CHAPTER II

RATIONAL AND THEORY

2.1 Skin

2.1.1 Human skin [11]

Skin, the human body's largest organ, is considered to be one of the most basic organs for regeneration. It has a surface area of 1.8 m^2 and makes up approximately 16% of our body weight. More importantly, the skin is a window through which the physician can "see" the entire body. Skin has several functions including: barrier to physical agents, protects against mechanical injury, prevents dehydration of body through fluid loss, reduces the penetration of UV Radiation, helps regulate body temperature, provides a surface for grip, acts as a sensory organ, acts as an outpost for immune surveillance, plays a role in Vitamin D production, has a cosmetic association

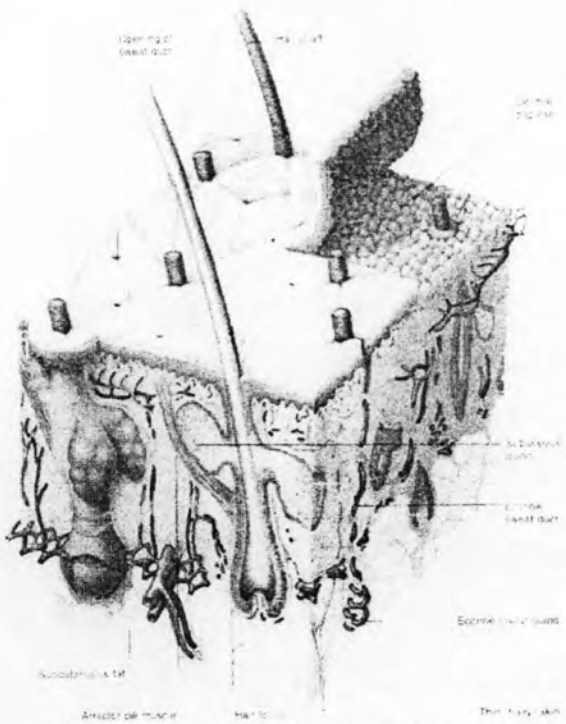

Figure 2.1: The microanatomy of human skin [11]

Skin is composed of two tissues, a connective tissue or dermis and a covering epithelium or epidermis connected together by the basement membrane that comprise the dermo-epidermal junction.

2.1.1.1 Epidermis [12, 13]

The epidermis is a stratified fusiform epithelium that mainly serves as a protective barrier. The epidermis is about 0.1 mm thick, but on the palms and soles, the thickness can be greater (0.8 -1.4 mm).

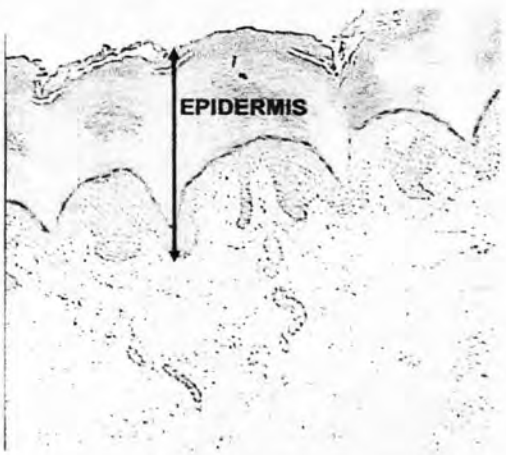

Figure 2.2: Epidermis layer [12]

The keratinocyte is the principal cell of the epidermis and it serves to produce the protein keratin. The basal or deepest epidermal cells are anchored to the basement membrane by adhesion molecules, namely fibronectin. There immature cells are continually dividing and migrating toward the surface to replace lost surface cells. Keratinocyte maturation can be represented in the 4 layers of the epidermis including:

1. Basal Cell Layer (stratum basale): The basal cell layer is comprised mostly of keratinocytes which are either dividing or non-dividing. The cells contain keratin tonofibrils and are secured by hemidesmosomes to the basement membrane. *Melanocytes* make up to 5-10% of the layer and make melanin which is transferred to neighboring keratinocytes via dendritic processes. Melanocytes are of neural crest origin and are most numerous on the face and other exposed areas of skin. Merkel cells can also be found in this layer and are closely associated with terminal filaments

of cutaneous nerves. *Merkel cells* have a role in sensation. Neuropeptide granules, neurofilaments, and keratin can be seen in their cytoplasm.

2. Prickle Cell Layer (stratum spinosum)

Daughter basal cells migrate upwards and differentiate into polyhedral cells in this layer. Desmosomes interconnect these polyhedral cells and give rise to the "prickles/spines" seen at light microscope level. *Keratin tonofibrils* form the cytoplasmic supportive network. *Langerhans cells* are mostly found in this layer. They are dendritic, immunologically active cells that play a role in antigen presentation.

3. Granular Cell Layer (stratum granulosum)

In this layer, cells become flattened and lose their nuclei. In the cytoplasm, there are keratohyalin granules as well as membrane-coating granules which expel their lipid contents into the intercellular spaces.

4. Horny Layer (stratum corneum)

This layer is composed of sheets of overlapping polyhedral cornified cells with no nuclei called *corneocytes*. This layer is thickest on the palms and soles. The flattened corneocyte develops a thickened cell envelope. Its cytoplasm is replaced by keratin tonofibrils in a matrix formed from keratohyalin granules. Whereas, the membrane-coating granules produce lipid glue that keeps the cells stuck together. This forms the hydrophobic barrier membrane that protects the skin and prevents the water loss.

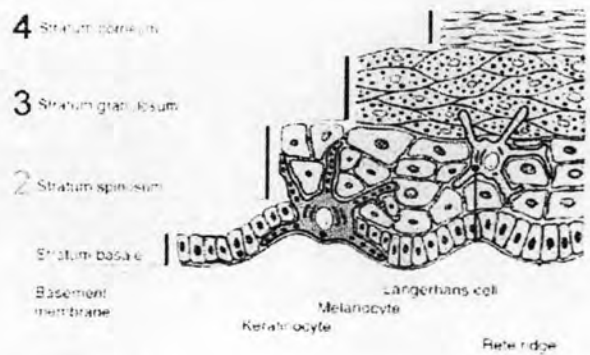

Figure 2.3: The four layers of epidermis [11]

Replacement of the epidermal layer by this regenerative process takes 2-3 days. Biological stimuli at the wound surface are necessary to direct proper orientation and mitotic response of the epidermal cells. Many of the cues come from dermal elements, especially the matrix proteins and matrix glycosaminoglycan.

