Dedication

To the love of my life, my beautiful wife Angela to whom I have been devoted for thirty six years

Michael R. Hamblin

To my parents and aunt, Seda, Debora, Esin, Berna, Begum and Ozan whose advise, encouragement and support have been genuine and precious

Pinar Avci

To my loving wife, Natalie, and to my son and my daughter, Jacob and Jasmine for their unwavering support and love

Tarl W. Prow

NANOSCIENCE IN DERMATOLOGY

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Preface

It has been said that dermatology is one of the slowest medical disciplines to embrace new technologies. In fact, some have even said that dermatology is a medical discipline that lags behind the technology curve. After developing this book, we have come to the conclusion that perhaps it is more accurate to say that clinical dermatology may lag behind the technology curve at present, but dermatology research is cutting edge and is indeed expected to be pushing new technologies into the clinic in the coming years. One example of the advent of this advanced technology is the use of innovative imaging techniques and automated image processing (as described in the companion book to this volume, *Imaging and Dermatology*). Clinical dermatology is incredibly visual in nature. The amount of imaging data being gathered by innovative modalities these days is unprecedented and growing. The driving force behind this growth has been the emergence of empowered patients with high-resolution mobile phones, not to mention the explosion in skin-specific "Apps". Patient empowerment is powerful, especially with the right mix of technology, accessibility, and the promise of improved healthcare. We are seeing similar growth in the field of nanotechnology with up-to-date researchers who have access to an impressive array of nanoparticle-based technologies that promise to change the dermatology drug delivery and imaging landscapes forever. One example of this is the nanoparticle-based imaging technology described in Chapter 6 that enables in vivo oxygen sensing. Imaging is one of the key technologies supporting nanoparticle research in dermatology. Chapters 9, 14, 16, 21, 22, and 24 all contain significant information about imaging nanoparticles on and in skin. We see that the combination of nanotechnology and advanced imaging is a powerful combination that supports a vast amount of research in this area.

A significant, but largely unappreciated, aspect of this wave of research is the establishment of toxicity testing through global networks. The single best example of this is the rollercoaster ride that our scientific and clinical community has witnessed over the last 5 to 10 years concerning zinc oxide nanoparticle research. Industry had generated organic sunscreens with ZnO as physical UV blockers and marketed them. They worked. Then the scientific community was gripped by uncertainty typified by conflicting reports concerning nanoparticle toxicity. We as a scientific and clinical research community had to go back to our roots and develop new ways to assess nanoparticle exposure and toxicity risk. Chapter 16 takes the reader through this journey where we now have much more sophisticated and relevant ways to test topical nanoparticles. As a community, we now know how to move more assuredly forward in the areas of nanotechnology in dermatology.

We have seen dermatology move rapidly to the cutting edge of medical technology with a huge number of people using nanotechnology on their skin in the form of sunscreens and cosmetics. The controversy and even confusion that this realization generated have had a ripple effect that has been felt in other high-impact areas, like nanotechnology in food science and environmental science. So, perhaps dermatology can be viewed as being a bit more "avant-garde" than it is usually credited.

As we journey through the table of contents for this book, it becomes clear that "nanodermatology" is now at the cutting edge of medical science. Why is that? Perhaps it is for the same reason that companies were able to confidently deploy tons of nanomaterials into the consumer market without too many negative consequences. The skin is an incredible barrier to the penetration of almost everything, and moreover the skin is easily accessible. Almost every chapter in this book describes a novel nanotechnology being tested in human skin, or at the very least in animal models. The amount of volunteer and patient testing in the field of nanotechnology in dermatology is unprecedented. Undoubtedly, this aspect of dermatology is on the vanguard with few competitors. The majority of this work is in the field of topical drug delivery.

Nanotechnology has had a significant impact on drug delivery in general, and there are constantly renewed promises of revolutionary improvements in drug bioavailability, controlled release, and sophisticated drug targeting. Chapter 4 describes the use of microneedle technology to improve topical drug delivery. Microneedles have been used to dramatically improve the delivery of large payloads like nanoparticles. This combination of micro- and nanotechnology in skin is another example of cutting-edge research in dermatology. Drug delivery is another major theme in this book. Several chapters, including Chapters 5–7, 9, 10–13, 15–19, and 23, all discuss roles for nanoparticles as drug carriers or nanoparticles as active agents for use as preventatives or therapeutics.

Readers may be somewhat surprised to see Chapter 25 by Attama and colleagues included in this book, as the chapter deals with ophthalmology, and the book is actually concerned with dermatology. Nevertheless, the editors felt that the delivery of nanomaterials into the eye and into the skin had much in common. Both organs are accessible for topical application of nanodrugs, and similar considerations of penetration and toxicology apply in both cases.

