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Alfonso Baldi
Paola Pasquali
Enrico P. Spugnini *Editors*

Skin Cancer

A Practical Approach

Humana Press

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Antonio Giordano, MD, PhD
Temple University
Philadelphia
United States

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Alfonso Baldi • Paola Pasquali
Enrico P. Spugnini
Editors

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Editors
Alfonso Baldi
Department of Environmental
Biological and Pharmaceutical Sciences
and Technologies
Second University of Naples
Naples
Italy

Enrico P. Spugnini, DVM, PhD
S.A.F.U. Department
Regina Elena Cancer Institute
Rome
Italy

Paola Pasquali
Department of Dermatology
Pius Hospital De Valls
Tarragona
Spain

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Contributors

Yasser A. Alqubaisy, MD Department of Dermatology and Cutaneous Surgery, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA

Peter E. Andersen DTU Fotonik – Department of Photonics Engineering, Technical University of Denmark, Roskilde, Denmark

Anna Maria Anniciello Pathology Unit, National Institute of Tumours Fondazione "G. Pascale", Naples, Italy

Federica Arginelli Department of Dermatology, University of Modena and Reggio Emilia, Modena, Italy

Enrico Baldessari Plastic Surgery, Casa di Cura Addominale EUR, Rome, Italy

Fondazione Futura-onlus, Rome, Italy

Alfonso Baldi Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples, Naples, Italy

Fondazione Futura-onlus, Rome, Italy

Feliciano Baldi Department of Biochemistry, Section Pathology, Second University of Naples, Naples, Italy

Christina Banzhaf Department of Dermatology, Faculty of Health Sciences, Roskilde Hospital, University of Copenhagen, Roskilde, Denmark

Raymond Barnhill Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, and Jonsson Comprehensive Cancer Center, Los Angeles, CA, USA

Alexander Bianchi Department of Biochemistry, Section of Pathology, Second University of Naples, Naples, Italy

Stefano Bizzi Advanced Computer Systems (ACS), Rome, Italy

Emanuela Bonoldi Pathology Unit, Hospital A. Manzoni, Lecco, Italy

Gerardo Botti Pathology Unit, National Institute of Tumours Fondazione "G. Pascale", Naples, Italy

Beniamino Brunetti, MD Plastic and Reconstructive Surgery Unit, Campus Bio-Medico of Rome University, Rome, Italy

Piergiacomo Calzavara-Pinton, MD Department of Dermatology, University of Brescia, Brescia, Italy

Elia Camacho Department of Dermatology, Pius Hospital De Valls, Valls, Spain

Stefano Campa Plastic and Reconstructive Surgery Unit, Campus Bio-Medico of Rome University, Rome, Italy

Emilia Caputo Department of Genetics and Biophysics, Institute of Genetics and Biophysics, Naples, Italy

Michele Caraglia, MD, PhD Department of Biochemistry and Biophysics, Second University of Naples, Naples, Italy

Sonal Choudhary, MD Department of Dermatology and Cutaneous Surgery, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA

Claudio Clemente Department of Pathology and Cytopathology, San Pio X Hospital, Milan, Italy

Department of Pathology and Cytopathology, IRCCS Policlinico San Donato, San Donato Group, San Donato Milanese, Milan, Italy

Alistair Cochran Departments of Pathology, Laboratory Medicine and Surgery, David Geffen School of Medicine at UCLA, and Jonsson Comprehensive Cancer Center, Los Angeles, CA, USA

Francesco Cognetti, MD Division of Medical Oncology "A", Medical Oncology Department, National Cancer Institute Regina Elena, Rome, Italy

Stefania Crispi, PhD Institute of Genetics and Biophysics, I.G.B., A.Buzzati-Traverso, CNR, Naples, Italy

Alfredo D'Avino Department of Biochemistry, Section of Pathology, Second University of Naples, Naples, Italy

Maria De Falco Department of Biology, Section of Evolutionary and Comparative Biology, University of Naples "Federico II", Naples, Italy

Antonio De Luca, PhD Department of Mental and Physical Health and Preventive Medicine, Section of Human Anatomy, Second University of Naples, Naples, Italy

Michele De Nuntiis Plastic Surgery, Casa di Cura Addominale EUR, Rome, Italy

Fondazione Futura-onlus, Rome, Italy

Emanuele Dragonetti Futura-onlus, Rome, Italy

Maria Elena Errico Pathology Unit, Paediatric Hospital Santobono-Pausilipon, Naples, Italy

Katherine M. Ferris, BA Department of Dermatology and Cutaneous Surgery, University of Miami Hospital, Miami, FL, USA

Angeles Fortuño-Mar, MD, PhD, MBA Eldine Patologia Laboratory, Valls, Tarragona, Spain

Renato Franco Pathology Unit, National Institute of Tumours Fondazione "G. Pascale", Naples, Italy

Anna Maria Frezza Medical Oncology, University Campus Bio-Medico, Rome, Italy

Riccardo Garcea Plastic Surgery, Casa di Cura Addominale EUR, Viale Africa, Rome, Italy

Joan Ramon Garcés, MD Department of Dermatology, Universitat Autònoma de Barcelona, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

Umberto Gianelli Department of Pathophysiology and Transplantation, University if Milan, Milano, Italy

Salvador Gonzalez Dermatology Service, Ramon y Cajal Hospital, Alcalá University, Madrid, Spain

Faculty of Dermatology Service, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Ira Gordon, DVM Department of Radiation Oncology Branch, National Cancer Institute, Bethesda, MD, USA

Anna Grimaldi Department of Biochemistry and Biophysics, Second University of Naples, Naples, Italy

Gregor B.E. Jemec Department of Dermatology, Faculty of Health Sciences, Roskilde Hospital, University of Copenhagen, Roskilde, Denmark

Barbara E. Kitchell, DVM, PhD, DACVIM Department of Small Animal Clinical Sciences, Center for Comparative Oncology, Veterinary Medicine Center, Michigan State University, East Lansing, MI, USA

Aleksandar L. Krunic, MD, PhD, FAAD, FACMS Department of Dermatology, University of Illinois at Chicago, Chicago, IL, USA

Department of Dermatology, Northwestern University Feinberg School of Medicine, River Forest, IL, USA

