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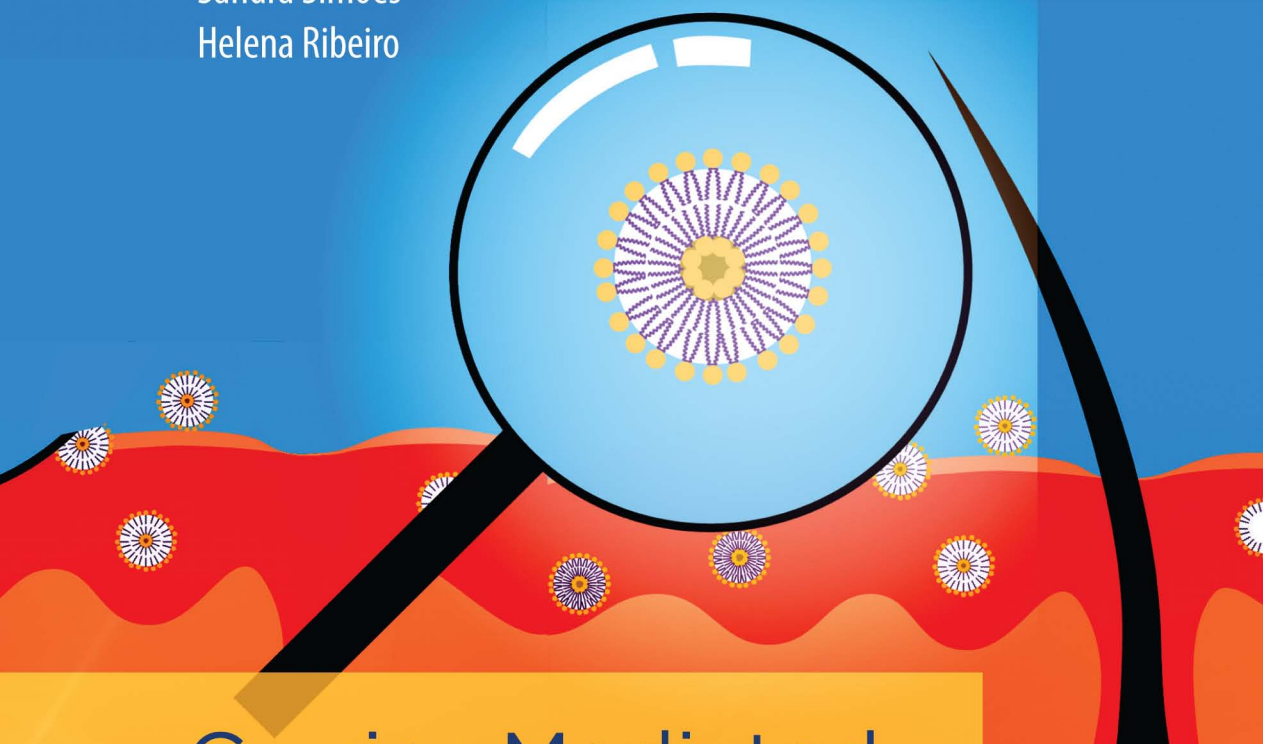
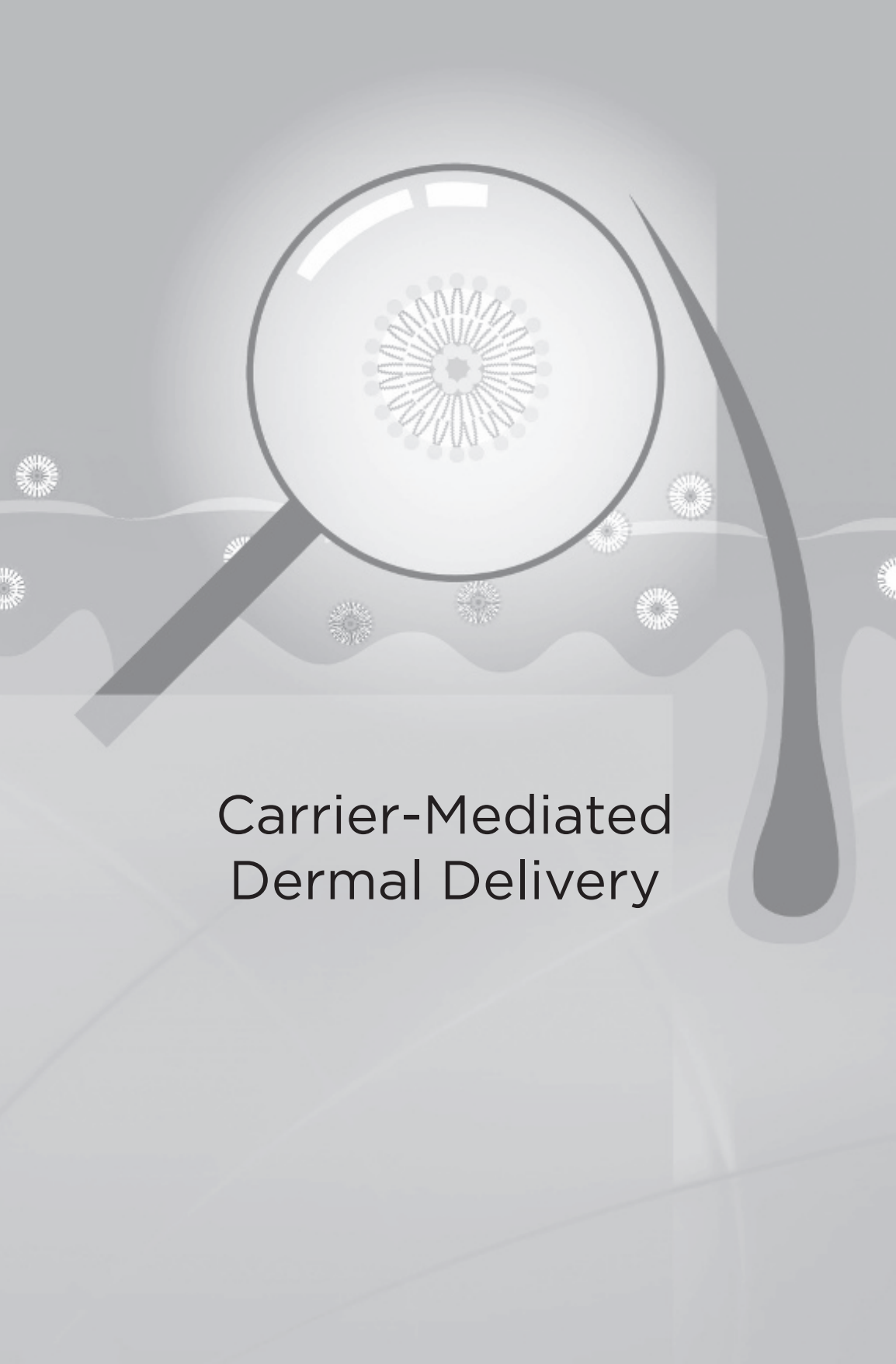