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CHAPTER

1

Anatomy and Function of the Skin

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OUTLINE

INTRODUCTION

The skin is the single largest human organ, with 2 m^2 of surface and 3.6 kg of weight in adults. It acts as a waterproof, insulating shield, protecting the body against environmental stresses. It also produces antimicrobial peptides that prevent infections, and hormones, neuropeptides, and cytokines that exert biological effects, not only locally on the skin but also systemically throughout the whole body.

The integumentary system develops from surface ectoderm and the underlying mesenchyme. It consists of the skin and the appendages, its derivative structures, which include hair follicles, nails, and sebaceous and sweat glands. The skin is composed of three layers: the epidermis, the dermis, and the hypodermis. It provides a life-sustaining interface between the body and the external environment, carrying out very important functions such as protection, preservation of water and electrolytes, regulation of temperature, and water and fat

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storage, and it plays a major role in the endocrine and immunological systems.

THE EPIDERMIS

The epidermis is the most external layer of the skin and can range in total thickness from 0.5 mm (eyelid) to 1.5 mm (palms and soles). It is formed by a stratified squamous epithelial layer and is composed basically of keratinocytes and melanocytes that form a binary system [1]. The epidermis harbors a number of other cell populations such as Langerhans cells (LCs) and Merkel cells, but keratinocytes are by far the most predominant cell type.

The epidermis is a perpetually regenerating tissue with cells continuously undergoing terminal differentiation and death. The total renewal time is approximately 2 months. The epidermis gives rise to other structures like nails, sweat glands, and pilosebaceous units. The epidermis penetrates down into the dermis through the rete ridges, while the dermis projects upward into the epidermis by the dermal papillae that occur between these rete ridges (Fig. 1.1). The epidermis is separated from the dermis by the basement membrane, which will be explained in this chapter.

