Chapter 2
Skin, Genetic Defects, and Aging

2.1 The Skin

The skin is the largest organ of the human body and comprises a total surface area of approximately 2 m\(^2\). The skin has multiple functions such as protection against external environmental factors, sensation, heat regulation, control of water evaporation, synthesis of vitamin D, and many more. At the cellular level, it comprises three layers, from top to bottom: epidermis, dermis, and hypodermis [1]. The epidermis is mainly composed of three kinds of cells: keratinocytes, melanocytes, and Langerhans cells [2]. The epidermis is the uppermost layer of skin and further contains several sublayers (Fig. 2.1) [3]. The epidermis functions as a barrier of the skin, which protects humans from invasion of pathogenic potentially infectious foreign particles into the body. It can have different degrees of thickness at various sites of the body. The epidermis is connected to the dermis via a basal membrane. The keratinocytes residing on the basal layer undergo cell division. As it progresses, the keratinocytes move upward through different layers named spinous, granular, and cornified layer (stratum corneum, SC), and eventually are lost from the skin surface by desquamation [4]. The epidermis does not have any primary blood supply, hence it receives all nutrients from the dermis.

The barrier function of the epidermis is provided by the topmost layer of protein-rich, flattened dead cells called stratum corneum [5]. The corneocytes, which are flattened dead bodies of keratinocytes, act as brick of the brick-and-mortar model. The intercellular lipid lamellae fill up the spaces between the dead cells, like mortar. Primary ingredients of the mortar portion are lipids: cholesterol, cholesterol esters, ceramides and long chains of fatty acids [4, 5]. The corneocytes contain largely packed keratin filaments aligned in an arrangement parallel to the skin surface. During the terminal differentiation corneocytes acquire a specialized structure called cornified envelope (CE) around them, which provides both chemical and mechanical protection to the skin against the environment. The CE is made of two parts, the protein and lipid envelopes [6–9]. The protein envelope (PE) contains
insoluble specific structural proteins crosslinked by sulphydryl oxidases and trans-glutaminases. Some of the structural proteins making up this region are indicated in Fig. 2.2, and include loricrin, involucrin, cystatin, small proline rich proteins (SPR), etc. The periphery of the PE is surrounded by a 5 nm thick lipid envelope (LE). This envelope contains a layer of ω-hydroxyceramides with uniquely long chained fatty acyl moieties \([10]\). These are covalently attached by ester bonds through their ω-hydroxyl group to selected glutamines on some of the structural proteins \([11–13]\). The uppermost granular layer has lamellar granules which stack newly synthesized lipids within them. These bodies fuse with cell membranes and release multilamellar lipid sheets filling the spaces between the corneocytes \([14, 15]\).

Intracellularly, the SC contains a dense network of keratin filaments which help to keep the skin hydrated. Keratin 5 and keratin 14 are the main structural protein products of proliferating cells in the basal layer, which assemble together in the form of keratin intermediate filaments as an addition to the preexisting cytoskeleton of microtubules and microfilaments (actin). Keratin 5 and 14 are the main proliferation products of the basal keratinocyte cells. During cornification, keratin 1 and keratin 10 are the first proteins to be expressed and they replace keratin 5 and 14. Other keratins are expressed at specific locations: keratin 9 in palms and soles and keratin 2e in areas with thickened skin \([16]\). There is another class of structural proteins called filaggrins, which aggregate with the keratin intermediate filaments

![Fig. 2.1 Structure of the skin [3]](image)
and convert them into tight bundles [17]. Both keratin intermediate filaments and filaggrin constitute together 80–90% of the protein mass in mammalian epidermis. Originally, filaggrins are synthesized as giant precursors called profilaggrins which are highly phosphorylated and insoluble. These are the main constituents of the keratohyalin granules in the granular sublayer (Fig. 2.2) [17]. These profilaggrins become activated to filaggrins via dephosphorylation. The monomeric filaggrins bind to the keratin intermediate filaments thus resulting in macrofibril formation. The enzyme transglutaminase further catalyzes crosslinking between them, resulting into a highly insoluble keratin matrix. This matrix acts as a protein scaffold for the attachment of the CE proteins and lipids that, together, form the SC [18]. The intraepidermal interactions are mediated by integrin proteins. Integrins are heterodimeric transmembrane proteins, which interact mainly with the actin filaments facilitating cellular adhesion and migration.

