2, upregulates the expression of many genes  
- Stimulates keratinocyte and fibroblast motility | Trials of topical application underway |
| VEGF Vascular endothelial growth factor | - Potent angiogenic factor | VEGF administration improves granulation tissue formation  
Potential being explored in salvage of ischaemic flaps and in tissue expansion |
| EGF Epidermal growth factor        | - Directs epithelialisation in an autocrine fashion  
- Stimulates fibroblast collagenase secretion  
- Inhibits foetal wound contraction | Aged dermal fibroblasts have a decreased EGF-receptor expression  
EGF may contribute to the scarless repair seen in utero |
Table 2.3: Profile of selected cytokines in wound healing

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Physiological effects</th>
<th>Clinical and experimental correlates</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα (Tumour necrosis factor α)</td>
<td>• Leucocyte chemotaxis</td>
<td>Elevated levels linked to insufficient collagen deposition and poor healing</td>
</tr>
<tr>
<td></td>
<td>• Monocyte maturation</td>
<td>Levels high in chronic nonhealing wounds</td>
</tr>
<tr>
<td></td>
<td>• Macrophage activation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Inhibits fibroplasias</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Stimulates IL-B by fibroblasts</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Increases activity of MMP-2 and MMP-9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Potentiates VEGF production</td>
<td></td>
</tr>
<tr>
<td>Interleukin-1</td>
<td>• Leucocyte and fibroblast chemotaxis</td>
<td>High levels associated with delayed healing</td>
</tr>
<tr>
<td></td>
<td>• Macrophage activation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Angiogenic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Stimulates fibroblast MMP production</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Stimulates keratinocyte migration</td>
<td></td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>• Inhibits extracellular matrix breakdown</td>
<td>High levels associated with poor healing</td>
</tr>
<tr>
<td></td>
<td>• Stimulates fibroblasts to secrete TIMP</td>
<td>Peaks at 24 h</td>
</tr>
<tr>
<td>Interleukin-8</td>
<td>• Leukocyte chemotaxis</td>
<td>Parallels IL-6, peaking at 24 h</td>
</tr>
<tr>
<td></td>
<td>• Enhances epithelialisation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Keratinocyte migration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Upregulates plasminogen activator in keratinocytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Inhibits fibroblast induced contraction of collagen</td>
<td></td>
</tr>
<tr>
<td>Interleukin-4 and interleukin-10</td>
<td>• Inhibit leucocyte chemotaxis</td>
<td>IL-10 exhibits bimodal wound levels peaking at 3 h and 72 h</td>
</tr>
<tr>
<td>(The anti-inflammatory interleukins)</td>
<td>• Down regulate expression of many pro-inflammatory cytokines</td>
<td></td>
</tr>
<tr>
<td>Interferons</td>
<td>• Inhibitory to fibroblasts</td>
<td>IFN γ reduces keloid size when administered intradermally</td>
</tr>
<tr>
<td>(The antifibrogenic cytokines)</td>
<td>• Inhibit post-translational changes to collagen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Increase collagenase activity</td>
<td></td>
</tr>
</tbody>
</table>

2.1.4.b. ii. Endocrine hormones

Several hormones may enhance or alter healing. Growth hormone has shown to increase angiogenesis and myofibroblast differentiation in wounded mice. It also stimulates the production of collagen. Its deficiency severely limits bone growth and hence the
accumulation of bone mass\textsuperscript{118}. Treatment with oxytocin activated the process of vasculogenesis, proliferation of endotheliocytes that in turn resulted in the effective clearance of the wound and optimal granulation tissue formation\textsuperscript{119}. Leptin accelerates the healing of colonic anastomoses\textsuperscript{120}. Insulin induces accelerated wound healing associated with diminished inflammation and increased collagen deposition in rats\textsuperscript{121}. Injury evokes the secretion of hormones. The adrenocorticotropic hormone released from pituitary or exogenously administered cortisone exert a deleterious effect on the wound healing process, which has been suggested to result from the anti-inflammatory action of these steroids. The corticosteroids lower transforming growth factor – beta (TGF-beta) and insulin like growth factor –1(IGF – 1) levels and tissue deposition in wounds\textsuperscript{122}.

2.1.4.c. Drugs

2.1.4.c.i. Corticosteroids
Steroids are known to inhibit all aspects of wound healing like tensile strength of closed wound, rate of epithelization and appearance of new blood vessels in healing tissue. The impaired healing, results from derangements in cellular function induced by steroids. A primary feature of wounds in steroid treated individuals is a deficiency in inflammatory cell function. Inflammatory cells, particularly macrophages mediate essentially all aspects of healing through cytokines. By diminishing supply of cytokines, steroids and other immunosuppressive agents profoundly impair all aspects of healing. Macrophage migration, fibroblast proliferation, collagen accumulation and angiogenesis are among the processes diminished by steroid administration\textsuperscript{123,124}.

All anti-inflammatory steroids have an inhibitory effect on wound healing. The effect of cortisone on healing is that it prevents the inflammatory phase, which is essential to the healing process. Cortisone and other anti-inflammatory steroids increase the integrity of the lysosome, the subcellular particle that contains an assortment of acid hydrolases\textsuperscript{123}. It is known that lysosomal enzymes take a prominent part in the inflammatory process. Glucocorticoids represent the most important and frequently used class of anti-inflammatory drugs. These are widely used for the treatment of
various diseases despite known side effects like skin atrophy and immunosuppressive effects. These are known to adversely affect wound healing in experimental skin models.

2.1.4.c.ii. Nonsteroidal anti-inflammatory drugs (NSAIDS)
Nonsteroidal anti-inflammatory drugs, are being used to suppress postoperative inflammatory edema and pain. The usefulness of nonsteroidal anti-inflammatory drugs is limited due to adverse gastrointestinal tract events. Adverse effects like gastric mucosal injury are known to be caused by aspirin and to retard gastric wound healing. NSAIDS such as aspirin, indomethacin and ibuprofen are the most widely used drugs for pain, arthritis, cardiovascular diseases and more recently for the prevention of colon cancer and Alzheimer’s disease.

2.1.4.c. iii. Anticancer Agents
Anticancer agents are antiproliferative and adversely affect the healing process. Hence they can delay wound healing when used perisurgically in cancer. Cyclophosphamide interferes with wound contraction, epithelization, tensile strength and granulation tissue formation. Supplementation of vitamin A reverses the anti-healing effect. The 5-fluorouracil (5-FU) and Colchicines (COL) also suppress wound healing. The drug probably decreases the collagen content by affecting the fibroblast proliferation and hence decreases the tensile strength. Cyclosporine is an immunosuppressant drug which causes a significant reduction in tensile strength of healing incision wound and granulation tissue weight of dead space wound and does not affect the wound contraction and epithelization period of excision wound. Prevention of impaired wound healing and reduction the adverse effects of radiation can be done using vitamin A supplementation.

2.1.4.c. iv. Drug resistance: Antimicrobial resistance is the ability of microbes, such as bacteria, viruses, parasites or fungi to grow in the presence of a chemical (drug) that would normally kill it or limit its growth. In 1928 while working with Staphylococcus bacteria, Scottish scientist Alexander Fleming noticed that a type of mold growing by accident on a laboratory plate was protected from, and even repelled, the bacteria. The
active substance, which Fleming called penicillin, was literally an antibiotic - it killed living organisms. Thus began the age of using natural and, later, synthetic drugs to treat people with bacterial infections. Though not widely popular until the 1940s, antibiotics and other antimicrobials (medicines that kill or slow growth of a microbe) have saved countless lives and blunted serious complications of many feared diseases and infections. The success of antimicrobials against disease-causing microbes is among modern medicine's great achievements. Soon after its use however, some strains of Staphylococcus Aureus began to produce the enzyme penicilinase inactivating the antimicrobials like ampicillin, other penicillins and cephalasporins. Methicillin, used to treat S. aureus, was the first penicillinase–resistant semisynthetic penicillin. Late 1960 and 1970’s saw the emergence of MRSA, (Methicillin resistant Staphylococcus aureus) with the first report out breaks in both acute and long-term care facilities. Emergence of MRSA in wound was of concern because of resistance to other antimicrobials. A gene on the bacterial chromosome that codes for abnormal penicillin binding protein (PBP) carries resistance to Methicillin. This abnormal PBP has minimal affinity for all penicillins, so very little Methicillin binds to it. Hence, all penicillin is ineffective against MRSA. Some strains of MRSA are becoming resistant to other antimicrobial agents like vancomycin. Treatment of MRSA wound infection involves antimicrobial therapy and prevention of cross contamination. An alternative method to control MRSA in wound is the use of ultraviolet rays. The emergence of antibiotic resistance is a major problem in antimicrobial therapy.

Mechanisms causing antibiotic resistance:

1. Permeability: Some microorganisms change their cell permeability to the drug, possibly by alteration in the chemical nature of outer membrane; example is tetracycline resistant to Pseudomoans Aeruginosa.

2. Production of enzymes: Penicillin resistant Staphylococcus Aureus produces an enzyme β lactamase that destroys the penicillin.

3. Aminoglycoside attaches with 30 S subunit of ribosome but resistant bacteria develop an altered receptor.

4. Altered metabolic pathway by altering the metabolic pathway, bacteria bypasses the reaction inhibited by the drug. Sulphonamide resistant bacteria utilize preformed
folic acid and do not require the conversion of PABA (Para-Aminobenzoic acid) to folic acid (a reaction inhibited by sulphonamides).