Carrier-Mediated Dermal Delivery

Applications in the Prevention and Treatment of Skin Disorders



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Contents

Preface

We are honored to present to the readers the book entitled *Carrier-Mediated Dermal Delivery: Applications in the Prevention and Treatment of Skin Disorders*, which is based on the systematic revision of the most recent findings. This book contains several contributions on new approaches for the management of skin aging and photocarcinogenesis and topical formulations based on nanocarrier systems for skin disorders. These chapters discuss the structure and skin morphology in detail. Cosmeceuticals, laser and photodynamic therapy, and melatonin-based treatments are presented as important strategies for photoaging management. Photodynamic therapy and melatonin can be also used in the context of photocarcinogenesis. Therefore, the inclusion of this strong antioxidant in sunscreen products could be a promising approach. The book discusses the safety and efficacy of sunscreen products as well.

Topical formulations, including emulsions (conventional formulations and emulsions stabilized by solid particles), nail films, and nanocarriers used for different actives delivery, are reviewed concerning certain skin and nail diseases context (e.g., acne, psoriasis, atopic dermatitis, fungal diseases, leishmaniasis, skin cancer). Finally, several nanocarriers are introduced, such as lipid vesicles (from the first generation of conventional liposomes until the more recent deformable vesicles), liquid crystalline nanodispersions, and gelatin and solid lipid nanoparticles. Their composition, formulation process, characterization, and examples of topical applications are discussed in detail for each system. In fact, these nanocarrier systems can be useful as topical and/or transdermal delivery systems attending to a higher skin penetration and permeation profiles, besides contributing to improving technological drawbacks (e.g., formulation stability) and increasing the therapeutic index.

Although this is a quite broad topic, the most important (nano)pharmaceutical formulations are presented in the book. Future perspectives are also discussed in some chapters. This book will be a useful reference for researchers and professionals interested in nanotechnology in the skin delivery context.

Part 1

New Approaches for Management of Skin Aging and Photocarcinogenesis

Chapter 1

New Trends in Anti-Aging Skin Care

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1.1 Introduction

Life expectancy has never been so high in developed countries according to the World Health Organization. Nowadays, the desire to maintain a young image and healthy appearance is omnipresent in both genders. Skin aging is generally the main concern with a marked social impact. In fact, the skin properties and functions based on maintaining the organism homeostasis and protecting it from external harmful agents [1–3] will decrease over time.

Several techniques have been developed that aim to prevent, slow, or revert the skin aging process with diverse successful outcomes alone or in combination with others. Those techniques can be surgical, such as eyelid surgery and facial lifting or non-surgical procedures, for example, botulinum toxin injection, filler injection, laser treatment, dermabrasion, and chemical peelings [3].

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Some of these non-surgical techniques will be mentioned in this review, especially injectable and laser-based treatments.

Although the skin nervous system has not been fully understood and somewhat underexplored, the possibility of manipulating neuropeptides to improve the skin appearance by delaying or treating its aging has becoming a recent research area with a significant potential. On the other hand, laser therapy is an excellent and safe technique. Both advantages and limitations of these techniques will be addressed as well as future perspectives in this context.

1.2 Skin

The integumentary system includes the skin and accessory structures (hair, nails, and glands). The skin or the integument covers the body externally, being its largest organ (about 15% to 20% of total body weight in adults). It possesses several functions, such as (i) **protective,** as a physical barrier against potential pathogens and chemicals as well as thermal, mechanical, and ultraviolet radiation damages; (ii) **sensorial,** as sensory skin receptors are able to detect heat, cold, pressure, touch, and pain, allowing skin to monitor the environment and regulate interactions with it; (iii) **thermoregulatory,** through water loss mechanisms or insulation ability; (iv) **endocrine** and **metabolic,** by secreting hormones, cytokines, and growth factors and synthesizing vitamin D (indispensable for calcium metabolism and bone formation); (v) **excretory,** through the skin pores and glands [4–6].

1.2.1 Structure

The skin is formed by three main layers: the epidermis, an epithelial layer of ectodermal origin; the dermis, a layer of mesodermal connective tissue; and the hypodermis, or subcutaneous tissue, a layer of loose connective tissue that connects the skin to the muscles or bones. Additionally, skin can have different thickness, being thick on the palms and soles and thin on the rest of the body [4–6].

1.2.1.1 Epidermis

Epidermis, the most superficial layer of the skin, is mainly formed by stratified squamous keratinized epithelial tissue. The living cells receive nutrients and excrete waste products by diffusion through the epidermis layer and the capillaries of the dermis [4, 5].

Different types of cells are present in the epidermis layer:

- **Keratinocytes,** which produce keratin, a protein that contributes for cells strength;
- **Melanocytes**, the melanin producers. These cells are accumulated in the *stratum basale* (1 melanocyte for every 5–6 basal keratinocytes), being attached to the basal lamina. Melanin synthesis starts with a reaction catalyzed by tyrosinase that converts tyrosine into 3,4-dihydroxyphenylalanine (DOPA) that will be transformed and polymerized in different forms of melanin. Melanin is stored in cell vesicles until they form melanosomes that are transported and taken up by keratinocytes. This molecule will protect the DNA (deoxyribonucleic acid) of living cells from the ionizing mutagenic effects of ultraviolet radiation (UVR);
- **Langerhans cells**, the antigen-presenting cells with an important role in the skin immune system. These cells are mostly present in the *stratum spinosum*;
- **Merkel cells,** which are tactile epithelial cells associated with the nerve endings, being responsible for perceiving light, touch, and superficial pressure. These cells are present in a higher percentage in the sensitive skin [4, 5].

Regarding the **epidermis renewal**, new keratinocytes are produced in the deepest layer of epidermis, pushing the older cells to the surface, and changing their shape and chemical composition along the way (keratinization process). During this process, the cells will die and the outer layer of dead cells will provide resistance to abrasion, forming a barrier with relative permeability [4].

