CHAPTER II

REVIEWS OF RELATED LITERATURE AND RESEARCH

1. The Skin

The skin is the body's largest tissue. It accounts for 12-16 % of body weight. The skin plays an important role in the body, due to its functions such as responsible for sense of touch and also as a barrier to the environment. The skin provides a number of vital functions including protection, interaction with the environment, production of hormones (vitamin D), movement of electrical forces, and homeostasis. It protects the body from external aggression, either of bacterial or mechanical origin. It keeps the body at a constant temperature and envelopes a volume conductor and resonant cavity. The skin of an adult weights an average 4 kg and covers an area of 2 m^2. Water accounts of 70% of the chemical composition. Skin deforms and reacts neurologically in response to the application of forces and when removed, recoils. This helps the body interact with its environment. When these abilities are altered by trauma, disease or ageing, the skin is altered or compromised. Therefore, a thorough understanding of both normal and abnormal skin presentations provides necessary support for clinical diagnosis and treatment. The skin is composed of three compartments: epidermis, dermis and hypodermis (Figure 1) [11, 12].

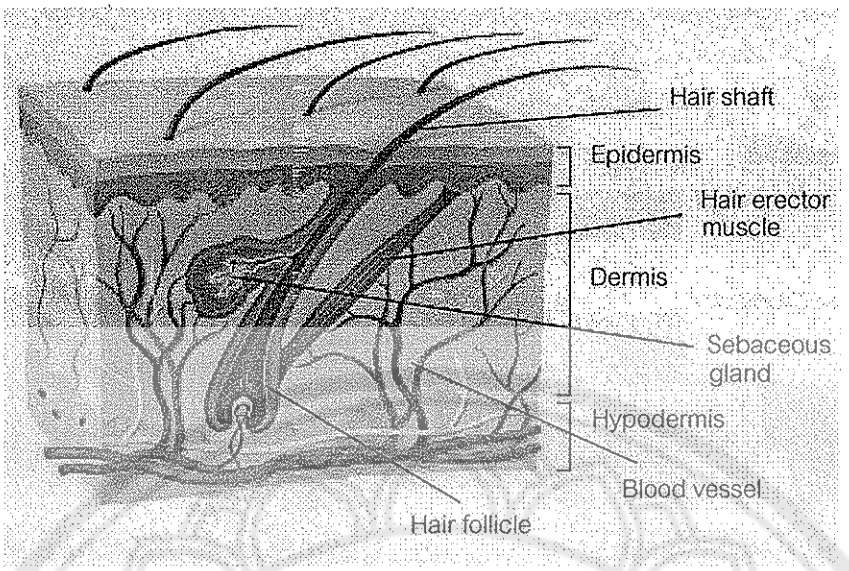


Figure 1 Cross section of skin

(obtained from http://www.eucerin.co.uk/skin/skincell_1.html).

1.1 The Epidermis

The epidermis forms the external structure of the skin, which is formed from the surface ectoderm. The thickness of this layer is in the range of 0.05-1.5 mm (its thickness average 0.1 mm) and especially thick on the palms of the hands and the soles of the feet. There are no blood vessels in the epidermis but its deepest layer is supplied with lymph fluid [13, 14]. The outermost layer of the epidermis forms a barrier against attacks from the environment, infectious agents and substance with which the skin may come into contact. It is the ultimate result of a 4-6 weeks migration by keratinocytes during which these cells. The most numerous keratinocytes in the epidermis divide and undergo a process of differentiation accompanied by structural and biochemical modifications migrate to the surface. This process is called keratinization. The epidermis is differentiated into five layers (Figure2) [12].

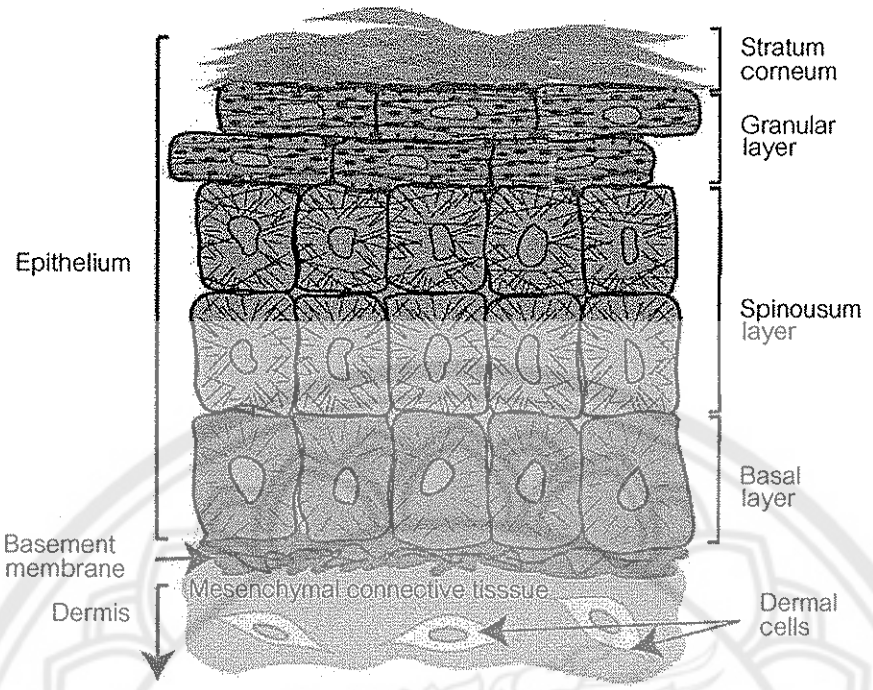


Figure 2 Keratinocyte migrations from the basal layer to the horny layer [15].

