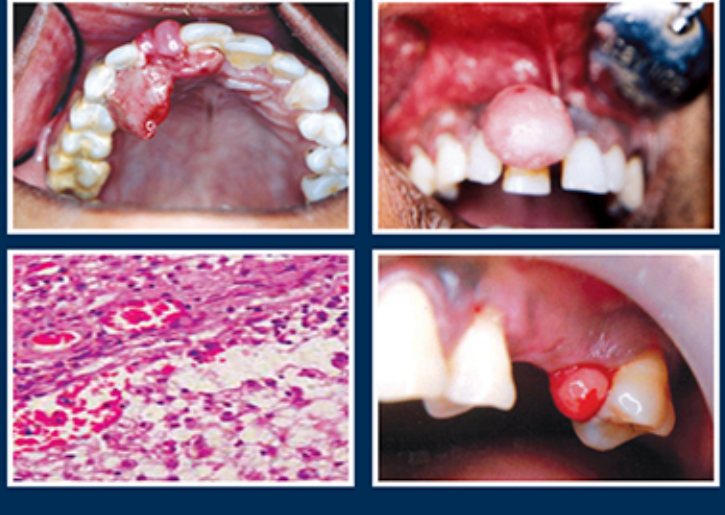
SECOND EDITION

Textbook of ORAL PATHOLOGY



Editors
ANIL GOVINDRAO GHOM
SHUBHANGI MHASKE (JEDHE)

JAYPEE

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6 Healing of Wound

Shubhangi Mhaske (Jedhe)

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INTRODUCTION

Oral wounds are relatively common mucosal wounds; healing in these tissues can reflect the susceptibility of other mucosal tissue to repair and infection. Wound repair is a well orchestrated and highly coordinated process that includes a series of overlapping phases: inflammation, cell proliferation, matrix deposition, and tissue remodeling.

This involves a complex, dynamic series of events including: clotting, inflammation, granulation tissue formation, epithelialization, neovascularization, collagen synthesis, and wound contraction. The knowledge of the physiology of the normal wound healing process through the phases of hemostasis, inflammation, granulation and maturation provides a framework for an understanding of the basic principles of wound healing.

Wound healing is the physiologic response to tissue trauma. It is related to tissue reconstitution which is the process by which the body replenishes cells that are being lost by normal physiologic events. In both processes similar events occur in varying degrees. The same basic molecular mechanisms governing growth and differentiation are active to a different extent. Without these being properly in place, even old wounds may be become subject to "re-opening".

FACTORS AFFECTING THE WOUND HEALING

There are many factors that can affect wound healing which interfere with one or more phases in this process, thus causing improper or impaired tissue repair. These multiple factors can lead to impaired wound healing. Factors may be considered in one of two categories depending on their source.

Extrinsic factors: Impinge on the patient from the external environment.

Intrinsic factors: Directly affect the performance of bodily functions through the patient's own physiology or condition.

In general terms, the factors that influence repair can be categorized into *local* and *systemic*. Local factors are those that directly influence the characteristics of the wound itself, while systemic factors are the overall health or disease state of the individual that affect his or her ability to heal (Table 6.1). Many of these factors are related, and the systemic factors act through the local effects affecting wound healing.

Local Factors

Location of Wound

Wound in an area which has a good vascular bed heals more rapidly than wounds in an area which is relatively avascular. Immobilization is important in healing of a fracture. If the wound is in an area that is subjected to constant movement and so the formation of new connective tissue is disrupted (in the corner of mouth) thus delaying the healing process.

Poor Circulation and Oxygenation

It has been shown in numerous clinical studies that typical wound partial pressures of oxygen are markedly reduced and may be the rate limiting process in wound repair. Oxygen is essential for maintaining cellular integrity, function, and repair when tissues are injured. Oxygen not only plays an important role in energy metabolism, but also is very important in polymorphonuclear cell function, neovascularization, fibroblast proliferation, and collagen deposition. Though in cases of acute hypoxia, healing will occur as long as other factors such as nutrients, blood flow, and immune function remain adequate, to allow regeneration of capillaries and restoration of nutrient delivery. Thus, other factors collaterally play a role in situations where oxygen delivery is impaired and chronic nonhealing wounds may develop.

Dressings and Local Infection

Wound infection delays collagen synthesis and causes granulation tissue to become more fragile and prone to bleeding. Wound Infection also delays healing as microorganisms compete for oxygen and nutrients with macrophages and fibroblasts. The use of dressings, which adhere to the wound bed, and the inappropriate usage of antiseptics can all lead to the hindrance of wound healing.

Foreign Bodies

Foreign bodies in the wound may be due to the presence of grit, parts of old dressings, suture material, staples, etc. These set-up an inflammatory response, which may increase the length of the inflammatory phase. Presence of foreign body also hampers the process of response of cellular events, mesh formation and collagen integrity subsequently leading to delayed healing.

Wound Temperature

This will inevitably slow down the healing process. The optimum temperature for cellular activity and division is 37°C and with a drop of 1°C it will take up to three hours for mitotic cell division to restart. Frequent dressing changes, application of cold solution and leaving the wound exposed can decrease the local temperature.