The human epidermis is renewed in 15 to 30 days, depending on several factors (age, region of the body, etc.). During the process of keratinization, the number of cell layers in each

stratum is influenced by the body location. The deepest stratum, **stratum basale** or **germinativum**, rest on the basal lamina and is formed by a single layer of keratinocyte steam cells (basophilic cuboidal or columnar cells) that go through mitotic divisions. **Hemidesmosomes** join these cells to the basal lamina and **desmosomes** bind the cells together on their lateral and upper surfaces. The **stratum spinosum**, or the spinous layer, lies on the stratum basale. Additional keratin fibers and lamellar bodies are formed inside the larger and polyhedral keratinocytes of this layer. Following these two strata, there is the **stratum granulosum** formed by flattened diamond-shaped cells. Keratohyalin granules are produced in this layer. These granules are basophilic masses rich in cystine and histidine proteins, the precursors of filaggrin, which aggregates the keratin filaments on the stratum corneum (SC). It also facilitates the lipids' release by lamellar bodies from the cells creating an impermeable layer around it. At this stage, the cells die. The **stratum lucidum** is above the stratum granulosum being constituted by some layers of dead cells with indistinct borders. It is normally present in thick skin and absent in thin skin. The most superficial layer is the **stratum corneum** formed by dead cells, which have a hard protein envelope filled with keratin (a mixture of keratin fibers and keratohyalin) providing structural strength. The released lipids from lamellar bodies are responsible for the skin permeability. At the end of the keratinization process, the cells are fully keratinized or cornified (squames) and shed from the epidermal surface as the desmosomes and lipid-rich cell envelopes break down [4–6].

The **basement membrane** or **basal lamina** separates the epidermis from the dermis. This irregular junction and projections (dermal papillae) merge with invaginating epidermal ridges, which contribute to a stronger adhesion of these two layers [4, 5].

1.2.1.2 Dermis

The **dermis** provides most of the structural strength of the skin. Its connective tissue is formed by collagen, elastic and reticular fibers. Several cells and structures can be found in dermis, such as fibroblasts/fibrocytes/myofibroblasts, adipocytes, macrophages, monocytes, mast cells, Langerhans cells, T

lymphocytes, dendritic cells, nerve endings, hair follicles, smooth muscle cells, and glands, besides the presence of blood and lymphatic vessels. It is divided into two sub-layers: (i) the **papillary layer**, which includes the dermal papillae, made up of loose connective tissue with collagen types I/III and collagen type VII, which connects the dermis to the epidermis fibrils and to the basal lamina, respectively; (ii) the **reticular layer,** which is a dense and irregular connective tissue mainly constituted by elastic fibers (more fibers than cells compared with the papillary layer) and collagen type I surrounded by proteoglycans rich in dermatan sulfate. Dermis thickness differs with the region of the body, reaching a maximum of 4 mm on the back [4–7].

Two major plexuses are formed by nutritive vessels on the dermis. The **microvascular subpapillary plexus** lies between the papillary and reticular dermis layers, and its extensions form a capillary matrix below the epidermis. The **deep plexus** lies between the dermis and the subcutaneous layer and is formed by larger vessels. These plexuses are connected by blood vessels [5, 7].

1.2.2 Innervation

The skin innervation is represented by a neural matrix formed by cholinergic and adrenergic nerves and sensory fibers. This dense innervation extends to the superficial layers of the epidermis, including the stratum corneum. Immunolabeling (with an antibody against protein gene product 9.5) is the best method for the visualization of skin innervation [8]. The skin possesses an enormous diversity of receptors, channels, neurotransmitters, and modulators, including various types of sensory receptors, motor nerve endings at the blood vessels, pili muscles, and sweat glands. Nevertheless, there is also contact between nerve fibers and keratinocytes, melanocytes, Langerhans cells, mastocytes, and dendritic and endothelial cells. The nerve endings differ within different body parts, reflecting their distinct functions [6–9].

1.2.2.1 Sensory receptors

Sensory receptors transduce and transmit pain (nocireception) and can be free or encapsulated nerve endings (or fibers). Sensory axons are morphologically categorized as myelinated (A-fibers)

or unmyelinated (C-fibers). Myelination degree increases the axonal signal transmission velocity. A-α, -β, and -δ fibers are highly, moderately, and poorly myelinated with a conduction velocity ranging from 70–120 m/s (highly myelinated) to 4–30 m/s (poorly myelinated). C-Fibers are unmyelinated and small fibers having a low conducting velocity, approximately 0.5–2 m/s [4, 5, 7, 8, 10].

Free nerve endings include (i) *Merkel cells,* which are associated with the recognition of continuous light touch and texture, consisting of A-fibers; (ii) *nerve terminations* in the papillary dermis, also extended to lower epidermal layers; these nerve terminations respond to extreme temperatures, pain and itching, consisting in A-fibers and C-fibers; (iii) *root hair fibers complex* localized around the hair follicles bases and *Haarschiebe touch domes*; these last structures are specialized in pressure reception, localized near the hair follicles and their neurite plexus, containing both A and C-fibers [4–6, 10].

Encapsulated nerve endings comprise (i) *Meissner corpuscles*, which are sensory axons (A and C-fibers) winding among Schwann cells located in the dermal papillae, mainly responsible for perceiving light touch or low frequency stimuli; their number decreases gradually after puberty; (ii) *Pacinian corpuscles*, which are *myelinated nerve endings (A-fibers)* encircled by a capsule (formed by flattened Schwann cells and collagen) and deeply located in the reticular dermis and hypodermis. It mostly detects pressure changes and vibrations applied on the skin; (iii) *Rufinni or bulbous corpuscles*, which surround A-fibers, have a thin collagenous fusiform capsule anchored to the surrounding connective tissue. It typically responds to tension and torque applied to the skin and intravenous; (iv) *Krause end bulbs* that have a very thin collagenous capsule penetrated by a nerve A-fiber, being generally associated with low frequency vibrations [4–6, 10].

1.2.2.2 Non-sensory receptors

Non-sensory receptors are expressed in **diverse types of skin cells**, and their signal transduction lead to diverse manifestations. Some non-sensory receptors are as follows:

- **Cholinergic receptors**, found in sweat glands and ducts, keratinocytes, sebocytes, fibroblasts and melanocytes. Their

activation on keratinocytes stimulates adhesion, motility and differentiation. Furthermore, acetylcholine may be a promoter in sebocytes differentiation, sebum production and hyperpigmentation. In addition to the presence of the cholinergic receptors in a non-neuronal manner, cholinergic neurons (sympathetic and parasympathetic) supply innervation to blood and lymphatic vessels, pili muscles, hair follicles and glands play a crucial role in the regulation of the body temperature [7, 10–12].

