Chapter 9
Extracts and Bioactives from Microalgae (Sensu Stricto): Opportunities and Challenges for a New Generation of Cosmetics

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Abstract The cosmetic industry, which increasingly aims to develop products that affect body appearance, prevent aging and promote skin and hair well-being, has changed over the last decades. The increased sensibility of consumers to the ethics of green economy drew the attention of this industry to microalgae as novel source of active ingredients. Microalgae, which are often improperly considered as inclusive of prokaryotic microorganisms, i.e. cyanobacteria, are eukaryotic microorganisms capable of synthesising biologically active molecules that affect human metabolism.

Many classes of beneficial compounds, including carotenoids, polyphenols, vitamins and polysaccharides, can be obtained from microalgae cultivated with sustainable and environment-friendly techniques. Microalgal extracts are already commercialised in products that claim several biological activities, such as hair growth stimulation, prevention of solar radiation damages, modulation of skin pigmentation, skin tightening and anti-aging. However, their mechanisms of action and metabolic effects are not fully understood, and the related beneficial effects are probably underestimated. This contribution aims to review the state-of-the-art cosmetic applications of microalgae with a critical discussion of the experimental methods adopted and potential perspectives.

Keywords Microalgae · Natural extracts · Cosmetics · Skin · Pigmentation · Dermis · Keratinocytes · Melanogenesis · Sebogenesis · Hair follicle · Biological activity · Oxidative stress · Transdermal delivery

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Abbreviations

ACTH	adrenocorticotropic hormone
Akt	Protein kinase B
AP-1	Activator protein 1
ARE	Antioxidant response elements
ATX	Astaxanthin
BAD	Bcl-2-associated death promoter
Bax	Bcl-2-associated X
Bcl-2	B-cell lymphoma 2
βC	β-Carotene
Casp	Caspase
CE	Cornified envelope
COX-2	Cyclooxygenase-2
CRH	corticotropin-releasing hormone
CT	Carotenoid
CTX	Canthaxanthin
cyt-c	Cytochrome-c
DGDG	Digalactosyl diacylglycerol
DP	Dermal papilla
DW	Dry weight
ECM	Extracellular matrix
EPA	Eicosapentaenoic acid
SEP	Sulphated exopolysaccharide
ERK	Extracellular signal-regulated kinase
FB	Fibroblast
FoxOs	Forkhead box, class O family member proteins
FT	Ferritin
FXT	Fucoxanthin
GABA	γ-Aminobutyric acid
GAG	Glycosaminoglycan
GCL	Glutamate-cysteine ligase
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GSR	Glutathione-disulphide reductase
h	Human
HA	Hyaluronic acid
4HNE	4-Hydroxy-2-nonenal
HO-1	Haeme oxygenase (heat shock protein fam.)
hSE	3D human skin equivalents
hFTS	Human full-thickness skin
HF	Hair follicle
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IR	Infrared rays
JNK	c-Jun N-terminal kinase

KC	Keratinocyte
Keap1	Kelch-like ECH-associated protein 1
L-DOPA	3,4-Dihydroxy-L-phenylalanine
LF	Lipofuscin
MA	Microalga
MAs	Microalgae
MAA	Mycosporine-like amino acid
MAPK	Mitogen-activated protein kinase
Mcl-1	Induced myeloid leukemia cell differentiation protein
MGDG	Monogalactosyl diacylglycerol
MITS	Microphthalmia-associated transcription factor
MMP	Matrix metalloproteinase
MW	Molecular weight
NEP	Neprilysin or neutral endopeptidase
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NMF	Natural moisturising factors
Nrf2	NF-E2 p45-related factor 2
NQO-1	NAD(P)H dehydrogenase (quinone) 1
NT	Neurotrophin
PKCδ	Protein kinase C-δ type
PMA	Phorbol myristate acetate
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
5α-R1	5α-reductase type-1
SAGE	Semisynthetic glycosaminoglycan ether
SC	Stratum corneum
SEP	Sulphated exopolysaccharide
SOD	Superoxide dismutase
SPR	Small proline-rich protein
STAT3	Signal transducer and activator of transcription 3
TGF-β	Transforming growth factor-β
T-Iso	Tahitian *Isochrysis*
TNFα	Tumour necrosis factor-α
UCA	Urocanic acid
UV	Ultra violet (UVR: rays; UVA: type A; UVB: type B; UVC: type C)

1 Introduction

In the biological world, all organisms are endowed with enzymes necessary for essential metabolic cell processes, but some have also developed enzymes that produce special secondary metabolites, especially amongst prokaryotes, plants and fungi. These metabolites, which include antioxidants or some toxins against preda-

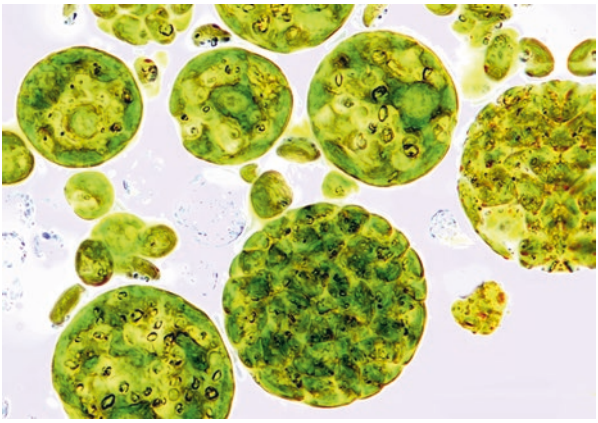

Fig. 9.1 *Chlorococcum minutum* (Source: courtesy of Cutech Srl)

tors, generally protect against potential damages from the external environment. Amongst autotrophic organisms, they comprise vitamins, special macromolecules (e.g. long-chain unsaturated fatty acids) and some accessory pigments for photosynthesis, e.g. carotenoids (CTs), which are able to work as free radical scavengers in humans (Zhang et al. 2014). Some of these compounds are irreplaceable dietary vitamins and micronutrients for animals and humans. The cosmetic industry is increasingly getting aware that the microorganism biochemistry offers numerous compounds to preserve youth and beauty. Amongst the richest sources of active ingredients, microalgae (MAs) have become the object of special attention due to their extraordinary capability to synthesise and store bioactives that are still relatively unknown (Plaza et al. 2009). In this regard, some clarifications are appropriate, because with the term MAs (sensu lato), very heterogeneous microorganisms are generally indicated (see Andersen 2013 for a concise systematic overview). For instance, prokaryotes belonging to the vast group of cyanobacteria (also called blue-green algae) are generally included. Cyanobacteria have biological traits that are typical of bacteria, including prokaryotic polysaccharides in their cell walls and special pigments with high biological activity (phycocyanins). The MAs sensu stricto, however, have a nucleus; therefore, they belong to the domain Eukaryota and have a biochemical composition of their own, which can sometimes include molecules in common with macroalgae or even higher plants depending on the case. This contribution will refer to MAs sensu stricto (Fig. 9.1 shows an eukaryotic microalga of cosmetic interest).

1.1 The Exploitation of Microalgae in Cosmetics: A Recent Story

The exploitation of MAs for industrial processes has become significant due to marine aquaculture, from which most of the knowledge that has inspired recent studies was derived. The need to cultivate MAs in monoculture was born at the

beginning of the last century for nutrition of microinvertebrates (Allen and Nelson 1910) and assumed commercial applications when microalgal cultures were used for the rearing of bivalve molluscs intended for human consumption (Bruce et al. 1940; Rhyter and Goldman 1975).

Many steps forward, such as the selection of monospecific strains for the nutrition of bivalves and phytophagous larval phases of prawns, were made in the following decades (De Pauw et al. 1984). However, in these pioneering experiences, MAs were intended for animal species that physiologically feed on MAs. The use of MAs as live food was an obvious choice, whereas the biological value of their biochemical composition for other applications was established only later. In the 1970s, the first attempts to reproduce and breed valuable marine fish had little success due to the low survival of early larval stages, which fed on zooplankton (rotifers), and the high rate of malformation suffered by fingerlings that reached weaning. These difficulties were overcome only when some researchers realised that the high nutritional requirements of many marine fish larvae could be satisfied by administering 'enriched' rotifers, i.e. fed with some MAs. The high content of polyunsaturated fatty acids (PUFAs) and other essential nutrients in MAs was recognised as the key factor. Japanese aquaculture gave a significant contribution (Watanabe et al. 1983) to the achievement of the first successes. Since the 1970s, fish farmers have selected many microalgal strains endowed with some fundamental properties, such as the presence of secondary metabolites with high biological value, absence of toxicity and adaptability to intensive culture conditions, which are useful for exploitation in cosmetics. Indeed, these three elements made phytoplankton cultivation an ideal source of natural ingredients for the cosmetic industry.