Table 6.1 Local and systemic factor affecting the wound healing

Local factors	Systemic factors	Social factors
• Location of wound • Poor circulation and oxygenation • Dressings and local infection • Foreign bodies • Wound temperature • Saliva (in case of oral wounds) • Mechanical stress • Desiccation or dryness of wound	• Nutritional status • Age and gender • Smoking and alcohol drinking • Drugs • Vascular and oxygen supply • Surgical techniques • Stress • Obesity • Infection • Diseases • Sex hormones • Immunocompromised conditions, cancer, radiation therapy, AIDS	• Poverty • Lifestyle • Housing • Cultural beliefs

Saliva (in Case of Oral Wounds)

Besides prevention of wound infections through the above antimicrobial effects, saliva plays other roles in the healing of oral wounds as well. Salivary EGF speeds up the healing process by its angiogenetic and cell proliferating effects. EGF promotes re-epithelialization of oral mucosa.

In addition to EGF in saliva, many other growth factors including insulin-like growth factor (IGF), transforming growth factor (TGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), insulin like growth factors and nerve growth factor (NGF) produced at the wound also contribute to the healing process.

Insulin-like growth factor (IGF-1), an isoform of IGF, stimulates chemotaxis of endothelial cells and proliferation of keratinocytes and fibroblasts, which promote re-epithelialization and extension of wound.

Transforming growth factor -beta1, an isoform of TGF-beta, promotes chemotaxis of monocytes, macrophages, neutrophils, lymphocytes, keratinocytes and fibroblasts, and production of growth factors from those cells.

These accelerate vascularization, deposition of extra-cellular matrices and inhibition of degradation of extra-cellular matrices.

Furthermore, saliva contains several blood clotting factors (IXa, VIII, XI) at a level comparable to plasma, and saliva can replace platelets in the thrombin generation.

This property of saliva is highly important in the oral wound healing because, although saliva dilutes blood-clotting factors of blood origin, blood-clotting can be initiated.

A relatively high amount of salivary kallikrein is suggested to play a role in vasodilatation around mucosal injuries to facilitate healing and defense of the injured area.

Hence, any condition which affect the salivary secretion any delay the wound healing.

Mechanical Stress

Mechanical stress can disturb the growth of granulation tissue which is an essential part of wound healing particularly in the process of healing by secondary intension. This is because mechanical stress interrupts the proliferation of new capillary growth.

These new capillaries carry the oxygen that is needed for epithelialization and without the capillaries the wound will be unable to heal up due to the hypoxic conditions.

Desiccation or Dryness of Wound

Dry wounds do not heal well due to the formation of eschar which slows down the migration of cells in the epidermis.

This is because migrating cells are forced deep into the dermis beneath the scab and healthy tissue becomes devitalized.

In turn healing is delayed due to the enlargement of the wound. This is more in case of skin/cutaneous wound. Therefore the patient must not become dehydrated.

Systemic Factors

Nutritional Status

Wound healing requires an adequate supply of macro and micronutrients. Deficiencies can result in poor wound healing, reduced tensile strength, wound dehiscence, and increased vulnerability to infection and poor quality scars.

Age

In advancing age, many processes slow down. The inflammatory response is reduced; therefore the risk of infection is increased. Collagen metabolism is reduced with the resulting scar being more fragile and there is less support for blood vessels thus making them more prone to damage. It is more likely that other medical problem, which are common in the elderly, slow down the healing process more than age itself.

Smoking and Alcohol Drinking

Smoking and alcohol abuse which leads to damage of the Liver and digestive system which can indirectly lead to delayed wound healing. Smoking can also affect epithelialization rates and cause problems with scarring.

The pathophysiological effects are multidimensional, including arteriolar vasoconstriction, cellular hypoxia, demineralisation of bone, and delayed revascularization.

Drugs

There are a variety of drugs that can impair the healing process. Medication can have a delaying effect on healing as well as an accelerating effect depending on the nature of the drug. Steroids are used as anti-inflammatory and immunosuppressant which will reduce the body's response to damage, delaying the healing process. For example, Corticosteroids and nonsteroidal inflammatory drugs reduce the normal inflammatory response and by

suppressing the synthesis of fibroblasts and collagen and slowing down epithelialization.

Antibiotics can enhance wound healing as they kill infective agents allowing healing to proceed. For example Aspirin and anticoagulants may cause excessive bleeding with the potential of a hematoma if not given in the correct dosage. Immunosuppressive drugs reduce leukocyte activity which reduces the inflammatory response and increases the risk of infection. Cytotoxic drugs interfere with cell proliferation including cells needed for wound healing.

Vascular and Oxygen Supply

A good blood supply is needed for wounds to heal. Taking excessive quantities of caffeine (coffee, cola drinks, or chocolate) or a high nicotine intake (smoking) can lead to vasoconstriction and lead to reduced tissue perfusion of the wound area.

Shock, hypoxia, diseases such as anemia and chronic obstructive airways disease, or an impaired arterial blood supply may cause a reduced supply of oxygen getting to the wound.

Although angiogenesis is stimulated by hypoxia, an adequate oxygen supply is required by the wound. Without it collagen synthesis and epithelialization are impaired.