- **Adrenergic receptors** of several α and β subtypes and are expressed in melanocytes, keratinocytes, and eccrine epithelial cells. Their activation leads to keratinocyte differentiation stimulation; α and β receptors are found in dermal blood vessels, and their stimulation leads to vasoconstriction and decreasing of the vascular permeability [7, 10].
- **Corticotropin-releasing hormone (CRH) and urocortin receptors** expressed in dermal fibroblasts, endothelial cells, hair follicle, smooth muscle of blood vessels, keratinocytes, melanocytes, and mast cells. CRH can act as a pro- or anti-inflammatory agent, and antinociceptive and wound-healing accelerator. Urocortin and CRH are able to inhibit the proliferation of keratinocytes and induce their differentiation as well as stimulate or inhibit melanoma cell proliferation depending on culture conditions [7, 10, 13].
- **Melanocortin receptors (MCR)** activated by the adrenocorticotropic hormone (ACTH) and some melanocyte-stimulating hormones (MSH). These receptors have been detected in melanocytes, keratinocytes, monocytes, sebocytes, fibroblasts, and endothelial, epithelial, Langerhans, and dermal immune cells. They are responsible for differences in skin color. The stimulation of melanogenesis and its switching to eumelanogenesis from pheomelanogenesis is the most recognized phenotypic effect of ACTH and MSH peptides [7, 10].
- **Opioid receptors**, m-opioid receptors identified in keratinocytes, hair follicles, sebaceous glands and sweat glands, and z-opioid receptors identified in epidermal keratinocytes. Opioid peptides can be divided into endorphins, enkephalins, and dynorphins. Generally,

the activation of opioid receptors leads to inhibition of neuronal excitability. Met- and leu-enkephalins can inhibit the differentiation of human keratinocytes *in vitro*. β-Endorphin and enkephalins have antinociceptive and immunomodulatory properties [7, 10].

- **Growth hormone receptor (GHR)** detected in the human skin epidermis, hair follicle, eccrine glands, dermal fibroblasts, keratinocytes, adipocytes, melanocytes, and in Schwann and muscle cells. GH may directly modulate keratinocyte and fibroblast function [7, 14].
- **Prolactin (PRL) and luteinizing hormone/chorionadotropin receptors (LH/CG-R)** also expressed in the skin. In humans, hyperprolactinemia has been associated with hirsutism [7].
- **Neurokinin receptors (NKR)** activated by substance P (SP) or neurokinins A and B (NKA and NKB), leading to the stimulation of keratinocytes, fibroblasts, and endothelial cell proliferation and neovascularization. In addition, they are also related with the modulation of pro-inflammatory processes. Their expression has been detected in keratinocytes, endothelial cells, mast cells, fibroblasts, and Merkel and Langerhans cells [7, 10].
- **Calcitonin gene-related peptide receptors (CGRP-R)** present in mast cells, keratinocytes, melanocytes, smooth muscle, blood vessels, and Merkel and Langerhans cells. CGRP has several functions, such as increasing the vascular permeability, producing dermal edema, stimulating endothelial cell, keratinocyte, and melanocyte proliferation, and regulating cytokine production [7, 10].
- **Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide receptors (PACAP/VIP-R or PVR)** found in sweat glands, keratinocytes, smooth muscle, and immune and endothelial cells. VIP stimulates the keratinocyte proliferation and sweat production, while PACAP is correlated with inflammation and neurotransmission in the skin. Through these receptors, peptide histidine-methionine (PHM) and GH-releasing factor (GFR) lead to keratinocytes proliferation [7, 10].
- **Neurotrophin (NT) receptors**, which are receptor proteins of the tyrosine kinase (Trk) and p75 panneurotrophin

(p75NTR) families expressed in epidermal and follicular keratinocytes, epidermal melanocytes, specialized dermal fibroblasts, mast cells, immunocytes, and cutaneous sensorial nerves. Nerve growth factor (NGF) stimulates dendrite formation. NTs take part in apoptosis, cutaneous nerve growth, and development [7, 10].

- **Parathyroid hormone (PTH) and/or PTH-related protein (PTHrP)** receptors are expressed in dermal fibroblasts. When stimulated, these receptors respond by producing cytokine and keratinocyte growth factor (KGF) [7].
- **Receptors for the active form of vitamin D [1,25-dihydroxyvitamin D (1,25-(OH)2D or calcitriol] (vitamin D receptor (VDR))** expressed in epidermal and follicular keratinocytes. Epidermal synthesis of vitamin D3 from 7-dehydrocholesterol is dependent on sun exposure [15]. Vitamin D is involved in keratinocyte differentiation, cell proliferation, and hair growth [7]. Additionally, it has a significant role in calcium homeostasis and metabolism, as is well known.
- **Glucocorticoid receptors (GR)** expressed in epidermal and follicular keratinocytes, epithelial cells from glands, sebocytes, melanocytes, immune cells, dermal fibroblasts, and smooth muscle, while **mineralocorticoid receptors (MR)** have been identified in epidermal keratinocytes, hair follicle, and sweat and sebaceous glands. They are linked to those cell functions exerting anti-inflammatory effects [7, 16].
- **Androgen (AR) and estrogen (ER) receptors** disseminated in the skin. AR promotes hair growth and sebum secretion depending on their location. ER are located in epidermal epithelial cells, hair follicle, sebaceous, glands, melanocytes, and fibroblasts [7].
- **Thyroid hormone receptors (THR)** activated by triiodothyronine (T3), which participates in the epidermal differentiation, increasing its response to growth factors. THR also has a role in the functioning of sebaceous and other glands, hair growth, and production of aminoglycans. Changes on the skin in the presence of hyper- or hypothyroidism reveal the functions of this receptor [7].

1.2.2.3 Neuropeptides

Skin neuromodulators are now starting to be seen as possible targets to prevent and treat skin aging. Neuropeptides are neurotransmitters and chemical messengers present in the skin. Their location in skin can be quite variable. Accordingly, Schulze *et al.* studied the distribution of some neuropeptides (VIP, NPY, CGRP, SP, NKA and CR) through different skin layers of both face and forearm by immunohistochemical detection. These authors have reported the following observations: VIP was present in dermis and subcutis layers of both face and forearm; NPY was only detected on the forearm subcutis; CGRP and SP were identified in all skin layers on both sites; NKA was only absent on the face epidermis while CR was absent on the epidermis of both sites [10, 17].

Additionally, neuropeptides can be produced by **resident and circulating skin cells** (e.g., gastrin-releasing peptide, somastostatin, NPY, atrial natriuretic peptide, PHM/PHI (peptide histidine isoleucine), galanin, neurokinins, substance P, neurotensin, CGRP, VIP, bradikinin, cholecystokinin, endothelins, CRH, urocortin, α-, β-, and γ-MSH, ACTH, β endorphin, enkephalins), and/or **in nerve endings** (e.g., substance P, neurokinins, neurotensin, CGRP, VIP, somatostatin, NPY, atrial natriuretic endothelins, α-, β-, and γ-MSH, β endorphin, CRH, urocortin, dynorphin, enkephalins) [7].

There is a linear reduction of epidermis innervation and content of NGF with **skin aging**, while neuropeptide levels and their receptor expression may increase or decrease. For example, epidermal resident cells suffer an age-related density decrease leading to more or less marked consequences on skin structure and appearance. As already stated, neuropeptides bind to skin receptors and produce several cellular responses related to skin functions. Consequently, the alteration of their levels is particularly relevant for skin cells survival, maintenance, and regeneration [18–20]. The list of some neuropeptides and their effect on the skin intrinsic aging is given below:

- **Corticotropin-releasing hormone (CRH)**: neuropeptide hormone that coordinates neuroendocrine and behavioral responses to stress. Aging leads to the dysregulation of CRH system subjecting the skin to a continuous stress

environment [10, 18]. Stopping this over-activation of the stress environment would be beneficial to skin health.