The spinous layer is located above the basal layer and has these filaments that cross the intercellular spaces and make contacts between cells. These connections are known as the desmosomes. The desmosomes are broken by proteases during desquamation. As the cells mature, they produce molecules like keratohyalin and the lamellar granules. As mentioned above, the granular layer matures and subsequently leads to a stronger skin barrier [2].

The epidermal layer is connected to dermis via the basal membrane (BM) [18]. This is made of four different components: one or more laminin proteins, type IV collagen, nidogen, and the proteoglycan perlecan. This BM has a gate keeping functionality, which controls the traffic of cells and biomolecules between epidermis and dermis.

![Expanded sublayers of the epidermal layer](image)

**Fig. 2.2** Expanded sublayers of the epidermal layer [17]
The dermis contains two layers, papillary and reticular. The upper layer (papillary) contains loosely arranged collagen fibers. The reticular layer (deep) contains dense collagen overlying a subcutaneous fat layer (Fig. 2.1). Overall, it contains 97.5% collagen and 2.5% elastin as fibrous proteins [19]. In the dermis, collagen fibers are mainly of two kinds: type I and III. The collagens run parallelly to the skin, whereas elastins form a network in the dermis. The dermis contains fibroblast, macrophage, and adipocyte cells. It also contains blood vessels, lymph vessels, and arrector pili muscles. The dermal matrix comprises a high amount of elastins which impart elastic properties to the skin.

The layer underneath the dermis is the subcutis, also known as hypodermis. It mostly contains adipocyte cells. This layer conserves heat and maintains the temperature of the body. It contains veins, lymph vessels, autonomic and sensory nerve fibers, and sensory corpuscles. Hair muscles and cutaneous glands are also contained in this deep layer of the skin.

Hair follicles are specialized structures of the skin, made of multiple layers of tissues (Fig. 2.1). Hair follicles contain two compartments, epidermal and dermal (mesenchymal) [20]. Interactions between these two compartments are important for morphogenesis and growth of the hair follicles. The dermal compartment further divides into the dermal papilla (DP) and the dermal sheath (DS). The dermal papilla is located at the base of the hair follicle. In the hair follicle cycle, DS is a reservoir of DP cells. It is hypothesized that stem cells reside in the DS. The DS contains similar cells, which can regenerate DP after loss of DP [21, 22]. A normal hair follicle, after being fully developed, enters three hair cycles: anagen (growing phase), telogen (resting phase) and catagen (regressing phase). During illness, hair follicles may prematurely enter the telogen phase which can further induce the loss of hair in people [1, 23].

2.2 Genetic Defects in the Skin Barrier

*Lamellar ichthyosis* (LI) is a genetic disorder with abnormalities in the cornified layer of the skin. At birth, some babies are born with a transparent sheath covering their skin, called colloidal membrane [16, 24, 25]. This membrane peels off in the first few weeks and affected babies have scaly skin. About 50% of these patients carry mutations in the gene encoding the enzyme transglutaminase I, between the N-terminal and the catalytic domain, area important for their catalytic activity. The loss of the transglutaminase function results in incomplete crosslinking of the keratin intermediate filaments hence impaired barrier function of the skin. Similarly, the *Vohwinkel syndrome* (VS) and the *progressive symmetric erythrokeratodermia* (PSEK) are associated with defects in the structural protein loricrin [26, 27]. Loricrin is a key structural protein in the cornified envelope, contributing 70–80% of its total mass. It is a substrate for many transglutaminases and undergoes various types of crosslinking in the epidermis. The patients with VS have hyperkeratosis on the palms and soles and develop constricting bands on the fingers, which can lead to
autoamputation. Single nucleotide deletion on the loricrin gene leads to frame shift mutation. The mutant has extended terminal sequence of arginine instead of glycine residues. Additionally, many crosslinking sites of glutamine and lysine residues are lost. The patients with PSEK have a CE thinner than normal and have less loricrin in their skin.