Susanne Lange-Asschenfeldt Department of Dermatology, Charité – Universitätsmedizin, Berlin, Germany

Stephan Lautenschlager, PhD Dermatologisches Ambulatorium Stadtspital Triemli, Zurich, Switzerland

Outpatient Clinic of Dermatology, Triemli Hospital, Zurich, Switzerland

Angela Lombardi Department of Biochemistry and Biophysics, Second University of Naples, Naples, Italy

Miguel Alejandro López Dermatologic Surgery and Cutaneous Oncology Section, Department of Dermatology, Hospital Militar "Dr. Carlos Arvelo", Caracas, Venezuela

Amalia Luce Department of Biochemistry and Biophysics, Second University of Naples, Naples, Italy

Joseph Malvehy Dermatology Department & CIBER-ER, Melanoma Unit, Hospital Clínic, Barcelona, Spain

Marco Manfredini Department of Dermatology (ACS), University of Modena and Reggio Emilia, Skin Center, Modena, Italy

Mario Manganaro Advanced Computer Systems, Rome, Italy

Ashfaq A. Marghoob Hauppauge Dermatology Section, Memorial Sloan-Kettering Skin Cancer Center Hauppauge, Long Island, NY, USA

Federica Zito Marino Pathology Unit, National Institute of Tumours Fondazione "G. Pascale", Naples, Italy

Sebastian Marschall DTU Fotonik – Department of Photonics Engineering, Technical University of Denmark, Roskilde, Denmark

Michael P. McLeod, MS Department of Dermatology and Cutaneous Surgery, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA

Martin C. Mihm Jr. Pathology and Dermatology Department, Harvard Medical School, Boston, MA, USA

Melanoma Program, Department of Dermatology, Brigham and Women's Hospital, Boston, MA, USA

Melanoma Program, Dana Farber and Brigham and Women's Cancer Center, Boston, MA, USA

Mette Mogensen, MD, PhD Department of Dermatology, Faculty of Health Sciences, Roskilde Hospital, University of Copenhagen, Roskilde, Denmark

Department of Dermatology, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark

Eduardo K. Moioli Section of Dermatology, University of Chicago, Chicago, IL, USA

Raffaele Murace Futura-onlus, Rome, Italy

Paola Muti Juravinski Hospital and Cancer Centre, Hamilton, ON, Canada

Department of Oncology, McMaster University, Hamilton, ON, Canada

Giovanni Francesco Nicoletti Dipartimento di Scienze Ortopediche, Traumatologiche, Riabilitative e Plastico-Ricostruttive, Second University of Naples, Naples, Italy

Keyvan Nouri, MD Department of Dermatology and Cutaneous Surgery, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA

Department of Dermatology, Sylvester Comprehensive Cancer Center/ University of Miami Hospital and Clinics, Miami, FL, USA

Carmen Nuzzo Division of Medical Oncology "A", Medical Oncology Department, National Cancer Institute Regina Elena, Rome, Italy

Pablo Luis Ortiz-Romero, MD, PhD Department of Dermatology, Instituto de investigación i +12, hospital 12 de Octubre, Facultad de Medicina. Universidad Complutense, Madrid, Spain

Giuseppe Palmieri, MD Department of Cancer Genetics, Institute of Biomolecular Chemistry, National Research Council (CNR), Sassari, Italy

Evangelia Papadavid Department of Dermatology, Athens University Medical School, Attikon University Hospital and Andreas Syggros Cutaneous Lymphoma Clinic, Athens, Greece

Paola Pasquali Department of Dermatology, Pius Hospital De Valls, Cambrils, Tarragona, Spain

Paolo Persichetti Plastic and Reconstructive Surgery Unit, Campus Bio-Medico University of Rome, Rome, Italy

Maria Simona Pino Division of Medical Oncology "A", Medical Oncology Department, National Cancer Institute Regina Elena, Rome, Italy

Michele M. Pisano Department of Molecular, Cellular and Craniofacial Biology, University of Louisville Birth Defects Center, Louisville, KY, USA

Susana Puig, MD, PhD Melanoma Unit, Dermatology Department, Hospital Clínic, Barcelona, Spain

Maria Teresa Rossi Department of Dermatology, University of Brescia, Brescia, Italy

Paulo Vilar-Saavedra Center for Comparative Oncology, Veterinary Medicine Center, Michigan State University, East Lansing, MI, USA

Raffaella Sala Department of Dermatology, University of Brescia, Brescia, Italy

Virginia Sanchez Dermatology Service, CEU University, Madrid, Spain

Daniele Santini Medical Oncology, University Campus Bio-Medico, Rome, Italy

Peter Sarantopoulos Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, and Jonsson Comprehensive Cancer Center, Los Angeles, CA, USA

Alessandra Scarabello Istituto Dermatologico San Gallicano, Rome, Italy

Alon Scope, MD Department of Dermatology, Sheba Medical Center, Ramat Gan, Ganey Tikva, Israel

Stefania Seidenari Department of Dermatology, University of Modena and Reggio Emilia, Skin Center, Modena, Italy

Enrico P. Spugnini, DVM, PhD S.A.F.U. Department, Regina Elena Cancer Institute, Rome, Italy

Stefania Tenna Plastic and Reconstructive Surgery Unit, Campus Bio-Medico of Rome University, Rome, Italy

Lotte Themstrup Department of Dermatology, Faculty of Health Sciences, Roskilde Hospital, University of Copenhagen, Roskilde, Denmark

Giuseppe Tonini Medical Oncology, University Campus Bio-Medico, Rome, Italy

Martina Ulrich Department of Dermatology, Charité – Universitätsmedizin, Berlin, Germany

Job Paul van der Heijden, MSc Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Bruno Vincenzi Medical Oncology, University Campus Bio-Medico, Rome, Italy

Leonard Witkamp, MD, PhD KSYOS TeleMedical Center, Amstelveen, The Netherlands

Ximena Wortsman, MD Department of Radiology and Dermatology, Faculty of Medicine, Institute for Diagnostic Imaging and Research of the Skin and Soft Tissues, Clinica Servet, University of Chile, Providencia, Santiago, Chile

Embryology and Anatomy of the Skin

1

Maria De Falco, Michele M. Pisano, and Antonio De Luca

Key Points

- Skin embryology: the embryology of the both epidermis and dermis is described.
- Epidermal development: the development of the epidermis beginning the third week of fetal life and its regulation is treated.
- Skin structure: the structure and the ultrastructure of adult skin are detailed.