2.1.1.2 Basement membrane [14]

Basement membrane is a broader of epidermis layer and dermis layer, provided strength connection with these two layers. Basement membrane connected with epidermis via hemidesmosome and connected with dermis via anchoring fibril and plague. It can be divided into 3 layers

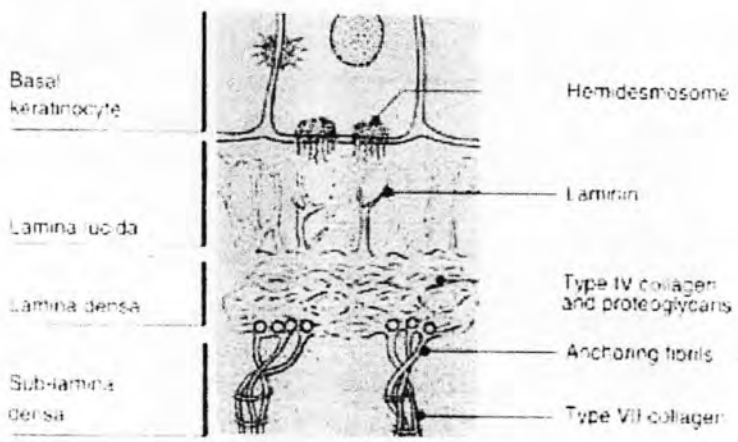

Figure 2.4: Basement membrane layer between epidermis and dermis [14]

1. Lamina lucida: (20-40 nm) the upper layer connected with epidermis via hemidesmosome and composed of laminin protein.

2. Lamina densa: (40-50 nm) the middle layer composed of collagen type IV.

3. Reticular layer: the lower layer composed of anchoring fibril anchored into dermis and rich of collagen type VII.

2.1.1.3 Dermis [13]

The dermis is a tough supportive connective tissue matrix containing numerous specialized structures. The dermal thickness varies being thinnest (0.6 mm)

on the eyelids and thickest (3 mm or more) on the back, palms, and soles. The dermis is intimately connected with the epidermis. *The papillary dermis*, the thin upper layer, lies directly below and interdigitates with the epidermal rete ridges. The papillary dermis is composed of loosely interwoven collagen. Found deeper is the thicker *reticular dermis* with its coarser and horizontally running bundles of collagen.

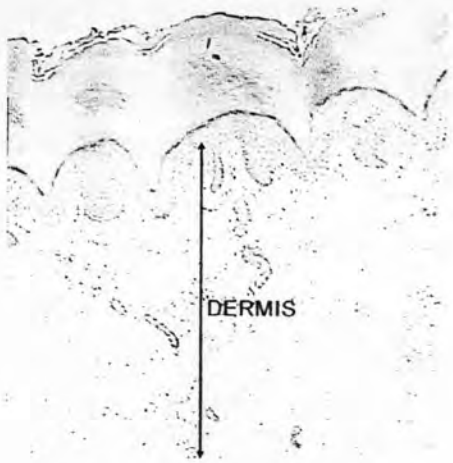

Figure 2.5: Dermis layer [13]

The papillary dermis is the major factory for the proteins providing direction for epidermal replication. The upper dermis also contains the highest blood flow. The primary cell type is the fibroblast which produces the key structural extra cellular matrix (ECM) protein collagen and elastin as well as the dermal ground substance.

Collagen fibers: make up 70% of the dermis and give structural toughness and strength. Collagen is the predominant protein in dermis, mainly collagen Type 1.

Elastin fibers: are loosely arranged in all directions and give elasticity to the skin. They are most prevalent near hair follicles and sweat glands in the papillary dermis.

The dermal ground substance: consists of a semi-solid matrix of glycosamino-glycans (GAG) which impart movement to some dermal structures.

Dermal dendrocytes: which are dendritic cells with immune function, mast cells, macrophages, and lymphocytes.

Blood vessels supply nutrients to the dividing cells in the basal layer and remove any waste products. They also help maintaining body temperature by carrying

more blood when the body needs to lose heat from its surface. They narrow and carry less blood when the body needs to limit the amount of heat lost at its surface.

Specialized nerves in the dermis detect heat, cold, pain, pressure and touch and relay this information to the brain.

A sebaceous gland opens into each hair follicle and produces sebum, a lubricant for the hair and skin that helps repelling water, damaging chemicals and microorganisms.

Sweat glands occur on all skin areas. When the body needs to lose heat, these glands produce sweat which is a mixture of water, salts and some waste material such as urea. Sweat moves to the surface of skin via the sweat duct, and the evaporation of water from the skin has a cooling effect on the body.

Hair follicles are embedded in the dermis and occur all over the body, except on the soles, palms and lips. Each hair follicle has a layer of cells at its base that continuously divides, pushing overlying cells upwards inside the follicle. These cells become keratinized and die, like the cells in the epidermis.

It is also important for maintenance of the skin's overall integrity by providing biological support to the overlying epidermis via extracellular matrix and cell factors. The epidermis in turn provides biological feedback to the dermis through the release of cytokines and other mediators. Fibroblast is important for the production and maintenance of the structural elements of skin, including collagen and elastin combined with non-fibrous substances such as glycosaminoglycans (GAGs) to form an extracellular matrix (ECM). Fibroblasts appear at the injury site at a very early stage and proliferate rapidly as wound healing progresses. They accelerate the healing process by relation of matrix deposition (collagen type I, type IV, elastin, laminin), epidermal differentiation and dermal regeneration. In addition, fibroblasts synthesize various growth factors; insulin growth factor (IGF), keratinocyte growth factor (KGF), Platelet derived growth factor A (PDGF-A), transforming growth factor (TGF), vascular endothelial growth factor (VEGF) and cytokines that stimulate wound healing [14]. For collagen, its turnover is normally low but it occurs at a higher rate during damage repair. Furthermore, the vascular network, which is difficult to replace, is quite critical to skin regeneration. Without an adequate blood

supply, repair is inhibited, and if revascularization cannot be achieved, the result of healing is scar formation.