Another pivotal element of innovation was introduced by the development of intensive phytoplankton cultures in photobioreactors, which allow very intensive production without using pesticides, are eco-sustainable and can be upscaled to industrial production with fully traceable and certifiable processes. However, although the use of modern photobioreactors has reduced production costs (Molina Grima et al. 2003; Tredici et al. 2016), the final price of MA biomasses remains relatively high (Barsanti and Gualtiero 2018).

The cosmetic industry is amongst the sectors that can exploit the biochemical characteristics of MAs, because value-added products can be developed using a relatively small quantity of biomass.

1.2 A New Concept of Cosmetic Treatment Based on Bioactives

Cosmetics are traditionally classified into skin care, makeup, body and hair care, oral cosmetics and fragrances (Mitsui 1997). Before the 1980s, they were principally used to beautify or cover minor, visible imperfections or, at the most, improve the structure of the skin and its annexes. The main biological activities were due to physicochemical properties of some ingredients, e.g. emollients and moisturisers. The use of beneficial natural ingredients has always been present in cosmetics, but

this aspect has become predominant only recently along with environmental sensibility. A new range of cosmetic products, which are designed as a vehicle of natural principles endowed with biological activity, have been developed (Kumar 2005; Paye et al. 2009). The concept of cosmeceutics, a neologism obtained from the fusion of the word 'cosmetics' and 'pharmaceutical' introduced by dermatologist Albert Kligman (Tsai and Hantash 2008), was born. Cosmeceutics is defined as '… *topical formulations which were neither pure cosmetics, like lipstick or rouge, nor pure drugs, like corticosteroids. They lay between these poles, constituting a broad-spectrum intermediate group*' (Kligman 2005).

The search for active ingredients suitable for the cosmetic sector received great impetus, and natural extracts are the main sources of inspiration due to their wealth of molecules that are already known as ingredients of traditional herbal medicines. The cosmetic exploitation of MAs is part of this sociocultural and industrial trend due to their richness of active compounds with a strong commercial appeal.

2 Bioactives from Microalgae and Their Applications in Cosmetics

2.1 Microalgae as Novel Sources of Active Compounds

Traditional medicine and, to a lesser extent, cosmetics have exploited the biological properties of plants since time immemorial to obtain natural extracts containing molecules that are active on human wellness. In terrestrial plants, many metabolites are often concentrated or stored in different organs (e.g. roots, leaves, flowers and fruits), depending on their specific functions, so that only that part is used for the preparation of cosmetic ingredients. Meanwhile, MAs are single-celled organisms with a whole library of enzymes and metabolites concentrated in the same cell. Sometimes cells are organised in colonial forms, such as in many Bacillariophyceae; however, metabolic self-sufficiency is generally maintained in each cell. Therefore, the microalgal biomass is homogeneous and entirely used for the extraction of active ingredients, whose composition depends on cell characteristics, solvent used and extraction procedure (Chojnacka and Kim 2015).

Some strains were identified as sources of specific compounds that were accumulated in large amounts, especially if cultivated under appropriate environmental conditions. Indeed, the prevailing approach in the exploitation of MAs was to use them as biofactories for the production of compounds known for their beneficial properties (Barclay et al. 1994; Jin et al. 2003; Spolaore et al. 2006; Catalina Adarme-Vega et al. 2012; Priyadarshani and Rath 2012; Guarnieri and Pienkos 2015; Guedes et al. 2011; Koller et al. 2014; Wobbe and Remacle 2015; Singh et al. 2017; Islam et al. 2017). Representative examples are the extraction of astaxanthin (ATX) from cysts of *Haematococcus pluvialis* (Guerin et al. 2003), CTs from *Dunaliella salina* (Jin and Melis 2003; Pisal and Lele 2005; Del Campo et al. 2007) and omega-3 fatty acids from *Phaeodactylum tricornutum* (Reis et al. 1996), *Porphyridium cruentum* (Asgharpour et al. 2015), *Crypthecodinium cohnii* (Mendes

et al. 2009) and *Nannochloropsis* spp. (Forján Lozano et al. 2007; Chini Zittelli et al. 1999).

Unfortunately, this approach is affected by competition with traditional sources of the same molecules, such as fish oil and chemical industries. However, an interesting alternative way to exploit the richness of MAs is to develop multifunctional extracts, which allow to achieve beneficial effects by acting simultaneously on various metabolic processes of the treated tissue. This topic will be discussed in more detail below, but it is worth noting here that the extraction of specific bioactives from the biomass involves relevant purification costs, whereas the multifunctional approach takes advantage of a simplified extraction process. Extracts with a broad spectrum of action require more research effort to characterise their effects on the tissues or organs, but their industrial production is less expensive and their composition cannot be synthetically reproduced by commercial competitors.

2.2 Effects on the Skin and Its Accessory Structures

Historically, the most investigated application is probably in the prevention of aging and reduction of wrinkles. Aging causes the overall loss of structural organisation of the skin, with particularly evident consequences in the dermis, whose biomechanical properties are attributable to the fibrous and amorphous connective tissue (extracellular matrix, ECM), which is composed of proteins, proteoglycans and glycosaminoglycans (GAGs). Many cosmetic products therefore claim the ability of stimulating the synthesis of the dermis ECM and protecting it from degradation processes.

To delay aging, however, modern cosmetic science has also developed a wide range of active products aimed at improving other aspects of skin metabolism, e.g. state of hydration, smoothness of the stratum corneum (SC), modulation of sebum production and melanogenesis. Many extend to the treatment of problems that are borderline with pathological disorders, such as melasma, acne, seborrheic dermatitis, various forms of dermatitis or psoriasis and solar erythema. To date, preparations obtained from MAs showed various activities on the skin and its annexes, confirming that they are a valuable source of active compounds of high cosmetic interest. However, an appropriate exploitation of them requires a deep comprehension of skin biology and molecular signals that regulate its metabolism.

2.2.1 Skin Anatomy

The skin is the largest organ of the body (1.5–2 m^2) and has an average thickness of 1–2 mm, but with variation from 0.5 mm of eyelids to over 6 mm between the shoulder blades (Saladin 2007) (Fig. 9.2).

The skin is composed of epidermis and dermis, whereas the underlying adipose panniculus (called hypodermis or subcutis) is generally considered distinct even though it is closely connected to the skin both anatomically, because some fat cells

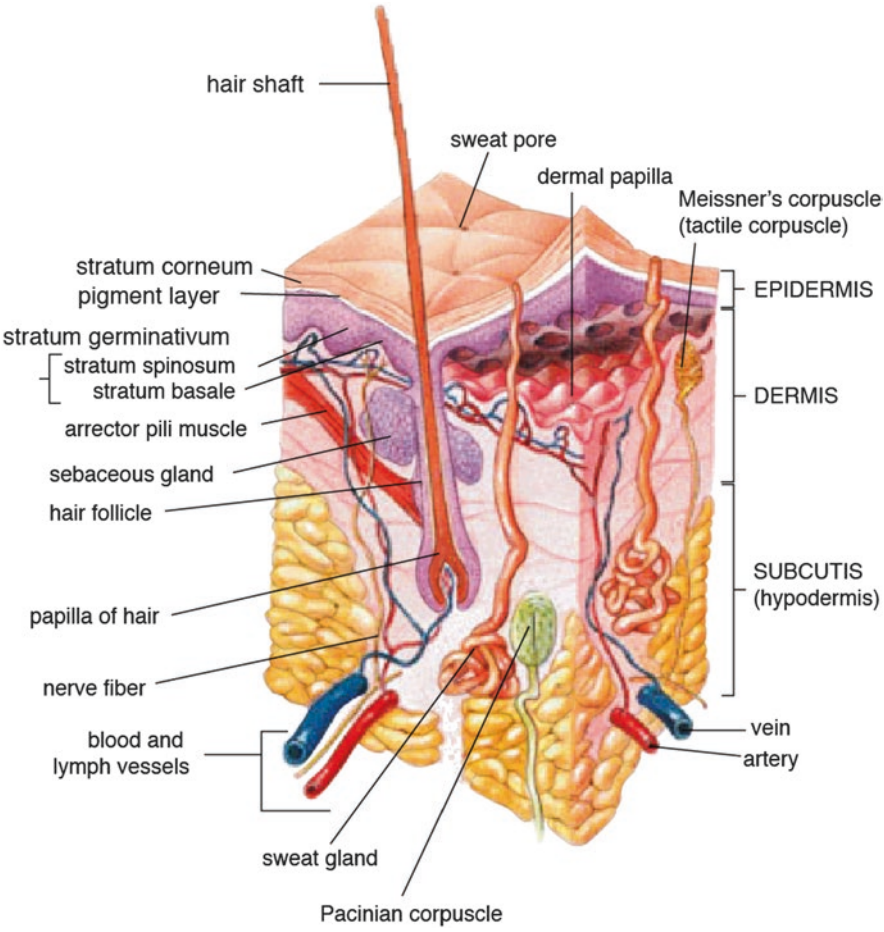

Fig. 9.2 A cross section of the skin and its underlying structures (image contributed by Wikimedia Commons, USGOV (Public Domain), from: Anatomy, Skin (Integument), Epidermis (Yousef and Sharma 2019), book distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/))

can be dispersed in the deep dermis, and functionally through a consistent exchange of cytokines. The epidermis is the superficial layer and is divided from the underlying dermis by the basal lamina, which is a dense planar-reticular structure mainly consisting of glycoproteins (e.g. fibronectin and laminin) and collagen (COL) type IV (COL-IV). Epidermis is a densely cellularised epithelial tissue composed of keratinocytes (KCs), which account for about 95% (McGrath et al. 2010) and proliferate starting from the primary basal layer, called *stratum basale*. Amongst KCs of the stratum basale occur the melanocytes, which are specialised pigmentary cells of neuroectodermal origin that synthesise melanin in special subcellular organelles called melanosomes. Melanocytes can assume a dendritic shape and develop temporary cell projections, known as pseudopodia, which carry melanosomes away

from the centre of the cell. KCs can engulf the tips of the melanocyte pseudopodia through phagocytosis, receiving a certain quantity of melanin (Nordlund et al. 1989). This process modulates the skin pigmentation and is stimulated by exposure to solar radiation (skin tanning).