Introduction

Skin Structure

The integumentary system is formed by skin and several skin appendages (glands, hair, nails, and teeth) [1]. The skin is the largest organ in the body (in adults it weighs from 3 to 5 kg) and represents both its border and its intermediary with environment [1, 2]. The skin covers the whole outer surface of the body, including the wall of the outer auditory canal (meatus). It proceeds with the mucosae of the alimentary canal and respiratory and urinary-genital ways [3]. Its total thickness varies from 1.5 to 4.0 mm [3]. Human skin is formed by two distinct layers: the outer epidermis, a stratified pavement epithelium, and the underlying dermis consisting of connective tissue, principally dense at interlaced bundles (Fig. 1.1).

The two skin layers are interconnected with each other through epidermal-dermal junctions. These are undulating in section and formed by ridges of the epidermis, known as rete ridges, that project into the dermis. The junction provides mechanical support for the epidermis and acts as a partial barrier against exchange of cells and large molecules. Below the dermis, there is a fatty layer, the panniculus adiposus, usually designated as "subcutaneous." This is separated from the rest of the body by a vestigial layer of striated muscle, the panniculus carnosus [4].

There are two main kinds of human skin. Glabrous skin (non-hairy skin), covering the palms and soles, is grooved on its surface by continuously alternating ridges and sulci. It is characterized by a thick epidermis divided into several layers, including a compact stratum corneum; by the presence of encapsulated sense organs within the dermis; and by a lack of hair follicles and sebaceous glands. On the contrary, hair-bearing

M. De Falco
Department of Biology,
Section of Evolutionary and Comparative Biology,
University of Naples "Federico II",
Naples, Italy

M.M. Pisano
Department of Molecular,
Cellular and Craniofacial Biology,
University of Louisville Birth Defects Center,
Louisville, KY, USA

A. De Luca, PhD (✉)
Department of Mental and Physical Health and
Preventive Medicine, Section of Human Anatomy,
Second University of Naples,
Via L. Armanni 5, Naples, 80138, Italy
e-mail: antonio.deluca@unina2.it

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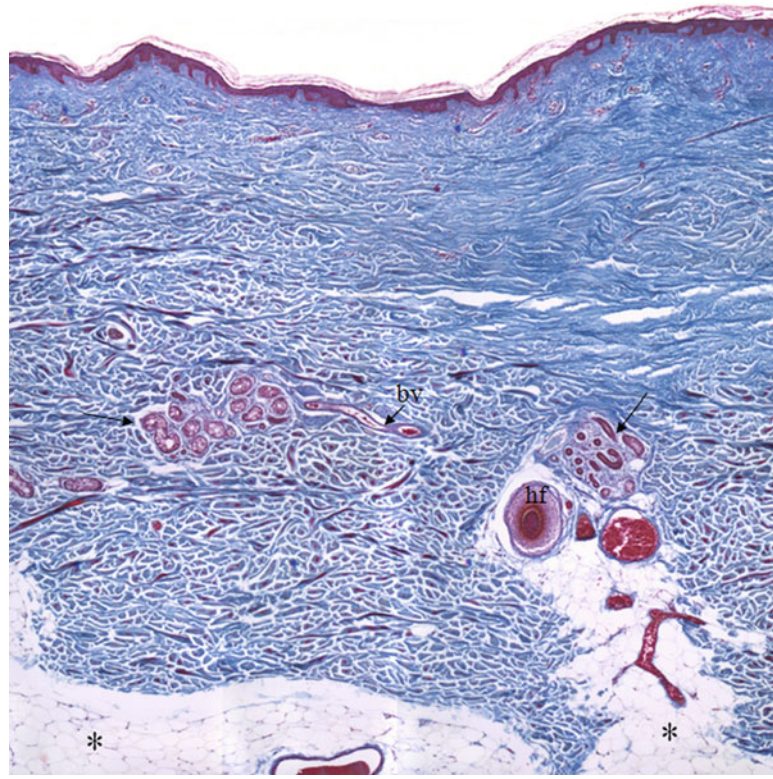

Fig. 1.1 Histological section of human skin. The human skin is composed by a superficial upper layer, the epidermis covering a deeper layer, the dermis. Inside the dermis, numerous glands (*arrows*), blood vessels (*bv*), and hair follicles (*hf*) are evident. Below the dermis is visible the subcutaneous fat (*stars*). Mallory trichromic stain. Original magnification 2.5×

skin has both hair follicles and sebaceous glands but lacks encapsulated sense organs [4].

The integumentary system has not only principally protection functions but also thermoregulation, respiration, and perception functions [1]. The skin is the first line of defense against environmental (mechanical, chemical, osmotic, thermal) insults and microbial infection, as well as water and electrolyte loss [2, 3]. It confronts these attacks by undergoing continual self-renewal to repair damaged tissue and replace old cells [5]. Stem cells are located in the adult hair follicle, sebaceous glands, and in the basal layer of the interfollicular epidermis [5, 6]; they have the function to maintain tissue homeostasis, regenerating hair and repairing the epidermis after injury [5].

Embryology of the Skin

The skin develops by the juxtaposition of two embryological elements: the prospective epidermis, which originates from a surface area of the early gastrula, and the prospective mesoderm, which is brought into contact with the inner surface of the epidermis during gastrulation [4, 7, 8].

The epidermis originates almost completely from the covering ectoderm, and only few cells (melanocytes and Langerhans' cells) migrate from other areas. On the contrary, the dermis develops from two different mesenchymal areas; the larger part arises from somatopleure (lateral mesoderm) and the smaller part arises from somites (paraxial mesoderm) [1]. Both components of the skin should be considered as donors and receptors of information. Morphogenesis of the skin depends on a careful and constant dialogue between them [9]. Before skin morphogenesis, several cell interactions take place, in order to specify first the formation of dermal progenitors [10, 11] and second their densification inside the sub-ectodermal space [9]. These two first steps lead to the formation of the embryonic skin, formed by an upper epidermis overlying a dense dermis. The next step is the initiation and organization of regular repetitive appendage primordia. Finally, the final step is the organogenesis of the epidermal primordia (placode) in a complete, mature appendage [9].