2.2 Connective Tissue [16]

The connective tissues are responsible for providing and maintaining form in the body. Functioning in a mechanical role, they provide a matrix that serves to connect and bind the cells and organs and ultimately give support to the body. The major constituent of connective tissue is its **extracellular matrix (ECM)**, composed of protein fibers, and amorphous ground substance, and tissue fluid, the latter consisting primarily of bound water of salvation. Embedded within the extracellular matrix are the connective tissue cells.

Connective tissue serves a variety of functions. Its most conspicuous function is structural. The capsules that surround the organs of the body and the internal architecture that supports their cells are composed of connective tissue. This tissue also makes up tendons, ligaments, and the areolar tissue that fills the spaces between organs. Bone and cartilage are specializes types of connective tissue that function to support the soft tissues of the body.

The role of connective tissue in defense of the organism is related to its content of phagocytic and immunocomponent cells and also cells that produce pharmacologically active substances important during imflammation. Phagocytic cells engulf inert particles and microorganisms that enter the body. Specific protein called, antibodies are produced by plasma cells in the connective tissue. These combine with foreign proteins of bacteria and viruses and combat the biologic activity of these harmful agents. In addition, connective tissue matrix components provide a physical barrier, preventing the dispersion of microorganisms that pass through the epithelia. The connective tissue in nutrition is due to its close association with blood vessel. The connective tissue matrix serves as the medium through which nutrients and metabolic wastes are exchanged between cells and their blood supply.

2.2.1 Ground Substance: The amorphous intercellular ground substance is colorless, transparent, and homogeneous. It fills the space between cells and fibers of connective tissue. It is viscous ad acts as a lubricant and also as a barrier to the penetration of the tissue by foreign particles. Because of its high content of water and amorphous appearances, it is difficult to study in both fresh and fixed material. The ground substance is formed mainly by 2 classes of components: *glycosaminoglycans* and *structural glycoproteins.*

2.2.1.1 Glycosaminoglycans: are linear polysaccharides formed by characteristic repeating disaccharide units usually composed of a uronic acid and a hexosamine. The term, acid mucopolysaccharides, was used originally to designate hexoamine-rich acid polysaccharides extracted from connective tissue. The hexosamine can be glucoamine or galactosamine, and the uronic acid can be glucuronic or iduronic acid. With the exception of hyaluronic acid, these linear chains are bound covalently to a protein core forming a proteoglycan molecule. In cartilage, these proteoglycan molecules have been shown to be bound to a hyaluronic acid chain, forming larger molecules. Owing to the abundances of hydroxyl, carboxyl, and sulfate groups in the carbohydrates moiety of most proteoglycans, they are intensely hydrophilic and act as polyanions. Hyaluronic acid is the only nonsulfated glycosaminoglycan. In proteoglycans, the carbohydrate portion is preponderant and constitutes 80-90% of the weight of this macromolocules. Because of the aforementioned characteristics, proteoglycans can bind to a great number of cations by electrostatic bonds, and they are intensely hydrated structures with a thick layer of salvation water surrounding the molecule. When fully hydrated, these molecules fill a much larger volume than in their anhydrous state.

The main proteoglycan are composed of a core protein asso ciated with the glycoaminoglycans know as dermatan sulfate, chondroitin sulfate, keratan sulfate, and heparin sulfate. Dermatan sulfate is sound mainly in dermis, tendons, ligaments, and firous cartilage, all structures that contain collagen fibers (collagen type I). Chondroitin sulfate predominates in hyaline and elastic cartilages, which are rich in collagen type II. Heparan sulfate seems to be associated with reticular fibers, which

are composed of collagen type III. Proteoglycans bind to collagen owing to electrostatic interaction between their acidic groups and the basic amino acid residues of collagen. The degradation of proteoglycans is carried out by several cell types and depends on the presence of lysosomal enzymes. The turnover of these compounds is rapid 2-4 days for hyaluronic acid and 7-10 days for sulfated proteoglycans. Several disorders have been described in which, owing to a deficiency in lysosomal enzymes, glycoaminoglycan degradation is blocked, with a consequent accumulation of these compounds in tissues. Lack of specific hydrolases in the lysosomes has been found as the cause of several disorders in humans, including Hurler's syndrome, Hunter's syndrome, Sanfilippo syndrome, and Morquio's syndrome.

Glycoaminoglycans have a lubricating function in connective tissue, but their main function is probably structural, interacting with collagen fibrils to bind these structures together.

2.2.1.2 Structural glycoproteins are compounds containing a protein moiety to which carbohydrates are attached. In contrast to proteoglycans, the protein moiety usually predominates, and these molecules do not contain linear polysaccharides formed by disaccharide containing hexosamines. Rather, the carbohydrate moiety of glycoprotein is frequently a branched structure. Although the presence of glycolproteins in the ground substance has been known for several years, recent studies have contributed to rapid progress in knowledge about the biologic importance of these compounds.

Glycosaminoglycan	Repeating Disaccharides		Distribution	Electrostatic Interaction With Collagen
	Hexuronic Acid	Hexosamine		
Hyaluronic acid	D-Glucuronic acid	D-Glucosamine	Umbilical cord, synovial fluid, vitreous humor, cartilage	
Chrondroitin 4-sulfate	D-Glucuronic acid	D-Galactosamine	Cartilage, bone, cornea, skin, notochord, aorta	High levels of interaction, mainly with collagen type II
Chrondroitin 6-sulfate	D-Glucuronic acid	D-Galactosamine	Cartilage, umbilical cord, skin, aorta (media)	High levels of interaction, mainly with collagen type II
Dermatan sulfate	L-Iduronic acid or D-glucuronic acid	D-Galactosamine	Skin, tendon, aorta (adventitia)	Low levels of interaction, mainly with collagen type I
Heparin sulfate	D-Glucuronic acid or L-iduronic acid	D-Galactosamine	Aorta, lung, liver, basal laminae	Intermediate levels of interaction, mainly with collagen types III and IV
Keratan sulfate (cornea)	D-Galactose	D-Galactosamine	Cornea	
Keratan sulfate (skeletal)	D-Galactose	D-Glucosamine	Cartilage, nucleus pulposus, annulus fibrosus	

Figure 2.6: Composition and distribution of glycoaminoglycans in connective tissue and their interaction with collagen fibers [16]

Several glycoproteins have been isolated from connective tissue and evidence shows that they play an important role not only in the interaction between neighboring cells but also in the adhesion of cells to their substrate. Fibronectin (MW 220,000-240,000) is a glycoprotein synthesized by fibroblasts and some epithelial cells. This molecule has binding sites for cells, collagen, and glycoaminoglycans. These interactions help mediate normal cell adhesion and migration. Cancer cells do not synthesize fibronectin, and this may account for their increased capacity to invade other tissues. Laminin is a large glycoprotein composed of at least 2 polysaccharide chains. It has been detected in basal laminae and is partially responsible for the adhesion of epithelial cells to the type IV collagen present in these structures. Condronectin is present in cartilage, where it mediates the adhesion of chondrocytes to type II collagen.