1.1.1 Stratum basal (or Basal layer)

This layer is the lowest layer of the epidermis, which is formed a single row of keratinocytes linked by desmosomes, and to subjacent dermis by hemi-desmosomes (Figure 3). This layer is very important as no other layer can form these new cells which gradually move towards the outer layers of the skin as the stratum corneum is abraded or shed. The new cells gradually change in form as they move upward to the outer layers, becoming keratinized in the process [13]. In this layer, the keratinocytes divide to give two daughter cells. One of them migrates towards the upper layer while the other remains and enters a new process of cell division to give rise more daughter cells and so on. During migration from the basal layer the keratinocytes begin to differentiate. Their shapes is changed from flattening become corneocytes. These flat non nucleate, keratin filled cells are linked by corneodesmosomes which ensure compact stacking of cells. The cohesion and flexibility of the structure is strengthened by a wide variety of lipids (ceramides, cholesterol, fatty acids) in the extracellular environment arranged between the cells [11-13].

1.1.2 Stratum spinosum (or Prickle-cell layer)

This layer is above the basal layer. In this layer is the first time for visibility of the keratinosomes, membrane bounded vacuoles. They contain the precursors of the epidermal lipids in the form of disk-like (lamellar) lipid bilayer membrane [14].

1.1.3 Stratum granulosum (or Granular layer)

The cornification or keratinization of keratinocytes is begun in this layer. The granules are also appeared. It contains the precursor of keratin, flaggrins, which is the intercellular cement of the skin structure [12, 14].

1.1.4 Stratum lucidum

The cells have been extremely flattened and closely packed. This layer is also translucent or transitional layer. The nuclei and other organelles are not visible. The cytoplasm is mostly made of keratin filaments [14].

1.1.5 Stratum corneum (or horny layer)

The outermost of epidermis is called the stratum corneum (or horny layer). This surface layer is composed of twenty-five to thirty sub-layers of flattened scale-like cells, which are continually being cast off by friction and replaced by the cells of the deeper epidermal layers. This surface layer is the real protective layer of the skin. These cells are commonly called keratinized cells because the living matter inside the cell (termed protoplasm) is changed to a protein (keratin) that helps to give the skin its protective properties [13, 14]. At the outermost of the skin, the corneocytes lose adhesion between cells though the action of specific enzyme which detaches them one after the other in an imperceptible way in normal skin.

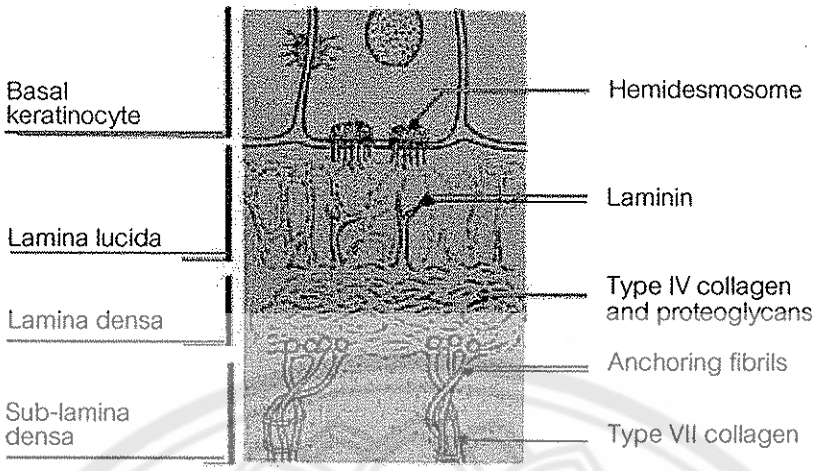


Figure 3 Skin layer (obtained from http://www.eucerin.co.uk/skin/skincell_1.html).

1.2 The cells in epidermis layer

1.2.1 Keratinocyte

Keratinocyte is derived from ectoderm cell that constitutes at least 80 percent of the epidermal cells. Keratinocyte is stratified which provides a barrier between the skin and environment. It prevents the entry of toxic substances from the environment and loss of important constitutes from the host. Keratinocyte differentiation is the epidermis progression from basal layer to the skin surface. The normal renewal time for keratinocyte is about 28 days [11]. Proteins synthesized in the granular layer are important in the final stages of epidermal differentiation and include profilagrin, lorcrin, involucin, and cornifin. These molecules are important in the formation of the stratum corneum, the outermost layer of the epidermis [11, 12].

1.2.1.1 Regulation of epidermal proliferation

Keratin

All keratinocytes contain cytoplasmic keratin intermediate filaments in their cytoplasm and form desmosomal junctions within the epidermis. Keratin filaments are a marker of the keratinocyte and acts as a cytoskeleton of the keratinocyte. It can be demonstrated directly and can be observed indirectly [12, 16]. Keratins are α-helical

molecules and divided into families, type I (acidic keratins) and type II (basic keratins). Keratin is assembly from acidic pair with basic keratin to form heteropolymers which are subsequently assembled into filament (Table 1) [12, 13].

Table 1 Keratin location.

Type I (acidic)	Type II (basic)	Location
K10	K1	suprabasal epidermal keratinocytes
K9	K1	palmoplantar suprabasal keratinocytes
K10	K2e	granular layer of the epidermis
K12	K3	cornea
K13	K4	nonkeratinizing stratified squamous epithelia
K14	K5	basal layer keratinocytes
K15	K5	basal layer of non-keratinizing epithelia
K16	K6a	outer root sheath (hair), hyperproliferative keratinocytes, oral epithelium
K17	K6b	nail bed, myoepithelium, inflammatory conditions
	K7	various partners in transformed cells
K18	K8	simple epithelia
K19		bulge cells (hair follicle), simple epithelia
K21		intestinal epithelium

Integrin

Growth factors, cytokines, and neuropeptides act in concert with cell-cell and extracellular matrix influences to control epidermal homeostasis. An association of matrix molecules with basal keratinocytes through integrin receptors, a family of transmembrane glycoprotein matrix receptors, is important. More than 20 integrins receptors are assembled as heterodimers from one α and one β subunit. The combination of subunits generally specifies the binding ligand [12].