Surgical Techniques

If tissue is handled roughly during surgery, it may become devitalized and provide a focus for infection. A hematoma may form if hemostasis is not achieved which can cause tissue damage by exerting pressure at the wound edges and is also a perfect environment for bacteria to grow. If sutures or staples are put in too tightly then tissue will become damaged with a poor cosmetic result.

Stress

Stress can be caused by a variety of things such as hypoxia, hypothermia, pain, and psychological issues. Irrespective of the cause, the overall effect is the stimulation of the sympathetic nervous system which leads to increase in levels of nor-adrenaline causing vasoconstriction and a diminished perfusion to the wound. Glucocorticoid has inhibitory effect on fibroblast activity, collagen synthesis and granulation tissue formation which results in delay in wound healing.

Obesity

Wound dehiscence and wound infection is increased in an obese person due to a decrease in perfusion to the wound.

Infection

Healing is delayed as bacteria compete with macrophages and fibroblasts for oxygen within the wound. There can be further tissue damage from this inflammatory response and abscesses may be formed. As the wound returns to the inflammatory phase, healing is slowed down.

Diseases

Various diseases have an influence on the healing process such as:

- **Diabetes:** There is a high-risk of infection in diabetic patients. High blood glucose levels will encourage invading microorganisms to multiply and hyperglycemia has a damaging effect on phagocytosis. Both of these will increase the risk of infection.
- **Malignancy:** Patients may have chemotherapy or radiotherapy. Radiotherapy can produce local skin damage and slows down healing. It has a fibrosing effect on local blood vessels as well as reducing the amount of fibroblasts and endothelial cells (Cutting, 1994).
- **Respiratory disease** (e.g. chronic obstructive airways disease): The amount of oxygen to the wound bed is diminished so causing a hypoxic state.

Social Factors

Poverty

The black report found that people who were living in poverty were more likely to become ill than those that were fairly affluent. Poverty can lead to a poor nutritional intake, which is important for wound healing. It can also affect the patient's ability to afford to have sufficient heating during cold weather, so causing peripheral vasoconstriction and a decreased blood supply to the wound.

Lifestyle and Habits

The lifestyle of the patient can influence wound healing, especially if the person smokes, drinks excessive amounts of alcohol or abuses drugs.

Housekeeping

Poor housekeeping can mean a lack of cleanliness, thereby increasing the risk of wound infection.

Cultural Beliefs

These may have an influence on diet, e.g. fasting or which dressings can be used as some contain porcine or bovine within them. Some patients may also have objections to being 'cared for' by members of the opposite sex.

Table 6.2 General and cellular events in wound healing

General events	Factors or cellular events
• Rapid hemostasis • Inflammation • Mesenchymal cell differentiation, proliferation, and migration to the wound site • Appropriate angiogenesis • Re-epithelialization • Proper synthesis and desired alteration of collagen to provide strength to the healing tissue	• Coordinated cell activation • Cell division • Chemotaxis • Migration • Differentiation of many cell types

CASCADE OF WOUND HEALING

Wound healing can be described as a complex and dynamic cascade of events initiated by injury. The initial or primitive response to injury is essential occurs in phases and can be called as an innate host immune response for the restoration of tissue integrity. The events of each phase should happen in a precise and regulated manner. Delayed wound healing occurs if there are interruptions, aberrancies, or prolongation in the process. These phases and their biophysiological functions must occur in the proper sequence, at a specific time, and continue for a specific duration at an optimal intensity (Table 6.2).

All the cellular events are mediated by locally released growth factors and cytokines, which may act in an autocrine or paracrine manner. Research work on acute wounds in an animal model shows that wounds heal in four phases. It is believed that chronic wounds must also go through the same basic phases.

Phases of Wound Healing
- *Hemostasis*
- *Inflammation*
- *Proliferation or granulation*
- *Remodeling or maturation.*

Hemostasis

The first action the body takes immediately after wounding is to control bleeding as damaged blood vessels must be sealed. Hemostasis serves as the initiating step and foundation for the healing process. Inflammation results in vasodilatation and increased vascular permeability.

The blood vessels themselves constrict in response to injury but this spasm ultimately relaxes. The platelet is the cell which acts as the important utility worker sealing off the damaged blood vessels. The platelets secrete vasoconstrictive substances to aid in this process but their prime role is to form a stable clot sealing the damaged vessel.

The injured blood vessel vasoconstricts, and the endothelium and nearby platelets activate the intrinsic part of the coagulation cascade. Under the influence of ADP (adenosine diphosphate) leaking from damaged tissues the platelets aggregate and adhere to the exposed collagen. They also secrete factors which interact with and stimulate the intrinsic clotting cascade through the production of *thrombin*, which in turn initiates the formation of *fibrin* from fibrinogen.

The fibrin mesh strengthens the platelet aggregate into a stable hemostatic plug. The clot that forms is made of collagen, platelets, thrombin, and fibronectin, and these factors release cytokines and growth factors that initiate the inflammatory response. Finally platelets also secrete cytokines such as *platelet-derived growth factor* (PDGF), which is recognized as one of the first factors secreted in initiating subsequent steps. The fibrin clot also serves as a scaffold for invading cells, such as neutrophils, monocytes, fibroblasts and endothelial cells. The clot also serves to concentrate the elaborated cytokines and growth factors in wound healing.