- **Somatostatin**: neuropeptide related to Alzheimer's disease, suggesting that lower values of somatostatin with aging may have some neurodegenerative effects (including those on the skin) [18, 21]. Thus, the maintenance of normal values may be a way to prevent this neurodegenerative effect.
- **Melanocortins** (pro-opiomelanocortin or POMC, derivatives —ACTH, α–, β–, and γ–MSH, γ–lipotropin, and β–endorphins): neuropeptides with anti-inflammatory properties, regulating cortisol levels and melanogenesis process, also involved in pigmentary responses (with CRH) and cornification of the skin. POMC system is greatly disturbed with aging. On one hand, peptides levels seem to increase. On the other hand, receptors for ACTH (MCR-2) and β-endorphin (I-opioid receptor 1) decrease with skin aging. However, it has been reported that *Achillea millefolium* extract is able to upregulate their expression. Consequently, this non-endogenous MCR agonist is able to increase skin thickness, which is quite beneficial for skin aging management. [20, 22, 23].
- **Endothelin**: induces collagen synthesis and is involved in tissue remodeling and fibrogenesis [10]. Thus, it could be also for used for the anti-aging process.
- **Pituitary adenylate cyclase-activating polypeptide (PACAP)**: involved in neuroprotective, regenerative, and immunomodulatory functions. However, high levels of this peptide are associated to neurogenic inflammation and lesions (e.g., psoriasis). In addition, an intravenous injection of PACAP leads to a long-lasting flush [10]. Consequently, this peptide should be kept at its normal levels instead of stimulating its synthesis.
- **Substance P (SP)**: a stress-related neuropeptide that can induce cytokines release, the major mediators in skin inflammatory processes and itch. SP is highly increased in sun-aged skin, promoting fibroblast and keratinocyte proliferation. Additionally, it is also involved in wound healing due to fibroblast proliferation with simultaneous collagen deposition and angiogenesis. However, SP can

enhance the virulence of bacteria normally present on the skin, which can limit its applicability to anti-aging methods [9, 10, 18, 24–26].

- **Neuropeptide Y (NPY)**: takes part in the skin defense mechanism owing to its antimicrobial activity. However, NPY should be used cautiously considering that it is highly expressed in melanoma [18].
- **Calcitonin gene-related peptide (CGRP):** expression decreases with age, being associated with the reduced survival and proliferation rate of keratinocytes. In addition, this peptide is able to stimulate melanocytes proliferation, which can be also changed in the aged skin. Moreover, CGRP has anti-inflammatory activity as well [18, 20, 27].

Neuropeptides levels and/or their effects can be conditioned by respective receptors and peptidases levels, which in turn could be affected by aging. For example, the level of neutral endopeptidase-24.11 (NEP, EC 3.4.24.11), which hydrolyzes bioactive regulatory peptides in the skin, significantly increases with aging. There is also an increase of elastase-type endopeptidase activity, leading to the degradation of the extracellular matrix [28, 29]. Regarding photoaging, there is an increase on the densities of dermal and intraepidermal nerve fibers, neuropeptidergic sensory nerve fibers in the dermis, neuropeptides levels (e.g., SP and CGRP), and NGF. The severity of the photodamage is closely related to the subjacent epidermal innervation changes [30].

Neuropeptides (mainly SP and CGRP) have been successfully used to accelerate and modulate **wound healing** in a dose-dependent manner, and since this process is delayed with aging, this could be another application of neuropeptides. In addition, they can be also potential **topical painkillers** (e.g., capsaicin excites C-fibers, releasing tachykinins and CGRP, and then those fibers become desensitized due to the lack of the same neuropeptides) [25, 31–34].

Moreover, neuropeptide modulation can be also used to treat other **skin disorders**, such as sensitive skin. Accordingly, an *in vitro* study with Sensiline® complex showed its influence on neuropeptides (mainly SP) levels in sensitive skin [35].

Incorporation of neuromediators in pharmaceutical formulations is quite difficult due to their fragile nature. However, agonists or antagonists of those neuromediators can be easily

formulated in order to control their release (e.g., botulinum toxin and capsacine), and prevent their degradation or modulate their synthesis [9]. Therefore, it is possible to determine the neuromediators effect on skin functions by modulating its levels, leading to **new approaches** toward skin disorder (including skin aging) and disease management.

1.2.3 Skin Changes with Aging

The skin suffers several **stru**
with aging in both neuronal and non-neuronal structures.

In general, the **epidermis** becomes thinner due to a progressive decline on the renewal rate of epidermal cells. Furthermore, repair mechanisms slow down and the dermal-epidermal junction becomes smoother, leading to an increase of skin fragility and decrease of nutrient transfer between the dermis and epidermis layers. Additionally, corneocytes have a tendency to accumulate on the skin surface, creating a rough appearance and texture [3–5, 36, 37].

Normally, there is a reduction of melanocytes function except on more exposed regions, such as the face and hands, where there is a production of age spots correspondent to melanin accumulation. Therefore, older skin is more susceptible to sunburn and skin cancer owing to a thinner epidermis and deficiency of melanocytes. Simultaneously, the absence of melanin production (since melanocytes from the bulb are lost) leads to gray or white hair [3, 4, 37].

In the **dermis** layer, the quantity of collagen and elastic fibers is decreased due to a reduction in fibroblast percentage and its synthetic ability and an increased expression of enzymes able to degrade the collagen matrix. Collagen fibers also become denser. These changes combined with a loss of adipose subcutaneous tissue and lymphatic deregulation lead to skin sagging, loss of elasticity and wrinkling (with volume loss). Gravity and disappearance of substance of facial bones and cartilage as well as muscular contractions influence the wrinkle appearance and degree of expression. Additionally, there is a loss of fibers in sweat glands and Meissner corpuscles. The activity of both sweat and sebaceous glands is also lower. The progressive reduction of blood supply affects the deterioration of these appendages [3–5, 36–39].

The skin immune system is also affected by the aging process. Langerhans cells may decrease up to 50% in late adulthood, lowering the skin immune surveillance level, and leading to a higher risk of skin cancer and infections [37].

Besides the **growth factors**, skin aging is also affected by **hormones**. The activity of several hormones declines with time, mainly estrogen, testosterone, and dehydroepiandrosterone (DHEA), but also melatonin, cortisol, thyroxine, and growth hormone [3].