Growth factor

Growth factors regulate epidermal growth and proliferation by autocrine and paracrine mechanisms. There are several growth factors in the epidermis such as epidermal growth factor (EGF), transforming growth factor (TGF-α) and keratinocyte growth factor (KGF). KGF, a member of the fibroblast growth factor family, is a potent mitogen for keratinocytes. Although the transcripts for KGF in skin are found only in the dermis, the receptor for KGF (KGFR) is expressed on keratinocytes [12]. Keratinocyte growth factor (KGF) stimulates proliferation of the keratinocyte therefore KGFR plays an important role in binding with KGF.

1.2.2 Other cells

Other cells are in the basal layer of the epidermis: melanocytes (dendritic cells). Melanocytes synthesize melanin pigments in form of melanosomes. Langerhans are other cells in epidermis, which are divided from heamopoietic stem cells in bone marrow and occur in the differentiation layer. Langerhans cells are also dendritic cells and form essential elements of the organism's defense mechanism.

1.3 The Dermis

The dermis is just under the epidermis layer and thicker than it. The dermis is the skin supporting tissue since it produces an extracellular matrix, which provides the epidermis with great strength and elasticity. The extracellular matrix is made from collagen and elastin fibers produced by fibroblasts, the main cells of dermis. Collagen secreted from fibroblast is type I and III collagen, however, type I is the main collagen of extracellular matrix. A number of capillaries run through the matrix and deliver growth factors and nutrients to the keratinocytes of the epidermis [17].

In young skin, the dermal-epidermal junction has the appearance of mountain range, and it called the dermal papillae. On the epidermis side, this structure allows the anchoring of the epidermal keratinocytes to the papillary dermis. The anchoring fibers (Figure 3) interact with the basal membrane forming a network which

traps collagen fibers. The basement membrane at the dermal-epidermal junction is known to be composed of laminins, integrins, type I collage, type VII collagen [14].

When the skin is damaged, fibroblast will proliferates and synthesizes a new collagen containing matrix called granulation tissue. Migration of fibroblasts at the edges of the wound initiates contraction and resulting in scar formation. Scar tissue, which is less flexible than physiologically normal dermis, can lead to restricted motion at a joint [1].

1.4 The Hypodermis (or Subcutis)

Hypodermis refers to the fat tissue below the skin. It composes of spongy connective tissue interspersed with energy storing adipocytes.

2. The Extracellular matrix

Most cells from extracellular structures, collectively called the extracellular matrix that carry out a variety of functions. In animals the extracellular matrix ranges from the tough, elastic structures of tendons, cartilage, and bone to the clear, crystal like cornea of the eye. The pattern and type of extracellular materials laid down in animals also regulate cell division, adhesion, cell motility and migration, and differentiation during embryonic development. It also plays an important role in the reactions to wounding and disease. In spite of their diverse types and functions, the extracellular structures (Figure 4) of eukaryotes compose of two elements, fibers and a surrounding network, fiber are long, semicrystalline elements that provide resistance to stretching and other tensile force. The network, a more elastic, interlocked place and resists compression by trapping and retarding the flow of water molecules. The degree of hardness and elasticity of extracellular structures depend on variations in kinds of fiber and network molecules, the degree of crosslinking, the amount of trapped water, and the types of added substances. The extracellular matrix of animal cells contains glycoprotein molecules called collagen as the primary fiber [12, 17].

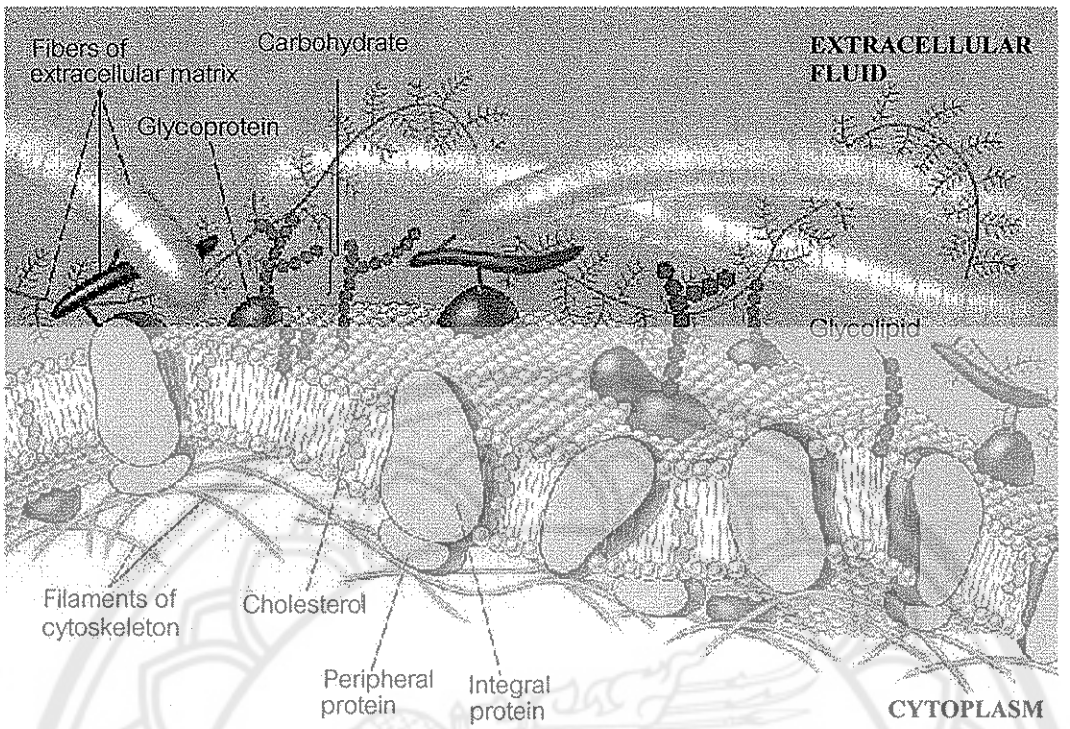


Figure 4 Structure of extracellular matrix

(obtained from http://telstar.ote.cmu.edu/hughes/tutorial/cellmembranes).