Genetic basis of resistance:
1. Chromosomal resistance: This occurs, because of spontaneous mutation. In clinical practice, mutational resistance is of great importance in tuberculosis. The antimicrobial drugs selectively suppress susceptible organisms but resistant mutants will multiply unchecked. On using two or more anti-tubercular drugs for treatment, resistant mutant does not occur, as, a mutant resistant to one drug, will be destroyed by another drug.
2. Extra chromosomal resistance: This occurs by transfer of plasmids and genetic material. Transferable drug resistance mediated by the R factor is the most important. R factors are plasmids that contain genes that code for drug resistance against one and often several antimicrobial drugs. Inactivation of drugs occur by plasmid coded enzymes example β lactamases destroy the β –lactam ring which is responsible for the antibacterial action of β lactam antibiotics like penicllins and cephalosporins.134,136

One group of β lactamases that is occasionally found in certain species of gram-negative bacilli usually Klebsiella pneumoniae and Escherichia coli. These enzymes are termed as extended spectrum β lactamases (ESBL) because they confer upon the bacteria the additional ability to hydrolyze the β-lactam rings of cefotaxime, ceftazidime, aztreonam and these enzymes have developed resistance to antibiotics like penicillin134. ESBL enzymes can also be found in bacteria such as Salmonella, Proteus, Morganella, Enterobacter, Citrobacter, Serratia, and Pseudomonas. Enzymes are proteins produced by living organisms possessing the ability to speed up biochemical reactions. In most cases, the body successfully fights off ESBL-producing bacteria. However, because of the enzymes' ability to fight off antibiotics, people with weak immune systems are at risk like children, the elderly and people with other illnesses.
The drug resistance in India is particularly grim due to various factors. Generally, there is little control on the use of antibiotics. Community awareness of the issues involved in antibiotic therapy is poor and this is compounded by over-the-counter availability. Coupled with primitive infection control in hospitals and weak or deficient sanitation, the conditions are suited for transmission and acquisition of antibiotic resistance. The facility with which enteric pathogens spread widely in India illustrates this point. On the other hand countries with a good sanitary infrastructure are hardly bothered by the import of cholera cases. Large parts of the country do not have the technical infrastructure to generate useable data on the ground. Thus, the contribution of infectious diseases is greater in impoverished societies. In the absence of a Central Monitoring Agency, the national scene in India with regard to antimicrobial resistance is unknown. The two probable exceptions are *M. tuberculosis* and *Leishmania donovani*. *Staphylococcus aureus* has developed resistance to newer antibiotics over the years. Methicillin resistance is quite frequent approaching and at time exceeding 50% in tertiary care centers. Vancomycin resistance has been very low. However, the reckless use of the antibiotic may alter the scenario. This coupled with the emergence of Community Acquired MRSA would pose serious clinical problems with global ramifications\textsuperscript{137-139}. Likewise, coagulase negative staphylococci have acquired multiple resistance and become important nosocomial pathogens\textsuperscript{140}. *Vancomycin Resistant Enterococci* (VRE) are being isolated in Indian hospital laboratories and tertiary care centers, one centre in north India reported to isolate 38% of blood culture was positive for VRE in Intensive Care Unit\textsuperscript{141}.

UVR is very effective against resistant microbial in very less duration, only in seconds\textsuperscript{142}. Research showing the effectiveness UVR will be discussed in detail in the next section.

### 2.1.5: Types of wound infection

Infection occurs when virulence factors expressed by one or more microorganisms in a wound compete the host natural immune system and subsequent invasion and dissemination of microorganisms in viable tissue provokes a series of local and systemic host responses. Characteristic local responses are a purulent discharge or
painful spreading erythema indicative of cellulitis around a wound\textsuperscript{143}. A multitude of microbial and host factors like the type, site, size and depth of wound, the extent of nonviable exogenous contamination, level of blood perfusion to wound, general health and immune status of the host, the microbial load, combined level of virulence expressed by the microorganism involved, progresses a wound to an infected state. Most acute and chronic wound infections involve mixed populations of both aerobic and anaerobic micro-organisms\textsuperscript{2,144}.

Different types of wound infections are described as follows:

\textbf{2.1.5. a: Surgical wound infections.} The risk of infection in surgical wound is generally based on its susceptibility to microbial contamination. Clean surgery carries 1 to 5\% risk of postoperative wound infection, and some procedures may be significantly more susceptible to endogenous contamination, a 27\% risk of infection has been estimated\textsuperscript{145}. With the exception of clean operative procedures, surgical wound infections are recognized as having a polymicrobial etiology, involving both aerobic and anaerobic microorganisms and intra-abdominal infections normally reflect the microflora of the resected organ\textsuperscript{2}. A study conducted in India on 1125 postoperative patients the commonest etiologic agents found were \textit{S. aureus} (33.3\%; \(n=45\)) of which 14.0\% (\(n=19\)) were methicillin-resistant \textit{S.aureus} strains (MRSA) and \textit{Enterococcus faecalis} 33.3\% (\(n=45\)) of which 1.4\% (\(n=2\)) were vancomycin-resistant \textit{Enterococci} (VRE). \textit{Pseudomonas aeruginosa} (24.4\%; \(n=33\)), \textit{Escherichia coli} (7.4\%; \(n=10\)) and \textit{Klebsiella} spp. (1.4\%; \(n=2\)) were also isolated\textsuperscript{146}.

Minimizing the incidence of postoperative wound infection relies on adequate asepsis, antiseptics and preservation of the local host defenses. UVR has been used to reduce the postoperative wound infection. Shimomura et al\textsuperscript{20} studied the effect of UV irradiation to the skin around the catheter exit site in 68 patients undergoing continuous ambulatory peritoneal dialysis. Their observation was, despite the strict disinfection of the catheter exit site with povidone iodine, 23\%-45\% of the cases were found to be microorganism positive. The most prevalent microorganism was, in order of highest to lowest prevalence, \textit{Staphylococcus epidermidis}, \textit{Staphylococcus aureus} and \textit{Pseudomonos aeruginosa}. UV irradiation was performed on 18 cases that constantly revealed bacteria
on culture. Ten cases became culture negative, 3 cases showed microbial decrease and 5 remained unchanged. It was concluded that UV irradiation can be used to eliminate bacteria and prophylactically.

Another study conducted by Taylor\textsuperscript{147} on total joint arthroplasty procedures to ascertain the effect of UVR. Bacteria was counted concurrently in the air and wounds during the first 20 min of total joint arthroplasty procedures in two theatres: a conventional plenum ventilated theatre with ultraviolet C (UVC) tubes installed and a filtered vertical laminar flow theatre. Four theatre environments were tested: conventional theatre and clothing; conventional theatre with UVC protective clothing, with UVC set to produce 100 or 300 µW/cm\(^2\)/s irradiation; and filtered vertical laminar flow air with staff wearing cuffed cotton/polyester clothing. When used, the UVC was activated 10 min after starting an operation to assess the effect of UVC clothing alone, and of UVC radiation on bacteria already present in the wound. Compared with conventional theatres, UVC clothing reduced air counts by 38\% with UVC at 100 µW/cm\(^2\)/s, by 81\% at 300 µW/cm\(^2\)/s by 91\%, and laminar flow by 92\%. Wounds counts fell correspondingly by 66\% with UVC clothing, 87\% with UVC at 100 µW/cm\(^2\)/s and 92\% both with UVC at 300 µW/cm\(^2\)/s and laminar flow. In conventional and laminar flow theatres air and wound counts correlated closely but in UVC theatres wound counts were lower than levels expected from prevailing air counts suggesting that UVC kills bacteria in wounds as well as in air.

\textbf{2.1.5.b: Acute soft tissue infections:} Acute soft tissue infections include cutaneous abscesses, traumatic wounds, and necrotizing infection. Microbiological investigations have shown that \textit{S.aureus} is the single causative bacterium in approximately 25 to 30\% of cutaneous abscesses\textsuperscript{148}. However, other studies have demonstrated that approximately 30 to 50\% of cutaneous abscesses\textsuperscript{148,149}, 50\% of traumatic injuries of varied etiology\textsuperscript{150}, and 47\% of necrotizing soft tissue infections\textsuperscript{151} have a polymicrobial aerobic-anaerobic microflora.

Necrotizing soft tissue infections occur with different degrees of severity and speed of progression; they involve the skin, (e.g., Clostridial and nonclostridial anaerobic
cellulitis), subcutaneous tissue, muscle fascia (necrotizing fasciitis), and muscle tissue (streptococcal myositis and clostridial myonecrosis). In two patients with rapidly progressing necrotizing fasciitis of the lower extremity, the single pathogen described is *S. aureus* and in a study of necrotizing fasciitis in eight children, Brook reported the presence of pure *Streptococcus pyogenes* in two patients and a mixed predominance of *Peptostreptococcus* spp., *S. pyogenes, B. fragilis, C. perfringens, E. coli*, and *Preotella* spp. in the others. Similar findings were reported by other authors also.

### 2.1.5. c: Bite wound infections

The reported infection rate for human bite wounds ranges from 10 to 50% depending on the severity and location of the bite, and up to 20% of dog bites and 30 to 50% of cat bites become infected. Brook reported that 74% of 39 human and animal bite wounds contained a polymicrobial aerobic-anaerobic microflora, with *S. aureus, Peptostreptococcus* spp., and *Bacteroides* spp. being the predominant isolates in both wound types. Due to the complex nature of the oral microflora in humans and animals, the majority of bite wounds harbor potential pathogens, many of which are anaerobes. As well as the common anaerobes in both human and animal bite wounds, such as *Bacteroides, Prevotella, Porphyromonas*, and *Peptostreptococcus* spp, less common potential pathogens such as *Pasteurella multocida, Capnocytophaga canimorsus, Bartonella henselae*, and *Eikenella corrodens* may also be involved. Management of bite wounds is likely to involve high-pressure irrigation to reduce the microbial load, debridement of devitalized tissue, and antibiotic treatment for high-risk wounds such as punctures.