There are more than 10 diseases which are associated with genetic defects in the keratin intermediate filaments [28–30]. For example *epidermolysis bullosa simplex* (EBS) is associated with a mutation in the gene encoding keratin 5 or keratin 14 that leads to a fragile keratin skeleton in the basal cells. The patients with EBS develop blisters with mild physical trauma like rubbing or scratching on the skin. *Bullous congenital ichthyosiform erythroderma* (BCIE) has hyperkeratosis characteristics and is associated with mutations in the gene encoding keratin 1 and keratin 10. Similarly, *non-epidermolytic palmar-plantar keratoderma* is associated with the mutation of a single lysine in the head domain of keratin 1 [4, 31]. This is associated with thickening of the *stratum corneum* of palms and soles. Lysine residues on these proteins help keratin intermediate filaments to crosslink to the protein envelope. The loss of this lysine residue interferes with the structure of corneocytes [32]. More keratin associated diseases are summarized in reference [28].

Effective barrier properties are important for any healthy skin. Deficiencies in barrier function result in ichthyosisiform diseases. Even minor depletion in the lipid barrier can lead to a dry skin. Lipid metabolism or essential fatty acid deficiency can lead to ichthyosisiform symptoms. Genetically, these are caused by impaired lipid synthesis. For example *X-linked ichthyosis* is characterized by thick dark scale and corneal opacities. Genetically that happens due to defects in an enzyme called steroid sulphatase [33]. Loss of functionality of that enzyme leads to accumulation of cholesterol sulfate in the extracellular spaces of the epidermal layer and inhibits desquamation. It also inhibits catalytic activities of transglutaminases and crosslinking of involucrin to the protein envelope as well as to the lipid envelope [34].

LI-5 (*nonbullous congenital ichthyosiform erythroderma*) is due to mutations in the gene of lipoxygenase-3 or 12(R)-lipoxygenase responsible for oxygenation of free polyunsaturated fatty acids and their corresponding esters to produce the respective hydroderivatives [16, 35]. The *Sjogren–Larsson syndrome* is due to mutations in the gene encoding microsomal fatty acid dehydrogenase, an enzyme which oxidizes long chain aliphatic aldehydes to the corresponding acids [36]. Gaucher Disease is due to mutations in the gene of an enzyme called β-glucocerebrosidase that catalyzes the hydrolysis of glucosylceramides to ceramides [37]. Ceramides are key components of intercellular lamellae and mediate the skin barrier function. Another defect, called the Chanarin–Dorfman syndrome, is due to a mutation in the CGI-58 protein that contains three sequence motifs corresponding to the catalytic triad present in lipases, esterases, or thioesterases [38, 39].

Proteases also play an important role in the cornification process. The *Netherton syndrome* is due to a mutation in the SPINK 5 gene which corresponds to the protease inhibitor LEKTI [40]. This disease is characterized by congenital erythroderma, a specific hair shaft abnormality. Mutations in the gene encoding cathepsin C lead
to a skin disease called the *Papillon–Lefevre syndrome* characterized by hyperkeratosis of palms and soles [41]. These diseases show abnormalities in the lipid structure of the cornified layer.

### 2.3 Skin Aging

Skin aging is characterized by wrinkling, sagging, dryness, and incremental laxity of the skin. There are two processes in skin aging: extrinsic and intrinsic. The extrinsic aging is due to premature induction by environmental factors such as exposure to ultraviolet radiation, pollution, cigarette smoking, and significant alcohol consumption [42]. These factors also add to intrinsic aging. Intrinsic aging occurs naturally depending upon inherent genetic makeup. That leads to loss of structural integrity and loss of physiological function as well.