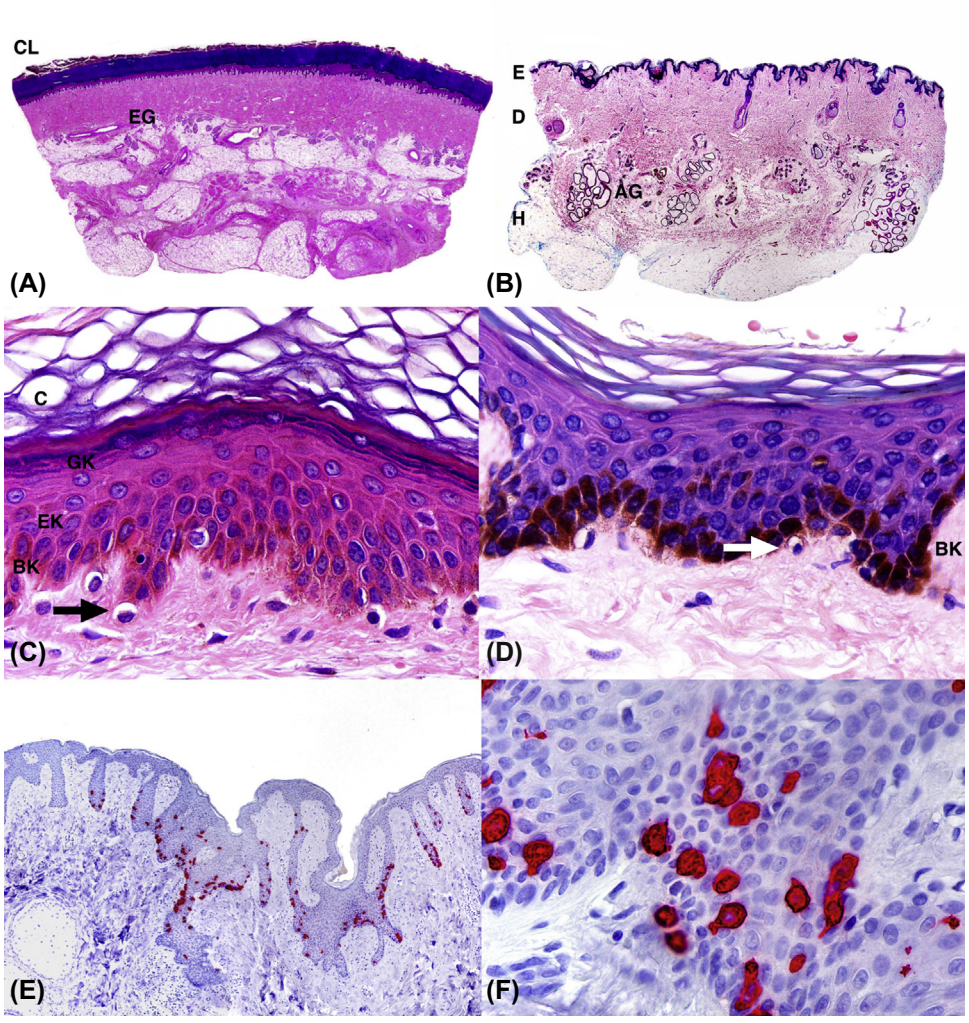

FIGURE 1.1 Normal epidermis. (A) Biopsy of the sole skin, where a thick stratum corneum and multiple eccrine glands are observed in the interphase between the dermis and the hypodermis. *CL*, Cornified layer; *EG*, Eccrine glands. (B) Scanning view of the skin of the axilla; unlike the sole in the axilla, the stratum corneum is thinner, there are multiple apocrine glands, and all the skin layers can be observed. *E*, Epidermis; *D*, Dermis; *H*, Hypodermis; *AG*, Apocrine glands. (C) Skin of a phototype II person; the arrow points the melanocytes located at the dermo-epidermal junction. *BK*, Basal keratinocytes; *EK*, Espinous cell layer keratinocytes; *GK*, Granular cell layer keratinocytes; *C*, Corneocytes. (D) Black skin with a higher degree of melanization, the presence of larger melanosomes, and a greater amount of them among basal cell keratinocytes (BKs). The arrow shows a melanocyte, with the clear halo around it. (E) Scanning view of the epidermis with scattered Merkel cells stained with inmunohistochemical stain CAM 5.2. (F) Higher magnification showing the oval-shaped Merkel cells with cytoplasmic processes extending into and between keratinocytes. *Courtesy of Prof. Luis Requena.*

Structure of the Epidermis

The epidermis is usually divided into four layers, taking into account the morphology and location of the keratinocytes (Fig. 1.1). Resting directly on the basement membrane is the basal layer, which is formed in part by the rapidly proliferating cells. Some cells leave this layer to continue differentiation by ascending up to the next stratum, the stratum spinosum or prickle cell layer, but other cells die by apoptosis either as a consequence of an intrinsic program or as a consequence of an imbalance of signaling molecules [2]. The next outer layer, the granular cell layer (stratum granulosum), is the last stratum that contains living cells. In the final steps of differentiation, the keratinocytes suffer a transformation into flat, anucleated dead cells, the corneocytes that form the stratum corneum, the most superficial layer of the skin, which functions as the main element of the skin barrier.

Keratinocytes

Keratinocytes represent ectodermal derived cells, which make up 80% of the total cell populations in the epidermis. Keratinization is defined as cytoplasmic events that take place in keratinocytes that move through the different layers of the epidermis to finally differentiate into corneocytes. Keratinization has two different phases: one of them is related to synthesis of keratin, and the other is related to degradation of keratin [3]. When cells that are destined to differentiate reach the stratum spinosum, their cytoplasm becomes larger and several bundles of keratin intermediate filaments are formed inside them. The main function of these keratin filaments is to provide the stiffness to the cells that allows them to withstand the environmental stress that is inflicted upon them when they perform their role as an environmental barrier [4]. It is believed that each keratin intermediate filament is formed by approximately 20,000–30,000 individual polypeptides. There are more than 30 different types of tissue-specific keratins, of which about 20 are epithelial keratin proteins and 10 are hair keratins [5,6]. Epithelial keratins are classified depending on their molecular weight and their isoelectric point into two types; type I keratins, which are acidic and have lower molecular weight, and type II keratins, which are neutral or basic with a higher molecular weight. Besides this, keratins are further subdivided numerically. Type I keratins include molecules numbered from K10 to K20, and type II keratins are those from K1 to K9 [5,6]. Except for K15 [7], each type I keratin is expressed alongside a type II keratin as a molecular partner. Depending on the keratin type, its location within the epidermis varies. For example, in the basal layer the keratinocytes contain K5 and K14, while in the suprabasal layers they contain K1 and K10. These keratin filaments converge at the plasma membrane, forming intercellular junctions called *desmosomes*. Mutations in the genes that encode keratin proteins have been implicated in the pathogenesis of numerous skin diseases, mainly in ichthyosis, some types of keratoderma, some types of hereditary bullous epidermolysis, and some pigmentation disorders [8].

Epithelial Stem Cells

The homeostasis of the epidermis relies heavily upon stem cells to replenish and repair wounds and continuously replace the many cells that die and are shed from the surface. Stem cells are characterized by their ability to self-renew and differentiate into the different cell lineages characteristic of their tissue of origin.

There are two types of proliferating cells in the basal layer of the epidermis: the epidermal stem cells and the epidermal progenitor cells. The stem cells are characterized by very slow division rates (4–6 times per year), have a long life span, and express high levels of integrin alpha 6. The progenitor cells in the basal layer express involucrin, divide much faster (once per week), and have a shorter lifespan [9].

The hair follicle stem cells are responsible for the growth of hair in the hair follicle and regeneration, and they reside in the lower part of the permanent portion of the hair follicle called the bulge. These cells express CD34, Lgr5, and keratin 15 as markers [10].

Recently, at least three more types of epithelial stem cells have been identified in the skin: stem cells homing to the sebaceous glands, the infundibulum, and the sweat glands. The sebaceous glands are maintained by unipotent stem cells expressing Lgr6 [11]. Stem cells from the infundibulum are multipotent and are characterized by the expression of Lrig1 as a marker [12]. Finally, four different types of progenitor cells have been identified in the epithelium of the sweat glands [13].