### 2.1.5.d: Burn wound infections

Thermal destruction of the skin barrier and concomitant depression of local and systemic host cellular and humoral immune responses are pivotal factors contributing to infectious complications in patients with severe burns. The burn wound surface (in deep partial-thickness and in all full-thickness burns) is a protein-rich environment consisting of avascular necrotic tissue (eschar) that provides a favorable niche for microbial colonization and proliferation. The avascularity of the eschar results in impaired migration of host immune cells restricts delivery of systemically administered antimicrobial agents to the area, while toxic substances
released by eschar tissue impair local host immune responses. Infection is a major complication in burn wounds, and it is estimated that up to 75% of deaths following burn injury are related to infection. Although exposed burned tissue is susceptible to contamination by microorganisms from the gastrointestinal and upper respiratory tracts, many studies have reported the prevalence of aerobes such as *P. aeruginosa*, *S. aureus*, *E. coli*, *Klebsiella* spp., *Enterococcus* spp., and *Candida* spp. In other studies involving more stringent microbiological techniques, anaerobic bacteria have been shown to represent between 11 and 31% of the total number of microbial isolates from burn wounds. While the aerobes isolated in the latter studies were similar to those reported previously, predominant anaerobic burn wound isolates were *Peptostreptococcus* spp., *Bacteroides* spp., and *Propionibacterium acnes*. Mousa H.A. also reported the presence of *Bacteroides* spp. in the wounds of 82% of patients who developed septic shock and concluded that such microorganisms may play a significant role in burn wound sepsis. Management of infection in burn wounds involves the use of topical and systemic antimicrobial agents, aggressive debridement of dead tissue, maximization of the immune response, and provision of adequate nutrition.

2.1.5. **Diabetic foot ulcer infections.** Plantar ulcers associated with diabetes mellitus are susceptible to infection due to the high incidence of mixed wound microflora, and the inability of the PMNs to deal with invading microorganisms effectively. However, with optimal treatment involving debridement of devitalized tissue, the use of appropriate dressings, and pressure relief, wound infection can be minimized. Boulton reported an infection rate of 2.5% in diabetic wounds treated with a moisture-retentive hydrocolloid dressing, compared with a 6% infection rate under a traditional gauze dressing. As in most wound types, *S. aureus* is a prevalent isolate in diabetic foot ulcers, together with other aerobes including *S. epidermidis*, *Streptococcus* spp., *P. aeruginos*, *Enterococcus* spp. and coliform bacteria. 70% of drug resistant organisms are commonly found in diabetic wound. With good microbiological techniques, anaerobes have been isolated from up to 95% of diabetic wounds, the predominant isolates being *Peptostreptococcus*, *Bacteroides*, and *Prevotella* spp. Thai et al. has shown effectiveness of UVC in treating diabetic foot infection.
with MRSA) in terms of reduction of bioburden and wound reepithelisation and marked improvement in pressure sore status.

2.1.5.f: Leg and decubitus (pressure) ulcer infections. The microflora of chronic venous leg ulcers is frequently polymicrobial, and anaerobes constitute approximately 30% of the total number of isolates in uninfected wounds. Although S. aureus is the most prevalent potential pathogen in leg ulcers, Bowler and Davies reported a significantly greater frequency of anaerobes (particularly Peptostreptococcus spp. and pigmenting and nonpigmenting gram-negative bacilli) in clinically infected leg ulcers than in non infected leg ulcers (49 versus 36% of the total numbers of microbial isolates, respectively)\textsuperscript{144}. The same investigators also suggested that aerobic-anaerobic synergistic interactions are likely to be more important than specific microorganisms in the pathogenesis of leg ulcer infection. Decubitus ulcers develop, because of continued skin pressure over bony prominences; they lead to skin erosion, local tissue ischemia, and necrosis, and those in the sacral region are particularly susceptible to fecal contamination. The opportunity for microbial synergy in many decubitus ulcers was demonstrated by Brook\textsuperscript{149}, who reported mixed aerobic and anaerobic microflora in 41% of 58 ulcers in children; S. aureus, Peptostreptococcus spp., Bacteroides spp. (formerly members of the B. fragilis group), and P. aeruginosa were the predominant isolates. Although localized wound care is normally sufficient to facilitate primary healing in decubitus ulcers, reports of occasional necrosis of adjacent soft tissues leading to necrotizing fasciitis has been recorded. Initial management of infected decubitus ulcers normally involves aggressive surgical debridement and broad-spectrum antimicrobial coverage\textsuperscript{2}.

2.1.6: Monitoring of wound repair through different wound models
To study the rate and extent of several processes that occur during wound healing a variety of wound models are employed. Wound repair is a complex phenomenon having various phases like granulation, collagenation, collagen maturation; scar remodeling, scar maturation, wound contraction and epithelization. Some of these phases are independent while many of them are sequential. Quite often, these phases progress concurrently.
2.1.6.a. Resutured incision wound
This is the much routinely used wound model. Skin is split through its full thickness and after securing haemostasis; the wound edges are approximated by interrupted sutures and wound allowed to heal by first intention. One of the important aspects of incision wound healing is the rate at which the wound gains it strength (tensile strength or breaking strength). The ability to resist rupture can be assessed in various ways and has been studied for centuries. Paget\textsuperscript{166} introduced the measurement of tensile strength to assess the progress of incisional wounds. The breaking strength required to disrupt the healed wound at various time intervals or at a given age of the wound, can be converted into tensile strength if cross sectional area of a wound edge is taken into account. The breaking strength is measured by applying tractional force on wound edge. Instead of traction face, if a pressure is applied to break the wound of a wall of viscus or skin it is called bursting strength. This wound model can also be employed to study histological and biochemical parameters and is not suitable for study on wound contraction and epithelization.

2.1.6.b. Dead space wounds
In this wound model, granulation tissue is harvested over a subcutaneously implanted foreign body. To produce dead space wounds workers have used various foreign body implants like cotton pellets\textsuperscript{167,168}. Propylene\textsuperscript{130} and silicon implants\textsuperscript{169} of uniform thickness and weight can be placed in dead space made in the axilla and groin regions. After a definite period, these pellets or tubes can be taken out, granulation tissue harvested on it, carefully dissected and tensile strength of the tissue measured. The dry granulation tissue weight can be estimated. This granulation tissue can be used for histological studies and estimation of hydroxyproline content of collagen.

2.1.6c. Excision wound
Conventionally a wide-open dermal wound (of known dimensions) is created by cutting away a piece of skin in its full thickness. To study physical aspects of wound healing, excision wounds, of different shapes and sizes in different locations has been employed. Rat, rabbit and guinea pig are used for this purpose. Hunt\textsuperscript{170} et al have studied the effect of steroids on 2 cm\textsuperscript{2} full thickness wounds made on depilated back of each rabbit.
Morton JP and Malone MH\textsuperscript{171} have used circular wound of 2.5 cm diameter made on depilated dorsal thoracic region in rats. This wound permits monitoring of wound contraction and epithelization; and it is possible to differentiate the process of contraction and epithelization in this wound model study by physical attribution. Repeated wound tracings are used to study the rate of contraction. Results are expressed as percentage of closure of their original wound size for the purpose of comparison.

\subsection*{2.2. PHYSICAL THERAPY MODALITIES IN WOUND HEALING}

Various physical therapy modalities are used in accelerating the wound healing. Among them popularly known are low-level laser therapy, therapeutic ultrasound, electrical stimulation, thermal and non-thermal diathermy, ultraviolet rays.

\subsubsection*{2.2.1 Low level laser therapy:}

Cold laser is also referred in the literature as low-level cold laser, low-level infrared laser, or monochromatic infrared photo energy (MIRE). Low-energy laser treatment uses light in the infrared spectrum. Low-level laser may modulate biologic processes in either a stimulatory (photobiostimulation) or inhibitory (photobioinhibition) manner. The process by which this modulation occurs is via interaction of low-level laser light with naturally occurring chromophores (pigments located in plant and animal cells that absorb light). These chromophores are part of the powerhouses of the cell (mitochondria), and absorption of low-level laser light from the visible and infrared region of the electromagnetic spectrum results in heightened cellular metabolism\textsuperscript{135}. Wavelengths of radiant energy between 600 and 1200 nm are capable of penetrating the dermis and interacting with chromophores in human tissues. Chromophores in cells that can absorb this low-level laser light include respiratory chain enzymes, melanin, hemoglobin, and myoglobin. Longer wavelengths of light (infrared) such as those produced by the gallium-arsenide (GaAs) laser can interact with a wide variety of cellular chromophores. In comparison, shorter wavelengths of light (visible and near infrared) such as those generated by the helium-neon (HeNe) laser interact with a limited number of chromophores (melanin, hemoglobin, and myoglobin). The biologic effects of lasers are numerous and varied\textsuperscript{172,173}. Additionally, research indicates that
compromised cells from damaged tissue are more responsive to these energies than their normal counterparts. Tissues that are inflamed, edematous, and ischemic, benefit from low-level laser energy. The cells in these tissues appear to have a lower threshold for excitation or energy transfer. Biologic effects produced by photoenergy in this wavelength range include increased cellular proliferation and differentiation, increased mitochondrial production of ATP, increased RNA synthesis, and increased release of chemotactic factors from mast cells. A number of other positive biologic effects of low-level laser have been identified that may enhance tissue healing. These effects include enhanced leukocyte infiltration, increased macrophage activity, increased fibroblast proliferation, increased growth factor release, enhanced epithelialization rates, and improved tissue tensile strength. Cochrane review on effect of laser therapy on venous leg ulcers (published online 1999 and assessed up to date until 2001) concluded that no evidence of any benefit associated with low-level laser therapy on venous leg ulcer healing. A combination of laser and infrared light may promote the healing of venous ulcers is suggested by a study, however, more research is needed. There were 4 eligible trials. Two RCTs compared laser therapy with sham, one with UVR and one with non-coherent unpolarised red light. Neither of the 2 RCTS comparing laser with sham found a significant difference in healing rates, there was no significant benefit for laser evident when the trials were pooled together (88 patients in total). Significantly more ulcers completely healed in the group receiving laser and infrared light compared with non-coherent unpolarised red light. A fourth trial (6 patients) compared laser and ultraviolet light and found no significant difference. Authors concluded no evidence of any benefit associated with low-level laser therapy on venous leg ulcer healing. Laser and infrared light may promote the healing of venous leg ulcer is suggested by a study; however, more research is needed.