Following these important cell interactions in the basal layer, the KCs move towards the epidermal surface undergoing a process of differentiation that leads to the formation of the SC.

Keratinocyte Differentiation

KCs proliferate from the basal layer and undergo differentiation, thereby forming the following layers (Fig. 9.3): *stratum basale* or *stratum germinativum*, *stratum spinosum*, *stratum granulosum* and *stratum corneum*. In palmoplantar skin is observed an additional electrolucent stratum, called stratum lucidum, interposed between the granulosum and corneum (McGrath et al. 2010). The differentiation process is called cornification and is regulated by the concentration of Ca^{2+}, which increases from the stratum basale to the SC (Eckhart et al. 2013). KC differentiation

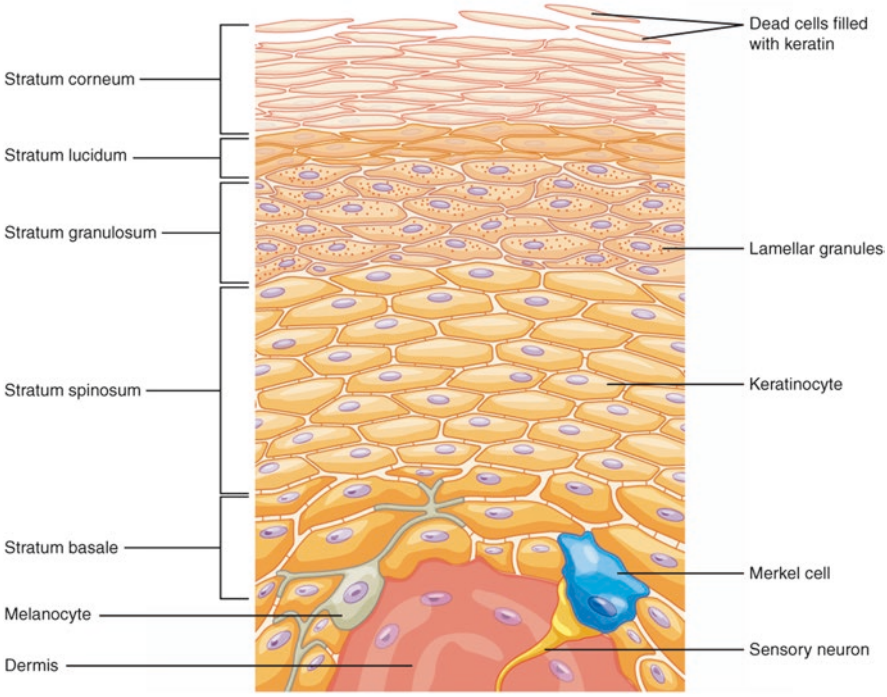

Fig. 9.3 Layers of the Epidermis. The epidermis of thick skin has five layers: stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum (Access for free at https://openstax.org/books/anatomy-and-physiology/pages/1-introduction) (license conditions at https://creativecommons.org/licenses/by/4.0/)

occurs with synthesis of cytoskeletal scleroproteins, organised in the cornified envelope (CE) and lipids, which are confined in lamellar bodies (for review see Candi et al. 2005 and Eckhart et al. 2013). The main CE proteins are loricrin (the most abundant), involucrin, filaggrin (which aggregates keratin filaments into tight bundles), elafin (a serine proteinase inhibitor) and small proline-rich repeat proteins (SPRs) that have antioxidant properties (Steinert and Marekov 1995; Rinnerthaler et al. 2015). These proteins constitute about 7–10% of the mass of the epidermis (Candi et al. 2005) and are synthesised at different phases of KC differentiation to be cross-linked by transglutaminases, especially transglutaminase-1 and transglutaminase-3, which are Ca^{2+}-dependent enzymes that catalyse ε-(ϒ-glutamyl)lysine cross-linking reactions (Terazawa et al. 2015).

In the stratum granulosum, KC develops lamellar bodies, which are derived from the Golgi apparatus and filled with phospholipids, glucosylceramides, sphingomyelin and cholesterol (Feingold 2007; Rinnerthaler et al. 2015). In the final phase of differentiation, KCs collapse into dead cells called corneocytes that are connected by keratin bridges, whereas the lamellar bodies are secreted in the extracellular space, where some enzymes complete the process of maturation of the corneal matrix. Part of filaggrin is degraded by caspase-14 (Casp-14) into amino acids, some of which act as natural moisturising factors (NMF). Filaggrin is also a major source of histidine, which is further metabolised into the potent UVB scavenger urocanic acid (UCA) in the cornifying layers (Eckhart et al. 2013).

The mature SC is a complex model of structure called ‘brick and mortar barrier’, wherein the lipid matrix is the mortar and the corneocytes are the bricks (Nemes and Steinert 1999). Interestingly, the pyknotic cytoplasm of the corneocyte is occupied by NMFs, i.e. amino acids and their derivatives and salts, which contribute to the hydration and elasticity of SC. The epidermis is superficially lubricated by sebum, which contributes to the proteolipid barrier, interacts with the microbiome and regulates the SC’s exfoliation process.

The Dermis

Under the basal lamina lies the dermis, which is composed of proteins and polysaccharides of the ECM, in which fibroblasts (FBs) and cells of the immune system are dispersed. Amongst the proteins, COL contributes 70–80% to the dry weight and confers tensile properties, followed by elastin (2–4% of the dermis per volume), which provides resilience and softness (Waller and Maibach 2006). The most abundant GAG is hyaluronic acid (HA), followed by several derivatives of chondroitin sulphate. Although GAGs represent only 0.1–0.3% of the dry weight of skin, they can bind up to 1000 times their own volume in water (Bernstein et al. 1996), thereby regulating the state of hydration and plumpness of the organ. The intrinsic and photoinduced aging processes determine the alterations of all these structural molecules, thereby compromising the mechanical properties of the skin and significantly reducing its ability to maintain water in the bound state (Waller and Maibach 2006). FBs are responsible for the synthesis of ECM, but together with immune cells, they also participate in its degradation, releasing matrix metalloproteases (MMPs) and other proteases and hyaluronidases (Pittayapruek et al. 2016).

Skin Appendages

The main skin appendages are the sebaceous glands (SGs), sweat glands, hair follicles (HFs) and nails. These organs are strongly integrated with the surrounding skin environment but have their own metabolism. SGs are holocrine glands comprising sebocytes that change into lipid-producing cells from the undifferentiated basal layer, which finally die to secrete the oily and waxy substance called *sebum* (Mitsui 1997). Sebum consists of squalene, esters of glycerol (glycerides) and wax, free fatty acids and free and esterified cholesterol (Picardo et al. 2009; Wertz 2009). It is excreted through SG ducts to the skin surface, almost always by way of the HF infundibulum, or HF canal, because HFs and SGs are anatomically associated in the so-called pilosebaceous unit.

HFs of scalp and body have an enormous impact on the appearance and related psychological, social and cultural implications. For this reason, the hair care market has huge commercial value. HF is a complex organ characterised by continual and cyclical transition amongst growth stage (anagen), in which the development of the hair is observed; a subsequent regression stage (catagen), in which the apoptosis of a considerable part of HF cells takes place; and a stage of quiescence at the end (telogen), following which the HF returns to the anagen stage with the formation of a new hair shaft. This life cycle is repeated over time with different rhythms depending on the region of the body (for more information on hair biology, see Paus and Peker 2003) and is controlled by the dermal papilla (DP), an inner region of the basal bulb comprising specialised FBs. The DP is in close contact with the *matrix*, a population of special KCs with high proliferative activity that occupies the upper part of the basal bulb and from which the hair develops.

During the telogen phase, the DP enters a resting phase, the basal bulb degenerates and the hair shaft remains in the scalp until it is pushed out by the growth of a new anagen hair (*exogen*). The telogen ends when DP releases signals that activate follicle regeneration, a process that starts from stem cells stored in a specialised follicular region called *bulge region*.