Hemostasis occurs within minutes of the initial injury unless there are underlying clotting disorders such as deficiency of Factor XIII (the fibrin-stabilizing factor) is associated with impaired wound healing secondary to decreased chemotaxis or decreased adhesion of cells in the inflammatory area.

Hemostasis → Blood vessels constrict → Platelets → Secrete vasoconstrictive substance → ADP influence aggregation of platelets → Intrinsic cascade → Thrombin secretion → Fibrinogen stimulates → Fibrin formation → Fibrin mesh entangles platelets to form hemostatic plug.

Inflammation

Clinically inflammation is the second stage of wound healing add marked as erythema, swelling and warmth often associated with pain. This stage usually lasts up to 4 days post injury.

In wound healing, it is important to clean up the debris. Neutrophils arrive via the blood and migrate into the tissue spaces of the wound within the first 24 hours. The neutrophils phagocytize debris and microorganisms and provide the first line of defense against infection. They are aided by local mast cells.

As fibrin is broken down as part of this clean-up the degradation products attract the next cell involved. Monocytes also migrate into the wound after about 24 hours. Once in the tissues, these cells also phagocytose bacteria and dead tissue, this causes them to grow and they become large cells called *macrophages*.

Macrophages provide a second line of defense. They also secrete a variety of chemotactic and growth factors such as *fibroblast growth factor (FGF), epidermal growth factor (EGF), transforming growth factor beta (TGF-beta) and interleukin-1 (IL-1)* there by coordinating much of the healing process.

An activated macrophage is important for the transition into the proliferative phase. These locally acting chemicals stimulate the regrowth of epithelium, new capillaries and the migration of fibroblasts.

At least 20 different growth factors are involved in normal wound healing. In the absence of monocytes, there are no growth factors to stimulate mitosis in adjacent healthy tissues. This means that regeneration of damaged tissues cannot occur.

Proliferation or Granulation

To replenish the damage part is prime importance for successful healing of wound. This is achieved by the cell induction, proliferation and migration. This leads to granulation tissue formation as well as epithelialization. The granulation stage starts approximately four days after wounding and usually lasts until day 21 in acute wounds depending on the size of the wound.

It is characterized clinically by the presence of pebbled red tissue in the wound base and involves replacement of dermal tissues and sometimes subdermal tissues in deeper wounds as well as contraction of the wound.

Epithelialization begins shortly after wounding and is first stimulated by inflammatory cytokines (IL-1 and TNF-alpha up regulate KGF gene expression in fibroblasts).

Epithelial cells located on the skin edge begin proliferating and sending out projections to re-establish a protective barrier against fluid losses and further bacterial invasion.

The stimulus for epithelial proliferation and chemotaxis is EGF and TGF-beta produced by activated platelets and macrophages (fibroblasts do not appear to synthesize TGF-beta). Epithelialization begins shortly after wounding and is first stimulated by inflammatory cytokines (IL-1 and TNF-beta) up regulate KGF gene expression in fibroblasts).

In turn, fibroblasts synthesize and secrete keratinocyte growth factor (KGF)-1, KGF-2, and IL-6, which simulate neighbouring keratinocytes to migrate in the wound area, proliferate, and differentiate in the epidermis. Also there is marked increase in new blood vessel formation called *angiogenesis*. This serves purpose of providing the nutrition to the cells which help in wound healing.

Fibroblasts and endothelial cells are the predominant cells proliferating during this phase. Endothelial cells located at intact small capillaries are attracted by VEGF (secreted predominantly by keratinocytes on the wound edge, but also by macrophages, fibroblasts, platelets, and other endothelial cells) to begin forming new capillary tubes.

Fibroblasts travel into the wound site from the surrounding tissue, become activated and commence producing collagen, and proliferate. PDGF and EGF are the main signals to fibroblasts and are derived from platelets and macrophages. PDGF expression by fibroblasts is amplified by autocrine and paracrine signaling.

Fibroblasts already located in the wound site (termed "*wound fibroblasts*") begin synthesizing collagen and transform into myofibroblasts for wound contraction (induced by macrophage- secreted TGF-beta 1).

Remodeling or Maturation

The main feature of this phase is the deposition of collagen in an organized and well-mannered network.

Once the basic structure of the house is completed interior finishing may begin. So too in wound repair the healing process involves remodeling the dermal tissues to produce greater tensile strength. The principle cell involved in this process is the *fibroblast.*

Early in wound healing, the matrix is thin and allows fibroblasts, neutrophils, lymphocytes, and macrophages to easily maneuver through it.

Initially, the matrix is composed mainly of *fibrin* and *fibronectin* (arising from the efforts for hemostasis and by macrophages).

Glycosaminoglycans, proteo-glycans, and other proteins (such as secreted protein acidic rich in cysteine, or SPARC) are synthesized next by the fibroblasts. As the matrix becomes denser with thicker, stronger collagen fibrils, it becomes stiff and less resistant. The fibroblasts are capable of "*adaptive response*" to the changing mechanical loading on the matrix as it matures.

The remodeling of the accommodating matrix depends on the cell migration throughout the matrix and proteolysis of the matrix proteins.

Keloid: In case of defect in extracellular matrix formation (from diet or disease), then the wound's strength is greatly compromised. In contrast, presence of excessive collagen synthesis, a hypertrophic scar or keloid can result.