Photoaging, due to exposure to solar UV radiation, is a primary factor in extrinsic aging. Solar UV radiation reaching the earth’s atmosphere can be divided into three categories: UVA (320–400 nm), UVB (290–320 nm) and UVC (200–290 nm) [43]. The UVC are the most dangerous radiations which can lead to various mutations and enhance the risk of skin cancer. However, the earth’s atmosphere is mostly protected by the ozone layer against this type of radiation. On the basis of similar irradiation doses, UVB radiation can have more impact on the skin compared to UVA. Solar UVA radiation can reach earth’s atmosphere more easily without being absorbed. The ratio of UVA/UVB is around 10/1 when the sun is at the overhead position. This ratio increases as the sun goes down. UVB is mostly absorbed in the epidermis by biomolecules and cannot reach the dermis. UVB can do direct or indirect damages to biomolecules and is responsible for sunburn of the skin and various other damages [44]. UVA can penetrate from epidermis to dermis. Due to the high intensity of UVA, damage to the extracellular matrix (ECM) in the skin can occur. Dark skin pigmentation can protect against damage via absorption of energy by chromophores. Neither UVA nor UVB can interact with molecular oxygen directly. However, epidermal proteins can absorb UVB radiation via excitation of aromatic amino acids like tryptophan, tyrosine or of disulfide bonded cysteine indirectly producing reactive oxygen species (ROS) responsible for various damages [45]. Also DNA/RNA bases can absorb UVB radiation, which can further lead to various mutations, arrest of the cell cycles, and apoptotic death of the cells.

Intrinsic aging is associated with decreased replication ability of the cells and increased degradation of the ECM [46]. Intrinsic aging leads to accumulation of toxic ROS from the oxidative metabolism which can mutate DNA, oxidize proteins (which further lose their functions), and oxidize lipids in membranes. Hence that can affect the transportation and signaling mechanisms of epidermal cells [47, 48].

Skin is composed of cellular and extracellular components. The extracellular component is mainly made of two types of macromolecules: polysaccharide glycosaminoglycans (GAG) and collagens. GAGs are polar, unbranched chain polysaccharide structures composed of glucosamine, galactosamine, hexuronic, iduronic
acid, and galactose units with sulfate groups at various positions. Their hydrophilic characteristics allow free diffusion of water. When these molecules complex with proteins they are transformed into proteoglycans. The UVA and UVB do not affect these molecules directly, but ROS may induce indirectly oxidative damage to these molecules during the process of photoaging. Non-sulfated hyaluronic acid (HA) is one of the members of the GAG family. It has been determined that chronic exposure of UVB radiation to mouse skin leads to loss of HA in the dermal tissues [49].

Collagens are abundant structural proteins present in the skin. They are synthesized and secreted by fibroblast cells. The dermal tissues mainly contain type I and type III collagens, which provide support and strength to the tissues. UVA and UVB induce indirectly production of ROS which further can induce production of matrix metalloproteinases (MMPs) which can degrade the ECM. MMPs are a family of zinc-containing endopeptidases. There are mainly four MMPs responsible for degradation: collagenase (MMP1), gelatinase A (MMP2), stromelysin (MMP3) and gelatinase B (MMP9). Collagenase is the only MMP which can degrade intact fibrillar collagen [50, 51]. The other three MMPs can further degrade already fragmented collagen.

Elastins are other abundant structural proteins providing elasticity to the skin. During aging elastin proteins are degraded by enzymes called elastases produced by macrophages and neutrophils, and which are MMPs [52].

The SC has a barrier function to the skin, against pathogens and foreign particles. With aging the size of the SC does not change significantly but the size of the viable epidermis, and epidermal-dermal junction becomes smaller. This affects the transfer of nutrients to the epidermal layer. Breakage of collagen and elastin proteins is associated with dermal aging. Overall, the skin becomes thinner with aging.

References

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