2.2.2 Effects of Microalgae Extracts on Epidermis

The effects of MA extracts on KC differentiation have been often studied using some protein markers indicative of CE development, but the findings obtained can be considered representative of the whole differentiation process, including the formation of the lamellar bodies. KC differentiation is of relevant cosmetic interest because its anomalies affect the proteolipid barrier with consequences on softness and smoothness of the epidermis.

Tests performed on human skin cultivated ex vivo showed that some extracts of *Tetraselmis suecica* can stimulate the synthesis of involucrin and filaggrin in KCs (Pertile et al. 2010). Involucrin modulation was obtained on the same experimental model by treatment with extracts of *Monodus subterraneus* and *Chlorococcum* sp., but with different results depending on the solvent used for the preparation of the extracts (Zanella et al. 2012). An aqueous extract of *Chlorella vulgaris*

(Dermochlorella, Codif) stimulated CE proteins, SPRs and elafin (Morvan and Vallee 2007). Thus far, little is known about the effects of MAs on the lipid composition of SC.

The cosmetic industry is also very interested in preventing or repairing the damages induced by ultraviolet radiation (UV), which causes photoaging. Nizard et al. (2004) showed in vivo that extracts of *P. tricornutum* stimulate the protective activity of 20S proteasome in KCs, preventing the increase of oxidised proteins and improving the protection of cell from UVB damages. Other molecules of interest for the same application are mycosporine-like amino acids (MAAs), which are secondary metabolites characterised by a cyclohexenone or cyclohexenimine chromophore conjugated with one or two amino acids (Cardozo et al. 2007). These compounds do not regulate epidermis metabolism but protect from UV due to their screening action in absorptions from 309 to 360 nm (Hartmann et al. 2015). In Table 9.1, some potential microalgal sources of MAAs are listed, but researchers need to beware, because this list also includes some toxic species not suitable for cosmetics (e.g. *Alexandrium tamarense*).

2.2.3 Activity of Microalgae Extracts on Dermis

The dermis is an object of special attention in the cosmetic field because its structure plays a primary role in determining the tensile properties and plumpness of the skin. Alterations connected to aging determine flaccidity and the formation of wrinkles. Intrinsic aging occurs physiologically, but it is accelerated by oxidative stress induced by solar radiation (photoaging) and by other stressful factors related to lifestyle and environment.

Chung et al. (2001) showed by in vivo analysis that intrinsic aging leads to a reduction of COL synthesis by FBs, whereas chronic photoaging leads to an increase, which does not compensate for the increased degradation due to the secretion of collagenases (MMP1 and MMP2) by the same cells. In both processes, the dermis COL undergoes qualitative and quantitative decrease with aging (Waller and Maibach 2006). This has decidedly oriented cosmetics towards the search for natural preparations suitable for promoting the production of COL, especially the type I (85–90% of this protein) and type III (10–15%) (Cheng et al. 2011). Amongst the preparations obtained from MAs, an aqueous extract of *Nannochloropsis oculata* exerted strong protection from oxidative stress and stimulated the production of COL in FB cultures (Stolz and Obermayer 2005). A similar preparation obtained from *D. salina* also stimulated COL production and cell proliferation (Stolz and Obermayer 2005). The already mentioned extract of *C. vulgaris* marketed under the name *Dermochlorella* was tested on cultures of primary FBs and KCs, as well as in clinical trials, and produced the following anti-aging and anti-inflammatory effects (Morvan and Vallee 2007):

– Stimulation of the synthesis of COL-I, COL-III, elastin, collagenase inhibitors and *plasminogen activator inhibitor-2*

Table 9.1 Mycosporine-like amino acids with UV shielding properties identified as compounds of microalgal species. Nomenclature according to www.algaebase.org (re-elaborated from Llewellyn and Airs 2010, Priyadarshani and Rath 2012, Flaim et al. 2014 and Suh et al. 2014)

Compound	Microalgae source
Sporopollenin	*Characium terrestre*, *Coelastrum microporum*, *Enallax coelastroides*, *Scenedesmus* sp., *Scotiella chlorelloidea*, *Scotiellopsis rubescens*, *Spongiochloris spongiosa*, *Dunaliella salina*, *Chlorella fusca*
Mycosporine glycine	*Chlamydomonas hedleyi*, *Alexandrium tamarense*, *Karlodinium venificum*, *Gymnodinium galatheanum*, *Prorocentrum lima*, *P. micans*, *P. cordatum*, *Scrippsiella trochoidea* and *Oxyrrhis marina*
Shinorine	*Chlamydomonas hedleyi*, *Peridinium aciculiferum*, *Chlorarachnion reptans*, *Alexandrium tamarense*, *Karlodinium venificum*, *Gymnodinium galatheanum*, *Kryptoperidinium foliaceum*, *Prorocentrum lima*, *P. micans*, *P. cordatum*, *Scrippsiella trochoidea* and *Oxyrrhis marina*
Porphyra-334	*Chlamydomonas hedleyi*, *Peridinium aciculiferum*, *Thalassiosira weissflogii*, *Alexandrium tamarense*, *Karlodinium venificum*, *Gymnodinium galatheanum*, *Prorocentrum micans*, *P. cordatum* and *Scrippsiella trochoidea*
Palythene	*Peridinium aciculiferum*, *Alexandrium tamarense*, *Karlodinium venificum*, *Prorocentrum lima*, *P. micans*, *P. cordatum* and *Scrippsiella trochoidea*
Palythine	*Peridinium aciculiferum*, *Alexandrium tamarense*, *Karlodinium venificum*, *Gymnodinium galatheanum*, *Prorocentrum lima*, *P. cordatum* and *Scrippsiella trochoidea*
Palythic acid	*Karlodinium venificum*, *Gymnodinium galatheanum*, *Kryptoperidinium foliaceum*, *Prorocentrum lima*, *P. micans*, *P. cordatum* and *Scrippsiella trochoidea*
Asterina-330	*Peridinium aciculiferum* and *Heterosigma akashiwo*
Undetermined mycosporine-like amino acids	*Ankistrodesmus spiralis*, *Chlorella minutissima*, *Chlorella sorokiniana*, *Dunaliella tertiolecta*, *Pelagococcus subviridis*, *Porphyridium purpureum*, *Rhodomonas maculata*, *R. salina*, *Cryptomonas reticulata, Cryptomonas baltica, Scotiella chlorelloidea*, *Isochrysis* sp., *I. galbana*, *Pavlova gyrans*, *Emiliania huxley*i, *Corethron criophilum*, *Thalassiosira tumida*, *T. weissflogii*, *Porosira pseudodenticulata*, *Stellarima microtrias, Alexandrium catenella, Euglena gracilis, Nannochloropsis oculata and Nephroselmis rotunda*

– Inhibition of the expression of collagenase activators: *tissue plasminogen activator* and *urokinase plasminogen activator*
– Increased synthesis of the antioxidant enzyme *thioredoxin-2*

The extracts of *Chlorococcum* sp., *Chaetoceros* sp. and *M. subterraneus* stimulated COL-I production in primary FB cultures (Zanella et al. 2012). The extracts of *Porphyridium purpureum*, *Rhodosorus marinus*, *Chlorella pyrenoidosa* and *D. salina* showed high inhibitory effect on hyaluronidase, the enzyme that degrades the polysaccharide fraction of ECM (Fujitani et al. 2001).

2.2.4 Effects of Microalgae Extracts on Skin Pigmentation

MAs offer opportunities in the development of novel cosmetics for skin pigmentation. Products that inhibit melanogenesis (skin lighteners) and stimulate it (skin darkeners or tanners) are both appreciated. Skin lighteners are used to obtain a lighter complexion or to treat unwanted hyperpigmentation (e.g. lentigo solaris and melasma), whereas skin darkeners promote a tan without exposure to solar radiation or prepare the skin for sun exposure, thereby preventing erythema or burns.

Many microalgal compounds exert an activity on tyrosinase, the key enzyme that controls melanin synthesis (Nordlund et al. 1989). Tyrosinase catalyses melanin synthesis by hydroxylation of L-tyrosine to *3,4-dihydroxy-L-phenylalanine* (L-DOPA) and by oxidation of L-DOPA to dopaquinone followed by further conversion to melanin (Godin and Touitou 2007). Some microalgal compounds, particularly fatty acids and CTs, have been shown to exert an activity on tyrosinase. Interestingly, although saturated fatty acids often stimulate melanogenesis by delaying the degradation of tyrosinase, PUFAs have a predominantly inhibitory effect (Table 9.2) by downregulating the activity of the enzyme and by accelerating its degradation (Ando et al. 1998; Chiang et al. 2011). Since MAs are major producers of PUFAs, most preparations obtained from their extracts are expected to act as skin lighteners. In fact, extracts of *Nannochloropsis gaditana* showed an inhibition of tyrosinase (Letsiou et al. 2017), and extracts of *T. suecica* (Pertile et al. 2010), *Chaetoceros calcitrans* f. *pumilus*, *M. subterraneus*, *Chlorococcum minutum*, *Thalassiosira pseudonana* (Zanella et al. 2012) and *Nannochloropsis* sp. (Zanella and Pertile 2016) acted as skin lighteners in ex vivo skin cultures. Kurfurst et al. (2010) showed that *T. pseudonana* reduces melanin synthesis and inhibits its delivery to KC by downregulating the expression of *Myosin-X protein*, a protein involved in this process. Finally, a hydro-alcoholic extract of *Chlamydomonas reinhardtii* inhibited melanogenesis in melanoma cell cultures and in 3D human skin equivalent (hSE) (Lee et al. 2018).