The final phase of wound healing involves wound contraction and the remodeling of the ECM produced by fibroblasts during the proliferative phase. The fibronectin produced in the formation of granulation tissue diminishes over time as the matrix is remodelled. This process involves the induction of MMPs 1-3, enzymes which are each involved in the catabolism of different ECM components.

These enzymes are controlled by natural tissue inhibitors of matrix metalloproteinase (TIMPS), and the balance between MMP and TIMP expression is crucial for normal matrix remodeling. Remodeling can take up to 2 years after wounding.

During wound contraction phenotypic changes to the fibroblasts populating the wound occur, with a switch for a pro-fibrotic myofibroblasts phenotype, characterized by an increase in expression of α-smooth muscle actin. The attachment of these cells to each other and the surrounding matrix then facilitates wound contraction, with the myofibroblasts contracting pseudopodia attached to the ECM. This remodeling of the ECM in the skin leads to scar formation. However, some marked differences in the oral mucosa wound healing may be due to intrinsic characteristics of the tissue.

HEALING OF BIOPSY WOUNDS

Biopsy: It is the removal of tissue for the purpose of microscopic examination and diagnosis.

Primary Healing

Healing by primary intention or healing by first intention is that type of healing which occurs after excision of a piece of tissue with close apposition of the edges of the wound.

When the edges of the wound are brought together into contact and held in place by sutures, the blood clots, and in a matter of hours, *numerous leukocytes are mobilized in the area*.

Connective tissue cells in immediate vicinity undergo transformation into fibroblasts which in turn undergo mitotic division. New fibroblasts begin to migrate into and across the line of incision. These cells form thin, delicate collagen fibrils which intertwine and coalesce in a general direction parallel to the surface of the wound.

Endothelial cells of capillaries begin to proliferate and small capillary buds grow out and across the wound. These buds eventually form new capillaries which fill with blood and a rich network of young capillaries and capillary loops are formed.

Secondary Healing

It occurs when there is a loss of tissue and the edges of the wound cannot be approximated like in the palate or on the alveolar mucosa. The material which fills the defect in secondary healing is called *granulation tissue*.

After the removal of the lesion, *blood clots* and the repair process begins. It is basically identical with the healing by primary intention except that the fibroblasts and capillaries have a greater distance to migrate, more granulation tissue must form and healing is slower.

Cellular proliferation begins around the periphery of the wound and fibroblasts and endothelial cells grow into the clot along the fibrin strands. In addition, polymorphonuclear leukocytes, mononuclear phagocytes and later the lymphocytes migrate into the granulation tissue from the adjacent vessels and tissues.

Large numbers of leukocytes also accumulate on the surface of the wound. As granulation tissue matures, it becomes more fibrous through condensation of collagen bundles and the surface of the lesion becomes epithelialized. Lesions become somewhat avascular.

HEALING OF EXTRACTION WOUNDS

The healing of extraction wound does not differ from the healing of other wounds of the body except as it is modified by the peculiar anatomic situation which exists after the removal of tooth.

Immediate Reaction Following an Extraction

After the removal of a tooth, *blood which fills the socket coagulates*, red blood cells get entrapped in the fibrin

meshwork and the ends of blood vessels in the periodontal ligament are sealed off.

Hours after the tooth extraction, if blood clot is dislodged, healing may be greatly affected and may be extremely painful. However, if the healing is normal, within 24 to 48 hours, there is *vasodilation and engorgement of blood vessels* in the remnants of periodontal ligament and there is mobilization of leukocytes to the immediate area around the clot.

Surface of the blood clot is covered by a thick layer of fibrin. It is important to recognize that the collapse of unsupported gingival tissue into the opening of fresh extraction wound is of great aid in maintaining the clot in position.

First Week Wound

Proliferation of fibroblasts from connective tissue into the remnants of periodontal ligament is evident and these fibroblasts begin to grow into the clot around the periphery.

The clot is gradually replaced by granulation tissue. Epithelium at the periphery of the wound exhibits evidence of proliferation seen in the form of mild mitotic activity.

Crest of the alveolar bone, which marks up the margin or neck of the socket exhibits beginning of osteoclastic activity. Endothelial proliferation signals the beginning of capillary growth. During this period, blood clot begins to undergo organization by in-growth of fibroblasts and occasionally by small capillaries from the residual periodontal ligament.

An extremely *thick layer of leukocytes* gathers over the surface of the clot and the edges of the wound continue to exhibit epithelial proliferation.

Second Week Wound

During the second week, after extraction of the tooth, the blood clot is becoming organized by *fibroblasts growing into the clot* and forming *fibrin meshwork*.

At this stage, new delicate capillaries have penetrated to the center of the clot. Remnants of periodontal ligament have been gradually undergoing degeneration. Walls of the bony socket appear slightly frayed. In some cases, trabeculae of osteoid can be seen extending outward from the walls of the alveolus.

Epithelial proliferation over the surface of the wound is extensive. Margins of the alveolar socket exhibits prominent osteoclastic resorption and fragments of necrotic bone are seen in the process of resorption or sequestration.