3. The Collagens

Collagen is semicrystalline, fibrous molecules that hold cells in place, provide tensile strength and elasticity to the extracellular matrix, and serve other functions related to cell motility and development. Collagen is the major component of the extracellular matrix in mammalian skin, cartilage, bone, and other connective tissue. It acts as a scaffold for the bodies, and controls cell shape differentiation, migration, and the synthesis of a number of protein. Collagen makes up 70% of the skin [12, 17].

3.1 Collagen structure

The collagen is insoluble glycoprotein characterized by a high content of glycine and two modified amino acids, hydroxylysine and hydroxyproline. About 10% of the total weight of collagen is carbohydrate. Collagen contains moderated amounts of

positively and negatively charged groups which are derived from the diamino and dicarboxylic acids built into the molecule. Individual collagen molecules are composed of three alpha-helical polypeptide chains known as α-chains. Three of the α-chains twist together into a right-handed triple helix that form about 95% of the rigid, rod like central portion of the molecule. Depending on the collagen type, the triple helix may be almost continuous and completely uninterrupted, or it may contain segments in which the triple helix gives way to less ordered conformations. The less structure segments acts as hinges that give the central region greater flexibility. The entire structure is held together by hydrogen bonds between the α-chains in both the triple helix. The hydroxyl groups added to the modified forms of proline and lysine are particularly important as hydrogen bonding sites that stabilize the triple helix. In human genome there are as many as 36 different genes encoding α-chains with variable amino acid sequences; these α-chains correspond to at least 21 different types of collagen with shown in Table 2 [12]. The molecules associated through both noncovalent bonding to produce collagen fibers of various structures and dimensions [12, 17].

Type I, II, and III collagen molecules make up the main fibers of animal extracellular structures. In the remaining collagens the central triple helix has more interruptions and is more flexible. All these more flexible collagens, with the exception of type IV, are minor constitutes of supportive structures.

Type I collagen, the primary component of bone, skin, and tendons, form about 90% of the body's collagen. In skin and tendon collagen fibers contain type I in combination with lesser amounts of type III and V molecules. Type I collagen is the most common of collagen in human and the major component of the skin. In the skin, collagen occurs in clear, crystalline layer overlapping the next at regular 90° angle. In all locations, collagen fibers combine with greater or lesser quantities of other molecules forming the surrounding network of the extracellular matrix. Collagen fibers containing type I, II, III, V, and XI molecules show a regular pattern of cross striations in the electron microscope. The pattern is believed to reflect a packing arrangement in which the triple helices of individual collagen molecules line up in overlapping, parallel rows [17].

Table 2 Genetic heterogenetic of collagen [12].

Collagen type	Chain composition	Molecular characteristic/ supramolecular assembly	Tissue distribution/ cell source
I	$[\alpha1(I)]_2\ \alpha2(I)$	Fibrillar	Skin, bone, tendon
I - trimer	$[\alpha1(I)]_3$	Fibrillar	Tumor, skin
II	$[\alpha1(II)]_3$	Fibrillar	Cartilage
III	$[\alpha1(III)]_3$	Fibrillar	Fetal skin, blood vessels, gastrointestinal tract
IV	$[\alpha1(IV)]_2\alpha2(IV)$	Basement membrane	Ubiquitous
V	$[\alpha1(V)]_2\alpha2(V)$; $[\alpha1(V)]_3$	Fibrillar	Ubiquitous
VI	$\alpha1(VI)\ \alpha2(VI)\ \alpha3(VI)$	Microfibril	Ubiquitous
VII	$[\alpha1(VII)]_3$	Anchoring fibril	Papillary dermis of the skin and other epithelial tissue
VIII	$[\alpha1(VIII)]_3$	Network forming	Endothelial cells
IX	$\alpha1(IX)\ \alpha2(IX)\ \alpha3(IX)$	FACIT	Cartilage
X	$[\alpha1(X)]_3$	Network forming	Hypertrophic cartilage
XI	$\alpha1(XI)\ \alpha2(XI)\ \alpha3(XI)$	Fibrillar	Cartilage
XII	$[\alpha1(XII)]_3$	FACIT	Tendons, ligaments, perichondrium, periosteum, cornea
XIII	$[\alpha1(XIII)]_3$	Transmembrane	Ubiquitous including epidermis
XIV	$[\alpha1(XIV)]_3$	FACIT	Skin, tendons, cornea
XV	Unknown	Basement membrane	Ubiquitous
XVI	$[\alpha1(XVI)]_3$	FACIT	Skin, internal organs, cartilage
XVII	$[\alpha1(XVII)]_3$	Transmembrane	Hemidesmosomes of basal keratinocytes in dermal-epidermis junction of the skin
XVIII	Unknown	Basement membrane	Ubiquitous
XIX	Unknown	FACIT	Basement membrane
XX	Unknown	FACIT	Corneal epithelia, embryonic skin, sternal cartilage, tendon
XXI	Unknown	FACIT	Smooth muscle cell in blood vessel walls, developing heart

Type IV collagen molecules assembles into very fine, unstriated fibers in which either end can link to points near the tips of other type IV fibers, this linkage pattern enables type IV collagens to set up a fine, sheet like layer, the basal laminin, which underlies the skin and epithelia covering internal organs and linking body activities.