Development of the Epidermis

After gastrulation, the embryo surface emerges as a single layer of neuroectoderm, which will ultimately specify the nervous system and the skin epithelium [5]. The covering ectoderm develops from epiblast during the third week of fetal life and represents the ectoderm that does not differentiate in nervous tissue. During the fourth week, the covering ectoderm separates from neural tube and closes on nervous system, forming a continuous coating on embryo surface. At the crossroads of this decision is Wnt signaling, which blocks the ability of ectoderm to respond to fibroblast growth factors (FGFs) [5]. In the absence of FGF signaling, the cells express bone morphogenetic proteins (BMPs) and become fated to develop into epidermis [5] (Fig. 1.2). Initially, the covering ectoderm is composed by a single layer of undifferentiated, cuboidal, and glycogen-filled cells [4, 12] (Fig. 1.3a). At this early stage, cell proliferation is the dominant process [13]. At the end of the fourth week, the epidermis forms a second layer that lies outer and originates simple squamous epithelium called periderm, a purely embryonic structure, which is unique to primates [4] (Fig. 1.3b). Its cells flatten, cornify, and eventually spread to several times the diameter of the deeper cells. The inner, basal layer includes stem cells and represents the germinal layer (stratum germinativum) of epidermis, whereas the periderm establishes the protection barrier on contact with amniotic fluid [1].

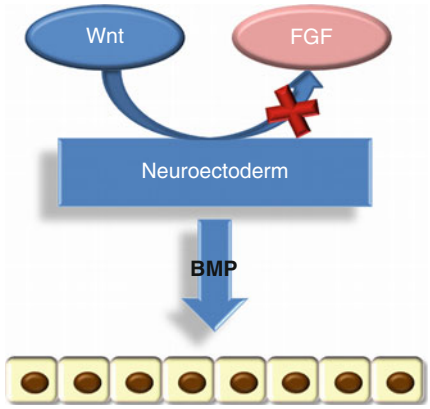

Fig. 1.2 The epidermis formation. Wnt signaling blocks FGF activity on the neuroectoderm that can express BMP proteins so developing the epidermis

Between 8 and 11 weeks, the germinal layer actively proliferates and originates a third

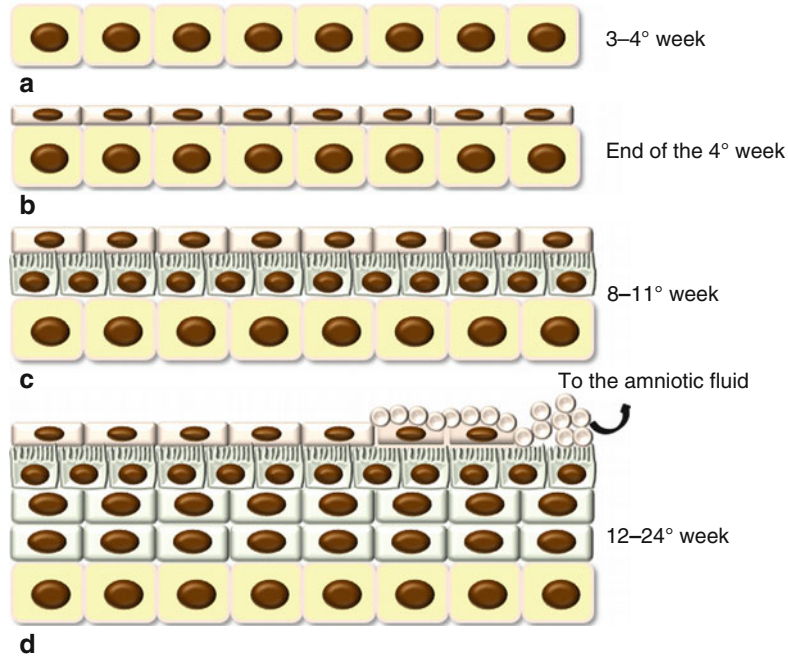

Fig. 1.3 Development of the epidermis. (**a**) The covering ectoderm is formed by a single layer of cuboidal, undifferentiated cells; (**b**) the epidermis is composed by a second superficial layer called periderm; (**c**) the germinal basal layer originates a third middle layer, the intermediate layer, whose cells are characterized by the presence of microvillus projections at the surface of the periderm; (**d**) during the fifth month, the intermediate layer proliferates, forming one or more other layers. The periderm cells form numerous blebs and get away in the amniotic fluid (See more details in the text)

middle layer, the intermediate layer (Fig. 1.3c). Development of this layer is associated with asymmetric cell division of embryonic basal keratinocytes [14–16]. Glycogen is abundant in all layers, and a few microvillous projections occur at the surface of the periderm. The surface cells are flat and polygonal [4, 17]. These three layers persist about a month and then the epidermis later on evolves. During the fifth month (12–16 weeks), there are one or more intermediate layers (Fig. 1.3d). These cells contain mitochondria, Golgi complexes, and a few tonofilaments, as well as abundant glycogen both within and between cells. Microvilli become much more numerous [4]. From this stage onward, dome-shaped blebs start to project from the centers of the periderm cells. At first, the blebs are simple, but later their surface becomes dimpled and infolded [4]. The periderm gets away, and in some weeks (by 24 weeks), it is removed in the amniotic fluid, forming, together with shed lanugo, sebum, and other materials, the vernix caseosa [4]. The periderm may be no more than a protective coating for the fetus before keratinization of the epidermis. On the other hand, features such as the abundant microvilli, raised blebs, coated- and smooth-membrane vesicles, and increasing cell size suggest that it may have an important function such as the uptake of carbohydrate from the amniotic fluid [4, 17].

In the same time, like basal keratinocytes, intermediate cells undergo proliferation and/or differentiation. The loss of their proliferative capacity is associated with the maturation of intermediate cells into spinous cells [14, 16, 18]. The basal layer together with the spinous layer forms the Malpighian layer [19]. The spinous layer cells undergo further maturation to form the granular layer (stratum granulosum) and the outer cornified layer (stratum corneum) [1, 18].