In connective tissue, in addition to the amorphous substance, there is a very small quantity of fluid called, tissue fluid that is similar to blood plasma in its content of ions and diffusible substances. Tissue fluid contains a small percentage of plasma proteins of low molecular weight that pass through the capillary walls as a consequence of the hydrostatic pressure of the blood. Under normal conditions, the quantity of tissue fluid is insignificant.

2.2.2 Fibers: There are 3 main types of connective tissue fibers: Collagen fibers, reticular fibers, and elastic fibers. Collagen and reticular fibers are known to be formed by the protein collagen, whereas the elastic fibers are composed mainly by the protein elastin. These fibers are distributed unequally among the different connective tissue. In many cases, the predominated fiber type is responsible fro conferring specific properties on the tissue.

2.2.2.1 Collagen

Collagen is the most abundant protein of the human body, representing 30% of its dry weight. Improvements in methodology have shown that the collagens of vertebrates are a family of proteins, produced by several cell types that are distinguishable by their chemical composition and by their different morphologic and pathologic features, distribution in tissues, and functions. Although many different types of collagen have been described, the most common, most important, and best studied are the collagen type I, II, III, IV and V.

- Collagen type I: is the most abundant and has a widespread distribution. It occurs in tissues as structures classically designated as collagen fibers that form bones, dentin, tendon, organ capsules, and dermis.
- Collagen type II: is present mainly in hyaline and elastic cartilage. Only very thin fibrils are formed.
- Collagen type III: is usually associated with collagen type I in the tissues and is the collagenous component of reticular fibers.
- Collagen type IV: is present in the basal lamina. This type of collagen does not form fibrils or fibers.
- Collagen type V: is present in fetal membranes, in blood vessels, and in small amounts in other tissues. Its structure and function is still a subject of controversy and intensive investigation.

Recent studies on collagen biology have shown that collagen synthesis, an activity though originally to be restricted to fibroblasts, chondroblasts, osteoblasts, and odontoblasts, is actually very widespread and many cell types produce this

protein. The principal amino acids composing collagen are glycine (33.5%), praline (12%), and hydroxyproline (10%). The amount of collagen in a tissue can thus be determined by measurement of its hydroxyproline content. Collagen contain 2 amino acids that characteristic of this protein – hydroxyproline and hydroxyllysine. These amino acids are not incorporated as such in the protein molecule but result from the hydroxylation of praline and lysine of nascent collagen polypeptides in the rough endoplasmic reticulum during collagen synthesis.

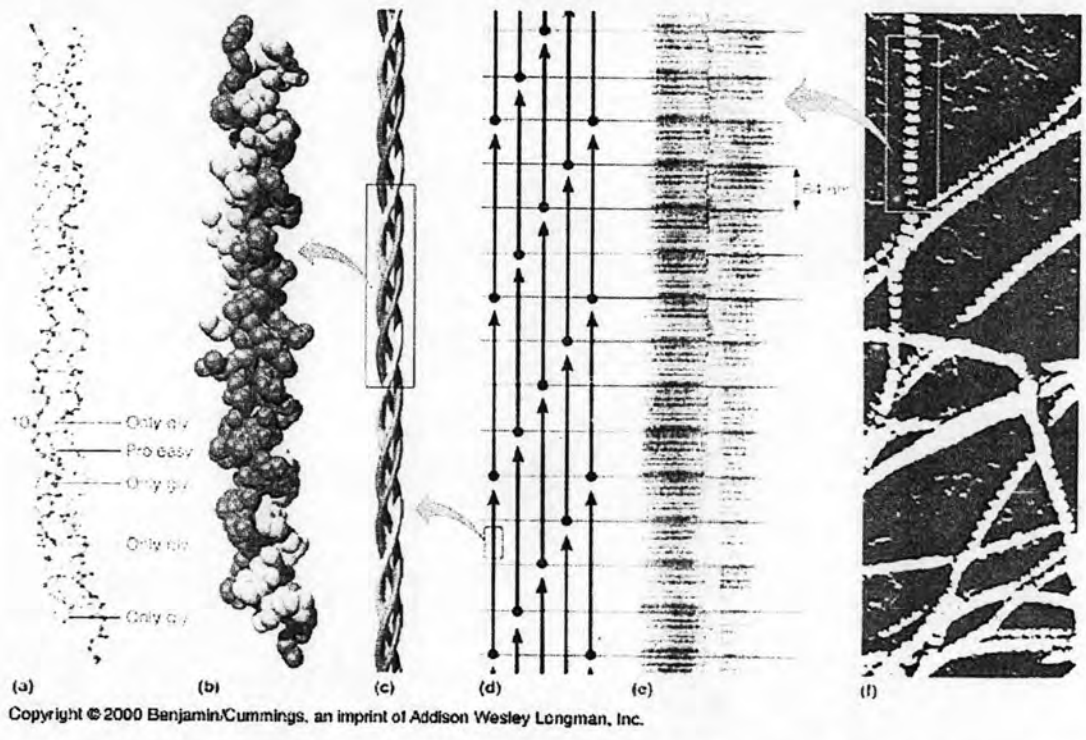

Figure 2.7: Diagram show the model of triple helix collagen [17]

The protein unit that polymerizes to form collagen fibrils is the elongated molecule called, tropocollagen, which measure 280 nm in length and 1.5 nm in width. Tropocollagen consists of 3 subunit polypeptide chains are responsible for the different types of collagen.

In collagen type I, II, and III, tropocollagen molecules aggreagate into microfibrillar subunits that are packed together to from fibrils. Hydrogen bonds and hydrophobic interactions are important in the aggregation and packing of these units.

In a subsequent step, this structure is reinforced by the formation of covalent cross-links, a process catalyzed by the activity of the enzyme lysyl oxidase.