The modifications already referred are **intrinsic modifications** dependent on time and individuals genetic. In addition, numerous **extrinsic factors** can also affect and accelerate skin aging, such as ultraviolet radiation from sunlight, ionizing radiation, severe physical and physiological stress, pollution, severe weather, alcohol intake, overeating and tobacco smoking. For example, if the skin is usually exposed to sunlight, the normal elastic fibers can be replaced with an elastic-like material, the number of collagen fibers lowers, keratinocytes division is compromised, and the lymphatic vessels become also damaged [3, 4, 37, 38]. Photoaging degree can be classified according to Glogau wrinkle scale (Table 1.1).

Table 1.1 Glogau wrinkle scale [40–42]

Type	Age reference (Years)	Photoaging degree
I	20 – 30	Early
II	30 – 40	Early to moderate
III	50 – 60	Advanced
IV	60 and over	Severe

1.3 Rejuvenation Procedures

The rejuvenation procedures have as a major goal the **reversion** or **delay** the dermal and epidermal signs of aging. Therefore, collagen, elastin, and glycosaminoglycans are the main targets of these procedures, which can have a preventive or therapeutic approach [43, 44].

Skin aging prevention may be achieved by essentially using daily skin care products (cleaning, moisturizing, among others)

and a **sunscreen** (besides the sun avoidance and the use of protective clothing).

Skin aging treatment can be divided into **invasive or non-invasive procedures**. **Topical application of anti-aging agents** is usually one of the most common non-invasive procedures. These agents are predominantly antioxidants and cell regulators. The use of **antioxidants** (vitamins, mainly C, B_3, and E, and botanical compounds such as flavonoids, carotenoids, and polyphenols) will reduce and neutralize free radicals, diminishing collagen destruction, and repairing oxidized membranes. On the other hand, the use of **cell regulators** (vitamin A and its derivatives/retinols, peptides and growth factors) will stimulate the production of collagen and elastic fibers and also act on collagen metabolism [37, 43, 44].

At least, **invasive procedures** are based on the removal of the damaged epidermis, as described in Table 1.2).

Table 1.2 Invasive rejuvenation procedures

Rejuvenation technique	Compounds/methods
Injectable	Hyaluronic acid
	Autologous fat
	Autologous platelet-rich plasma
	Botulinum toxin
Skin Resurfacing	Chemical peels
	Dermabrasion
	Ablative Lasers
	Ablative Fractional Lasers
	Non-ablative procedures

1.3.1 Injectable Techniques

Microinjections in the superficial dermis can contribute to **recover an ideal physiologic environment** by improving the synthesis of collagen, elastin and hyaluronic acid, cell activity, and skin hydration. These microinjections can contain one or more biocompatible active components [43, 45].

Dermal fillers are products that are injected into the skin in order to improve its physical features by soft tissue augmentation,

one of the most common minimal invasive procedures of skin rejuvenation. These fillers can be categorized according to their source and permanence in the tissue. Regarding their origin, fillers can be **autologous**, i.e., from the person himself (e.g., fat tissue) or **heterologous**, such as collagen derived from human, porcine or bovine tissue cultures, animal or synthetic hyaluronic acid, synthetic or pseudo-synthetic implants, polymers, etc. According to the time that fillers remain in the tissue, they can be **temporary** (results visible only for a few months), **semi-permanent** (results visible for 1 to 2 years) or **permanent** (results visible for more than 2 years). Permanent fillers are usually non-biodegradable unlike non-permanent fillers, which are eliminated eventually through digestion or metabolism [43, 46–49]. The use of temporary fillers is more common in rejuvenation procedures since skin aging is a dynamic process and time adaptation is inevitable. These fillers are associated with a low incidence of secondary effects and complications. Nevertheless, the fillers can produce redness, inflammation, pain, bruising, edema, erythema, presence of visible material in the form of papules or nodules, tissue necrosis (a rare complication due to alterations on the blood flow), infections, hypersensitivity reactions, etc. [43, 46–48].

Hyaluronic acid (HA) is a glycosaminoglycan disaccharide composed of an alternating and repeating unit of D-glucuronic acid and *N*-acetyl-D-glucosamine. It is an important component of the interstitial matrix of the dermis layer and has become the "gold-standard" for skin rejuvenation through injectable technique. It is a space filler, lubricant, cell regulator (on proliferation and locomotion) and promotes the stabilization of the connective tissue. HA also increases skin hydration due to its hydrophilic ability, and activates fibroblasts, leading to skin augmentation/rejuvenation. As a temporary filler, its effect can last from 3 to 12 months, depending on the type of HA used [43, 45, 47, 49].

The distribution of subcutaneous fat between compartments becomes more evident with aging in the form of the loss of fat volume in facial skin. **Autologous fat** can be used as a safe and natural filler since no rejection or allergic reaction would be expected. Although this material can be easily obtained from thighs, abdomen, or buttocks, it requires an operation room to be

extracted. The duration of its effect is unpredictable; it can last from months to several years [45, 47].

Autologous platelet-rich plasma (PRP) from the fresh whole blood contains a high concentration of platelets and various growth factors that can regulate several processes, such as cell migration, attachment, proliferation, and differentiation. It can promote collagen synthesis and stimulate fibroblasts activation, also leading to skin rejuvenation [43].

Botulinum toxin (BTX) is a neurotoxin produced by different strains of *Clostridium botulinum.* There are seven subtypes, neurotoxins A–G, from which only A, B, and F subtypes are available for clinical use, the A subtype being the most potent. Although BTX can slow down the skin aging process, it cannot discontinue this process. The BTX mechanism of action consists of blocking the presynaptic release of acetylcholine resulting in temporary chemical denervation at the neuromuscular junction and leading to striated muscular flaccid paralysis. This process occurs through different steps as follows: (a) The neurotoxin binds to a specific receptor on the presynaptic cholinergic neuron; (b) the toxin/receptor complex suffers internalization through endocytosis into nerve terminals, and (c) the formed vesicle is lysed, preventing the acetylcholine release from inside the cell. A significant wrinkle reduction will be obtained through this transitory and reversible paralysis state. Although BTX effects are temporary and localized, repeated injections may lead to a long-term effect**.** Nevertheless, the side effects are related with BTX's mechanism of action. Therefore, BTX is not indicated under conditions that may be exacerbated by the toxin (e.g., pre-existing neuromuscular disorders, psychiatric disorders, local infection, etc.). Complications are mild and may include pain, edema, erythema, ecchymosis, headache, and short-term hypoesthesia [43, 44, 50, 51].

The **ideal dermal filler** should present several characteristics, including biocompatibility; absence of immunogenicity; being not carcinogenic, infectious, teratogenic neither non-migratory; easy to obtain and store; inexpensive; removable (if required); with reproducible results, etc. [44, 49].

These techniques have been shown to be effective individually. Anyway, the effectiveness of combinations has been studied

in order to obtain better and prolonged results with fewer side effects. The combination of two or more complementary techniques may be the answer to achieve an optimal, adaptable, and durable result for skin aging management without compromising the skin health or the patient comfort.