In the epidermis of the hand and foot, among granular and cornified layers, a thin additional layer, called bright (glossy) layer (stratum lucidum) for its refraction property, lies. This layer is formed by cells containing the fluid eleidin that replaces the granules. All the epidermis layers are formed by cells called keratinocytes because they contain keratin by 14 weeks [4]. The germinal layer continuously produces cells that differentiate in keratinocytes, move toward the upper layers, degenerate, and finally are eliminated in the environment. During this migration among layers, keratinocytes pass through several maturation phases and their structural transformations originate morphological differences. Hemidesmosomal and desmosomal proteins are already detectable in the basal keratinocytes at 10 weeks [4].

Epidermal cells must undergo growth arrest before they can initiate a differentiation program [13]. Moreover, the morphological changes that are a hallmark of epidermal stratification are associated with changes in the expression of keratin differentiation markers [16, 20]. In fact, during normal epidermal development, commitment to the epidermal lineage involves the repression of the non-epidermal keratin pair K8/K18 [16, 21] and the induction of the epidermal keratin pair K5/K14 [6, 16, 22, 23]. Keratinocytes belonging to the spinous layer are big and polyhedric cells, synthesizing high quantity of keratin and keratohyalin, the two specific proteins of the epidermis. In the granular layer, these proteins are organized in two several types of subcellular aggregates: keratohyalin granules and keratin bundles. Some derivatives of keratohyalins, particularly the filaggrin, used to tightly join cells together, are first detectable at 15 weeks [4]. Moreover, cells forming the layer just beneath the periderm become to express loricrin [13, 23–25]. Specifically, keratohyalin granules appear at 21 weeks in the uppermost layer [4]. The initiation of terminal differentiation results in the induction of K1 and K10 expression in the newly formed suprabasal keratinocytes [18, 26, 27]. In addition, cornified envelope proteins, which are rich in glutamine and lysine residues, are synthesized and deposited under the plasma membrane of the granular cells [5]. When the cells become permeabilized to calcium, they activate transglutaminase, generating γ-glutamyl-ε-lysine cross-links to create an indestructible proteinaceous sac to hold the keratin macrofibrils (including various keratins, involucrin, loricrin, and filaggrin) [5, 13]. In the higher part of granular layer, the cells start to show the first signs of terminal

differentiation and degeneration: flattening cell form, destruction of cellular organelles, dense chromatin granules, and breaking of the nuclear envelope. When the cells pass into the cornified layer, they completely degenerate, lose nuclei, and assume the shape of flattening sacs full of keratin, forming 15–30 layers of dead cells. Terminal differentiation is a slow process that requires many newly synthesized proteins in all layers of the epidermis [13].

The plane of union between epidermis and dermis is smooth until early in the fourth month when epidermal thickenings grow down into the dermis of the palm and sole. About 2 months later, corresponding elevations first appear on the skin surface. These epidermal ridges complete their permanent, individual patterns in the second half of fetal life [28].

The Regulation of Epidermal Development

The process during which the unspecified surface ectoderm adopts an epidermal fate is defined as epidermal specification [16].

Generally, keratinocytes take about 4 weeks to pass from the germinal layer to the outside of the body, and the epidermis structure depends on both their proliferation rate and differentiation processes. The fine balance among proliferation and differentiation is regulated by a complex system of interaction between many growth factors (Table 1.1). Some of these stimulate keratinocyte proliferation (epidermal growth factor, fibroblast growth factor, transforming growth factor-α, insulin, and interleukins), whereas others inhibit it (transforming growth factor-β1 and transforming growth factor-β2, interferons, and tumor growth factor) [1]. These pathways may be variably activated, both spatially and temporally, leading to a diverse series of transcribed genes [4].

TGF-α is made by the basal cells and stimulates their own division. If the TGF-α gene is linked to a promoter for keratin 14 (one of the major skin proteins expressed in the basal cells) and inserted into the mouse pronucleus, the resulting transgenic mice activate the TGF-α gene in their skin cells and cannot downregulate it. The result is a mouse with scaly skin, stunted hair growth, and an enormous surplus of keratinized epidermis over its single layer of basal layer [19, 29].

Table 1.1 Main growth factors involved in proliferation and differentiation of keratinocytes

Proliferative	Anti-proliferative
EGF	TGFβ1
FGF	TGFβ2
TGF-α	Interferons
Insulin	Tumor growth factor
Interleukins	NF-κB
KGF	

Another growth factor needed for epidermal development is keratinocyte growth factor (KGF), a paracrine factor produced by the fibroblasts of the underlying dermis. KGF is received by the basal cells of the epidermis and probably regulates their proliferation. If the gene encoding KGF is fused with keratin 14 promoter, the KGF becomes an autocrine factor in the transgenic mice. These mice have a thickened epidermis, baggy skin, too many basal cells, and no hair follicles, not even whisker follicles [19, 30].

Many studies have demonstrated that the dermis provides the initial signals required for epidermal specification [16]. In vertebrates, the acquisition of the epidermal fate is associated with the induction of p63 expression, which is the first transcription factor to be specified for the epidermal lineage [16, 31–36]. It has been demonstrated that mice that are deficient for *p63* gene function have truncated or absent limbs, poorly developed skin, and die shortly after birth, presumably due to dehydration [13, 37, 38]. p63 is involved in the development of the embryonic basal layer (Fig. 1.4a). In the epidermis, at least six p63 isoforms are expressed, which fall into two categories: those that encode proteins with an amino-terminal transactivation domain (TA isoforms) and those that encode proteins that lack this domain (ΔN isoforms) [13]. Among the TA or ΔN isoforms, alternative splicing gives rise to three different carboxyl termini that are associated with the designations of α,