Third Week Wound

Original clot appears almost completely organized by *maturation of granulation tissue*. Very young trabeculae of osteoid or uncalcified bone are forming around the entire periphery of the wound from the socket wall.

Early bone is formed by *osteoblasts derived from pluripotential cells* of the original periodontal ligament which assume osteogenic function.

Original cortical bone of alveolar socket undergoes remodeling so that it no longer consists of such a dense layer. Crests of alveolar bone have been rounded off by osteoclastic resorption. By this time, surface of the wound may have become completely epithelialized.

Fourth Week Wound

There is continuous deposition and remodeling, resorption of the bone filling the alveolar socket.

Due to this, crest of the alveolar bone undergoes considerable amount of osteoclastic resorption during the healing process and because of this, bone filling the socket does not extend beyond alveolar bone crest. It is obvious that crest of the healing socket does not extend above the alveolar crest.

Radiographic Changes in Healing Sockets

A tooth having been removed and after variable and unspecified length of time, there is gradual loss of density of the lamina dura and at the same time bone develops at the base and sides of the socket. By the time that the socket is filled with bone, all traces of lamina dura is gone.

Later the new bone consolidates and comes to resemble the adjacent bone. Most healed socket reveal slight or marked cortical bone formation at the surface of the alveolar process. Following the removal of teeth, the alveolar margins undergo some resorption. The surface usually becomes flat or slightly curved but smooth.

HEALING OF FRACTURES

Immediate Effect of a Fracture

After fracture, Haversian vessels of the bone are torn at the fracture site so are the vessels of periosteum and marrow cavity that happen to cross the fracture line. Due to disruption of vessels, there is considerable extravasation of blood in that area, but at the same time, there is loss of circulation and lack of local blood supply. The bone cells

or osteocytes of Haversian system die due to tearing of vessels at the fractured site.

Concomitant with the disruption of blood supply and the tearing of the blood vessels, there is death of bone marrow adjacent to the fracture line. The blood clot formation, play an important role in healing of the fracture, which later on is replaced by granulation tissue followed by its subsequent replacement by bone.

Callus Formation

It is a structure which unites the fractured ends of bone and is composed of varying amounts of fibrous tissue, cartilage and bone.

There are two types of callus:

1. **External callus:** It consists of new tissue which forms around the outside of the two fragments of bone.
2. **Internal callus:** It consists of new tissue arising from the marrow cavity.

Periosteum is an important structure in callus formation and ultimate healing of fracture. Cells of periosteum torn at the fracture line usually die, but peripheral to the area is found a flurry of cellular activity within hours of injury. Outer or fibrous layer of periosteum is relatively inert and actually lifted away from the surface of bone by proliferation of cells in osteogenic or inner layer of periosteum which assumes features of osteoblasts which in turn begins the formation of small amount of new bone at some distances from the fractured site. There is continuous proliferation of these osteogenic cells forming a collar of callus around or over the surface of fracture.

New bone which begins to form in the external callus usually consists of irregular trabeculae laid down at right angles to the surface. This differentiation of cells into osteoblasts and subsequent formation of bone occurs in the deepest part of the callus collar. In the rapidly growing area of collar, varying number cells of the osteogenic layer differentiate into chondroblasts rather than osteoblasts and actually form cartilage.

This cartilage fuses with bone and then begins to calcify by endochondroal bone formation. The calcified cartilage is gradually resorbed and replaced by bone. Internal callus forms from endosteum of Haversian canals and undifferentiated cells of bone marrow. Shortly after the fracture, endosteum begins to proliferate within a week to form new bone, which gradually unite and establish the continuity of bone. After this, both external and internal callus remodel to form indistinguishable bone.

HEALING OF OSSEOINTEGRATED IMPLANTS

Implants are of three types, i.e. endodontic, endo-osseous and subperiosteal.

Osseointegration is the term used for healing of bone around an endo-osseous implant. This results in an intimate interface between the bone and the implant. The healing process in the initial phases is same as of the healing of the extraction wound. Osteogenesis and remodeling are the prime features seen during the process.

For successful osseointegration, it is required that the implant should be totally free of any small amount of load or movement.

Healing process consist initially of deposition of the granulation tissue and woven bone. Complete healing requires a period of several months together. The process is completed with the deposition of cancellous or compact bone around the implant. Currently the implants used for support the restorative or rehabilitative oral procedures are biocompatible.

Biocompatible implants serve as a normal interface of the connective tissue and the implant as is with the dentogingival junction. Peri-implant disease is the term used for the pathologic changes seen around the implant. It involves periimplant mucositis peri-implantitis. The inflammation is seemed more at the coronal aspect; the apical osseointegration is maintained under favorable conditions.

HEALING OF PULP

Dental pulp regeneration is difficult task as it is enclosed in dentin without the collateral blood supply except through the apical foramen. With the advent of the concept of tissue engineering and discovery of stem cells regeneration of dental pulp have been a long quest. Moony and Rutherford were the pioneers in the regeneration of the pulp.

The regeneration can be cell based pulp/dentin tissue regeneration or non-cell-based regeneration. Dental pulp stem cells (DPSCs), Stem cells from human exfoliated deciduous teeth (SHED), Stem cells for apical papilla (SCAP) are potentially suitable cell source for dentin – pulp regeneration.