However, it is wrong to assume that the high concentration of PUFAs is a guarantee of skin whitening activity, because microalgal extracts are complex mixtures of bioactives, whose final effects depend on the overall balance of their combined effects. For example, the ethyl acetate extract of Tahitian *Isochrysis* (T-Iso), a cos-

Table 9.2 Activity of some fatty acids on tyrosinase

Compound	Activity	Reference
Palmitic acid (C16:0)	stimulating	Ando et al. (1998, 1999)
Stearic acid (C18:0)	stimulating	Ando et al. (1998)
Oleic acid (C18:1$n-9$)	Inhibiting	Ando et al. (1998)
Linoleic acid (C18:2$n-6$)	Inhibiting	Ando et al. (1998, 1999)
α-Linolenic acid (C18:3$n-3$)	Inhibiting	Ando et al. (1998)
Arachidonic acid (C20:4)	Inhibiting	Mishima et al. (1993)
Eicosapentaenoic acid (C20:5$n-3$)	Inhibiting	Mishima et al. (1993)
Docosahexaenoic acid (C22:6$n-3$)	Inhibiting	Balcos et al. (2014)

metic preparation marketed with the name *BIO1659* (Symrise AG), can stimulate melanogenesis in the skin and hair (Herrmann et al. 2012a, 2013), even though this Haptophyta is an excellent producer of PUFAs (Mishra and Mishra 2018).

Amongst the microalgal compounds that show activity on skin melanogenesis are CTs. Fucoxanthin (FXT) has been reported to decrease tyrosinase activity in UVB-irradiated guinea pigs, melanogenesis in UVB-irradiated mice and the mRNA levels of proteins linked to melanogenesis in skin cells (Sathasivam and Ki 2018). Also, orally administered lutein and zeaxanthin promoted skin lightening in a clinical trial involving 46 healthy subjects (Juturu et al. 2016). Zeaxanthin purified from *N. oculata* showed anti-tyrosinase action (Shen et al. 2011), thereby confirming that contributes to the skin whitening properties of this MA. Finally, ATX can inhibit skin pigmentation by interfering with the signaling of the *stem cell factor* released by KCs, which regulates different aspects of the melanocyte activity, including proliferation, differentiation and melanogenesis (Pillaiyar et al. 2017)

2.2.5 Preliminary Evidence of Microalgae Activities on Signals Released by the Peripheral Nervous System

A further aspect that should be considered for skin homeostasis and wellness is the effect of neurogenic inflammation, which is a challenging issue of great cosmetic interest due to its implications on irritation and itching. In vivo, skin responds to stress-induced brain nervous stimuli producing numerous local signals. KCs and melanocytes secrete *corticotropin-releasing hormone* (CRH), *adrenocorticotropic hormone* (ACTH) and catecholamines. Dermal FBs secrete ACTH, cortisol and prolactin. Skin nerve endings secrete adrenaline, noradrenaline and substance P. SGs secrete CRH and prolactin (Zmijewski and Slominski 2011; Alexopoulos and Chrousos 2016). In addition, cutaneous nerve endings and almost all skin cells share the ability to produce and respond to special cytokines called *neurotrophins* (NTs) in a paracrine and autocrine way. These affect many metabolic processes of the skin (e.g. FB migration, melanocyte response to UV stress and epidermal differentiation) and stimulate the development of nerve endings (Borroni et al. 2009; Truzzi et al. 2011). The NT signalling network is important in some inflammatory processes, such as atopic dermatitis and psoriasis, in which nervous stress plays a role. NTs can induce proliferation of cutaneous nerve endings with important consequences on symptoms, such as itching and pain (Grewe et al. 2000; Pavlovic et al. 2008). Studies on the activity of microalgal extracts on skin disorders due to NTs are limited, but some important evidences concerning compounds from MAs suggest that they might be very effective. Horváth et al. (2015) verified that βC and lutein are effective in the treatment of neurogenic inflammation induced on mouse ear by stimulation with mustard oil. These two CTs (but not lycopene) negatively modulated the expression of *transient receptor mustard oil potential ankyrin 1* in peptidergic nerve terminals. Sharma et al. (2018) showed that ATX inhibited neuropathic pain in rats subjected to thermal and mechanical trauma. This is consistent with the efficacy of ATX in the inhibition of *N*-methyl-D-aspartate receptors, which are also

implicated in the mechanism of action of pain from neurogenic inflammation (Kinkelin et al. 2000).

Scandolera et al. (2018) recently tested a *Rhodosorus marinus* extract (Rhodophyta) on in vitro cultures of human (h) KC, astrocytes and hSE, thereby demonstrating its effectiveness in reducing the secretion of *interleukin-1α* (IL-1α) and *nerve growth factor* following an inflammatory stimulus with phorbol myristate acetate (PMA). The same preparation inhibited PMA-induced overexpression of *transient receptor mustard oil potential vanilloid 1*, another receptor implicated in the inflammation induced by mustard oil. These activities were attributed to the γ-aminobutyric acid (GABA) and GABA-alanine derivatives contained in the tested MA.

2.2.6 Activity of Microalgae on Skin Appendages

The cosmetic industry is strongly interested in achieving novel natural principles suitable to modulate sebogenesis, because sebum overproduction affects the appearance of the skin and hair, making them shiny and oily. Sebum is important in skin wellness, because the hydrolipidic film derived from secretions of the sebaceous and sweat glands contributes in regulating water loss and in protecting the skin against mechanical damage and UV. Its composition is relevant for both UV-induced photo-oxidation processes and the effects on the skin inflammasome (Oyewole and Birch-Machin 2015), since the presence of oleic acid and other unsaturated fatty acids can irritate sensitive subjects (DeAngelis et al. 2005; Schwartz et al. 2012). In addition, it is the main source of tocopherol for the skin (Mackenna et al. 1950; Thiele et al. 1999) and one of the main sources of CTs (Darvin et al. 2011a).

Excessive sebum production can also lead to skin disorders, such as seborrheic dermatitis, acne and dandruff. Although these disorders have multifactorial causes that often involve the skin microbiome, excessive sebum is an important condition for their onset (DeAngelis et al. 2005; Schwartz et al. 2012). Few studies have addressed the exploitation of MAs for the treatment of skin appendages, but early findings are promising. A hydrophilic extract of *Galdieria sulphuraria* reduced the expression of *5α-reductase type-1* (5α-R1), an enzyme involved in testosterone metabolism, in immortalised hFBs and hKCs (Bimonte et al. 2016). The reduction of 5α-R1 was considered responsible for the downregulation of sebogenesis, which has been documented also in vivo. In fact, SG activity is largely affected by male hormones (Mitsui 1997, p. 18).

Extracts of *C. calcitrans* f. *pumilus*, *T. pseudonana*, *M. subterraneus*, *C. minutum* and *Nannochloropsis* sp. decreased sebum production in human SGs cultivated ex vivo and were found comparable with or superior to treatments with reference compounds, e.g. capsaicin (Zanella and Pertile 2016; Zanella et al. 2016). MA extracts can regulate sebum quantitative production, but to date, no information is available on their effects on sebum composition, despite both have relevant effects on the skin microbiome (DeAngelis et al. 2005; Byrd et al. 2018). For instance, two yeasts considered amongst those responsible for dandruff, *Malassezia globosa* and

M. restricta, grow only in areas of the scalp where sebum is overabundant (Schwartz et al. 2012). Concerning sebum composition, *Propionibacterium acnes*, a bacterium predominant in sebaceous follicles, metabolises some lipids to short-chain fatty acids that act as antimicrobials (Christensen and Brüggemann 2013). Since MAs regulate the quantitative production of sebum by SGs, it is likely that they may also influence its composition, but it has not been possible to find studies in this regard.

HF is another appendage of great interest in the cosmetic industry, but surprisingly, the disclosure of active ingredients from MAs still has few well-documented case studies. Amongst these, the methanolic extract of T-Iso marketed with the name *BIO1631* (Symrise AG), showed anti-hair loss effects due to prolonged HF anagen phase and a reduced ratio between apoptotic and proliferating KCs of the matrix (Herrmann et al. 2012b, 2013). Similar results were obtained in ex vivo HF cultures with ethanol extracts of *Chaetoceros* sp., *Chlorococcum* sp. and *M. subterraneus* (Zanella et al. 2012), whereas some extracts of *T. suecica* had been shown to reduce hair growth (Pertile et al. 2010). A number of patent applications have been filed for MA embodiments aimed at treating hair, protecting against environmental agents (e.g. UV and pollution) and increasing mechanical resistance (Table 9.3).