Collagen Type	Molecular Formula	Tissue Distribution	Optical Microscopy	Ultrastructure	Site of Synthesis	Interaction With Glycosaminoglycans	Function
I	$[\alpha 1(I)]_2\alpha 2(I)$	Dermis, bone, tendon, dentin, fascias, sclera, organ capsules, fibrous cartilage.	Closely packed, thick, nonargyrophilic, strongly birefringent yellow or red fibers. Collagen fibers.	Densely packed, thick fibrils with marked variation in diameter.	Fibroblast, osteoblast, odontoblast, chondroblast.	Low level of interaction, mainly with dermatan sulfate.	Resistance to tension.
II	$[\alpha 1(II)]_3$	Hyaline and elastic cartilages.	Loose, collagenous network visible only with picro-Sirius stain and polarization microscopy.	No fibers; very thin fibrils embedded in abundant ground substance.	Chondroblast.	High level of interaction, mainly with chondroitin sulfates.	Resistance to intermittent pressure.
III	$[\alpha 1(III)]_3$	Smooth muscle, endoneurium, arteries, uterus, liver, spleen, kidney, lung.	Loose network of thin, argyrophilic, weakly birefringent greenish fibers. Reticular fibers.	Loosely packed thin fibrils with more uniform diameters.	Smooth muscle, fibroblast, reticular cells, Schwann cells, hepatocyte.	Intermediate level of interaction, mainly with heparan sulfate.	Structural maintenance in expansible organs.
IV	$[proα1(IV)]_2 proα2(IV)$	Epithelial and endothelial basal laminae and basement membranes.	Thin, amorphous, weakly birefringent membrane.	Neither fibers nor fibrils are detected.	Endothelial and epithelial cells, muscle cells, and Schwann cells.	Interacts with heparan sulfate.	Support and filtration.
V	$[\alpha 1(V)]_2\alpha 2(V)$	Placental basement membranes.	Insufficient data.	Insufficient data	Insufficient data.	Insufficient data.	Insufficient data.

Figure 2.8: Main characteristics of the different collagen type [16]

Collagen fibrils are thin, elongated structures with variable diameters (ranging from 20 to 90 nm) that have a transverse striation of the collagen fibrils is determined by the overlapping arrangement of the subunit tropocollagen molecules. The dark bands retain more of the stain used in electron microscope studies because they have more fee chemical groups that react more intensely with the lead solution used as a "stain" than the light bands. In collagen types I and III, these fibrils associate to from fibers. In collagen type I, fibers can associated to form bundles. Collagen type II (present in cartilage) occurs as fibrils but does not form fibers.

Collagen type IV, present in basal laminae, does not from fibrils or fibers and probably occurs as unpolymerized or scarcely polymerized procollagen molecules. Collagen types I, II, and III from fibrils and are often referred to as

interstitial collagens to distinguish them, as a group, from collagen type IV and V, which do not form fibrils.

2.2.3 Connective tissue cells: There are a number of cells integrating in all kinks of connective tissue including: [16]

- *Fibroblasts*: Fibroblasts are the most common cell type found in connective tissue. The term "fibroblast" is commonly used to describe the active cell type, whereas the more mature form, which shows less active synthetic activity, is commonly described as the "fibrocyte". Fibroblasts are elongated, spindle-shaped cells with many cell processes. They have oval, pale-staining, regular nuclei with prominent nucleoli. Abundant rough endoplasmic reticulum and active Golgi bodies are found in the cytoplasm. Fibroblasts synthesize collagen, reticular and elastic fibers and the amorphous extracellular substance including the glycosaminoglycans and glycoproteins.

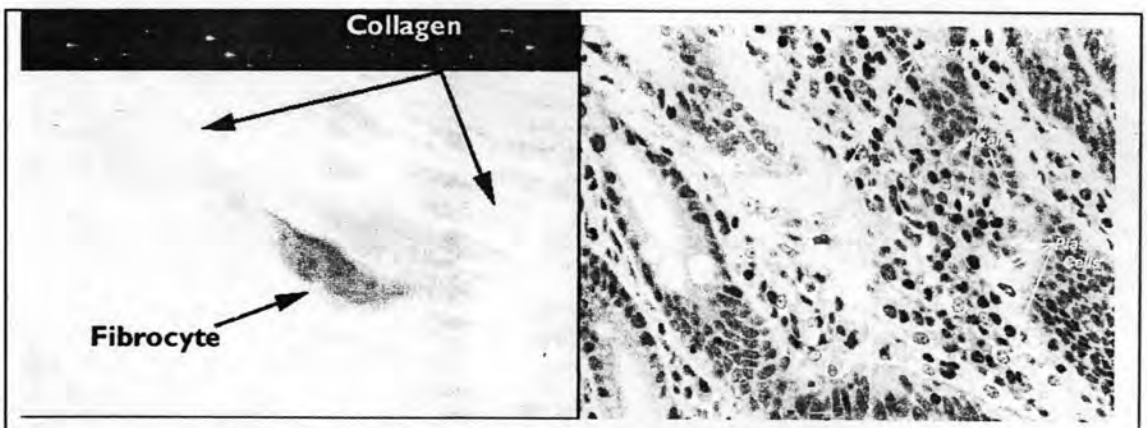

Figure 2.9: Many kinds of connective tissue cells [17]

- *Macrophages*: Macrophages show pronounced phagocytotic activity. This can be demonstrated following injection of vital dyes such as trypan blue or Indian ink and the uptake of the particulate matter. Macrophages originate from monocytes (from precursor cells in bone marrow), which migrate to connective tissue and differentiate into tissue macrophages. Today the various macrophages of the body are grouped in a common system called the Mononuclear Phagocyte System (MPS). Today a wide range of macrophages are included in the MPS and include: Kupffer

cells of the liver, alveolar macrophages of the lung, osteoclasts, and microglia. The main functions of macrophages are ingestion by phagocytosis of microorganisms such as bacteria, viruses, fungi, parasites, particulate matter such as dust, and they also participate in the breakdown of aged cells including erythrocytes. The intracellular digestion occurs as a result of fusion of lysosomes with the phagosome (ingested body). Macrophages are normally long-lived and survive in the tissues for several months. In some cases where a foreign body (such as a small splinter) has penetrated the inner tissues of the body, several macrophages may fuse together to form multinuclear "foreign body giant cells". These large cells accumulate at sites of invasion of the foreign body and sites of inflammation.