2.2.2. Therapeutic ultrasound:
Ultra sound (US) is sound wave with frequency of greater than 20 KHz. Therapeutic US is generally in the range of 1-3 MHz. When US is absorbed by the skin it can cause variety of effects. Continuous US can cause increase the tissue temperature and thereby increasing vasodilatation and blood supply. If there is poor circulation (in case of
chronic wound) with an excessive increase in the temperature it can cause burns. Whereas lower intensities of pulsed US does not raise the temperature, but may enhance healing by nonthermal effects. Nonthermal effects include stable cavitations, micro streaming and acoustic streaming. The following physiological changes in wound healing have been observed with application of ultrasound.

1. Mast cell degranulation, resulting in release of histamine and chemotactic factors into the peri wound tissue and an initiation of the inflammatory healing phase.

2. Increase in vascular permeability, resulting in an increase in flow of platelets, macrophages, leukocytes and mast cells, all of which are active during the inflammatory phase of healing.

3. Increase in phagocytosis of hematoma material by macrophages and neutrophils.

4. Stimulation of fibroblast activity, resulting in increased collagen synthesis, and in turn producing two positive healing effects - accelerated wound closure and stronger scar tissue.

5. Stimulation of endothelial cell activity, resulting in accelerated dermal repair.178

Basic science evidence and clinical research have established that skin repair and wound contraction can be accelerated, collagen secretion can be stimulated, elastin properties affected to strengthen scar tissue. Standard procedure for the treatment is to cover the wound with a sheet of hydrogel, or an application of amorphous hydrogel. A hand-held applicator is used to deliver US. Another option for treatment is to apply US transmission gel to the peri wound area and treat from this region in addition to or instead of the wound bed.14

A study conducted by Serena et al.179 to determine if noncontact, nonthermal, low-frequency ultrasound therapy is effective in controlling wound bacterial colony counts. They conducted a series of in-vitro experiments to ascertain the depth of penetration and bacterial reduction and in-vivo experiment to study the bacterial reduction in animal model and human model. Authors concluded that noncontact ultrasound could be used to reduce bacterial quantity and requirement of more controlled clinical studies to ascertain the efficacy of this treatment and to further elucidate its effects on various Gram-negative and Gram-positive bacteria.179
Another study conducted by Nussbaum et al\textsuperscript{21} to compare the effect of US/UVC and laser for pressure ulcer treatment in patients with spinal cord injury with skin wounds showed a significant improvement with US/UVC in terms of healing time and faster rehabilitation programs than laser alone.

Cochrane review on therapeutic US in treatment of venous leg ulcers aimed to assess the effectiveness of US in accelerating the wound healing. They concluded there is no evidence of benefit of low frequency ultrasound in treatment of venous leg ulcers. These conclusions are based on the results of only eight small studies of generally poor quality and therefore, should be interpreted with caution\textsuperscript{180}.

Cochrane review on therapeutic US in treatment of pressure sores included three studies on 146 patients, among them, two were RCT with sham control. Authors concluded that there is no evidence of benefit of ultrasound therapy in the treatment of pressure ulcers. However, the possibility of beneficial or harmful effects cannot be ruled out due to the small number of trials, some with methodological limitations and small numbers of participants. Further research is recommended by the authors\textsuperscript{181}.

2.2.3 Whirl Pool: Whirlpool can be classified as a means of cleansing and mechanical debridement. Despite at least a decade of investigation, with little evidence to support its use, whirlpool is still used for both nonselective mechanical debridement and for wound cleansing. However, many clinicians involved in wound care have decreased their use of whirlpool significantly with the evolution of wound care and subsequent publication of the Agency for Health Care Research and Quality (AHRQ) guidelines. The historical rationale for use of whirlpool was based on its use in deodorization, skin and wound cleansing, mechanical nonselective debridement, wound decontamination and infection control, and softening adherent necrotic tissue in preparation for debridement. Evidence-based rationale for changes in the use of whirlpool is based on a number of factors like contamination from waterborne pathogens, cross-contamination, dependent position can initiate or increase venous congestion and extremity edema, loss of endogenous fluids from the wound bed and heat loss affecting core body temperature and/or the local wound area. Whirlpool saturates wound tissue and surrounding skin,
creating the potential for maceration and skin breakdown and temporarily inactivates normal skin defenses through immersion. Thus, there is a potential to prolong inflammation and delay wound healing. Whirlpool can also increase heart and respiratory rates. Finally, the use of whirlpool is labor intensive and costly in terms of use of water, utilities, linen, and staff. Burke et al. studied effect of whirlpool on 42 patients with pressure ulcer of stage 3 and 4. Study group patients received conservative and whirlpool therapy, control group received only conservative treatment. Their findings support significant wound healing activity in these study groups.

2.2.4 Electrical stimulation:
The electrical stimulation (ES) is used to treat chronic, and more recently, acute wounds as well. ES is recommended to eliminate bacterial load, promote granulation tissue, decrease inflammation, reduce edema, reduce wound pain, and augment blood flow. Human skin, wounds and the cells that facilitate wound healing all have measurable electrical currents. ES affects various types of cells and their activities by supporting, altering, or providing electrical currents to accelerate wound healing. The available literature is diverse and instructive. ES can be applied by either direct or indirect method depending on the type of wound, and the condition of patient. Clinical decisions related to voltage, electrode placement, dosage, and other variables must be made on a case-by-case basis. A study conducted by Petrofsky J et al. on ten subjects with stage III and IV wounds and 8 controls, subjected to 5 minutes of biphasic ES (20 mA, pulse width 250 msec). Before, during, and after the treatment, blood flows, was measured using a Laser Doppler Imager. Author concluded that in wounds, where blood flow is high due to bradykinin and cytokine release, the vasoconstrictor tone is not present and electrical stimulation causes a large increase in circulation that lasts after stimulation is over.

A study conducted by Lawson D et al. to compare healing rates and skin blood flow (BF) of chronic stage III and IV wounds in people with diabetes (DM) and those without diabetes (WD) using a warm room and electrical stimulation. Skin blood flow was measured using a Laser Doppler Imager. Results showed an increase in BF not only during the stimulation (the increase in BF was greater for DM at 87% than WD at 6%)
at the outside of the wound but even at rest before stimulation started after the initial treatment creating a carryover effect. There was no increase in skin blood flow in the center of the wound. Healing rates over four weeks of up to 70% were seen in subjects with diabetes using biphasic current. Author concluded using stimulation in a warm room significantly increased healing and skin blood flow in these wounds.

2.2.5 Thermal and non thermal diathermy

Pulsed short wave diathermy (PSWD), continuous short wave diathermy (CSWD), and pulsed radiofrequency stimulations (PRFS) have been used successfully to treat chronic wounds, facilitating progress from one phase of healing to the next. These diathermy treatments utilize radio waves to provide thermal and non thermal effects respectively. All transmit radiation from an applicator head to the target tissues. PSWD heats superficial and deep tissues. CSWD heats deep muscle and joint tissues. PRFS can influence tissue at the cellular level. Wound sites treated with diathermy have demonstrated increased fibroblast proliferation, collagen formation, tissue perfusion, and metabolic rate. The clinical use of these modalities has increased since the publication of a number of studies regarding the non thermal effects of pulsed diathermy and the production of smaller treatment units for clinical use. Equipment needed for treatment includes a diathermy unit/electronic console, and one or two applicator heads. Treatment is usually delivered without touching the skin. Wounds should be carefully prepared before treatment according to guidelines. With newer units, the pad can be placed above the wound dressings, compression garments, and casts. Because the effects of heat may continue after the treatment patients should be observed carefully and protocol guidelines should be followed closely\textsuperscript{14}.

A study conducted by Hill J et al\textsuperscript{187} to investigated the influence of pulsed shortwave diathermy (PSWD) on fibroblast and chondrocyte cell proliferation rates and to establish the influences of different dosages applied. He conducted a four single-blind in-vitro study on human adult dermal fibroblast and chondrocyte cells which were plated at known concentrations and incubated for 5 days. Exposure to PSWD, twice daily, on days 2, 3, and 4. Author concluded PSWD is associated with increased rates of fibroblast and chondrocyte proliferation in-vitro, which is dose dependent. The study
conducted by Seaborne et al\textsuperscript{188} evaluated the effectiveness of four different electrostatic and electromagnetic fields on surface area of pressure sore. Author reported under these circumstances it has been postulated that the so-called non thermal biological effects that is changes in cell sensitivity, permeability and membrane potential, may predominate over the thermal effects. This study confirmed that PSWD at the low power densities are an effective treatment modality for reducing the pressure sores surface area.

2.3. ULTRAVIOLET RAYS IN WOUND HEALING

2.3.1 Background
Phototherapy, also known as light therapy, is a form of radiant energy utilized for healing purposes by humans since the time of primitive man. Radiant energy from the sun was used by both, the early Greco-Romans and the ancient Egyptians for its curative powers; including its effects on wounds and skin disorders. Herodotus known as the "father of history,"\textsuperscript{189} was one of the first individuals to surmise a relationship between sun exposure and a heightened biologic response. He was credited with having observed the difference in degree of calcification between the skull bones of vanquished Egyptian and Persian soldiers. He ascribed the increased thickness of Egyptians soldiers' skull bones to greater exposure because of the cultural practice of shaving the scalp from an early age. As a result of these observations, Herodotus and others became advocates of sun therapy and sunlight was prescribed for numerous ailments including epilepsy, paralysis, asthma, malnutrition and obesity.