Type I collagen molecule is composted of three polypeptide chains. The α-chain is a unique triple-helical structure. The polypeptide chains are a heterotrimer of two α1 and one α2 chains (Figure 5) [12, 17, 18]. Each peptide chain is repeated amino acid sequence, glycine-ploline-hydroxyproline. The fibrillar collagen synthesis is shown in Figure 6. Type I collagen is used in gelatin industry, several biomaterials, and medical research due to its involvement in human body. It is also biocompatible and biodegradable polymer. Therefore, collagen has a potential as an extracellular matrix.

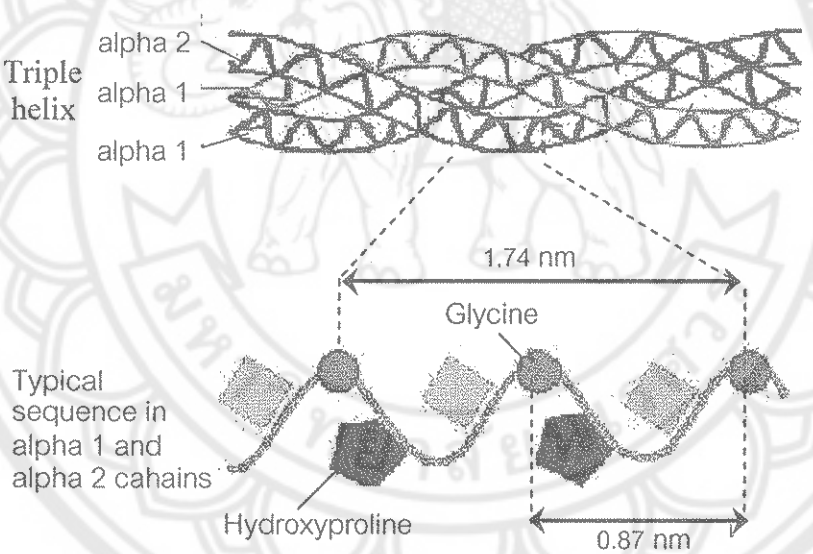


Figure 5 Molecular character of a collagen (obtained from www.ccmbel.org/Chap5.html).

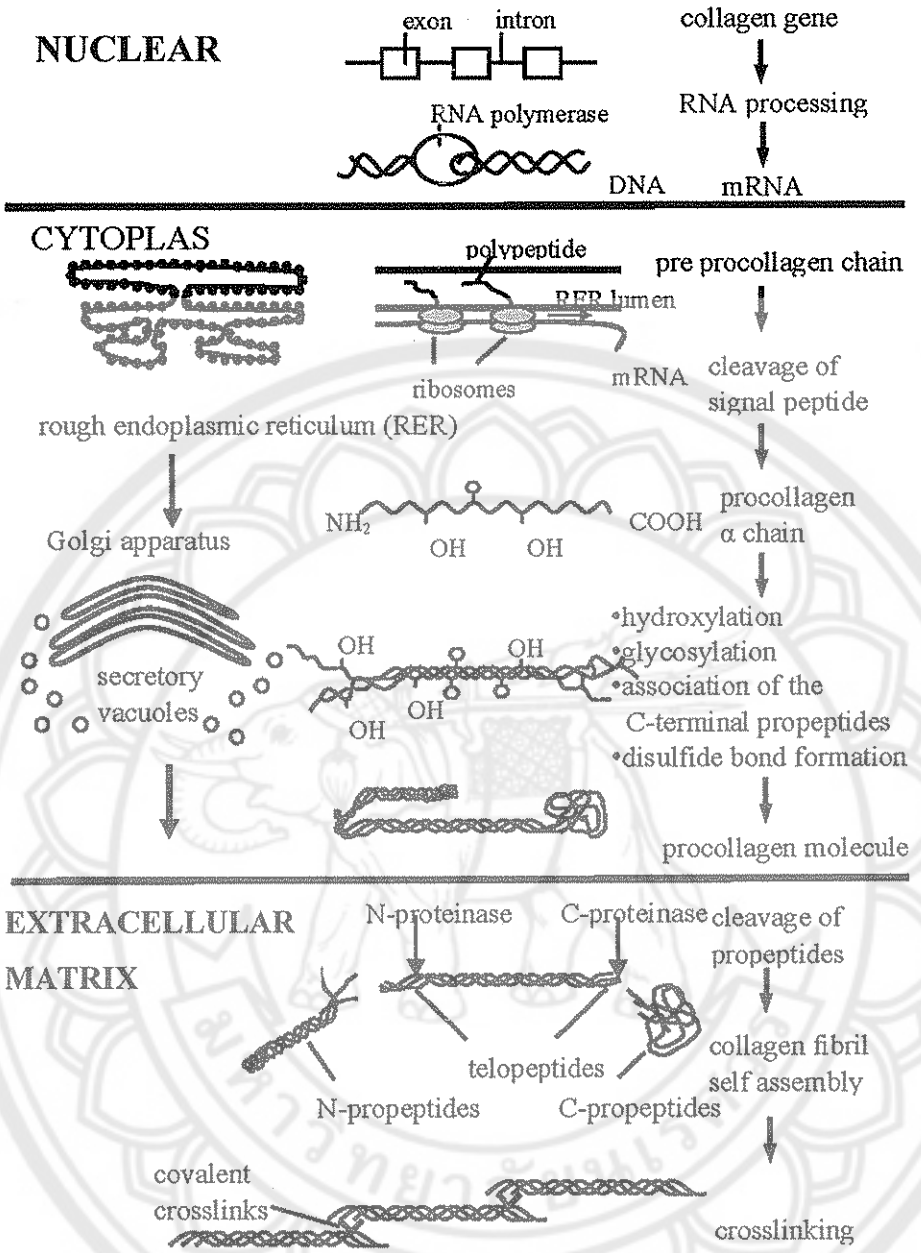


Figure 6 Synthesis of collagen fibrils

(obtained from http://herkules.oulu.fi/isbn9514272374/html/x183.html).