Basal Layer or Stratum Germinativum

The basal layer is formed by column-shaped keratinocytes that are attached to the basement membrane zone. They comprise a single layer of cells with dark-staining oval nuclei containing melanin pigment, which would have been transferred by melanocytes located right next to them [14]. Most authors admit the presence within the epidermal basal layer of a keratinocyte subpopulation that meets the functional definition of stem cells, but there is no consensus concerning their location, organization, and activity with regard to the whole epithelium [15]. To the best of our knowledge,

keratinocyte stem cells are considered to be the long-term guardians of integrity of the epidermis. Chadli et al. postulated that the basal layer contained both early progenitor cells, which were close to stem cells, and late progenitor cells, which can differentiate and undergo the keratinization process [16]. It takes about 26–42 days for the individual cells to migrate from the basal layer up to the granular layer, and then the transit through the cornified layer requires an additional 14 days.

Squamous Cell Layer, Also Called the Prickle Cell Layer or Stratum Spinosum

The squamous cell layer is formed by a variety of cells, which differ in shape, structure, and subcellular properties depending on their location. This layer is five to six cells thick [14]. The suprabasal cells are polyhedral with a rounded nucleus, while the upper spinous layer cells are larger and flatter, and have lamellar granules in their cytoplasm. These granules consist of a type of lysosomal vesicles containing glycoproteins, phospholipids, glycolipids, free sterols, acid hydrolases like lipases, proteases, phosphatases, and glycosidases. The granules are more active in the interphase between the stratum granulosum and corneum, but they appear for the first time in the upper spinous layer [17]. There are abundant desmosomes in the intercellular spaces between the spinous cells. Desmosomes are adhesive intercellular junctions that join adjacent cells closely together by linking desmosomal cadherins with the keratin intermediate filaments, which form the cytoskeletal network. They link these proteins together through densely clustered cytoplasmatic plaque proteins, including plakoglobin and plakophilin, and some other members of the plakin family like desmoplakin [18,19] (Fig. 1.2). These junctions are very important for the correct function of the skin and other organs; in fact, mutations in genes related with desmosomes cause cardiomyopathy and disorders of keratinization. Moreover, autoantibodies or bacterial toxins that selectively target desmosomal cadherins cause pemphigus or staphylococcal scalded-skin syndrome, respectively [20,21].

The Granular Layer or Stratum Granulosum

The granular layer is the most superficial layer of the epidermis that still has living cells. It is composed of flattened cells with abundant keratohyaline granules. These granules are basophilic and irregular in shape and size, and they result from the accumulation of newly synthesized proteins, the most relevant being profilaggrin (>400 kDa). This insoluble multiunit protein is dephosphorylated and degraded to produce monomeric filaggrin molecules in the stratum corneum and then further proteolyzed to release its component amino acids. Filaggrin is a histidine-rich protein that binds to keratins 1 and 10 and to other intermediate filament proteins within the keratinocyte cytoskeleton to form tight bundles, causing collapse of the granular cells that become flattened anuclear squames (thin flakes).