2.3 Role of Oxidative Stress in Skin Photoaging and Inflammation

Aging processes are closely linked to oxidative stress produced by highly reactive compounds, which are free radicals or, more correctly, reactive oxygen species (ROS) and reactive nitrogen species (RNS). These highly reactive compounds include a heterogeneous group of molecules, some electrically charged and others neutral, characterised by the presence of atoms with an unpaired electron in the outermost atomic orbital (Halliwell 2006). This condition makes them extremely unstable, since they attempt to lose or add an electron to restore the equilibrium of the orbital, reacting and modifying different molecules, such as glucides, proteins, lipids and DNA, with which they come into contact in the cellular environment. Some ROS are produced physiologically through cell metabolism in cytosol organelles, such as mitochondria, endoplasmic reticulum and peroxisomes (Rinnerthaler et al. 2015). For example, hydrogen peroxide is normally produced by some reactions of the mitochondrial respiratory chain or by lymphocytes in immune defence processes. For this reason, cells also have enzymes that can neutralise ROS, thereby protecting themselves from damage.

Unfavourable external factors, such as exposure to UV, aggressive agents of chemical or biological origin, inflammatory agents and atmospheric pollution, can increase ROS production up to a level that that overcomes cell defence and causes cell damage. These events are strongly connected with skin aging. Therefore, ROS toxicology has become a core issue for the cosmetic industry. Although many oxidised molecules can be repaired or catabolised and replaced, some instances of

Table 9.3 Some granted and pending patents concerning hair treatments with preparations or extracts obtained from MAs

MA species	Claim	Applicant		Reference
Porphyridium, *Rhodosorus*, *Chlorella*, *Dunaliella*, *Closterium*, *Phaeocystis*, *Pleurochrysis*, *Primnesium*, *Euglena*	5alpha-reductase inhibitor and hair-growing agent containing the same	Microalgae Corp	JP2002068943 (A)	Fujitani et al. (2002)
Chaetoceros gracilis	Scalp hair loss prevention and improvement	Park SH	KR20140062249 (A)	Park et al. (2014)
Chlorella	Grey hair prevention agent	Shiseido Co Ltd	JP2002212039 (A)	Suzuki et al. (2002)
Chlorella vulgaris	Hair papilla cell growth agent and vascular endothelial growth factor production promoter	Naris Cosmetics Co. Ltd	JP2006282597 (A)	Megata (2006)
Tetraselmis tetrathele	Improves scalp and prevents hair loss	Park SH	KR20150100302 (A)	Park et al. (2015)
Prototheca moriformis, *Chlorella protothecoides*	Increases hair shine, combability, strength, prevents UV and pollution damages, moisture loss or spit ends	Solazyme Inc.	US20150352034 (A1)	Schiff-Deb and Sharma (2015)
Chlorella	Hair growth and care	Nakano Seiyaku KK, Chlorella Ind	JPS63135315 (A)	Katsuyama and Obata (1988)
Porphyridium cruentum	Enhances beta-catenin activity and cell differentiation in dermal papilla cells	Radiant Co Ltd. (KR), Seoul Cosmetics Ltd. (KR)	KR101856480 (B1)	Kee et al. (2018)
Haematococcus pluvialis	Source of ATX for HF protection from oxidative stress and anti-hair loss	Cognis Deutschland GmbH & Co. KG.	WO03105791 (A1)	Eisfeld and Mehling (2003)
Isochrysis	Improves combability, strength, volume and stress resistance and reduces frizz and breakage	Symrise AG	WO2019037843 (A1)	Nakano et al. (2019)

damage are permanent and accumulate over time, thereby leading to many of the effects we observe in aged tissues. There are several environmental factors that produce chronic oxidative stress (e.g. air pollution and aggressive detergents), but solar radiation is the most relevant and studied. UVR associated with solar radiation comprises UVC (100–280 nm), UVB (280–315 nm) and UVA (315–400 nm). UVC is blocked by the atmospheric ozone layer, whereas UVB (<5% of the UVR), which does not penetrate far beyond the epidermis, produces DNA damage, burns and erythema (Svobodova et al. 2006). Oxidative damage at the epidermis, which as mentioned is densely cellularised, is mainly caused by UVB (Van Laethem et al. 2005).

Most of UVR is composed of UVA, which has lower energy content than UVB and requires doses of 600–800 times greater to produce erythema, but they are able to penetrate deeply into the dermis (Gilchrest 1996). ROS toxicology is extremely complex, and possible consequences in the cell environment strongly depend on several conditions, which include overall energy dose of the radiant spectrum, individual characteristics of the skin (e.g. pigmentation and epidermis thickness), diet and lifestyle of the subject.

Importantly, at low doses, solar irradiation stimulates autogenous defences against ROS by activating the transcription factors of *forkhead box, class O family member proteins* (FoxOs), which promote the transcription of antioxidant enzyme genes, e.g. *superoxide dismutase-2* (SOD2), *peroxiredoxins 3* and *5* and *catalase* (CAT) (Klotz et al. 2015).

2.3.1 Skin Inflammation by Oxidative Stress

Figure 9.4 summarises and simplifies some biochemical pathways through which ROS can induce oxidative damage. UVB activity is expressed at the level of epidermis KCs, whereas it affects the dermis only marginally. The absorption of energy can denature DNA by the formation of pyrimidine dimers, leading to apoptosis by p53 activation (Van Laethem et al. 2005). This signal activates the cytosolic protein *Bcl-2-associated-X-protein* (Bax), which is stimulated by ROS also through the activation of *mitogen-activated protein kinases* (MAPKs), particularly via the p38MAPK. In the activated form, Bax moves to the outer mitochondrial membrane, where it produces two effects: (1) inhibition of *B-cell lymphoma-2* (Bcl-2), an antagonistic signal that promotes cell survival by activating mechanisms of protection of mitochondrial integrity (Dewson and Kluck 2010), and (2) release of *cytochrome-c* (cyt-c), which is the main signal of apoptosis activation (Van Laethem et al. 2005).

Cyt-c activates Casp-9, which in turn activates Casp-3, an effector protease that is the primary performer of apoptotic death (Brentnall et al. 2013). Casp-3 cleaves and activates *protein kinase C delta type* (PKC-δ), a pro-apoptotic factor that cooperates in the activation of Bax by downregulating *induced myeloid leukaemia cell differentiation protein* (Mcl-1), a potent anti-apoptotic factor (D'Costa and Denning 2005). This cascade triggers a self-amplifying cycle that results in the expression of

pro-inflammatory signals IL-1α and IL-6 (Fig. 9.4). IL-1α acts as an autocrine signal stimulating the same KC to release *granulocyte-macrophage colony stimulatory factor* (GM-CSF) (Yano et al. 2008; Imokawa et al. 2015). GM-CSF and IL-1α are released into the surrounding tissue and penetrate the dermal layer, stimulating FBs to release neprilysin (also called *neutral endopeptidase*, NEP), a protein that degrades elastin, thereby favouring the formation of wrinkles (Imokawa et al. 2015).

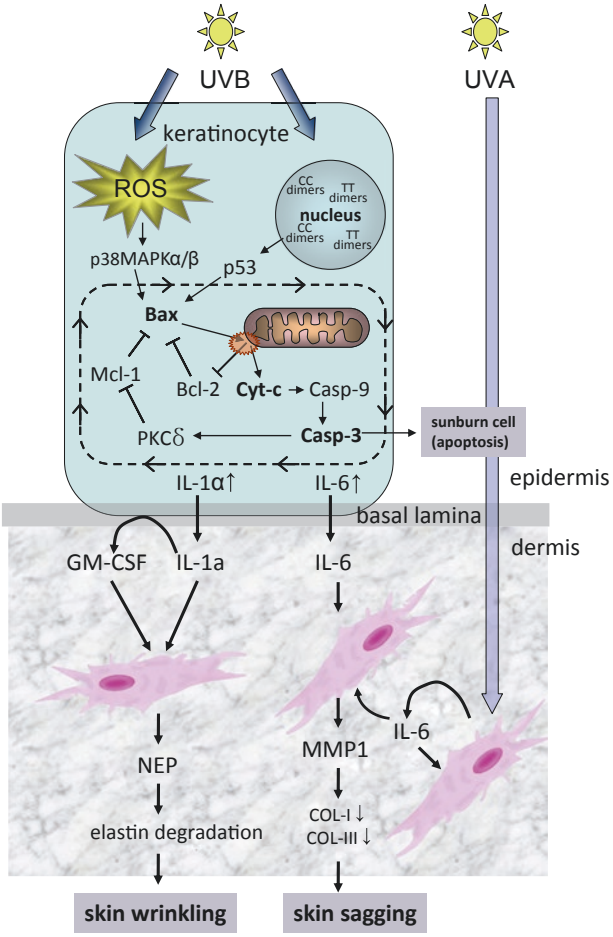

Fig. 9.4 Some major inflammatory and apoptotic routes in the skin epidermis. UVB induces DNA denaturation and release of p53, as well as the activation of p38MAPKs. These effects activate Bax, which in turn promotes a self-amplifying pro-apoptotic cascade of signals and effector proteins (schematised within the dashed line). Besides, Bax is sustained and amplified by the inhibition of some anti-apoptotic proteins (Bcl-2 and Mcl-1). This scenario leads to the KC death and release of inflammatory signals that induce dermis ECM degradation, especially by lysis of elastin. UVA reaches the dermis FBs and stimulate the release of inflammatory signals and MMPs, promoting mainly the degradation of COLs (Re-elaborated from Van Laethem et al. 2005 and Imokawa et al. 2015)

At the same time, IL-6 promotes the release of collagenases by FBs, particularly MMP1, which acts on COL-I and COL-III by promoting skin flaccidity (for more information on MMPs, see also Pittayapruek et al. 2016).