- *Mast cells*: Mast cells are oval or round cells (20-30mm diameter) in connective tissue characterized by cytoplasm packed with large round *basophilic granules* (up to 2mm diameter). The granules are stained metachromatically (purple after toluidine blue staining). Two of the main components of mast cell granules are histamine and heparin. The granules of mast cells are released in inflammatory responses. Mast cells are abundant in loose connective tissue (especially adjacent to blood vessels), in the dermis, and in the lamina propria of the respiratory and digestive tracts.

- *Plasma cells*: Plasma cells are responsible for *antibody production.* These large cells have eccentric nuclei, basophilic cytoplasm (much rough endoplasmic reticulum associated with protein synthesis) and well-developed Golgi bodies. Plasma cells are relatively short-lived (10-20 days) and are found in sites of chronic inflammation or sites of high risk of invasion by bacteria or foreign proteins (such as the lamina propria of the intestinal and respiratory tracts).

- *Leukocytes*: The white blood corpuscles are commonly found in connective tissue. They migrate from the blood vessels to the connective tissue, especially to sites of injury or inflammation.

Cell Type	Main Product or Activity	Main Function
Fibroblast, chondroblast, osteoblast, odontoblast	Fibers and ground substance production	Structural
Plasma cell, lymphocyte, eosinophilic leukocyte	Production of antibodies (humoral immunity) and of immunocompetent cells (cell-mediated immunity), phagocytosis of antigen-antibody complex	Immunologic
Macrophages, neutrophilic leukocyte	Phagocytosis of foreign substances, phagocytosis of bacteria	Defense
Mast cells, basophilic leukocyte	Liberation of pharmacologically active substances (eg, histamine)	Release of pharmacologically active substances
Adipose cell	Storage of neutral fats, heat production	Energy reservoir

Figure 2.10: Function of connective tissue cells [16]

2.3 Wounds

2.3.1 Classification of burn injuries [18]

The skin has several layers. The deeper the burn injury is, the greater the number of layers that are damaged. Sweat glands and the roots of hair follicles are in the deeper layers and will be destroyed with a deep burn. Deep injuries heal more slowly, are more difficult to treat, and are more likely to have complications than superficial injuries. The names given to burn injuries of various depths have changed.

- *Superficial (First degree burn)*: Injury to the top layer of skin (the epidermis) is now called a superficial burn, formerly called a first degree burn, which only affect the epidermis. They can be healed rapidly and generally don't require any medical attention. Superficial burns normally heal within 5 to 7 days. A common type of superficial burn is sunburn. Because the top layer of epidermis is very thin, about the thickness of a piece of paper, it is easily replaced. Even when skin is not injured, the skin completely replaces the epidermis every 45 to 75 days. Healing from a

superficial burn usually occurs without scarring, although there may be some temporary discoloration.

- *Partial thickness (Second degree burn)*: Injury to the second layer of skin (the dermis) is now called a partial thickness or dermal injury, formerly called a second degree burn. The dermis is 15 – 40 times thicker than the epidermis. As a result, the seriousness of a partial thickness burn depends on how much of the dermis has been injured. A deep and large partial thickness burn will usually be treated with skin grafting. Partial thickness burns usually leave scars. Second degree burns are considered minor if they involve less than 15% of the body's skin in adults and less than 10% in children. When treated with reasonable care, second degree burns will heal themselves and produce very little scarring. Healing is usually complete within three weeks.

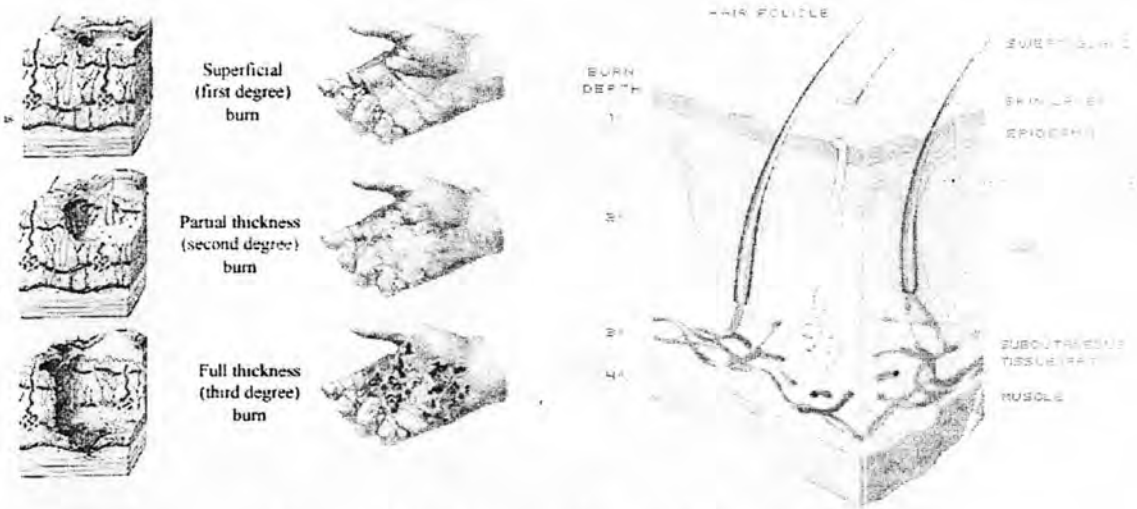

Figure 2.11: Degrees of burn injuries [18, 19]

- *Full thickness (Third degree burn)*: An injury that extends down the third layer, the subcutaneous tissue including fat, is called a full thickness injury, formerly called a third degree burn. Burns that damage muscles underneath the subcutaneous skin layer are described as full thickness burns with injury to the underlying muscle (sometimes formerly called fourth degree burns). A full thickness burn destroys all three layers of skin, resulting in the loss of not only the skin but also the hair follicles, sweat glands, and the region where new skin cells are formed. While

a third degree burn may be very painful, some patients feel little or no pain because the nerve endings have been destroyed. For these reasons, full thickness burns require skin grafts. As third degree burns heal, dense scars form.

2.4 Immunology of skins [20]

The immune response is genetically controlled. The genes controlling self-nonself discrimination is called HLA class I genes and the proteins they specify are called HLA class I antigens. The genes controlling the interaction of T cells with macrophages and B cells are called HLA class II genes, and the proteins for which they code are class II antigens.