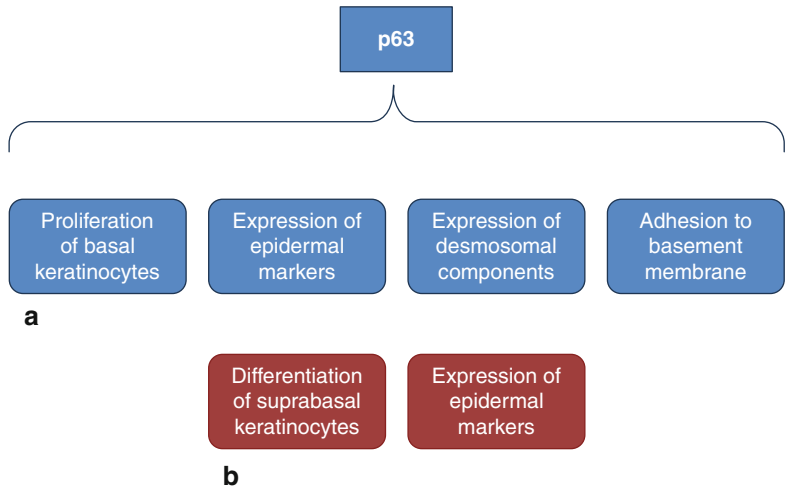

Fig. 1.4 Roles of p63 in the development of the epidermis. (**a**) p63 is involved in several processes important for the formation of the embryonic basal layer; (**b**) p63 isoforms also control terminal differentiation of keratinocytes (See more details in the text)

β, and γ [13, 39]. Regulation by p63 involves an intricate interplay between various p63 isoforms [13, 20]. Specifically, TAp63 isoforms are detected prior to the commitment to stratify and strongly localize to the nucleus, indicating that they may be required to initiate the epithelial stratification program in the developing embryo [13]. Particularly, the TAp63α isoform induces expression of AP-2γ, a transcription factor implicated in the regulation of K5 and K14 expression during epidermal morphogenesis [6, 16, 23, 36, 40–43]. Other than K14 expression, the epidermal fate is also associated to the expression of desmosomal components, important for cell-cell adhesion within the epidermis [18, 44] (Fig. 1.4a). One desmosomal component directly involved by p63 in the embryonic basal layer is Perp [6, 16, 45]. Moreover p63 is important for regulating the adhesion of keratinocytes to the basement membrane which is mediated by integrins, a family of transmembrane receptors for the basement membrane protein laminin [6, 46]. Specifically, it has been demonstrated that p63 induces expression of several integrin subunits, such as integrin α3 [6, 47, 48]. Furthermore, p63 controls basement membrane formation by directly inducing the expression of the basement membrane component Fras1 [6, 18].

The basal layer other producing keratinocytes that undergo terminal differentiation provides the epidermis with mechanical stability and barrier function (Fig. 1.4b). To this purpose, some keratinocytes terminally differentiated are ultimately sloughed off, whereas other cells which can continuously supply terminally differentiating keratinocytes must be maintained for the life of the organism [16]. In order to prevent premature terminal differentiation, basal keratinocytes must repress the expression of genes that initiate this process and, at the same time, must induce and maintain the expression of genes required for proliferation and K5/K14 expression [16]. It has been demonstrated that ΔNp63α can directly induce K14 expression, and it is probably involved in maintenance of K14 expression in keratinocytes [16, 43, 49]. Moreover, ΔNp63α is able to maintain keratinocyte proliferation by directly inhibiting the expression of two genes induced during epidermal terminal differentiation: $p21^{WAF/Cip1}$, a member of the cyclin-dependent kinase inhibitor (CKI) family [13, 50], and 14-3-3σ, a member of 14-3-3 family of intracellular signaling proteins [13, 16, 51–57]. ΔNp63α binds directly to the $p21^{WAF/Cip1}$ and 14-3-3σ promoters [55], so inhibiting their transcription and then favoring epidermal cell proliferation [13]. In addition, ΔNp63α inhibits p21 expression by preventing Notch signaling, an upstream regulator of p21 in the epidermis [16, 57–59]. Moreover, it has been hypothesized that Notch signaling is involved not only in growth arrest but also in cornification [13]. In fact, it has been

demonstrated that addition of JAG-1 to human keratinocytes lift cultures resulted in Notch activation; strong induction of loricrin, involucrin, and peroxisome proliferator-activated receptor-γ (PPARγ); and cornified envelope formation [13, 60] other than in enhanced levels of nuclear NF-κB [13, 61]. Furthermore, p63 also represses two cell cycle inhibitors Ink4a and Arf [6, 62] as well as induces the expression of genes required for cell cycle progression, including ADA and FASN [6, 55, 62–66]. Ink4a regulates cell cycle arrest by blocking phosphorylation of Rb family members, thereby maintaining them in their anti-proliferative states [20, 67]. On the other hand, it has been demonstrated that p63 is able to directly repress the expression of genes required for cell cycle progression including cyclin B2 and cdc2 [6, 16, 68]. This apparent controversy may be explained by supposing that p63 functions to maintain proliferation in early transit amplifying (TA) cells, whereas it acts inducing cell cycle exit in mature TA cells [6, 16]. In fact, it has been demonstrated that p63 directly induces $p57^{Kip2}$, a cyclin-dependent kinase inhibitor, in order to allow cell cycle exit and undergo terminal differentiation [6, 69, 70]. Specifically, whereas TAp63 isoforms inhibit terminal differentiation [36], ΔNp63 isoforms are first expressed after the single-layered epidermis has committed to stratification [13]. In vivo studies using transgenic mice suggest that one function of ΔNp63α during early epidermal morphogenesis is to counterbalance the effects of TAp63-induced inhibition of differentiation, allowing cells to respond to terminal differentiation program [13, 36]. ΔNp63α may block TAp63 isoforms directly, via a dominant-negative action of TAp63 [13]. As reported for p63 and Notch, another molecule involved in the switch from proliferation to growth arrest is NF-κB [13, 71]. In normal epidermis, NF-κB proteins localize in the cytoplasm of basal cells and then in the nuclei of suprabasal cells [13, 71]. Particularly, it has been shown that NF-κB in association with selective induction of $p21^{WAF/Cip1}$ induces growth arrest [13, 72]. As p63, also NF-κB acts by downregulating molecules that promote cell proliferation [13]. Specifically, during epidermal development, NF-κB functions by opposing the proliferative activity of TNFR1/JNK [13].

In humans, the earliest morphological sign of stratification during epidermal development is the formation of the intermediate cell layer [14, 16, 73]. The intermediate keratinocytes, which proliferate and express K1, populate the first suprabasal layer of the embryonic epidermis [14–16, 18]. ΔNp63α is the only transcription factor known to be required for the formation of the intermediate layer [16]. In fact, ΔNp63α synergizes with Notch to induce K1 expression [16, 57].