These stem cells can differentiate into specific tissue cells and can be used further for the other tissue defects after *in vitro* culture and characterization. Healing in pulp consists of initial response in form of acute pulpitis or pulpal

inflammation consisting of vasodilatation and capillary proliferation. This response is reversible or irreversible based on the nature of the stimulus. (Refer chapter Pulp and periapical diseases). The degree of inflammation, the time of irritation and infection, and the location of the exposure must be regarded as decisive factors for the healing of the inflamed pulp. The origins of newly differentiated odontoblasts and ability of precursor cells in the pulp to differentiate, particularly under the influence of bone morphogenic protein, a cytokine responsible for the differentiation of osteoblasts is main factor which will determine the healing or repair of pulp. In majority of case, the pulp is not uniformly affected by deep carious lesions.

After a week new collagen is formed against the necrotic zone and the beginnings of a calcifying front. Odontoblast-like cells orientate against the calcifying front and develop cellular extension around which bone-like tissue is deposited after 4 weeks. After 12 weeks, a hard tissue barrier with tubules is formed.

CEMENTUM

Cementum is considered to be avascular dental tissue. Then too it has the capacity to repair to a limited extent by the formation of cellular intrinsic fiber cementum. Cementum resorption may be by local or systemic factors. It is not considered to be a continuous process. There is alteration of the resorption and the repair process. Cementum repair requires presence of viable connective tissue. Cementum repair can occur in both vital and nonvital teeth. The cells for cemental repair are gained from the undifferentiated mesenchymal cells present in the periodontal ligament. The recruitment of the cementoblasts occurs after the stimulation by the various growth factors like the BMP-2, BMP-3, PDGF, IGF-1, TGF-beta, etc. The proteins like the bone sialoprotein (BSP), osteopontin (OPN), etc. are also involved in the differentiation of cementoblast progenitor cells to cementoblast. Repair and the regeneration of cementum is important as the principal fibers of the periodontal ligament are embedded into the cementum at one end. This prevents the tooth from being extruded and thus increases its stay in the oral cavity.

DENTIN

Reparative dentin is the tertiary dentin which is the dentin formed due to caries, attrition, cavity preparation and microleakage. This can be in the form of tubular or atubular dentin. The dentin has less and irregularly arranged tubules. There can be a demarcation in the normal dentin. Also the continuity is lost between the normal and tertiary dentin. This reparative dentin is formed by odontoblasts and sub-odontoblasts.

Types of Tertiary/Reparative Dentin

- Reactionary dentin:
  - Formed by damaged existing odontoblasts
- Reparative dentin:
  - Formed by odontoblast-like cells
- Sclerotic dentin:
  - Transparent dentin
  - Due to advancing caries and attrition
  - Octocalcium phosphate crystals and some derived from saliva.

ENAMEL

Although it was said earlier that repair or regeneration of enamel is not possible as the cell responsible for enamel formation during the tooth development, ameloblasts are not present lifelong. They are lost once the complete enamel formation occurs. But, now-a-days, various studies regarding the regeneration of enamel are being carried out. There can be remineralization of the subsurface of the enamel depending on the supply of calcium and phosphate ions from saliva. The use of fluoride is also done in remineralization of enamel. The enamel treated with fluoride becomes more resistant than the normal to further demineralization. Studies have been going on the use of nano hydroxyapatite crystals (20–40 nm) for repair of enamel.

SKIN HEALING AND ORAL MUCOSAL WOUND HEALING

Although cutaneous and mucosal wound healing proceed through the same stages of hemostasis, inflammation, proliferation, and remodeling, mucosal wounds demonstrate accelerated healing compared to cutaneous wounds. Mucosal wounds also generally heal with minimal scar formation, and hypertrophic scars are rare in the oral cavity.

Healing Response in Skin

The damaged epidermis is regenerated by two mechanisms:

1. Involves the activation of epidermal keratinocytes in the wound margin.

2. Involves the proliferation, simultaneously with this keratinocyte activation, of hair follicle cells, their migration into the skin, and their transformation to epidermal keratinocytes.

Phospholipids of the cellular membrane are essential for both the cellular function and morphological maintenance. Fatty acids, the major component of these phospholipids, are key factors in the regulation of cell proliferation and differentiation. Among the fatty acids known to constitute the cell membrane, palmitic acid (16:0) is a basic unit in the membranes of all human cells. Once the epidermis is damaged, then rapid cell proliferation becomes necessary, and the abundant palmitic acid (16:0) stored in hair follicle cells is used for wound epithelialization, i.e. wound healing in a dynamic environment.

Healing Response in Oral Mucosa

The oral mucosa shows earlier wound healing than does the skin, because "early wound healing" of the oral mucosa has been studied in terms of the "moisture environment in the oral cavity" but not from other aspects.

The oral mucosal epithelium (both the basal and suprabasal layers) demonstrated a significantly higher percent composition of palmitic acid (16:0) than did the epidermis, but with no difference in its distribution between the two layers. There is a much higher energy metabolism in the oral mucosa than in the skin.

The skin is significantly more dependent on essential fatty acids than the oral mucosa, thus suggesting that the skin is more susceptible than the oral mucosa to wound healing failure attributed to malnutrition.