The rejection of a transplanted tissue graft is immunological mediated. It has specificity and memory and is controlled by the MHC genes. Both class I and class II antigens are involved in these reactions. Class I antigens are the targets of the response since they are the non-self markers. Class II antigens are involved because they control initiation of the response.

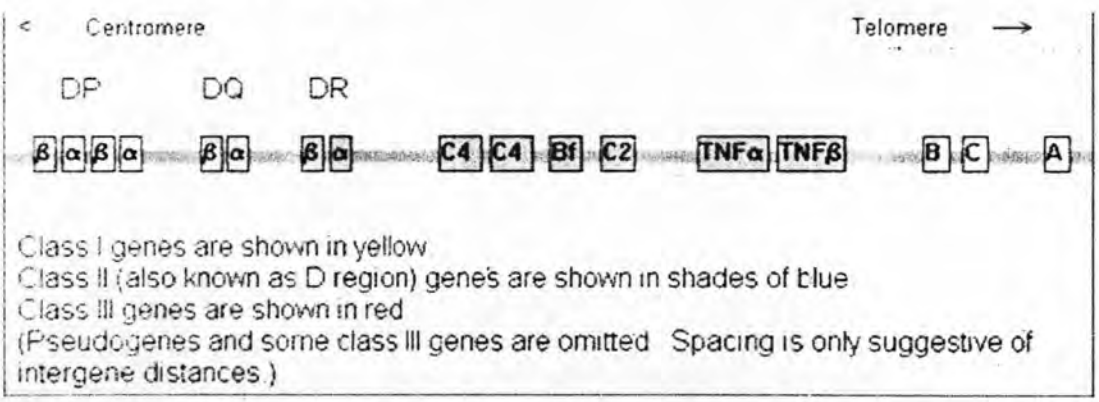

Figure 2.12: Major Histocompatibility Complex (MHC) on Human chromosome 6 [20]

The Major Histocompatibility Complex (MHC) on human chromosome 6. The genes on the left (called the D region) are those that code for the class II antigens. The three genes on the far right code for the class I antigens. Both classes are co-dominantly expressed, that is the copy on each chromosome of the pair will be transcribed, translated into protein, and transported to the cell membrane. Thus, each nucleated cell (except nerve cells) will have six different classes I proteins on its

surface if the person is heterozygous for all three antigens. The situation is more complicated for the class II genes, since they are heterodimers and all have multiple β-genes. Each chromosome could code for more than one allele of each of DP, DQ, and DR.

2.4.1 Molecular mechanisms of graft rejection

The molecular mechanisms of graft rejection are based on recognition of foreign transplanted cells by the expression of polymorphic, co-dominant genes. These genes code for protein molecules which are found on the surfaces of cells called antigens. Due to polymorphism, it is rare to find a donor and recipient with matching surface antigens. MHC molecules are responsible for the most rapid rejection reactions. They are encoded by the MHC complex. These MHC molecules which present foreign peptides are in turn presented to recipient T-cells in two different ways, direct and indirect presentation.

2.4.1.1 Direct presentation

Normally, host T-cell receptors (TCRs) recognize foreign peptides presented on self MHC molecules. In direct presentation, these host TCRs recognize foreign MHC-peptide combination presented by donor antigen presenting cells (APCs). The T cell is able to recognize and respond to the foreign MHC molecule because there are enough similarities between the self and non-self MHC molecules. The T cell, however, is able to determine that the MHC molecule is non-self. Studies have shown that TCR's are capable of recognizing foreign MHC molecules with some, but not other, bound peptides. This suggests that the particular peptide plays a role in determining whether or not its binding MHC molecule will be recognized.

Most rejection episodes are elicited through direct presentation. It is shown in many clinical studies that allograft are identified more readily, and responded to with more vigor than normal non-self antigens. This increased responsiveness is due in part to the fact that most host T-cells have the capability of recognizing a single foreign MHC molecule. This is due to several factors:

- The MHC is extremely polymorphic in nature; many different amino acid residues can be found on a single MHC molecule. If a T-cell recognizes even one of those residues, it can target that particular MHC molecule. Therefore, several different classes of T-cells may recognize a single MHC molecule.

- The surface of each foreign cell can have up to 105 copies of each MHC molecule with each molecule forming a complex with a distinct peptide. In this way, a single foreign cell has the potential to activate many different T-cells.

2.4.1.2 Indirect Presentation

Indirect presentation is the mechanism in which recipient APCs are able to process the donor antigens and present the resulting foreign peptides to T-cells using self-MHC molecules. The host APCs digests the foreign antigens through phagocytosis. The peptides from the digested complex are presented by host MHC molecules to either CD4+ or CD8+ T-cells. This intermediate pathway is termed "cross priming" because one cell, the host APC, presents the antigens of another cell from the graft, to activate, or "prime" T-lymphocytes. Due to the polymorphic nature of the MHC molecules, they are digested into a wide range of peptides, each of which is recognized by different host T-cells. In this case, the foreign MHC molecules are handled in a manner similar to other foreign antigen.

Other surface molecules that function in antigen presentation that are not included in the MHC group are classified as minor histocompatibility antigens. They are usually processed and presented indirectly to host T-cells by host APCs and MHC molecules.

2.5 Fluorescence Microscopy [16]

Fluorescence microscopy technique is based on the fact that when certain fluorescent substances are irradiated by light of proper wavelength, they emit light with a longer wavelength. In fluorescence microscopy, tissue sections are usually irradiated with ultraviolet light so that the emission is in the visible portion of the

spectrum. The fluorescent substances appear as brilliant shiny particles on a dark background. In this procedure, a microscope with a strong ultraviolet light source is used. Special filters that eliminate ultraviolet light are employed to protect the observer's eyes.