4. Tissue engineering

Tissue engineering is a life science which is development of biological substitutes for restoring, maintaining or improving a tissue function. As far as the skin

concerned, it consists of reconstructed skin models which include the functions of the epidermis and/or those of the dermis [2, 19].

If the region of damaged skin is not too extensive, skin grafts are used (xenografts, allografts or autografts). However, because of the antigenicity and limitation of donor site of the skin grafts cannot accomplish the purpose of the skin recovery. Loss of tissue or organ as well as the skin is one of public health problems. A number of studies concentrate on treatment of wound healing. Tissue engineering may approach overcomes the main limitations of the conventional therapies, which include a limited supply of donor tissues and limited function of synthetic prosthesis or mechanical devices. Tissue engineering has emerged as a route to treat the loss or damage of tissue or organ without the limitation of current therapies. This therapy is based on the principle that dissociated cells will reassemble *in vitro* into structures similar to the original tissue. Small tissue biopsy is isolated from patients and expanded *in vitro*. The cells are subsequently cultured on exogenous extracellular matrices, produced from biocompatible and biodegradable polymers. When the new tissue emerges, this tissue can be transplanted into patient. Completely natural new tissues will result follow by polymer degradation (Figure 7) [2].

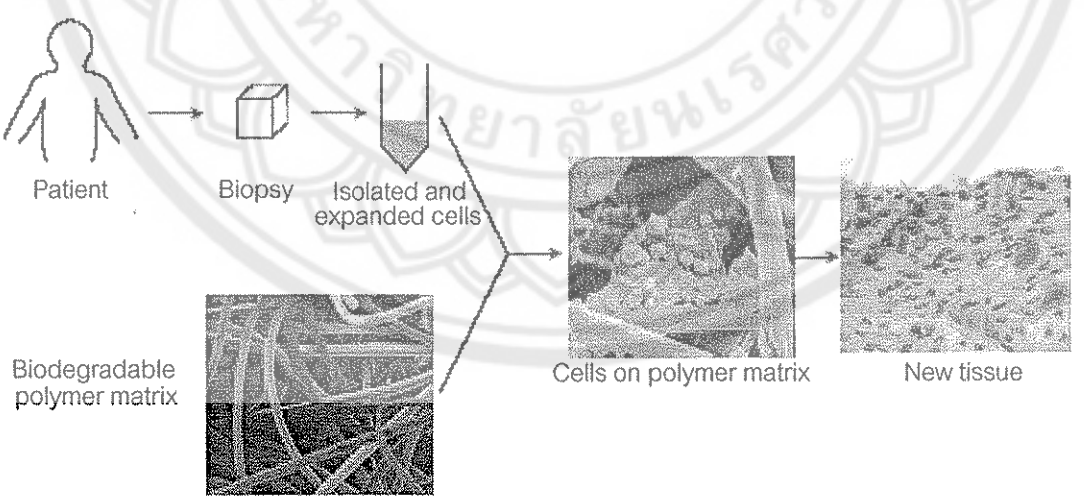


Figure 7 Overview of tissue engineering [2].

5. The Extracellular matrix for skin tissue engineering

The extracellular matrices act as the construction for supporting cells. The cells adhere and proliferate and differentiate over time into the targeted tissue or organ. Therefore, the extracellular matrices are the necessary factor for tissue engineering. The extracellular matrices for tissue engineering is designed to mimic the natural extracellular matrices both function and structure [2].

5.1 The Roles of extracellular matrix

All synthetic extracellular matrices used to engineer tissues have three primary roles. First, the matrices facilitate localization and delivery of cells to specific sites in the body. Second, they define and maintain a three-dimensional space for the formation of new tissues with appropriate structure. Finally, they guide the development of new tissues with appropriate function [2, 19].

5.2 The Extracellular matrix properties

The synthetic matrix for tissue engineering should provide temporary mechanical a support sufficient to withstand *in vivo* forces and maintained a potential space for tissue development components [2, 4, 20, 21]. In addition, the matrix should be biocompatible, biodegradable, and promotes cell adhesion and growth. It should be also degraded into non-toxic. The matrix should exhibit an appropriate microstructure and mechanical properties, such as high porosity (>90%) with usually diameter is 15-100 μm [22]. A degradation time of 25 days is suitable for healing acute wounds (burn and skin excision) [23, 24] or about 8 weeks for chronic wounds (diabetic ulcer, pressure ulcer) [25]. The cell composing the engineered tissue must express appropriate genes to maintain the tissue specific function of the engineered tissue. The function of seeded cells is strongly depend on the specific cell surface receptors used by cells to interact with the material, on interactions with surrounding cells and on the presence of soluble growth factor. These factors can be controlled by incorporating to integrating a variety of

signals, such as cell adhesion peptides and growth factor, into the synthetic matrix or subjecting it to mechanical stimuli [2].

5.3 The Matrix fabrication

There are a variety of ways to fabricate the extracellular matrix, such as, freeze drying, fiber bonding, foaming and salt leaching etc. (Figure 8). Design criteria for synthetic matrix may vary considerably depending on the specific strategy utilized to create a new tissue.

5.3.1 Freeze drying

This method is used to produce collagen based matrices for skin regeneration. The polymer solution is prepared by dissolving collagen from bovine tendon in acetic acid and blending with glucosaminoglycan from cow cartilage. And then, the mixture is freeze dried. Since ice has a low solubility for the co-precipitate, freezing the mixture forces the co-precipitate into the spaces between the growing ice crystals to form a continuous interpenetrating network of ice and co-precipitate. A reduction in chamber pressure causes the ice to sublimate, leaving a highly porous solid (Figure 8a). The size can be controlled by the rate of cooling. Typical pore sizes are in the range of 100-200 μm while typical porosities are in the range of 90-99% [1].