1.3.1.1 Skin resurfacing techniques: Chemical peelings and dermabrasion

There are several skin resurfacing methods, such as chemical peelings, dermabrasion, and laser treatment as follows:

Chemical peelings consist of a chemical ablation of the exposed tissue. Chemical exfoliating agents are applied to the skin destroying portions of the epidermis and/or dermis through epidermolysis, protein precipitation or tissue denaturation, which will lead to the activation of skin regeneration and repair mechanisms. Peelings can reach different skin depths, depending on the substance used, its concentration, pH of the formulation as well as the type and condition of the skin, mode and time of application, etc. Accordingly, they can reach the epidermal layers (**su**
dermis (**medium-depth peels**) or even to the mid/lower reticular dermis (**deep peels**) [43, 44, 52, 53].

Some skin modifications may be observed as a uniform distribution of melanocytes and melanin grains, a homogeneous thickness of the basal membrane, a fresh sub-epidermal band of collagen and network of elastic fibers [43, 44, 54].

Although all skin phototypes (Fitzpatrick scale) respond to all peel types, phototypes I to III are less predisposed to scar or suffer pigment changes while phototypes IV and V have a greater risk of post-treatment dyspigmentation [44, 52].

Dermabrasion is the process of uniform mechanical abrasion of the skin, including the epidermis and upper papillary dermis layers, and consequently removes or reduces superficial wrinkles. It can be performed by using a serrated wheel, diamond embedded fraises, wire brush or sterilized sandpaper as a cutting tool, which is attached to a rotating handpiece electrically powered. However, this technique is highly dependent of the operator, requiring specialized skills and experience since any inaccuracy may result in major scarring. The healing time can be extended to one month. Once more, patients with darker skin may

experience dyspigmentation. This technique tends to be replaced with or used simultaneously with resurfacing lasers [52, 55, 56].

1.3.1.2 Skin resurfacing techniques: Laser and light therapy

Light possesses particle and wave properties. Whereas wave properties comprise light behavior in space and large interfaces, such as skin and air, particle properties involve the tissue interactions on a molecular level. Lasers have a well-defined energy with the capability of ablating selected tissue while preserving the surrounding tissue. They may differ in wavelength, intensity, and duration of action, being the wavelength the responsible for the heat generation and, consequently, the tissue destruction. The extreme control of these parameters (mainly intensity and monochromaticity) permits a higher degree of precision compared with a non-laser source [52, 57].

The transformation of light or electrical energy into heat is the basic mechanism of photothermal and electrothermal skin rejuvenation procedures, respectively. The energy of a laser is proportional to its frequency and inversely proportional to its wavelength [57].

The light source produces a photon, which transfers its energy to a chromophore. The energy absorbed by the chromophore leads to an excited state, and to leave that state the chromophore may dissipate the energy as heat or fluorescence (reemission of light). It is possible to be selective toward the heating process by considering specific wavelengths and pulse duration as different chromophores may absorb at diverse wavelengths bands. The primary absorber is the water present in deeper skin layers. Water absorption peaks appear at 980, 1,480, 2,940, and 10,600 nm. The optical properties of the skin will determine the laser penetration, absorption, and internal dosimetry. Lasers act by selective photothermolysis, targeting water in the skin and stimulating collagen synthesis, and thus preventing skin aging [51, 55, 57, 58].

In fact, lasers have become quite popular for skin aging management. Ablative lasers were first used for this dermatologic approach. Then, fractional photothermolysis was introduced in order to obtain lesser side effects and lower risks [55, 58]. There was also an evolution from laser generation working at a continuous wavelength to recent pulsed lasers.

In particular, CO_2 and erbium:yttrium aluminum garnet (Erb:YAG) lasers, which operate at high wavelengths of 10,600 nm and 2,940 nm, respectively, are the main lasers used for skin rejuvenation. CO_2 laser is mainly absorbed by skin water, which favors its application for resurfacing techniques. However, Erb:YAG laser is around 10 times more selectively absorbed by water than CO_2 laser, leading to an extremely rapid heating with minimal damage to the surrounding tissue. In addition, it is more superficial and promotes the skin re-epithelialization earlier. These Erb:YAG lasers are extremely effective for all skin types, quite flexible, and produce from pure ablation craters to rapid superficial abrasion. Although several studies have compared these two laser types, no statistical differences were observed regarding the wrinkle reduction in most cases. Nevertheless, CO_2 laser seems to be more efficient for collagen synthesis, while Erb: YAG has milder effects. On the other hand, an improved outcome and decreased healing time has been shown with combined laser techniques [51, 52, 58].

Ablative lasers are hypothetically more effective than non-ablative lasers once the ablative technique removes the epidermis, causing a more extensive regeneration and prolonged effect, while the non-ablative technique keeps it intact. Consequently, dermal histological changes are more perceptible after the first procedure. On the other hand, the non-ablative approach presents lesser side effects [41, 58].

1.3.1.2.1 Ablative laser resurfacing

Ablative laser resurfacing consists of the controlled ablative removal of the superficial skin layers, based on the selective photothermolysis. An insignificant injury to the adjacent tissue can be observed, and the wound healing may occur from days to weeks. Whereas the laser penetration into tissue will depend on the selective absorption of skin water, the instantaneous tissue effects will depend on the laser potency, irradiation speed and treatment area. Possible thermal damage may occur due to an extended period of laser–tissue interaction. Nevertheless, the skin will be renewed by the re-epithelialization process, and damaged collagen, elastic fibers, and epithelial cells will be replaced with their normal homonyms [44]. There are two types of ablative laser, as already mentioned:

- **Carbon dioxide lasers (CO_2 resurfacing):** CO_2 lasers consist of unfractionated fully ablative laser emitting at 10,600 nm wavelength that deliver peak fluences above the ablation threshold of the cutaneous tissue and shorter tissue-dwell time. Although CO_2 lasers emitting a continuous wavelength were initially used, pulsed lasers are now more common. In fact, the first CO_2 laser generation (continuous wavelength lasers) emitted a quite high energy dispended for heating instead of ablating tissue. Therefore, this laser was limited by its risk of scarring, variable level of thermal damage, delayed healing, among other side effects. On the contrary, the last laser generation provides a precise control of tissue vaporization, hemostasis, and less residual thermal damage [41, 44]. CO_2 lasers ablate 20 to 60 μm of tissue per pass at the ablation threshold for cutaneous tissue (5 J/cm^2), resulting in a thermal damage zone up to 150 μm [41].
- **Erbium:yttrium-aluminum garnet (lasers Er:YAG)** were developed after CO_2 lasers, consisting of a laser emitting at 2,490 nm wavelength. These lasers can be used for more superficial resurfacing processes since they ablate 5 to 15 μm of skin tissue per pass at the ablation threshold resulting in a thermal damage zone smaller than 15 μm. This minimum thermal damage can be justified by their coefficient of water absorption, which is approximately 16 times higher than the CO_2 lasers. They also cause less pigmentation changes in people with higher skin phototypes (III and above) [41, 44].