Historical Perspective: The sun and the light that it provides was recognized as potential healing properties by many early cultures, among the Egyptians, Romans, Greeks, Sumerians, Chinese Indians, and Japanese\textsuperscript{190}. Because of the sun's integral role in ancient societies, many of the ancient gods were named after the sun. One such god was Helios, Greek god of physical light and sun\textsuperscript{191}. Evidence of the role that the Greeks believed that the sun god played a role in healing is recorded in ancient stone inscriptions. However, with the advent of Christianity, little was written about heliotherapy or sun therapy until the 18\textsuperscript{th} century. It was not until 1796 that significant
attention was refocused on the question of whether sunlight was beneficial to humans. At this time, a prize was offered by the University of Gottingen, for the best essay on the effects of light on the human body. The winning essay by Ebernaier was the first to propose a relationship between the lack of sun exposure and the development of rickets. Some years later, Niels Finsen prepared a paper on the influence of light on skin. Using his own forearm, he demonstrated the ability of sunlight to induce a delayed erythema on unprotected skin exposed to sunlight. It was around this time, that the germicidal property of sunlight was discovered. The bactericidal property of light was first demonstrated in 1877\textsuperscript{189}. Using an un-boiled Pasteur's solution, Downes and Blunt showed that sunlight could prevent the growth of bacteria. In later experiments, Downes and Blunt were able to demonstrate that light near the violet end of the electromagnetic spectrum had the greatest bactericidal potency. However, it was not until Duclaux in 1885 and Ward in 1892 demonstrated the bactericidal effects of sunlight in the absence of heat generation that the bactericidal properties of sunlight became generally accepted\textsuperscript{135}. Some years later, Bernhard and Morgan were the first to show that ultraviolet (UV) radiation below 329 nm was bactericidal\textsuperscript{189}. Between 1890 and 1909, UV energy was shown to be bactericidal to many bacteria, including Mycobacterium tuberculosis, Staphylococcus, Streptococcus, Bacillus, and Shigella dysenteriae. It was during this time that UV radiation became a common treatment for tuberculosis of the skin. In fact, the Nobel Prize for Medicine and Science was awarded to Finsen in 1903 for his work on the treatment of tuberculosis-induced skin lesions. In the following decades, much of the UV research focused on the use of UV radiation to control or prevent surgical wound infection\textsuperscript{135,189}. This interest continues today with several groups investigating the utility of using UV radiation to prevent or control infection of orthopedic surgical wounds\textsuperscript{192}. Additionally, with the emergence of antibiotic-resistant wound pathogens, the role of ultraviolet light in the C band (shorter wavelength ultraviolet) for the treatment of infected acute and chronic wounds is being re-examined along with its putative ability to stimulate wound healing processes\textsuperscript{19,142,193,194}. 
2.3.2.a: Therapeutic Radiant Energy Forms

A number of health-care professionals use radiant energy for a variety of purposes. For example, therapeutic radiation involves the use of very short wavelength, high-energy radiant energy (x-rays and gamma rays) by medical physicists, oncologists, and surgeons in the management of tumors.

Radiant energy from the mid-portion of the electromagnetic spectrum (UV, visible, and infrared radiation [IR]) is used commonly in the management of wounds.

Ultraviolet energy: As mentioned above, the three energy forms that have been used historically to provide phototherapy include UV, visible and IR. UV light is a form of radiant energy that falls between X-rays and visible light on the EMS (Fig:2.1).

![Electromagnetic spectrum](image)

**Figure 2.1: Electromagnetic spectrum**

However, UV light is a misnomer, as this portion of the EMS is largely invisible to the human eye. UV light is more appropriately described as UV energy or radiation. UV energy encompasses the wavelengths between 180nm and 400 nm and has been commonly separated into three distinct bands UVA, UVB, and UVC based on their wavelengths and associated biologic activities. According to the International Commission on Illumination (CIE), UV wavelengths can be subdivided as follows: 400-315 nm (UVA), 315-280 nm (UVB), and 280 nm - 100 nm (UVC)\(^{135}\). The World Health Organization (WHO) definition of the three UV bands differs somewhat as compared with the CIE. The WHO defines UVA as wavelengths 400-320 nm, UVB as
wavelengths 320-280 nm, and UVC as wavelengths 280-200 nm. Below 220 nm UVR is increasingly absorbed by the oxygen in the air to form poisonous ozone, which implies that there is no practical use for these shorter wavelengths outside the specialized laboratory\textsuperscript{195}. Recently, UVA has been subdivided into UVAI and UVA2, since UVA2 rays are thought to have actions more similar to those of UVB. UVAI encompasses wavelengths from 340 to 400 nm while UVA2 encompasses wavelengths from 320 to 340 nm\textsuperscript{135}. Long-wave UV radiation, or UVA, is referred to as black light or near UV radiation and is closest to the visible light portion of the EMS. The middle band of UV radiation, or UVB, is known as sunburn radiation and is thought to mediate most of the harmful effects of sunlight on human skin, including photo-aging and carcinogenesis\textsuperscript{196-198}. UVA and UVB account for approximately 6.3\% and 0.5\% of sunlight during the summer, respectively. On the other hand, UVC, radiation is known for its germicidal effects, and almost all of these rays are prevented from reaching the earth by the ozone layer\textsuperscript{135}.

2.3.2.b: Physical Science of Phototherapy Radiant Energy Forms
Specific wavelengths and frequencies characterize radiant energy forms. As the wavelengths of energy increase across the EMS, from cosmic rays to electric power, the frequency decreases. For example, gamma rays have short wavelengths but are high frequency compared with broadcast or electric power waves that are long wave and low frequency. In biologic tissues, longer wavelengths typically penetrate more deeply than do short ones. Therefore, longer wavelengths allow treatment of deeper structures. The density of the medium in which it travels affects the time to delivery of radiant energy. Radiant energy travels in a straight line at high speed while in a vacuum. However, as the density of the medium increases (skin, muscle and bone) the speed of the radiant energy wave decreases. Interaction with the medium affects the delivery of radiant energy to a biologic medium in the following ways:

1. Reflection (decrease in energy to the medium as some waves are reflected away from the medium)
2. Absorption (energy captured by the medium with a decrease in waves travelling completely through the medium)
3. Refraction (bending and dividing wavelengths into component parts)
4. Penetration or transmission through the medium (decreased absorption by the medium with loss of energy from the medium)

Three physical laws govern the use of radiant energy in the treatment of biologic tissues. The first, Grotthus-Draper law, states that the effect of a wavelength on a tissue is determined by the amount of energy that the tissue absorbs. Different wavelengths are known to produce different effects. The second law, the inverse square law, states that the intensity of the energy wave is inversely related to the square of the distance from the radiant source. The third law, the cosine law, states that energy absorption is at its greatest when energy waves strike a medium at right angles. All these laws must be taken into consideration for the proper and optimal application of radiant energy.\(^{15}\)

2.3.2.c. Transmission of UVR through plastic: UVR energy impinging upon a transparent medium or target is partly reflected and partly absorbed; the remainder is transmitted. The relative values are dependent upon the optical properties of the transparent object and UVR. Translucent materials such as polyethylene can also transmit the germicidal components of sunlight.\(^{199}\) A study by Mutwiwa\(^{200}\) used 2 small tunnels which were constructed and covered with either an UV-transmitting or UV-absorbing plastic film understand the effect of orientation and distribution behavior of the greenhouse whitefly, Trialeurodes vaporariorum. An in-vitro study conducted by Dai\(^{201}\) et al on catheter related infection, showed UVC when passed through the catheter was able to inactivate microorganism on the outer surface of central venous catheter. The study compared catheter made of silicone and poly urethane and found polyurethane had less absorption than the catheter made of silicone. Hence they selected polyurethane material catheter for the study. The transmission of the UVC through the prototype device catheter was calculated to be about 13.6%. When the catheter was exposed to UVC irradiation from a cold cathode fluorescent lamp inside the catheter lumen at a radiant exposure of 3.6 mJ/ cm\(^2\), more than 6-log\(_{10}\) of drug-resistant Gram-positive bacteria adhered to the outer surface of the catheter were inactivated. Three to 7-log\(_{10}\) of drug-resistant Gram-negative bacteria and 2.80-log\(_{10}\) of fungi were inactivated at a radiant exposure of 11 mJ/ cm\(^2\). UVC irradiation also offered a highly selective inactivation of bacteria over keratinocytes under exactly comparable conditions. After
11 mJ/cm² UVC light had been delivered, over $6-\log_{10}$ of bacteria were inactivated while the viability loss of the keratinocytes was only about 57%. Wallbank AM showed in his study that the bacteria and viruses were inactivated by the germicidal UV light as the water flowed through the Teflon pipe.

2.3.3.a. Biologic Effects

The three bands of UV radiation differ in their ability to penetrate human skin (Figure 2.2) and to produce certain biologic effects. The UVA band constitutes the longest wavelengths of the UV energy spectrum, and these rays penetrate human skin to the level of the upper dermis. In contrast, UVB rays penetrate only to the stratum basale, the lowermost level of the epidermis. UVC rays (which have the least ability to penetrate human skin) reach only the upper layers of the epidermis. Specific UV wavelengths have been associated with particular biologic responses. For example, germicidal effects of UV radiation are associated with UV wavelengths from 250 to 270 nm, while UV wavelengths of 254 nm and 297 nm have the greatest ability to induce an erythematous or reddening reaction in the skin. The tanning response, on the other hand, is predominantly associated with wavelengths of 254 and 299 nm.

![Figure 2.2: Depth of penetration of different wavelengths of UVR](image)

UV energy has been used to treat a variety of skin conditions, including open wounds, because of its biologic effects. UV radiation promotes exfoliation of the outer skin layers, enhance healing through the induction of an erythematous response in the skin, and inactivate a variety of microorganisms. The biologic effects of UV have been
classified as immediate or long term Stenback lists the induction of erythema and its accompanying inflammatory changes along with increased pigmentation as examples of acute skin reactions to UV exposure.