At the same time, it has been shown that ΔNp63α expression is reduced to approximately 25 % in suprabasal keratinocytes with respect to its high expression in basal keratinocytes [6, 74]. Its rapid downregulation in suprabasal keratinocytes is mediated by several processes: first, ΔNp63α transcripts are degraded by micro RNA-203 [6, 75, 76] which is expressed only in suprabasal keratinocytes [6, 75–77]; second, ΔNp63α protein is also actively degraded in suprabasal keratinocytes through the proteosomal pathways by the E3 ubiquitin ligase Itch and $p14^{Arf}$ [6, 78, 79].

The intermediate cell layer exists only transiently, since intermediate cells are replaced by postmitotic keratinocytes that form spinous layer during later developmental stages [14, 16]. Histological analysis has demonstrated that intermediate cells mature directly into spinous cells [16, 18, 73]. Despite ΔNp63α active degradation in suprabasal cell layers, the remaining protein is sufficient to control important aspects of keratinocytes differentiation and particularly seems to be involved also in this process. In fact, ΔNp63α critical target gene is IKKα [18], a previously identified regulator of epidermal, skeletal, and craniofacial morphogenesis [16, 80–82] which is a critical mediator of cell cycle exit during keratinocyte differentiation [6, 18, 83]. Intriguingly, the failure of intermediate cells to mature into spinous cells, lacking IKKα, also resulted in the aborted development of epithelial appendages and limbs [16, 84].

An important trigger of epidermal terminal differentiation is an increase in extracellular Ca^{2+}

concentration, which is involved in regulating the formation of the spinous layer, granular layer, and epidermal barrier [16]. A Ca^{2+} gradient is first established in utero, and in mature epidermis, an increasing gradient of extracellular Ca^{2+} is present from basal to the cornified layers [16, 85–87]. Specifically, protein kinase C (PKC) is activated during keratinocyte differentiation [16, 88]. During terminal differentiation, PKC proteins appear to function specifically in the transition from spinous to granular cells, by contributing to downregulate K1 and K10 expression [16, 89], a process that is associated with the transition from spinous to granular cells [27, 90]. In addition, PKC activation induces expression of loricrin, filaggrin, and transglutaminase, markers of granular keratinocytes [16, 89, 91].

The final step in epidermal stratification involves the formation of the epidermal barrier [16]. The best-studied transcription factor involved in the regulation of epidermal barrier formation is Klf4 which is expressed in the upper spinous and granular layers [16, 92, 93]. Another transcription factor implicated in this process is Grhl3/Get1 [94, 95] which downregulates many genes involved in lipid synthesis and metabolism [16]. Finally, it has been demonstrated that ΔNp63α is also important for the formation of the epidermal barrier by inducing at least two genes required for barrier formation: Claudin 1 and Alox12 [6, 96, 97]. Moreover, several in vivo and in vitro studies indicated that PPAR activators accelerate differentiation and cornification in fetal and adult epidermis [98–101]. It has been demonstrated that Notch signaling may be involved also in these processes. In fact, Notch may function upstream of PPARs to induce terminal differentiation [13]. Moreover, Notch also induces the caspase 3 in order to promote terminal differentiation during embryonic development of epidermis [13].

Dermis Development

The dermis is the skin layer under the epidermis. It arises from mesenchymal cells that come in part by somatopleure and, in lower size, by dermatomes. In the face and in wide regions of the neck, instead, the dermis arises from cells that migrate from neural crest of the skull [1]. Initially, dermis cells form several junctions between their cytoplasmic extensions and originate the *primordial dermis*, characterized by high cell density and by aqueous extracellular matrix, rich in glycogen and hyaluronic acid [1]. This primordial dermis changes into the *mature* or *definitive dermis*, during the third month, when a great part of its mesenchymal cells differentiate in fibroblasts, which secrete a fibrous extracellular matrix, formed by collagen fibers (especially collagen I and IV) and by elastic fibers [1]. This change gives dermis both a high resistance and notable resilience, allowing it to carry out one of its main functions: to give a physical stable support to the epidermis, essential to establish an effective protective barrier [1]. Other than to provide physical support, the dermis nourishes the epidermis, which is not vascularized. This function is allowed by the development of dense network of blood vessels in the stroma of the dermis [1]. Always during the third month, the surface that divides the dermis from the epidermis loses its primordial flatten shape and becomes highly wavy by the formation of crests and depressions that form very complicate drawing (dermatoglyphics). These waves originate both by epidermis ridges and by dermis eversions (dermal papillae). Interdigitations between ridges and papillae markedly increase adhesion of the epidermis to the dermis, especially in areas exposed to high mechanical efforts [1]. The first visible lines on the skin appear at the end of the third month in the hands and feet (forming fingerprints), and after several weeks, they cover the whole body surface, with drawing that varies from one side to the other [1]. From the fifth month, each region of the skin is marked by a specific network of lines and keeps this configuration also if it is transplanted elsewhere [1]. Formation of dermal papillae divide the dermis in two layers. An upper layer, close to the epidermis, is called papillary layer. It is a thin sheet that maintains many features of the looser connective tissue which forms the primordial dermis [1]. The deeper layer, instead, is much more thick and is called reticular layer, since here the collagen fibers assume an

arrangement at weave, or at a reticulum (network), that characterizes the compact connective tissue [1]. The reticular layer of the dermis, in its turn, is close to the hypoderm, or subcutaneous layer, the region which is addressed to become the main fatty storage of the body. Other than by the formation of connective fibers and blood vessels, the dermis development is characterized by appearance, in its stroma, of tactile receptors (Meissner, Pacinian, and Ruffini corpuscles), nerve endings (free or encapsulated), and skin adnexa (hairs and glands) [1].

Structure and Ultrastructure of Adult Epidermis

The epidermis is a complex, terminally differentiated, stratified squamous epithelium formed by one basal and several suprabasal layers of keratinocytes, which provides barrier functions to the skin [2]. The epidermis can be divided into four distinct layers: stratum basale or stratum germinativum, stratum spinosum, stratum granulosum, and stratum corneum [4] (Fig. 1.5). In palmoplantar skin, there is an additional zone, also electron lucent, the stratum lucidum between the granulosum and corneum [4]. Each epidermal layer contains keratinocytes at various stages of differentiation and proliferative potential [2, 102].