Ablative lasers are exceptionally versatile tools and can lead to surprising results with just one treatment. However, their side effects may limit their use, such as erythema (which can be maintained for several months mainly after treatment with CO_2 laser), permanent hypopigmentation, transitory hyperpigmentation, prolonged healing time and post-operative period, possible infections (bacterial, viral, or even fungal), and scarring [41, 43, 44, 51–53, 58, 59].

This technique is primarily contraindicated in people with several clinical conditions, including abnormal wound healing (e.g., keloids); vascular diseases; abnormal decrease of adnexal structures of skin (as occurs after radiation therapy); deep peels

or scars; active infections or immunosuppression; isomorphic diseases (e.g., vitiligo); uncontrolled hypertension; diabetes; and other significant medical conditions that may be compromised by the procedure or compromise the procedure itself [41].

The ideal laser skin resurfacing would provide skin vaporization with minimal side effects, relying considerably on both depth of ablation and energy fluency. Recent resurfacing methods allow the choice of different systems, and the treatment can be adjusted individually in order to improve its safety and efficacy [60].

1.3.1.2.2 Ablative fractional laser resurfacing

Fractional ablative lasers (CO_2 and erbium types) have been developed to diminish some side effects of fully ablative lasers. In this technique, only skin fractions are ablated, and the laser beams just damage or remove an array of microscopic columns of skin tissue leading to dermal controlled thermal damage. In addition, fractional ablative lasers deeply penetrate into the skin. This technique leads to the enhancement of elastin and collagen synthesis and to a shorter healing time since the healthy skin that surrounds the ablated zone will help in the healing process. Fractional ablative lasers differ from fully ablative forms by the pixelation of the laser beam [41, 44, 59].

Although the possible side effects may include those already mentioned for the fully ablative method (particularly if there is an excessive heating), their severity is much moderate. Additionally, immediate side effects may include burning sensation, discomfort, and redness, which disappear after a few days. On the other hand, this method is mainly contraindicated in people with a history of keloid scarring, immunosuppression, vitiligo, psoriasis, vasculitis, active infections, prior radiation, or recent oral retinoid treatment [41, 44, 59].

1.3.1.2.3 Non-ablative procedures

Non-ablative resurfacing methods have been developed in order to overcome the morbidity and complications (mainly permanent hypopigmentation and scarring) associated to ablative techniques. These methods employ visible, near-infrared, and mid-infrared wavelengths and a skin cooling system to protect it. In general,

less promising results may be obtained compared with ablative approaches. Nevertheless, the risk/benefit ratio between the different techniques should be taken into account for each case [41, 42, 44, 61]. Their **mechanism of action** is based on non-ablative fractional photothermolysis, in which the dermis is inflamed and thermally denatured in order to stimulate the healing process also improved by the surrounding healthy skin. During this process, collagen is synthesized and melanin is released leading to a pigmentary skin redistribution [42, 51, 61].

In contrast to fully ablative resurfacing methods in which the thermal damage is homogeneous at a specific depth, in this case (non-ablative fractional photothermolysis and fractional ablative photothermolysis) the thermal damage only occurs in microscopic skin columns called **microthermal zones** (MTZs). The extension and size of these columns are defined by the energy used (i.e., higher energy will provide wider and deeper columns). The MTZs are encircled with healthy tissue that will help the healing process, as already mentioned. This process is safer and quicker than the ablative skin resurfacing method. It is used to improve skin texture and dyspigmentation, being able to target both epidermis and dermis layers. Even though the results can be pronounced, it involves multiple treatments to achieve them. Side effects are usually mild and temporary, mainly mild redness, swelling, blistering, herpes activation, acne flare up, etc. Pigmentary changes might be observed in darker skin phototypes when compared with treatment with other wavelengths [44, 59, 61, 62].

Non-ablative **infrared lasers** were developed to rejuvenate the skin with higher safety. Mid-infrared laser heating can target dermal collagen by dermal water heating and stimulation of collagen synthesis concomitant with epidermal protection (cooling). Repigmentation is linked to melanocytes migration and proliferation, sideways with the release of cytokines and inflammatory mediators. Although this process has fewer and less severe side effects, they can still be present, mainly scarring and temporary hyperpigmentation due to an aggressive cooling [41, 44, 61].

Non-ablative fractional lasers also comprise **erbium** (1,410; 1,440; 1,540 and 1,550 nm), **neodymium-doped** (1,320), **diode laser** (1,450), and **thulium** (1,927 nm) [42, 61].

Pulsed dye lasers (PDL) which use oxyhemoglobin as the principal chromophore and intense pulsed light (IPL) are other non-ablative rejuvenation procedures based on selective photothermolysis. Additionally, less aggressive histological changes are observed after treatment with different IPL devices. The flash lamps of these devices produce a broad spectrum of light heating and denaturating the target tissue and, consequently, accelerating the epidermal turnover. Thus, new collagen in the papillary and reticular dermis is observed as well as an increase of the fibroblasts number. Although IPL absorption spectrum of skin chromophores is not monochromatic, a filter addition allows the adaption to specific applications. Nevertheless, the effectiveness is weaker compared with ablative results [42, 43, 57, 63].

In summary, the effects of different laser resurfacing methods are shown in Fig. 1.1.

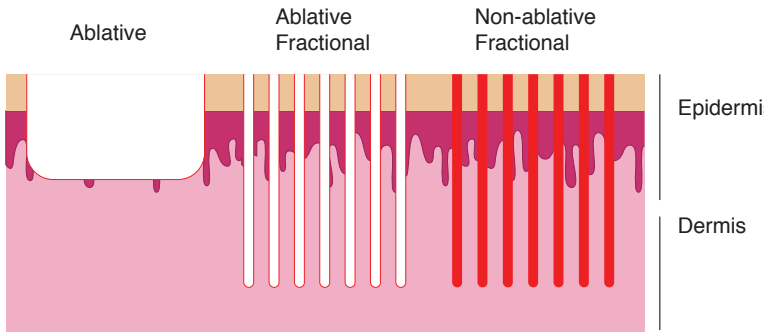

Figure 1.1 Effect of different laser resurfacing methods.

1.3.2 Cosmeceuticals

Cosmetic ingredients were once considered inert. Cosmeceuticals are topical products on the borderline between cosmetics and drugs, presenting a cosmetic effect associated to a physiological mechanism. The search for better and effective skin products with real bioactivity is increasing since consumers start getting interested in understanding the technology and mechanisms behind these products design. Therefore, there is a good acceptance to cosmeceuticals. It is important to use not only rejuvenation methods but also cosmeceuticals to delay and prevent skin aging.