**2.3.3. i)**: Early (0-60 hours)- Immediate hyperpigmentation, Epidermal hyperplasia, Inflammatory reactions. 
Late (60-336 hours)-Secondary hyperpigmentation, Hyperplasia, Fibrosis.

**2.3.3. ii)** Long standing (chronic exposure) - Elastosis, Carcinoma

**2.3.3. i)** Early Cutaneous Effects:
Skin reddening or erythema is a well-known effect of UV at certain exposure levels. UV radiation produces reddening of the skin via stimulation of an inflammatory response that leads to increased vascularity of the dermis. Erythema is most effectively produced by the 297 nm and 254 nm UV wavelengths. These wavelengths encompass both the B and C bands of UV. Erythema that results from the longer wavelengths has a greater latency lasting for a longer period than that produced by shorter wavelengths. However, the shorter wavelengths have a greater potency. UV-induced erythema is associated with a latent period of 2 to 3 hours. The exact mechanism that underlies this latent appearance of erythema is unknown, but several theories have been proposed. The latent development of the erythematous response has been ascribed to the production of some diffusible biologic mediator from damaged epidermal cells. It is assumed that this mediator then diffuses to the dermis, where it enhances blood vessel permeability. The identity of this diffusible mediator is unknown. Several substances including histamine, bradykinin, and prostaglandins have been implicated in this role. In the past, prostaglandins were considered the most likely candidate for this role as a diffusible mediator. However, work by Hensby utilizing prostaglandin antagonists has been inconclusive, and the role that prostaglandins play in mediating erythema is unclear. The study conducted by Brauchle have demonstrated a significant increase in vascular endothelial growth factor (VEGF) expression in cultured keratinocytes after irradiation with both sub lethal and physiologic levels of UVB. Irradiation of quiescent keratinocytes lead to both an increase in mRNA levels as
well as increased levels of VEGF. Since VEGF is known to enhance vascular permeability, it is thought that VEGF may be a potential target for the above-mentioned diffusible mediator. However, the identity of this diffusible mediator(s) remains unknown. Congruent with the findings of Brauchle, Holtz\textsuperscript{210} has shown that an intercellular edema in the stratum spinosum layer of the skin and an accumulation of white cells in local blood vessels accompany UV-induced erythema. The development of intercellular edema is also consistent with the separation of the upper and lower epidermal layers that occurs upon exposure to high-intensity UV radiation. These effects on the skin most likely underlie the ability of UV radiation to stimulate debridement. Debridement would result from this sloughing of the upper layers of the epidermis as well as recruitment of phagocytic white blood cells. Increase in lysosomal activity and leakage of lysosomal enzymes has been detected with UV exposure may also contribute to this debridement effect.

Another immediate effect of UV exposure includes skin thickening, or hyperplasia\textsuperscript{196}. UV rays induce a hyperplasia or cellular proliferation in the stratum corneum, or outermost layer of the skin. Protection against subsequent sunlight-induced skin damage by this process is assumed\textsuperscript{196}. Research indicates that DNA changes within 4 to 7 hours after irradiation of the epidermis. Epidermal cells in stratum basale accumulate glycogen at 12 hours, and increased RNA levels are seen at 24 hours in both the basal and stratum spinosum layer. These increased levels of RNA reflect an increased rate of transcription, indicating on-going repair. These increased levels of RNA are consistent with the finding that UV radiation stimulates the production of IL-I\textalpha by keratinocytes\textsuperscript{211}. IL-I\textalpha plays a role in enhancing wound epithelialization. Work by Kaiser et al\textsuperscript{211} demonstrated that UVB stimulated epithelialization, thus providing further evidence of a role for UVB in stimulating epidermal migration by enhancing IL-I\textalpha production. This work supports the current treatment approach of utilizing UV to enhance epithelialization, especially with indolent wounds that exhibit fibrotic edges. In these wounds, UV may stimulate or restart the epithelialization process.

When low-level UV radiation exposure occurs, the above-described effects confine to the upper third or one-half of the epidermis. As a result, no long-lasting effects occur as
the cells in these layers are differentiating in transit to becoming part of the outer dead layer of the skin. Therefore, these data among others lend support to the supposition that UV radiation stimulates repair processes and that these effects may be harnessed at a low enough level to prevent long-term damage. It is shown that high levels of UVB exposure affect Langerhans cells. These cells inhabit the middle region of the epidermis and appear to have an immune function. They are part of the macrophage lineage and are derived from the bone marrow. High-level UVB exposure is known to produce Langerhans cell necrosis within 24 hours. This pattern of cellular necrosis is seen in both experimental rodent models and humans. The destruction of these cells is thought to account for the immunosuppressive ability of high levels of UVB. Interestingly, harnessing the immunosuppressive effect of high-level UVB exposure to enhance graft take in individuals with burn wounds has demonstrated by researchers. However, since the Langerhans cells are derived from the bone marrow, no long-term effects are expected because of this local immunosuppression of the treated skin. Furthermore, the effects of short treatment times at low intensities with UVB or UVC are unknown. It is possible that these effects will be different from those seen with high-level UV radiation.

2.3.3. ii. Late Cutaneous Effects
Late cutaneous effects include elastosis or loss of skin elasticity, and carcinogenesis. Elastosis has been observed traditionally in the skin of individuals who labor in the sun for most of their lives. Degeneration of collagen and elastin fibers in the dermis characterizes this.

Histological analysis of skin, which exhibits elastosis, includes basophilic degeneration and enlarged, blunted elastic fibers. These changes are not found in adjacent skin areas that have been protected from prolonged exposure to the sun.

Prolonged or lifetime exposure to UV radiation, particularly rays in the B band, is well accepted as a causative factor in certain types of skin cancers. Supporting evidence for UVB-related carcinogenesis includes,

1. Increased number of skin cancers on sites exposed to the sun chronically
2. Decreased numbers of certain types of skin cancers with increased natural pigmentation
3. Increased incidence of skin cancer in light-skinned people, especially those who spend significant time outdoors
4. Increased incidence of skin cancer in light colored people who live near the equator
5. UVB readily produces skin cancer in experimental animal models with prolonged continuous exposure (hours)
6. Individuals with deficient DNA repair mechanisms in the skin are more prone to skin cancers.

However, these effects are associated with prolonged exposure to sunlight, particularly high-intensity UVB over a period of years\textsuperscript{135}.

2.3.3.b: i) Safety levels in humans: There are currently no recommendations for safe doses for human skin\textsuperscript{214}. The National Health and Medical Research Council (NHMRC Canberra)\textsuperscript{215} standard provides UVR exposure limit values for exposure of the eye or skin in the spectral region between 180 and 400 nm. The NHMRC recommends exposure limit values are to be used as guides to evaluate potentially hazardous exposure from both pulsed and continuous sources of UVR where the exposure duration is not less than 0.1 μsec. The exposure limit values in the NHMRC Canberra standard do not differ from the International Commission on Non-Ionizing Radiation Protection (ICNIRP)\textsuperscript{216}. Guidelines essentially restrict radiant exposure on unprotected skin to no more than 30 joules per square metre (30 J/m\textsuperscript{2}) for wavelength between 180-400nm. This means that for an eight-hour day, the effective irradiance in a second should not exceed one milliwatt per square meter (1 mW/m\textsuperscript{2}). Table (2.4) shows some artificial sources of UVR and the times taken to exceed the exposure limits of 30 J/m\textsuperscript{2} for an eight-hour day.
Table 2.4: Some artificial sources of UVR and the times taken to exceed the exposure limits\textsuperscript{217}.

<table>
<thead>
<tr>
<th>Category</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescent lamp</td>
<td>&gt; 8hrs</td>
</tr>
<tr>
<td>Quartz halogen lamp</td>
<td>~ 10 mins</td>
</tr>
<tr>
<td>UVA lamp</td>
<td>~ 17 mins</td>
</tr>
<tr>
<td>Germicidal (UVC) lamp</td>
<td>1 – 3 mins</td>
</tr>
<tr>
<td>Arc Welder</td>
<td>1 – 5 mins</td>
</tr>
</tbody>
</table>

The wavelength dependency of a given photobiological effect is demonstrated by its action spectrum, which depends on a variety of factors but is based on the absorption spectrum of the chromophore (UVR absorbing biomolecule) and the optical properties of the skin. It is estimated that only 5\% of 254 nm UV-C at the skin surface penetrates to the top viable cell layer, compared with 15\% for 365 nm (UV-A) and 50\% for 297 nm (UV-B). Action spectroscopy studies with different broad-spectrum sources show that UVB is much more effective than UVA for most acute endpoints studied in human skin. This includes erythema, delayed pigmentation, DNA photodamage and UCA (Trans urocanic acid) photoisomerization. In general, UVB is 3 to 4 times more harmful with same per unit physical dose (J/cm\(^2\)) than UVA, but this difference depends on the specific wavelengths/wavebands being compared. Action spectra for immunosuppression in human skin are not available. UVB is known to be immunosuppressive but the role of UVA is still not clear. The action spectrum for IPD (Immediate pigment darkening) shows that UVA is more effective than UVB. UVC is not an issue for terrestrial solar UVR because it is completely absorbed by the ozone layer. In any case, UVC is strongly attenuated by chromophores in the upper epidermis and UVC-induced DNA damage in the dividing basal layer of human epidermis is not readily detected which may explain why the dose response curve for UVC erythema in human skin is very much less steep than for UVB. It is unlikely that UVC from artificial sources presents an acute or long-term hazard to human skin since germicidal UVR is
absorbed by the dead outer layer. However, UVC is likely to cause acute photokeratitis since cornea has no such outer layer. Hence, the cells of the cornea have greater exposure to UV irradiation injury. Wavelength dependency is crucial in determining the biological effect of a given spectral region of a UVR source. UVC exposure is unlikely to cause acute or long-term damage to the skin but can cause severe acute damage to the eye and should not be permitted at all from any tanning device\textsuperscript{218,219}.