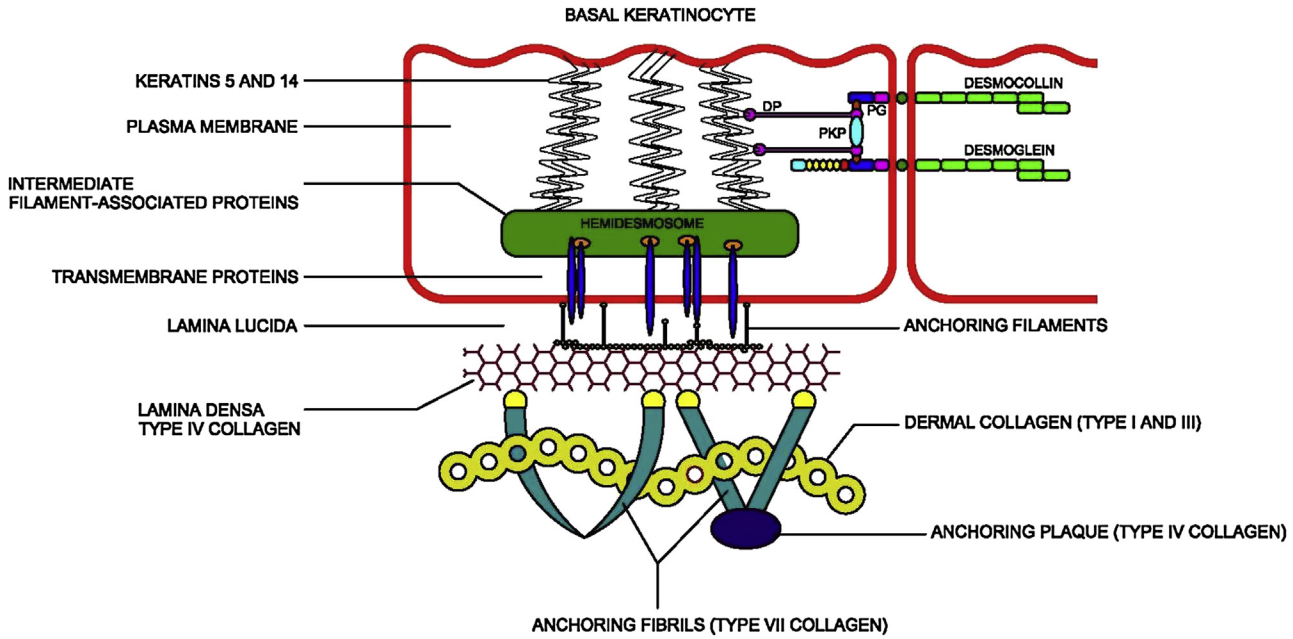

FIGURE 1.2 Basement membrane diagram representing its main components and the intercellular desmosome (not to scale). *DP*, Desmoplakin; *PG*, Plakoglobin; *PKP*, Plakophilin.

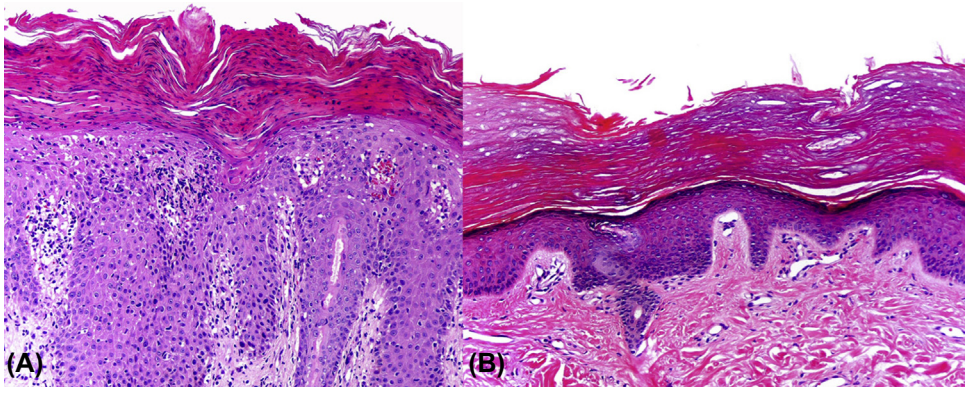

FIGURE 1.3 (A) Skin of a psoriasis patient without a granulous layer and with intense parakeratosis in the stratum corneum. (B) Skin with granulous layer and a compact orthokeratotic stratum corneum. *Courtesy of Dr Luis Requena.*

Filaggrin has a fundamental role in the development and maintenance of the skin barrier, and its alteration is implicated in numerous skin diseases such as atopic dermatitis or ichtiosis vulgaris [22,23]. Depending on the overlying horny cell layer, the stratum spinosum varies in thickness. In locations with a thin cornified layer like the trunk, it may only be one to three cells thick, whereas in locations like the palms and soles, which have a thicker cornified layer, it could be more than 10 times thicker. In some diseases like psoriasis, in which there is parakeratosis (the presence of cell nuclei within the cornified layer), there is an absence of granular cells (Fig. 1.3).

Cornified Layer or Stratum Corneum

The stratum corneum is formed by corneocytes that are dead cells linked together by corneodesmosomes. An insoluble barrier called the cornified envelope, which replaces the plasma membrane and is composed of proteins and lipids, covers the corneocytes. This cornified cell envelope is a critical structure for the skin barrier function formed by covalent cross-linking of component proteins such as involucrin, loricrin, and the small proline-rich protein. This coupling reaction requires transglutaminase, which is a calcium-dependent enzyme catalyzing the formation of an intermolecular isopeptide bond between proteins [24]. The physical and biochemical characteristics of corneocytes vary between the upper cells and the supragranulous cells in order to facilitate desquamation. During desquamation, corneodesmosomes undergo proteolytic degradation.

The intercellular lipids, the corneocytes, some amino acids, other salts from sweat and sebaceous secretions, and degradation products from corneal proteins besides lipids all contribute to the overall barrier effect, preventing loss of water and keeping the skin pH at its optimum condition (5.5).

Nonkeratinocyte Cells

Melanocytes

Melanocytes are cells derived from the neural crest, which migrate through the developing embryo to specific locations in the fetal body, mostly to the skin and hair follicles [25]. In the epidermis, they are confined predominantly to the basal layer, where they come into contact with keratinocytes through cytoplasmic extensions (dendrites). Melanocytes contain melanosomes, tissue-specific "lysosome-related" organelles characteristic of pigment cells in which melanin molecules are synthesized and stored. During the biosynthesis of melanin, toxic intermediates are generated. There are two different forms of melanin in mammals: eumelanin, which is black or dark brown, and phaeomelanin, which is yellow or red. The melanin is transferred from melanocytes to keratinocytes. Deeply pigmented skin may be due to an increased production of melanosomes, to a higher degree of melanization, to the presence of larger melanosomes, to a greater dispersion of melanosomes in the keratinocytes, or to slower degradation of melanosomes [26,27].