5.3.2 Casting

This technique is performed by casting the polymer solution into the Preti dish. The matrix is formed after the solvent is dehydrated. Casting technique is simply prepared in laboratory. The matrix prepared from this technique tends to stable and such matrix has been used for wound healing as well as skin healing. Moreover, it is independent of porosity and pore size, and it can control in the thickness. However, it is limited in interconnectivity of the porosity [20].

5.3.3 Fiber bonding

This technique has been used to make matrix for attachment of liver cell (hepatocyte). For an example, a non bonded structure of PGA fibers is immersed in a solution of PLLA. The solvent is evaporated, leaving an interpenetrating network of PGA and PLLA which is then heated to above the melting temperature of PGA to bond the fibers at their junctions. The PLLA is then dissolved in methylene chloride to give a porous matrix of PGA (Figure 8b) [1].

5.3.4 Foaming

Foaming can also be used to create a porous structure. Carbon dioxide gas is dissolved in a polymer under high pressure (800 psi and 25°C) and then expanded to form bubbles by releasing the pressure. The matrix with a porosity of 93% and the pore sizes of roughly 100 μm have been made this way (Figure 8c) [1].

5.3.5 Salt - leaching

This technique gives a microstructure similar to the foam. The mixture of polymer is evaporated. The remaining solid is then heated to above the melting point of the polymer to distribute it more uniformly. After cooling, the material is immersed in water to leach out the salt, leaving a porous structure (Figure 8d). Porosities in the range of 20-93% and pore sizes in the range of 30-120 μm have been achieved with this technique [1].

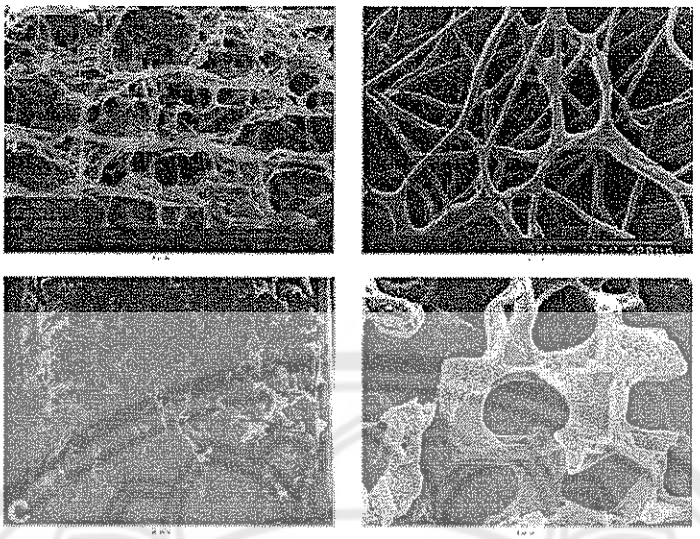


Figure 8 Micrographs of porous synthetic materials made by: (a) freeze drying (collagen-GAG; (b) fiber bonding (PGA); (c) foaming (PLLA); (d) salt-leaching (tyrosine-derived polycarbonate) [1].

The biomatrix may be derived from xenogeneic extracellular matrix that is natural polymers [24], such as collagen, gelatin and glycosaminoglycan etc. Recently, the natural polymers are widely used for tissue and organ regeneration. They guide cell proliferation and differentiation into target tissues or organs [22, 25].

6. The Polymer used in this experiment

The composition of the extracellular matrix is a complex mixture of structural and functional proteins, glucosaminoglycams, glycoproteins and small molecules arranged in a unique. It is a complex tissue specific three-dimensional architecture. The matrix for tissue engineering must have both suitable structural and functional properties.

6.1 Collagen

Collagen is abundant protein within the extracellular matrix. More than 20 different types of collagen have been identified [24]. The primary structural collagen in human tissues is type I collagen. This protein has been well characterized and is ubiquitous across the animal and plant kingdom. Collagen has maintained a highly

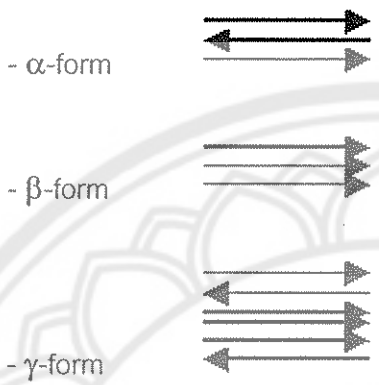
conserved amino acid sequence through the course of evaluation. For this reason, allogeneic and xenogeneic sources of type I collagen have been long recognized as a useful matrix for tissue repair with low antigenic potential [26, 27]. Type I collagen is abundant in tendon and ligament to provide the uniaxial and multiaxial mechanical loading to which these are commonly subject. For the same these tissues provide a convenient source of collagen for many medical applications. Bovine type I collagen is isolated from tendon and is perhaps the most widely used biologic matrix for therapeutic applications due to its abundant source and its history of successful use [24]. Moreover, bovine type I collagen is already an approved for implantation and used in the bioartificial skin product Apigraf$^{(R)}$ (Organogenesis Inc., USA), which has been approved by US Food and Drug Administration (FDA) [28].