UVA radiation acts mainly on dermal FBs inducing the secretion of IL6, which in turn promotes in autocrine and paracrine manner the production and release of MMP1, whereas NEP to a lesser extent (Imokawa et al. 2015; Wlaschek et al. 1993).

Imokawa et al. (2015) showed that UVB acts mainly on KCs favouring the signal cascade that promotes FB release of NEP and degradation of elastin, whereas UVA is less active on KCs and causes dermal FBs to secrete IL-6 and MMP1, with more intense degradation of COLs. According to these findings, UVB mainly favours the formation of wrinkles, whereas UVA is the main factor responsible for skin sagginess.

2.3.2 Activation of the MAPKs/AP-1 and PI3K/Akt Pathways by Oxidative Stress

Figure 9.5 shows two biochemical paths promoted by ROS as a result of alternative combinations of signals and transcription factors outlined below.

Activator Protein 1 (AP-1) pathway

ROS activates several MAPKs, which include p38, *extracellular signal-regulated kinase* (ERK) and *c-Jun N-terminal kinase* (JNK). These MAPKs cooperate by activating the nuclear transcription factor AP-1 (Berthon et al. 2017). The latter inhibits the *transforming growth factor-β* (TGF-β) that stimulates procollagen production (Pittayapruek et al. 2016) and promotes the expression of pro-apoptotic factors, such as *BCL2-antagonist of cell death* (BAD) and *signal transducer and activator of transcription 3* (STAT3). Besides, AP-1 induces ECM degradation by stimulating the secretion of MMPs (Akhalaya et al. 2014; Berthon et al. 2017).

Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells (NF-kB) pathway

ROS can activate *phosphoinositide-3-kinase* (PI3K) by triggering the sequential phosphorylation of *protein kinase* B (Akt), *IkB kinase* (IKK) and *inhibitor of kB* (I-kB). Akt also inhibits transcriptional functions of FoxOs reducing the expression of antioxidant factors, such as CAT and SOD (Berthon et al. 2017). I-kB loses its inhibitory function on *nuclear factor kappa-light-chain-enhancer of activated B cells* (NF-kB), which consist of two proteins, namely, p50 and p65. In the absence of inhibition, NF-kB moves from cytosol into the nucleus and stimulates the expression of various pro-inflammatory molecules, i.e. IL-1β, IL-6, IL-8, tumour necrosis factor-α (TNFα), *inducible nitric oxide synthase* (iNOS) and *cyclooxygenase-2*

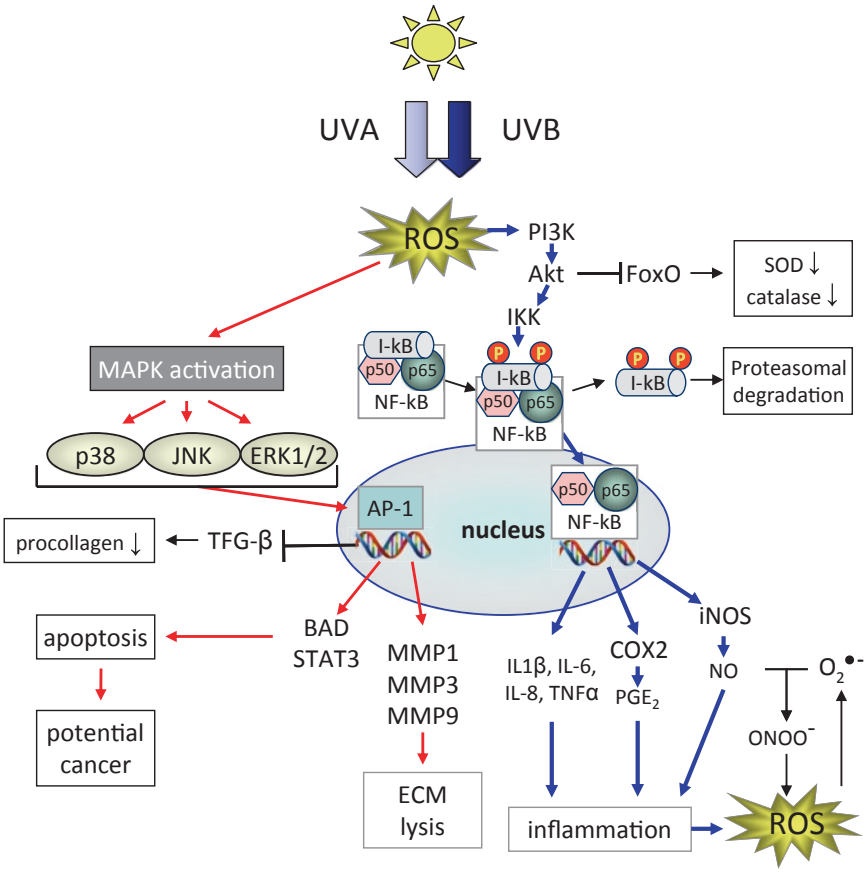

Fig. 9.5 Biochemical pathways governed by the key nuclear transcription factors AP-1 and NF-kB (re-elaborated from Chiou et al. 2011, Zhang et al. 2014 and Berthon et al. 2017)

(COX-2) (Zhang et al. 2014; Berthon et al. 2017). These signals and enzymes are involved in promoting inflammation and RNS production (Fig. 9.5), resulting in the enhancement of oxidative stress to which contribute also the immune cells stimulated by ILs and TNFα.

2.3.3 Cytoprotective Response Involving the Keap1/Nrf2 Pathway

The cell reacts to the formation of ROS by activating signals that enhance antioxidant defences and repair or degrade dysfunctional molecules. Emphasis is attributed to the regulatory factor *NF-E2 p45-related factor 2* (Nrf2), which controls the gene expression of *antioxidant response elements* (AREs) and a large range of other proteins related to cytoprotective function (Baird and Dinkova-Kostova 2011).

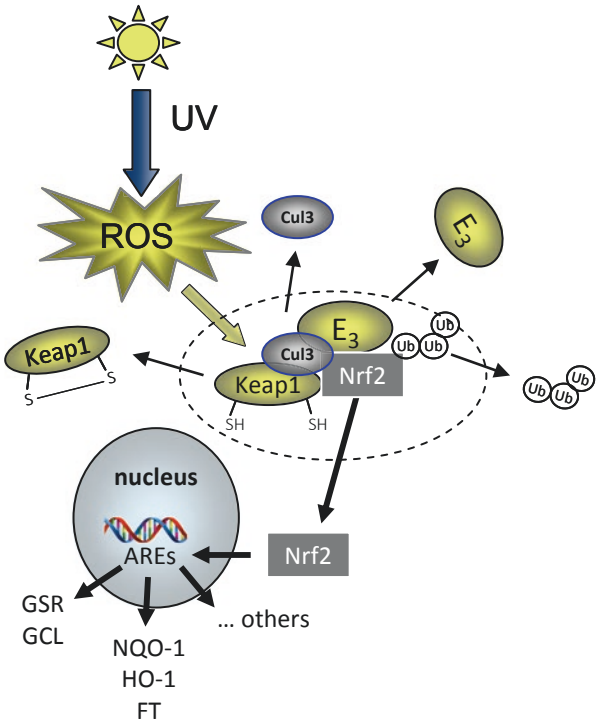

Fig. 9.6 Signalling pathway controlled by Nrf2/Keap1 complex. The basal repressed condition of Nrf2 depends on the molecular complex within the dashed line. The redox perturbation of the citosol triggers the detachment of Keap1 and consequently of Cul3 and ligase E3 (re-elaborated from Kobayashi et al. 2004, Baird and Dinkova-Kostova 2011 and Kansanen et al. 2013)