Langerhans Cells

LCs are antigen-presenting dendritic cells involved in several T-cell responses. They reside in the epidermis, constituting between 2% and 8% of the total epidermal cell population, and most of them are located in the squamous and granular layers. These dendritic cells are derived from the bone narrow and do not form intercellular unions with keratinocytes as do melanocytes. The birbeck granules are a unique organelle found in LCs, and they can be seen with the electron microscope as having a shape resembling that of a tennis racquet. The phenotypic hallmark of LCs is their expression of the C-type lectin receptor called langerin or CD207 [28].

Merkel Cells

Merkel cells were discovered by Friedrich Sigmund Merkel in 1875 [29]. They are found in the basal layer of the epidermis. Merkel cells are oval-shaped, and their membrane interacts with nerve endings in the skin with synapse-like structures. On their opposite side, they have cytoplasmic processes that extend into and between the keratinocytes and to which they are linked by desmosomes. Merkel cells function as type 1 mechano-receptors and can sense light touches. They are part of the tactile-end organs in the skin, which include Merkel discs, Pacinian corpuscles, Meissner's corpuscles, and Ruffini endings [30].

The Dermal-Epidermal Junction: The Epidermal Basement Membrane

The epidermal basement membrane is a thin extracellular matrix separating the epidermis from the dermal connective tissue, located in the dermo-epidermal junction [31]. It has four major components: basal cell plasma membrane, the lamina lucida, the lamina densa, and the sublamina densa fibrillar zone that includes the anchoring fibrils [32] (Fig. 1.2).

The plasma membrane and hemidesmosome plaques of the basal keratinocytes are the outer part of the basement membrane [31]. Keratin intermediate filaments (K5 and K14) insert into hemidesmosomes. There act as anchoring filaments through the plasma membrane, lamina lucida, and lamina densa.

The lamina lucida, a 35–40 nm wide zone that appears transparent under an electron microscope, is the weakest link in the dermal-epidermal junction. It is traversed by anchoring filaments, including laminin 5, laminin 6, uncein, laminin 1, nidogen, and epiligrin [33,34].

The lamina densa is an electron-dense zone between 35 and 60 nm thick, whose main component is a specific collagen protein, type IV collagen, produced by both keratinocytes and fibrocytes [35]. Other components of the lamina densa are laminin 1, percelan, nidogen, and chondroitin sulfate proteoglycan.

The sublamina densa is a less well-defined zone that could be considered to be the top of the papillary dermis. It contains distinct structures, including the anchoring fibrils; the collagen fibers type I and III, looping from the dermis in close apposition to the lamina densa; the microfibrils, components of elastic fibers in the lower dermis; the micro-thread-like fibers; and the anchoring plaques, which contain type IV collagen and laminin-1. Anchoring fibrils are composed of collagen VII and laminin 332, and their major function is linking the epidermis with the dermis through the lamina densa and anchoring plaques of the dermis [36]. Understanding this structure is of great importance because genetic alterations of their components and the presence of auto-antibodies produced against these proteins are responsible for most subepidermal bullous disorders.

DERMIS

Beneath the epidermis, a thick layer of fibrous and elastic tissue, called the dermis, provides structural and nutritional support. The dermis comprises two layers: the thin and superficial papillary dermis, and a thicker and deeper reticular dermis. The papillary dermis lies below the dermoepidermal junction and contains loosely arranged collagen fibers. The reticular dermis is formed by thicker bundles of collagen running parallel to the skin surface. The dermis contains stromal cells such as fibroblasts, fibrocytes, and structural cells of the blood and lymph vessels. In addition, many different populations of myeloid and lymphoid immune cells either reside in or traffic through the dermis.

Most of the constituents of the dermis are of mesodermal origin, except for the nerves, which, like melanocytes, derive from the neural crest [37]. Until week 6 of fetal development, the dermis is formed mainly by dendritic-shaped cells, which are precursors of fibroblasts. By week 12 the fibroblasts are producing collagen and elastic fibers, and by week 24 the vascular network and the hypodermis appear.