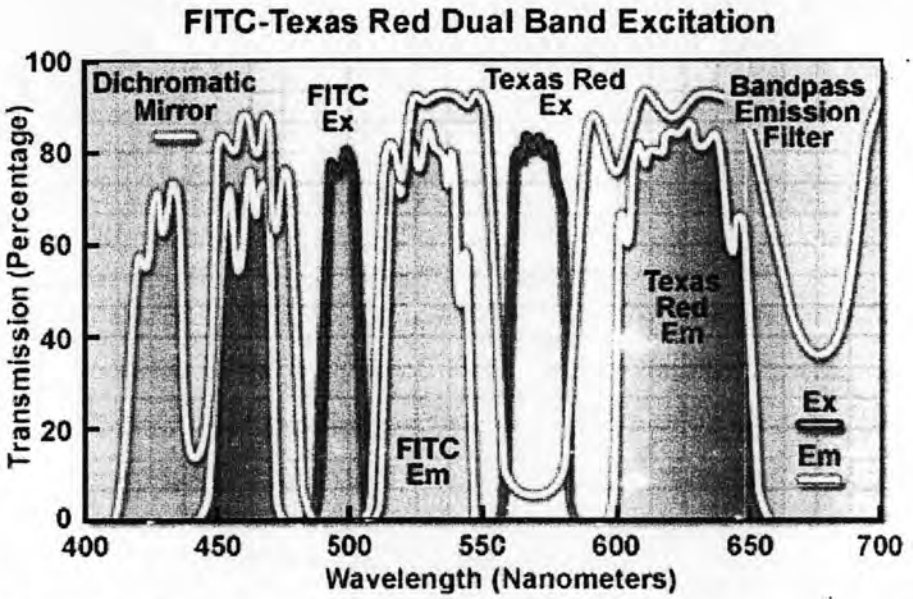

Figure 2.13: Excited and emitted wavelength of each fluorescent compound [21]

2.5.1 Fluorescent Compounds

Some naturally fluorescent substances are normal constituents of cells such as vitamin A, vitamin B, and porphyrins. Other fluorescent compounds that have an affinity for tissues and cells are used as fluorescent stains. Acridine orange is most widely used, because it can combine with DNA and RNA. When observed in the fluorescence microscope, the DNA-acidine orange complex emits a yellowish-green light, where as orange light. Thus, it is possible to identify and localize nucleic acids in the cells. Since cancer cells usually contain larger amounts of RNA than normal cells, acridine orange can be used to identify them in smears obtained from patients.

Fluorescence spectroscopy is a method of analyzing the light emitted by a fluorescent compound in micro-spectrophotometer. It can be used to characterize

several compounds present in cells and is of particular importance in the study of catecholamines.

2.5.2 Immunohistochemistry [16]

Specific amino acids or reactive groups can be identified by conventional histochemical methods, but these techniques cannot localize specific proteins. The fluorescent antibody method has proved most useful in localize specific proteins and certain other macromolecules. This test is based on the reaction of the body when exposed to foreign substance called *antigens* or *immunogens*. The body will respond by producing *antibodies* that react specifically and bind strongly to the antigen and result in neutralization of the foreign substance. Antibodies are proteins of the globulin group (immunoglobulins) that appear in plasma and tissue fluids after antigen injections. Their production enables the organism to oppose invasion by foreign microorganisms and to eliminate certain proteins and other foreign matter not recognized as self. Immunohistrochemistry is based on the coupling of immunoglobulins to substances that render them visible in the microscope without causing loss of biologic activity of the antibody. Since the labeled immunoglobulins bind specifically to their antigens, these compounds permit localization of specific antigens, these compounds permit localization of specific antigens in tissue specimens. When a tissue section containing certain antigens is incubated in a solution containing labeled antibodies to these antigens, the antibodies bind specifically to the antigens, the antibodies bind specifically to the antigens, whose location can then be visualized with either the light or electron microscope.

Three methods of labeling antibodies are frequently used

2.5.2.1 Coupling with fluorescent compound: This permits one to identify the site of specific antigens using a fluorescent microscope.

2.5.2.2 Coupling with an enzyme: This permits detection of the labeled antibody by conventional enzyme histochemistry. The enzyme most often used is

peroxidase, which can be detected by the method described above, using either the light or electron microscope.

2.5.2.3 Coupling to an electron-scattering compound that can be detected in the electron microscope: An iron-rich protein called *ferritin* or gold particles are often used as antibody markers. In the electron microscope, the location of ectron-dense ferritin- or gold-bound antibodies can be easily identified.

Three are both direct and indirect methods for antigen localizing by immunocytohistochemistry.

- *Direct method*: Sections of a tissue suspected of containing an antigen (protein X) are incubated with a labeled antibody to X, and the antibody will specifically combine with X. The excess antibodies are washed off, and the tissue is processed according to the methods outlined above. The location of the antigen is then detected in either the light or electron microscope.

- *Indirect method*: Antibodies to protein X (the antigen) are produced in an animal such as a rabbit. Rabbit immunoglobulins are, in turn, capable of inducing an antibody response in another animal, such as a sheep or goat. A tissue section containing protein X is incubated with unlabeled rabbit anti-X antibodies. After washing, labeled antirabbit antibodies are added, and the location of protein X can be visualized by a microscope technique appropriate for the label. This method has the advantage that the sensitivity of the technique is considerably increased over the direct method.

2.6 Review literature and related article

The role of skin substitutes [22]

In 2005 Shakespeare PG. has described what is required from the substitute will be different according to the nature of the wounds treated. Perhaps the general functions of individual substitutes can be identified as following.

Protection

Many substitutes can be used to provide an impermeable barrier at the wound surface. They have a benefit in restricting fluid loss and providing a barrier to wound colonization. Generally, an impermeable (occlusive) dressing may not be wholly desirable because the difficult management of exudates is then encounter. Impermeable membranes are often designed to have a measurable rate of vapor transmission although they will restrict access to the wound by microorganisms. If pooling of exudates occurs and accesses from the edge of the dressing, these dressings may cause problems in the management of subsequent wound colonization and infection.

Procrastination

Procrastination is perhaps not an obviously beneficial property. If autografts or other autologous materials are not readily available, the skin substitute will provide a stable situation in the wound bed until skin replacements become available. Skin substitute must be taken to protect the wound bed until the wound can be finally closed.

Promotion

A skin substitute should have the ability to provide a suitable environment to promote biological activities involved in the replacement of lost tissue and the salvage of damaged tissue. Substitutes may supply matrix components, cell growth, and growth factors to promote the rate of wound healing.

Provision

Provision of new elements should be incorporated into the healed wound. A skin substitute should supply an organized structure that forms the basis of a stable wound. Cells may be seeded to accelerate the process of tissue replacement or repair. Materials supplied may be designed to persist in the healed wound or to provide a substrate for neodermis remodeling.