Lower neutrophil, macrophage, and T-cell infiltrations were consistently observed in the intra-oral injury site. One possibility is that within the oral cavity, saliva provides many necessary growth factors, making macrophage function less critical.

Saliva containing abundant amounts of cytokines, growth factors, and protease inhibitors—is the primary factor that accounts for rapid oral wound healing. At sites of injury, the epithelium is a rich source of both pro-inflammatory mediators, such as IL-1, and TNF-α, as well as growth factors, such as vascular endothelial growth factor (VEGF).

The rapid healing and the absence of scars in oral mucosa are most directly related to intrinsic characteristics of the tissue and not to environmental factors such as temperature, salivary flow, the absence of a hemostatic plug, or microflora.

Notably, the mucosal epithelium differs from skin in that it lacks a stratum corneum and does not need to serve functionally as a barrier to water loss. Furthermore, mucosal healing occurs in a fully hydrated environment. In the skin, migrating keratinocytes at the wound edge express high levels of VEGF, suggesting that keratinocyte derived VEGF stimulates angiogenesis during wound healing.

Keratinocytes are also capable of modulating fibroblast behavior, including collagen synthesis, through the production and release of soluble factors. Keratinocytes from skin and oral mucosa respond differently to equivalent IL-1b stimulation. Keratinocytes from skin and mucosa maintain differential regulatory pathways that lead to differential responsiveness at sites of injury.

This fundamental difference in intrinsic genetic response to wounding between skin and mucosa, which makes mucosal wounds heal faster and with less inflammation and scar formation.

Excessive scarring such as hypertrophic scars and keloid scars have not been reported in the oral mucosa. Scalds to the oral mucosa do not result in contractures unlike scalding to the skin.

It was hypothesized that secretory leukocyte protease inhibitor *(SLPI; a cationic serine protease inhibitor with antimicrobial and anti-inflammatory properties)* found in large quantities in the saliva may have a role in mediating scarless healing in the oral mucosa.

Various reasons have been suggested for minimal scarring in the oral cavity, including distinct fibroblast phenotype, the presence of bacteria that stimulate wound healing and the moist environment and growth factors present in saliva.

Oral mucosal wounds also demonstrate a more highly regulated angiogenic response and have a differential expression of key profibrotic growth factors compared with skin, resulting in less scarring than in skin wounds.

Great differences have been observed in wound healing between the oral mucosa and the skin. Oral mucosa wound repair is characterized by rapid re-epithelialization as well as enhanced wound repopulation and matrix reorganization *in vitro*.

Oral mucosal wounds were shown to contain significantly lower levels of macrophages, neutrophils and T-cells when compared with dermal wounds

Research had demonstrated that oral fibroblasts are phenotypically distinct, and are capable of achieving a higher number of cumulative population doublings *in vitro* when compared to patient matched skin fibroblasts.

Table 6.3 Systemic and local aspects

Systemic aspects	Local aspects
• Optimize nutritional status - Consult with dietician - Ensure adequate protein and caloric intake, trace metals, vitamins • Examine psychosocial status - Depression inhibits compliance and wound healing - Pain control is extremely important • Optimize biochemical status - Acid base balance - Endocrinologic status, blood sugars, hypothyroidism - Correct renal failure, liver failure • Optimize perfusion and oxygenation - Cardiac status - Pulmonary status - Anemia - Vascular bypass surgery if required • Correct spasticity and other physical factors contributing to abnormal mobility • Examine and reduce all medications	• Optimize wound environment by - Thorough wound debridement - Eliminate foreign bodies, dead space and necrotic tissue - Elimination of infection systemic/topical antimicrobials Ph active agents - Decreased edema - Off-load pressure - Provide moist wound environment

Oral mucosal fibroblasts (OMFs) were shown to produce increased levels of epithelial mitogens keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF), produce lower levels of α-smooth muscle actin, all factors implicated in fibrotic wound healing.

Oral mucosal fibroblasts have also been shown to exhibit greater proliferation in the presence of basic fibroblast growth factor (beta-FGF) *in vitro*. OMF have increased ability to remodel ECM and contract a wound by use of fibroblast populated collagen lattices.

The matrix metalloproteinase activity of OMFs has also been shown to be superior to dermal fibroblasts, with increased levels of MMP-2 with a decreased production/activation of TIMPs.

A CLINICAL APPROACH TO OPTIMIZING WOUND HEALING (TABLE 6.3)

The multitude of factors affecting wound healing should be examined for every individual. In general, to provide for optimal wound healing one should strive to eliminate underlying causative and/or contributory factors and stimulate positive physiologic factors required for the healing process.

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Shubhangi Mhaske (Jedhe) has completed MDS in Oral Pathology from Government Dental College and Hospital, Aurangabad, Maharashtra, India in 2000 and has over 13 years of undergraduate and postgraduate teaching experience in oral and maxillofacial pathology. She is presently working as a Professor and Head, Department of Oral Pathology, People's Dental Academy, Bhopal, Madhya Pradesh, India. She has published many national and international articles. She has been external examiner in reputed national universities for undergraduate and postgraduate examinations. She is a member, Board of Studies of People's University, Bhopal, Madhya Pradesh, India, and also an editorial board member, People's *Journal of Scientific Research*; and *Journal of Orofacial Research*.

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