The stratum basale is a continuous layer directly in contact with a basement membrane. It is generally described as only one cell thick, but may be two to three cells thick in glabrous skin and hyperproliferative epidermis. The basal cells are small and cuboidal and have large nuclei that vary from euchromatic (in stem cells and in young keratinocytes) to eterochromatic (in older keratinocytes), dense cytoplasm containing many free polyribosomes, mitochondria, dense tonofilament bundles [4], included actin microfilaments and keratin filament bundles, and melanin granules [3]. Moreover, basal keratinocytes specifically express K5 and K14 [103]. The basal cells are linked to basement membrane through hemidesmosomes. Almost always, basal surfaces of basal cells are many pleated, interacting with basement membrane projections [3]. The basal layer is composed by dividing cells that give rise to several upper layers where keratinocytes progressively differentiate [4]. Close to the basal layer, the epibasal keratinocytes enlarge to form the spinous/prickle cell layer or stratum spinosum. This stratum contains more mature keratinocytes, disposed in more layers and closely packed. These cells show interacting surfaces through projections and indentations and

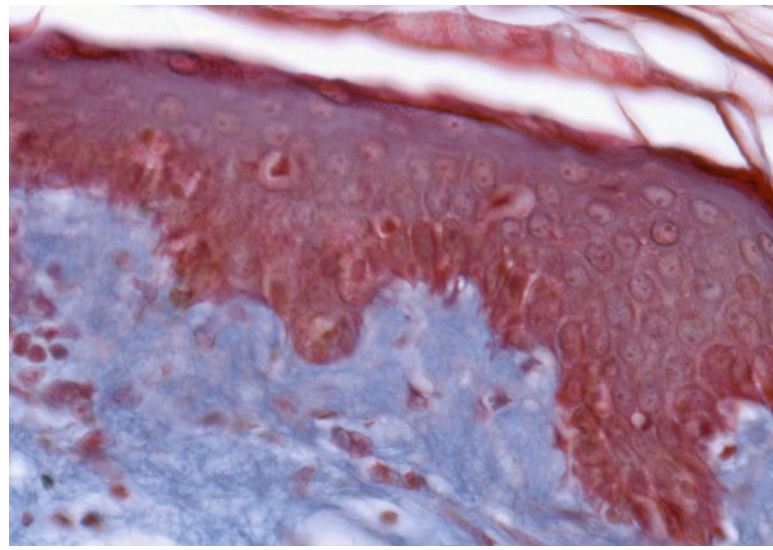

Fig. 1.5 Histological section of the mature adult epidermis. Mallory's trichrome stain. Original magnification 40×

are interconnected with each other through desmosomes. In their cytoplasm, there are many abundant keratin bundles integrated with desmosomes. This feature allows a strong cohesion to the epidermis and a high strength to traction [3]. The suprabasal layer (spinous) cells express K1 and K10 [103]. The cytoplasm of the cells of the stratum spinosum show moderately euchromatic nuclei, with large nucleoli, numerous polyribosomes, and typical vacuoles containing melanin granules (melanosomes) [3]. The melanin comes from melanocytes of the epidermis. Melanin granules are more numerous in the deeper part of stratum spinosum and progressively are degraded by keratinocytes, so that they lack in the superficial part of the stratum [3]. The stratum spinosum is succeeded by the stratum granulosum or granular layer, so called for the presence of the intracellular granules of keratohyalin inside keratinocytes cytoplasm [4]. At high magnification, the dense mass of keratohyalin granules from human epidermis has a particulate substructure, with particles of irregular shape on average 2 nm length and occurring randomly in rows or lattices [4, 104]. The granular cells also contain a highly phosphorylated protein, rich in histidine, called basic profilaggrin [3]. The cytoplasm of the cells of the upper, spinous layer and granular cell layer also contains smaller lamellated granules averaging 100–300 nm in size, which are known as lamellar granules or bodies, membrane-coating granules, or Odland bodies [4, 105]. These are numerous within the uppermost cells of the spinous layer and migrate toward the periphery of the cells as they enter the granular cell layer. They discharge their lipid components into the intercellular space, playing important roles in barrier function and intercellular cohesion within the stratum corneum [4]. The granular cells contain pyknotic nuclei and show degenerated organelles [3]. Granular cells also express and produce filaggrin, loricrin, and transglutaminase 3 (TG3) [103, 106].

In the palmoplantar region, the cells forming the stratum lucidum are still nucleated and may be referred to as "transitional" cells [4]. The outermost layer of epidermis is the stratum corneum where cells, now called corneocytes, have lost nuclei and cytoplasmic organelles. The cells become flattened and the keratin filaments align into disulphide cross-linked macrofibers, under the influence of filaggrin, the protein component of the keratohyalin granule, responsible for keratin filament aggregation [107]. The corneocyte has a highly insoluble cornified envelope within the plasma membrane, formed by cross-linking of the soluble protein precursor, involucrin [108], following the action of a specific epidermis transglutaminase also synthesized in the high stratum spinosum [109]. The process of desquamation involves degradation of the lamellated lipid in the intercellular spaces and loss of the residual intercellular desmosomal interconnections. The corneocytes which protect the viable cell layers are continually shed from the skin surface, and the rate of production of cells in the basal layer must match the rate of loss from the surface to produce the normal skin thickness, although increased rates of loss and cell division occur in pathological states [4].

Within the epidermis, there are several other cell populations, namely, melanocytes, which donate pigment to the keratinocytes; Langerhans' cells, which have immunological functions; and Merkel cells [4].

Structure and Ultrastructure of Adult Dermis

The dermis is composed of dense connective tissue at interlaced bundles formed by cells scattered in a supporting matrix (Fig. 1.6). The dermis consists of the following: (a) retiform interlacement of collagen fiber bundles, with a varying amount of elastic fibers; (b) a matrix containing proteoglycans, fibronectin, and other typical element of the matrix; (c) blood vessels; (d) lymphatic vessels; and (e) nerve endings. The matrix is constituted by proteins and polysaccharides linked to each other to compose macromolecules that provide a remarkable capacity for retaining water [4]. Inside the matrix, there are two kinds of protein fibers: collagen, the major constituent of the dermis, which has great tensile strength, and elastin, which makes up only a small proportion of the