The dermis is composed of a mucopolysaccharide gel held together by collagen and elastic fibers. Collagen fibers make up 70% of the dermis, giving it strength and toughness, while elastin maintains normal elasticity and flexibility and proteoglycans provide viscosity and hydration. This extracellular matrix of the dermis is constantly being degraded by proteolytic enzymes called matrix metalloproteinases (MMPs) and replaced by new matrix components. MMPs are a group of zinc-dependent extracellular proteinases that remodel the extracellular matrix. There are three predominant groups: collagenases, gelatinases, and stromelysins. The collagenases (MMP-1, MMP-8, MMP-13, and MMP-18) cleave interstitial collagen, with MMP-1 as the predominant one. Gelatinases (MMP-2 and MMP-9) degrade basement membrane collagens and denaturated structural collagens. The stromelysins (MMP-3, MMP-10, MMP-11, and MMP-19) degrade basement membrane collagens and proteoglycans, and matrix glycoproteins. Other MMP family members include membrane-type MMPs, matrilysins, elastase (MMP-12), and others [38]. MMPs are regulated by tissue inhibitors of MMPs (TIMPs), especially TIMP-1 and TIMP-2 [39]. The balance between MMPs and TIMPs is important in the maintenance of the dermal matrix structure. MMPs are mainly produced by keratinocytes, fibroblasts, neutrophils, and mast cells. Transforming growth factor-β (TFG-β) is an important regulator of the

expression of MMPs, stimulating their expression in keratinocytes and inhibiting cell growth [38]. The production of MMPs is increased in several physiological and pathological processes, such as wound repair, skin aging, or tumoral invasion.

Dermal blood and lymphatic vessels, nerves, sweat and sebaceous glans, hair roots, mast cells, and small quantities of muscle are embedded within the fibrous tissue.

Dermal Blood and Lymphatic Vessels

Blood and lymphatic vessels fulfill important homeostatic functions such as providing nutrients for the skin and regulating the immunologic processes. In the dermis, the blood vascularization is organized into a deep plexus and a superficial horizontal plexus, with capillaries arising from the latter one [40,41]. The lymphatic vessels also form two plexuses in proximity to the vascular blood system. Branches from the superficial lymphatic vessel plexus extend into the dermal papillae and drain into the larger lymphatic vessels in the lower dermis [42]. While the blood microvasculature is located immediately below the epidermis, the lymphatic vessels reside more deeply within the dermis [43].

Muscles

The involuntary muscles of the skin comprise the arrector pili, the muscle fibers of veins and arteries, and the glomus bodies. The fibers of the arrector pili are located in the upper dermis and are fixed to the hair follicle below the sebaceous gland, pulling the hair follicle into a vertical position during contraction. Glomus bodies consist of an arteriovenous shunt surrounded by a capsule of smooth muscle cells, and they are found on the digits (toes and fingers), palms, and soles. Their function is to regulate body temperature by shunting blood away from the skin surface when exposed to cold temperature, thus preventing heat loss, and allowing maximum blood flow to the skin in warm weather to allow heat to dissipate.

Voluntary muscles can be found in the skin of the face, as these muscles are responsible for facial expressions, and in the skin of the neck as platysma (a broad sheet of muscle fibers extending from the collarbone to the angle of the jaw).

Nerves

The skin is an important sensory organ, being the principal interface with the environment. The network of sensory nerves allows the perception of touch, temperature, pain, and itch [44]. The autonomous nervous system is of great importance in maintaining cutaneous homeostasis by controlling vasomotor functions, pilimotor activities, and glandular secretions.

Nerves in the skin contain both myelinated and unmyelinated fibers. In general, the myelinated (or type-A) fibers are motor neurons that interface with striated muscles and include a subgroup of sensory neurons, while unmyelinated (or type-C) fibers correspond to autonomic and sensory fibers. After losing the myelin sheaths, cutaneous nerves terminate as free nerve endings, either in association with receptors or as special nerve-end organs.

Meissner corpuscles mediate touch perception and are located in the dermal papillae. Vater—Pacinian corpuscles are located in the deeper dermis and are large nerve endings that create the perception of pressure. The unmyelinated nerve fibers are responsible for pain, temperature, and itch sensations.

The autonomic nervous system provides the motor innervation of the skin. Adrenergic fibers innervate the blood vessels, hair erector muscles, and apocrine glands, while cholinergic fibers innervate eccrine sweat glands. The secretion of sebaceous glands is not innervated by autonomic fibers but is regulated by the endocrine system.

Mast Cells

Mast cells are multifunctional immune cells that play an important role in both adaptive and innate immune responses. These cells attract other key players of the immune system by secreting cytokines and chemokines. Skin mast cells contain metachromatic granules, which release histamine, leukotrienes, prostanoids, proteases, and many cytokines and chemokines after activation by surface antigen binding or cytokine-dependent events. In addition to their role in allergic responses, mast cells have been implicated in other inflammatory responses, host defense, innate immunity, as well as cell growth and adhesion [45].

HYPODERMIS

Embryologically, at the end of the fifth month of gestation, fat cells begin to develop in the subcutaneous fetal tissue. The hypodermis serves as a reserve energy supply, protects the skin, and allows mobility by sliding over underlying structures. The hypodermis is primarily formed by adipocytes, which are organized into lobules defined by fibrous connective tissue (septa). Nerves, blood, and lymphatic vessels are located within the septa. The subcutaneous tissue stores energy through the following biological reactions: exotrophy, deposition, and endotrophy. Adiponectic is a specific mediator of subcutaneous adipocytes. In addition, subcutaneous tissue is considered to be an endocrine organ,

converting androstenedione into estrone by the aromatase enzyme. Moreover, the adipocytes produce leptin, a hormone that regulates body weight [46].