6.2 Chitosan

Chitosan, cationic biopolysaccharide, is the *N*-deacetylation derivative of chitin (Figure 9), although this *N*-deacetylation is almost never complete. Chitosan is a copolymer of β- (1⟶4) link D-glucosamine units and *N*-acetyl-D-glucosamine (Figure 10). Chitosan is a natural biomaterial found in fungus cell walls, insect exoskeletons and crustaceans shell such as shrimp, squid and crab. The main parameters influencing the characteristics of chitosan are its molecular weight and degree of deacetylation, representing the proportion of deacetylation units. Chitosan has both free amino group and hydroxyl groups on its backbone, which easily modified by many organic reactions, alkylation, carbocylation etc. [29-31]

The structure of chitosan is related to the structure of chitin due to chitosan derived from chitin. The different chitin sources are different in arrangement of polymer chains within chitin structure; therefore, the structure and physicochemical properties are also different. X-ray diffraction analysis suggested that chitin is a polymorphic substance that occurs in three different crystalline modifications, termed α-, β- and γ- chitin. They mainly differ in the degree of hydration, in the size of the unit cell and in the number of chitin chains per unit cell. In the α form, all chains exhibit an anti-parallel orientation; in

the β form the chains are arranged in a parallel manner; in the γ form sets of two parallel strands alternate with single antiparallel strands [32]. α-Form is found in shrimp and crab chitin. β-Form is found in squid chitin and γ-form includes chitin from mushroom and fugal mycelia.



The anti-parallel arrangement of chitin molecules in the α- form allows tight packaging into chitin microfibrils, consisting of ~20 single chitin chains that are stabilized by a high number of hydrogen bonds formed within and between the molecules. This arrangement may contribute significantly to the physicochemical properties of the chitin such as mechanical strength and stability. By contrast, in the β- and γ- chains, packing tightness and numbers of inter-chain hydrogen bonds are reduced, resulting in an increased number of hydrogen bonds with water. The high degree of hydration and reduced packaging tightness result in more flexible and soft chitinous structures [32, 33].

As a copolymer, chitosan is readily converted to fibers, films, coatings, and beads as well as powder and solutions, further enhancing its usefulness. Chitosan is great deal of interest for medical and pharmaceutical applications. The Interesting intrinsic properties of chitosan are the main reasons for using this polymer. Chitosan is known for being biocompatible allowing its use in various medical applications. Furthermore, it is metabolized by certain human enzymes, especially lysozyme, and is considered as biodegradable. Because of its positive charges at physiological pH, chitosan is also bioadhesive, which increase retention at the region of application. Chitosan also promotes wound healing [5, 6, 34]. Chitosan is cheap and large-scale availability as well as non toxic. It also supports biological activity of several cell types

[35-39]. Chitosan is able to induce adhesion, proliferation and differentiation of several cell types including keratinocyte and fibroblast [40]. Therefore, chitosan has been widely use as biomaterials for tissue engineering, drug delivery carriers, bone healing materials and wound dressing [41].

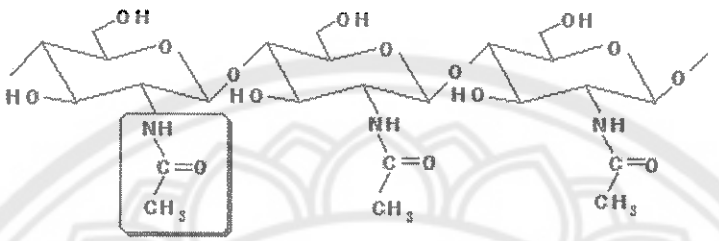


Figure 9 Structure of chitin.

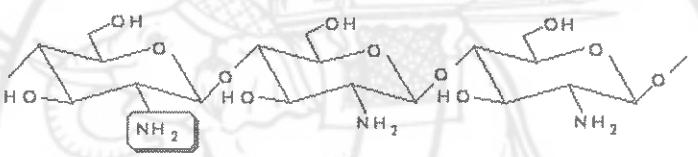


Figure 10 Structure of chitosan.

6.3 Polyvinyl alcohol (PVA)

PVA is a hydrophilic synthetic polymer with good film forming property: good tensile strength and flexibility. It is used as emulsifier, colloid stabilizer, sizing agent, coating in the textile industry and adhesive. PVA is a water soluble synthetic polymer and its solubility in water depend on both degree of hydrolysis and degree of polymerization with a maximum at 88% hydrolysis, and higher solubility for lower degree of polymerization. The changes in molecular structure of the basic polymer are strongly influenced by change temperature, pH, or solvent composition [42]. The structure of PVA is shown in Figure 11. The abundant hydroxyl groups on PVA can be simply modified to attach growth factors, adhesion proteins, or other molecules of biological importance. The available PVA shows good physicochemical and mechanical properties. In addition, PVA is bio-inert, therefore, relatively biodegradable [8-10]. The stability and degradation rate of PVA are dependent on the molecular weight [43]. PVA has been blended with

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natural polymers because of its hydrophilic characteristics that allows for good compatibility. PVA was blended with natural polymer such as collagen [44] and chitosan [45] is used for controlling drug delivery. Blending collagen with PVA results in stable and high porous hydrogel.

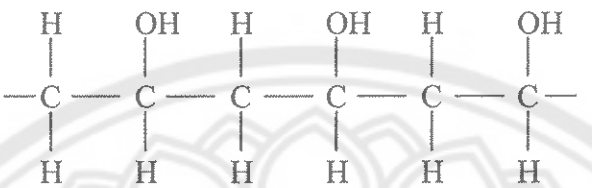

Figure 11 Structure of polyvinyl alcohol.

7. The Matrix for reconstruction of skin

There are several literatures on the use of synthetic matrix, especially chemically crosslink biologic matrix, for tissue engineering. The chemical crosslinked purified bovine type I collagen (Contigen™) [4], crosslink human dermis (Alloderm™) [24] and Apigraf® [28] are examples of such products currently available for use in human. Collagen-GAG porous matrix made by freeze drying process is another example, which is successfully being used to regenerate skin in burn patients; this material received FDA approval for clinical use in 1996 [1].