2.3.3.b: ii Safety levels in animals: Wavelength-dependent penetration depths of UV light were almost without exception taken from ex vivo or in-vitro measurements of human skin or even from ex vivo animal skin, which is said to display comparable characteristics\textsuperscript{220}. Because of these similarities extensive studies have been conducted in animals like Hybrid Fish, Monodelphis domestica, Mouse and Angora goat to induce carcinoma\textsuperscript{221}. Skin cancers have been induced experimentally with UVR in hairless Mice model, valuable for investigating the formation of SCC. These animals develop SCC after several/ chronic UV exposures. Solar UV exposure has been shown to produce cancers in domestic and food animals\textsuperscript{222}. Dai T et al also reported that mouse skin could tolerate a dosage of 6.48J/cm\textsuperscript{2} (UVC 254nm)\textsuperscript{223}. The action spectrum\textsuperscript{218} for carcinogenesis in the albino hairless mouse closely approximates the action spectra for UV-induced erythema in human skin shown in Figure 2.3.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{action_spectra.png}
\caption{The CIE (International Commission on Illumination 1987) reference action spectrum for erythema in human skin (red) and the estimated CIE (2000) action spectrum for human squamous cell carcinoma (blue) based on mouse studies.}
\end{figure}
2.3.4: Bactericidal effects

Research has shown that UV radiation from all three bands, A, B and C, has the ability to kill numerous microorganisms. As a result of its effectiveness in killing microorganisms, UV light has been used in a variety of ways, including water purification, serum sterilization, pharmaceutical clean room, and surgical theatre decontamination. A variety of skin infections and heavily contaminated wounds has been treated by this. Because of continuing issues with surgical infections, there has been renewed interest in the potential role of UV light in preventing surgical wound infections. Taylor et al\textsuperscript{147,191}. In the United Kingdom examined the effectiveness of UVC radiation on reducing bacterial numbers in individuals undergoing total joint arthroplasty. UVC energy was delivered by tubing placed overhead in a conventional plenum-ventilated surgical theatre. The UVC tubes were activated 10 minutes after the initiation of surgical procedure. Results of the study showed that the UVC application was effective in significantly reducing bacterial levels in both the theatre air and surgical wounds. Bacterial levels in the surgical wounds fell 87% with UVC delivered at 100 micro W/cm\textsuperscript{2} (N=18) and 92% with UVC delivered at 300 micro W/cm\textsuperscript{2} (N=13). In a similar study, Moggio et al\textsuperscript{214} also found that UV irradiation significantly lowered the average number of air born bacteria detected over the surgical site. The rate of infection for 1,322 individuals that underwent hip arthroplasties was found to be only 0.15% with application of eye. Similar findings were obtained by Berg et al\textsuperscript{225} when UVR application in operating rooms was compared with a sham blue light application. These authors concluded that the air quality was similar to that produced by ultra clean air ventilation systems. It is also interesting to note that both Berg et al\textsuperscript{225} and Taylor et al\textsuperscript{147} recorded no adverse effects of UV exposure on operating room personnel. A growing interest in the use of UV energy for treatment of established wound infections has also been seen in the past two decades. This renewed interest in UV light comes at a time when antimicrobial resistance is rampant among common wound pathogens and when the health-care community is increasingly under pressure to find the most cost effective and time-efficient method of treatment for various health-care problems. Many researchers using in-vitro testing have demonstrated the effectiveness of UV radiation in killing microbes. High and High\textsuperscript{17} demonstrated that broad-spectrum UV radiation
delivered by the Kromayer lamp (model 10), which produces wavelengths from all three UV bands, was effective in eliminating a wide range of wound pathogens in-vitro. Complete eradication of all wound pathogens tested was only obtained at an E4 dose. In contrast, Nordback et al\textsuperscript{226} did not find a difference in colonization levels in rats with acute surgical wounds that were exposed to broad-spectrum UV radiation. Since the UV radiation source emitted a broad spectrum of UV A, B, and C wavelengths and the proportion of each type of wavelength is not described, it is difficult to determine if the wavelengths that are known to have the greatest germicidal activity (UVC at 250-270 nm) were present at adequate doses.

Study conducted by Sullivan et al\textsuperscript{19,227} using a low - pressure argon gas/liquid mercury lamp, (Med Faxx, Inc., Raleigh, NC) that emits predominantly UVC have shown that a broad range of wound pathogens including those expressing antibiotic resistance can be effectively eliminated with short treatment times. They have shown that with selective emission at 254 nm it was possible to obtain a 99.99\% kill rate for all tested common wound pathogens. Sullivan et al\textsuperscript{227} shown that UVC irradiation is effective in eradicating both procaryotic organisms such as bacteria and eucaryotic organisms such as yeast or multicellular fungi at short exposure times. They found that UVC is effective in killing multicellular eukaryotic wound pathogens at treatment times shorter than currently advocated for procaryotic (bacterial) organisms.

Studies by Taylor et al\textsuperscript{228} using an in-vitro model the effects of UVC on bacteria alone and in combination with pulsed jet lavage was compared with commonly used antiseptics. All of the tested topical agents including 3\% hydrogen peroxide, 1\% and 10\%povidone-iodine, and 0.05\% chlorhexidine reduced bacterial numbers on agar. However, effective elimination of the bactericidal effects of only hydrogen peroxide and povidone-iodine when tested on muscle tissue treated with whole blood or plasma was obtained. The effects of UVC application and pulsed jet lavage were found to be additive, suggesting a clinical role for co-application of these modalities in treating wound infection.
2.3.5: UV Preclinical Studies

The effects of UVA and UVB radiation on wound healing has been examined using a number of different animal models, including the rat, hairless guinea pig, rabbit, and pig\textsuperscript{229-233}. Positive effects of UVB on wound healing were observed in both the rats\textsuperscript{226} and rabbit\textsuperscript{229} animal models but not in the hairless guinea pig\textsuperscript{230-232} model. Irradiation of the acute surgical wound bed of rats with a UVA and UVB energy source resulted in a significantly increased rate of wound closure between the fourth and fifteenth days of treatment compared with untreated controls on the contra lateral side of the animal\textsuperscript{226}. Additionally, no decrement in wound tensile strength was observed either at day 7 or 15 when compared to the untreated controls. The results from this study also suggest that the effects of UVA and UVB are localized and not systemic, as there was no enhanced healing of the contralateral wounds. Similarly, Batouty et al\textsuperscript{229} found a modestly higher rate of tissue regeneration in acute full-thickness wounds to the pinna of rabbit ears using a hot quartz lamp UVA and UVB. UV-treated wounds healed more rapidly than their untreated controls. Additionally, histopathological analysis demonstrated significant increases in epithelization rates and collagen deposition as compared with untreated controls.

In contrast, acute surgical wounds induced in hairless guinea pigs that had been pretreated with UVA or UVB radiation every other day for 16 weeks did not exhibit enhanced wound closure rates\textsuperscript{231,232}. Additionally, wound tensile strength significantly less in both the UVA and UVB-treated animals. Histopathologic analysis also demonstrated marked endothelial swelling and eosinophilic infiltration in the irradiated group. Similar findings using hairless guinea pigs pretreated with pure UVA radiation prior to wounding were observed for decreased wound tensile strength. Due to the extraordinarily long duration of treatment (16 weeks in the first study and 21 weeks in the second study) and the use of a pretreatment UV paradigm rather than UV treatment post wounding, the relevance of these findings are not clear. Furthermore, additional work by the same investigators found that there were no significant differences in tensile strength of wounds made to UV-treated versus untreated skin by recovery day 90\textsuperscript{231}. 
In the above-mentioned studies did not show a significant positive effect of UVC on wound healing rates in both pig and the rodent model. No significant effect of UVC radiation on wound tensile strength was detected in the porcine model. A study conducted by Sullivan et al on effect of UVC on rodent model showed a cleaner and smoother transition area between the peri-wound and the wound bed with no tissue curling. This treatment paradigm also produced a change in wound morphology due to altered wound contraction. The induction of a change in wound contraction by UVC is consistent with the findings of Morykaw et al. He conducted in-vitro study using cultured fibroblasts, demonstrated increased secretion of fibronectin into the culture medium after UVC irradiation. Fibronectin is an extracellular matrix protein that appears to play a role in wound contraction.

2.3.6: UV Clinical Studies
Although there is significant experimental data to suggest a positive role for UV radiation in enhancing wound healing, relatively few clinical studies have been conducted. However, positive effects are noticed in majority of the studies that are conducted. Literature, as far back as 1945, document positive effects of UVA and UVB on wound healing. Stein and Shorey detailing the increased rate of wound healing and reduction of wound infection in two soldiers, one with a resistant traumatic wound and the other with a resistant pressure wound prior to UVR. The traumatic wound healed within 10 days of the initiation of UV therapy, and the pressure wound healed in less than 2 months.

A randomized controlled trial examining the effects of both UVA and UVB energy on superficial pressure sores in the elderly has also demonstrated enhanced healing rates. In this study, UV-treated ulcers closed in an average time of 6.25 weeks compared with an average of 10 weeks for control wounds. Additionally, a clinical study by Crous and Malherbe also examined the effects of UVA and UVB on wound healing in individuals with venous insufficiency. Commonly accepted method of determining UV dosage by determining the degree of erythemal response by the skin upon exposure to successively longer treatment times was the basis for treatment parameters. The doses used for peri wound skin and granulation tissue and necrotic tissue were E1 and E4.
respectively. At these doses, UV radiation appeared to facilitate wound healing, with no wound closure in any of the wounds.