EPIDERMAL APPENDAGES

Besides the epidermis, there are some ectodermal-derived structures in the skin, called adnexa or epidermal appendages. They include pilosebaceous units, eccrine ducts, apocrine glands, and nails. Some authors claim that the skin appendages promote wound healing by encouraging re-epithelialization after injury through the migration of keratinocytes from the pilosebaceous units to the damaged epidermis [47].

Apocrine Glands and Eccrine Sweat Glands

Evolutionarily, apocrine glands were the first to appear. In humans, their presence has been limited to the periumbilical area, areola, axillae, mons pubis, labia, scrotum, foreskin, perianal region, free edge of the eyelids (Moll's gland), and ear canal (ceruminous gland) [48]. Eccrine glands appeared later phylogenetically and are found throughout the surface of the body; the only variation between different locations are their density, ranging from 100 to 600/cm^2. This makes eccrine glands the most abundant skin adnexa. The absence of eccrine glands in normal skin is found in a condition called anhidrotic ectodermal dysplasia. The embryonic origins of the eccrine and apocrine glands are not the same. Whereas apocrine glands appear first during embryonic development (third month) and are derived from the same epithelial germ cells from which the hair follicles originate, the eccrine glands originate later (fourth month) from different epithelial germ cells.

Histologically, the excretory duct structure is roughly similar in both glands and consists of bilayered cubic cells that are surrounded by a basement membrane, and a lack of myoepithelial cells (Fig. 1.4D). The cells of the inner layer are larger than the cells of the outer layer. Excretory ducts differ in their outlet portion; while the apocrine duct opens into the infundibulum of the pilosebaceous unit, the eccrine duct penetrates through

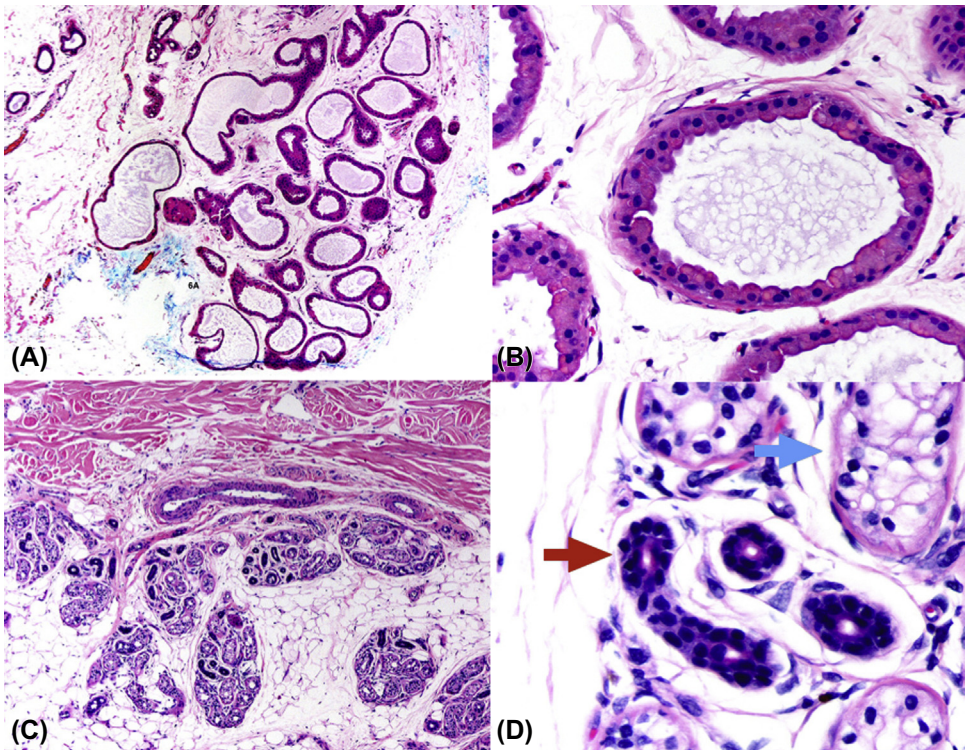

FIGURE 1.4 (A) Axillae skin with multiple apocrine glands with a large lumen. (B) The apocrine glands have one columnar secretory cell type with an oval basal nucleus arranged in more cylindric or cuboidal shapes surrounded by myoepithelial cells. (C) Eccrine glands and ducts located between the dermis and the subcutaneous tissue. (D) The blue arrow shows the secretory portion of the eccrine gland with the clear and dark cells, and the red arrow points ducts with bilayered cubic cells that are surrounded by basement membrane and lack of myoepithelial cells. Their cytoplasm delimits the lumen with a wide eosinophil density of tonofilaments (cuticle). *Courtesy of Prof. Luis Requena.*