The effects of UVC on chronic wound healing have also been examined. A study by Nussbaum et al.\textsuperscript{21} examined the effects of UVC combined with ultrasound on pressure ulcer healing. The treatments parameters included EI for clean/granulating wounds, E3 for purulent/slow healing wounds, E4 for heavily infected wounds, and 2E4 for necrotic wounds. Combination of the UVC and ultrasound treatment enhanced healing over that of cold laser or moist wound healing. However, it is difficult to ascribe the enhanced healing effects observed in this study to a UVC-mediated effect, because the UVC treatment in combination with ultrasound was employed. Therefore, it is not clear as to what effect either of the modalities had separately. The study conducted by Taylor R on 56 patients following skin infections with UVC tinea pedis, tinea capitis, sporotrichosis, and tinea corporis. The treatment times were between 2 and 5 minutes, with individuals receiving an average of 3.2 treatments over a period of 5 to 7 days. Fifty patients showed a good response to therapy, with most showing significant clearing of the infection within 1 week\textsuperscript{235}.

Recently, Thai et al.\textsuperscript{203} found that UVC was effective in decreasing bacterial numbers in chronic wounds. \textit{Pseudomonas aeruginosa}, \textit{Staphylococcus aureus}, and methicillin-resistant \textit{Staphylococcus aureus} numbers among other bacterial species were reduced with a single application of UVC for 180 seconds. Consistent with in-vitro testing, UVC was shown to be most effective in reducing the level of \textit{Pseudomonas aeruginosa} in chronic wounds. The author stated that multiple treatments of 180 seconds might be required for complete eradication of common bacterial pathogens from chronic wounds.

Another case study by Thai et al.\textsuperscript{142} on three patients with chronic wound infected predominantly with MRSA showed a reduction in bacterial load and facilitation of healing following UVC therapy. He further emphasized the need for well-designed randomized controlled trials to ascertain the efficacy of UVC and determine the optimal treatment dosage time and length of UVC treatment. He also emphasized the need for monitoring the effectiveness using semi quantitative swab results.
2.4.: LIMITED ACCESS DRESSING:

After 1960, apart from conventional closed dressing techniques, moist wound dressings and negative pressure dressings are commonly discussed in the literature. Vacuum assisted wound closure (VAC) is among the recent trends in wound care. Limited Access Dressing (LAD) was designed in an attempt to combine the advantages of both moist wound healing and negative pressure dressings. The design has notable advantages, while avoiding some major disadvantages such as an inaccessible, offensive smelling wound environment, and relatively high treatment costs.

It has been claimed that occlusive dressings promote rapid wound healing by preventing dehydration and scab formation, facilitating debridement, minimizing inflammation, reducing pain, increasing the rate of epithelialization, and diminishing scarring. However, there is concern that a moist environment may lead to bacterial proliferation and wound infection. Moist wound healing achieved by occlusive hydrocolloid produces offensive-smelling exudates and has raised some doubt about its effect on bacterial flora, specifically anaerobes.

In 1962, moist wound healing was first described in an experimental wound and later in a human. An occlusive dressing that traps moisture on intact skin can produce an explosive proliferation of bacteria. Occlusive hydrocolloids are impermeable to water and the colloid gel, formed by absorption of exudate, produces an absorption gradient that removes the toxic components of the wound exudate that the cellular and bacterial destruction produce. The bad odor that is produced has been explained as a result of either gelatin breakdown in the colloid gel or anaerobic infection.

Increasing evidence shows that the presence of many and varied bacterial species in chronic wounds does not adversely affect healing. The need for routine bacteriological culture swabs does not seem to be a necessity in chronic leg ulcers. This would result in considerable cost savings, and would avoid unnecessary use of antibiotics and toxic chemicals that may delay wound healing. The vacuum sealing techniques (VST) were first described by Argenta et al. and Fleischmann et al. Others have used VST for the treatment of acute traumatic soft tissue defects, soft...
tissue defects complicated by exposed bone and/or implants, and skin graft and flap resurfacing. The optimal topical negative pressure (TNP) regimen has not yet been established.

Nakayama et al\textsuperscript{25}\textsuperscript{5} have used negative pressure dressings on free skin grafts with an adhesive drape and a disposable suction drain. This method applies constant pressure on the graft and allows for easy inspection of possible hematomas and similar findings have been reported\textsuperscript{25\textsuperscript{6}}.

Infected groin wounds following lymph node dissection and groin lymphorrhea have been effectively treated by negative pressure dressing\textsuperscript{25\textsuperscript{7}}.

**Limited access dressing: The concept and applications:** How is LAD a combination of moist healing and negative pressure dressing? The Limited Access Dressing is a combination of intermittent negative pressure (for 30 minutes) and a moist wound dressing (for 31/2 hours without negative pressure) that is covered with a transparent polythene material (a total of 21 hours moist dressing and 3 hours negative pressure dressing in a 24- hour period). Negative pressure (up to -30 mmHg) is applied through naso gastric tube connected to a suction machine that is then placed under a polythene wound cover.

**LAD designs.** The material that contacts the wound LAD is classified into 2 groups: A. Hydrocolloid material contacts the wound (LAD I Figure 2.4)

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Figure 2.4: LAD I Applied to protect exposed tendons following dorsal skin necrosis following chemotherapy. Exposed tendons remained viable under LAD I for 6 weeks when they are then were covered with a radial artery forearm flap.
B. Polythene sheet contacts the wound (LAD IB Figure 2.5 and LAD II- Figure 2.6)

Figure 2.5: LAD IB for sacral pressure ulcer

Figure 2.6: Bag design of LAD II for amputation stump

Two problems associated with LAD I are liquefied hydrocolloid materials blocking the tube, and poor wound floor visibility for the initial few days until the suction removes any liquefied material. To avoid doubts and problems associated with LAD I, in LAD IB a sterile polythene sheet separates the wound along with tubes from hydrocolloid and in the Hydrocoll® (Hartmann, Heidenheim, Germany) a central hole was made to improve visibility. LAD I and LAD IB was used for smaller wounds (up to 10 cm x 10 cm given that the maximum size of Hydrocoll was 15 cm x 15 cm). In LAD II wounds are covered with larger polythene sheets, polythene tubes, and polythene bags after placing tubes (as in other LAD designs) and when sealing is achieved with pieces of Hydrocoll and the adhesive polyurethane film (OpSite™, Smith & Nephew, Largo,
This retains all the advantages of available dressing methods with additional advantages of better wound care, controlling hospital acquired infection and leech effect.

**Intra-LAD negative pressure:** There is a general belief among most physiologists that true interstitial fluid pressure in loose subcutaneous tissue is slightly less than atmospheric pressure (average value of this pressure is negative in relation to atmospheric pressure and is approximately -3 mmHg)\(^2\). When the skin cover is absent in wounds, the pressure will rise to zero mmHg (i.e., equal to atmospheric pressure). These increases in interstitial tissue pressure from -3 mmHg to 0 mmHg will also cause the lymph flow to increase 20-fold\(^2\) and the re-absorption of fluid to increase through capillaries. Hence, the chances of bacterial invasion and absorption of chemicals (toxins) through venules and lymphatics increases, when edema increases the interstitial tissue pressure or produces an open wound that is not sutured.

If the interstitial tissue pressure is slightly more negative than -6 mmHg, the lymph flow is slight and consequently, the absorption of interstitial fluid will be negligible. As a result, the risk of sepsis due to bacterial invasion and the risk of systemic inflammation syndrome (SIRS) due to absorption of pro-inflammatory cytokines will reduce considerably at -6 mmHg.

Surgeons generally believe that after wounding the wound remains contaminated for 6–8 hours, after which bacterial invasion occurs. If negative pressure (more than -30 mmHg) is applied every 3 1/2 hours for 30 minutes, chances of invasive wound sepsis and SIRS will be reduced considerably—the author determined this schedule by a trial and error method and through daily observation of changes in wound granulation appearance after applying the LAD in more than 1000 cases while generating a maximum -30 mmHg negative pressure through the suction machine. This level of pressure (-30 mmHg) not only appears to be safe for most of the tissues, but also produces a desirable negative pressure effect even if slight leakage occurs. Magnitude of negative pressure is directly proportional to the pain and discomfort produced.
Indications for LAD:

LAD is indicated to avoid and treat infection in acute, sub acute or chronic wounds. Since the mechanism of action of LAD is different from that of UVR, it is worth exploring the combined effect of LAD and UVR on wound infection.

Thus, we discussed physiological process involved in wound healing, factors responsible for delay in healing of wounds, different types of wound infections, organism frequently colonizing the wound, different physical therapeutic modalities for wound healing. And also along with this background, available literature on effect of UVR in, in-vitro, preclinical and clinical studies, still there is a paucity of data pertaining to

1. Few studies utilized clinical isolate of wound for assessing the antibacterial effect of UVR.
2. All the in-vitro and in-vivo studies have used considerable longer duration of exposure (180 second) which may lead to unnecessary exposure to UVA and UVB produced along with UVC source. Considering the benefits and exposure limit risk associated with UVR on humans, there is a need to explore the shortest effective exposure time (Inverse square law).
3. All the in-vitro and in-vivo studies have used the distance of 1 inch. If this distance for exposure is increased, increased temperature of the irradiated surface due to heat produced by UVR source may be reduced.
4. Few studies used non-infected experimental wound model for studying the effect of UVR. In addition, UVR treatment is given immediately or within 24 hours after creating wound.
5. Very few experimental studies have examined the effect of UVR on percentage wound contraction quantitative bacterial count and histopathology.
6. LAD is a new method of moist wound dressing that uses transparent polyethylene material, also the increase in the multi antibiotic resistance of bacteria in recent years and understanding the benefits of both LAD and UVR on bacterial growth, and hence it is worth to explore the synergistic/any other combined effect of UVR and LAD.