Under homeostatic conditions, Nrf2 is found at the inhibited state in the cytosol and bound to *Kelch-like ECH-associated protein 1* (Keap1), a cysteine-rich dimeric protein. It seems that Keap1 acts as an adapter for *cullin 3* (Cul3), a protein that interacts with *E3 ligase*, thereby resulting in the proteasomal degradation of Nrf2 by polyubiquitination (Kobayashi et al. 2004). Therefore, Nrf2 is characterised by a rapid turnover and is continuously synthesised, inhibited by Keap1 and degraded via proteasome. Due to its high cysteine content, Keap acts as a sensor for the alterations of the cellular redox environment (Baird and Dinkova-Kostova 2011). In the presence of ROS, Keap1 cysteine groups are oxidised to cystines, and through conformational changes and interactions that are not yet fully elucidated, Nrf2 is rapidly released and deubiquinated, thereby escaping degradation and moving into the nucleus (Fig. 9.6). Moreover, the newly synthesised Nrf2 continues to move into the nucleus as long as the cellular environment maintains Keap1 in a reduced form (Baird and Dinkova-Kostova 2011; Kansanen et al. 2013). In the nucleus, Nrf2 cooperates with *small Maf protein* (sMaf), thereby promoting the expression of over 600 target genes (Baird and Dinkova-Kostova 2011). Amongst these genes, the expression of AREs produces proteins of relevant protective impact, e.g. *NAD(P)H quinone dehydrogenase 1* (NQO-1), *haeme oxygenase 1* (HO-1), *glutamate-cysteine ligase* (GCL), *glutathione-disulphide reductase* (GSR), *leukotriene B4 12-hydroxydehydrogenase* (LB4DH) and *ferritin* (FT) (Baird and Dinkova-Kostova 2011; Kansanen et al. 2013). In the nucleus, Nrf2 undergoes a slow turnover due to non-proteasomal degeneration (Kobayashi et al. 2004).

2.3.4 Permanent Damages by Oxidative Stress in Human Skin

In the previous paragraphs, attention was on the signals that govern reactions to oxidative stress. Here, we focus on some oxidative damages to the enzymatic and structural components of the cell. Cells can survive oxidative damage by repairing or recycling processes, but some damaged molecules persist and tend to accumulate over time.

Oxidative stress frequently causes protein carbonylation and peroxidation of membrane lipids with formation of malonaldehyde and 4-hydroxy-2-nonenal (4HNE). The 4HNE groups of peroxidised lipids can give rise to non-enzymatic reactions and cross-link with carbonylated proteins, which in turn can contain proline and lysine modified into glutamic semialdehyde and aminoadipic semialdehyde, respectively (Castro et al. 2017). Such reactions, which establish covalent bonds between proteins and lipids, can prevent molecule unfolding, which is necessary for the enzymatic degradation and can favour aggregation in clusters of progressively increasing dimensions. Thus, heteropolymeric macroaggregates are formed; besides being unaffected by cytosolic proteases, they interfere with lysosomal functionality (Terman and Brunk 2004) and irreversibly bind to proteasomes, thereby blocking the activity of these organelles (Höhn et al. 2011). The inactivation of lysosomes and proteasome deprives the cell of its main tools for recycling dysfunctional molecules, thereby triggering a vicious cycle that favours the incorporation of other oxidised molecules into these 'ceroid' macroaggregates termed 'lipofuscin' (LF). LF has variable composition that includes proteins (30–58%), lipids (19–51%), carbohydrates (4–7%), metal ions and mineral elements, such as Fe, Cu, Al, Zn, Mn and Ca, which account for <2% (Terman and Brunk 2004; Jung et al. 2007). Being nondegradable, it accumulates indefinitely in postmitotic cells, occupying up to 75% of cell volume in motor neurons (Rinnerthaler et al. 2015). In proliferative cells, such as epidermal KCs, it ends up accumulating in the intercellular space upon cell death. The formation of age spots on the back of the hand is a common consequence of its accumulation. Age spots of the skin may have different origins, e.g. melanin overproduction, but the so-called senile lentigo or liver spot is due to LF (Skoczyńska et al. 2017), whose brownish colour is due to oxidation of lipids and metal ions. Wang-Michelitsch and Michelitsch (2015) reported interesting observations and hypothetical models to explain the isolation of LF in extracellular fibrotic capsules that increase in size and change shape over time.

2.3.5 Microalgal Products for Preventing and Treating the Oxidative Stress in Human Skin

The biochemical scenarios described above do not exhaust the possible reactions triggered by ROS but provide an idea of their complexity and highlight how these events are closely interconnected with inflammatory processes and skin aging. The

comprehension of these pathways is important for an appropriate interpretation of the antioxidant activity of many MAs. Herein, apart from their chemical activity of ROS scavenging, some MA metabolites regulate key factors that affect the inflammasome of skin cells.

On this regard, interesting reviews have recently reported extensive tables that summarise the cosmetic applications from microalgal compounds (Ariede et al. 2017; Mourelle et al. 2017) and list microalgal sources of active compounds of cosmetic interest (Berthon et al. 2017; Brunt and Burgess 2018; García et al. 2017). However, case studies based on extracts or preparation from MAs are limited, even if some of them have a relevant significance.

In addition to the aforementioned anti-oxidative activity of *N. oculata* (see Sect. 2.2.3), a hydro-alcoholic extract of *T. suecica* showed anti-stress properties with modulation of genes involved in the protection against oxidative damages (Sansone et al. 2017). An aqueous extract of *Scenedesmus rubescens* tested in vitro both on primary skin cells and ex vivo full-thickness skin (hFTS) exerted protective effects against UVR damages, stimulated COL, reduced DNA impairment (sunburns cells) and increased both mitochondrial efficiency and cell proliferation (Campiche et al. 2018).

An aqueous extract of *C. pyrenoidosa* showed intense protective activity against UVC damage in cultured FBs, reducing the expression of pro-apoptotic proteins, in particular *Fas-associated death domain-containing protein* and the activated Casp-3 (Shih and Cherng 2012). A similar preparation obtained from commercial *Chlorella* inhibited the expression of MMP1 and the pro-inflammatory signal *cysteine-rich angiogenic inducer 61* in cultures of FBs treated with UVB, thereby preventing the reduction of pro-COL (Chen et al. 2011). Finally, concerning the inhibition of inflammatory signals, the release of IL-1α by ex vivo hFTS stimulated with an irritant (SDS) was inhibited by treatments with lipophilic and hydrophilic extracts of *Nannochloropsis* to a comparable or greater extent than dexamethasone (Zanella and Pertile 2016).

However, most information concerning the potential anti-oxidative power of MAs is inferred from studies on the isolated compounds of which they are important sources. Subsequently, some relevant case studies are discussed below.

Carotenoids

CTs are yellow to red-brown pigments rich with unsaturated double bonds or phenolic rings, which are easily oxidised by ROS, thereby protecting cells from oxidative damage. They are polymeric molecules with isoprenic derivation and are divided into carotenes and xanthophylls. Carotenes, such as β-carotene (βC) and lycopene, lack bonds with oxygen. Xanthophylls, such as lutein, zeaxanthin, violaxanthin, canthaxanthin (CTX) and ATX, contain oxygen atoms. Ketocarotenoids, such as CTX and ATX, are synthesised in MAs and other microorganisms, but they are generally lacking in higher plants (Zhang et al. 2014; Safafar et al. 2015). Many green MAs synthesise mixtures of CTs, which are intermediate or final compounds

of complex biosynthetic pathways and are differently arranged amongst species depending on their enzymatic machinery (Jin and Melis 2003; Sathasivam and Ki 2018). However, each species is generally characterised by few prevailing CTs. For example, *Dunaliella bardawil* and *D. salina* produce mainly βC (Jin and Melis 2003), and *H. fluviatilis* is the richest source of ATX (Guerin et al. 2003). *Nannochloropsis* spp. synthesise several xanthophylls, amongst which violaxanthin and vaucheriaxanthin are prevalent, accompanied by antheraxanthin, zeaxanthin and other less abundant CTs (Antia and Cheng 1982; Lubián et al. 2000; Faé Neto et al. 2018).

CTs are chemically lipophilic and suitable to protect the integrity of cell membranes, as occurs with tocopherols. Aboul-Enein et al. (2003) quantified CTs, vitamin E and vitamin C of seven strains belonging to the genera *Dunaliella*, *Chlorella* and *Scenedesmus* and tested their extracts for the efficacy against lipid peroxidation of mice liver microsomes. In that case, the antioxidant efficacy was proportional to the microalgal concentration of active molecules. However, the efficacy of an antioxidant depends on the chemical structure of the ROS with which it reacts; hence, the ranking of extract strength from different MAs can change depending on the antioxidant test (Safafar et al. 2015). In Table 9.4, the scavenging efficiency of some CTs is shown, estimating their relative strength compared with some benchmark antioxidants (trolox, ascorbic acid and cysteine) (Rodrigues et al. 2012). The efficiency of each CT depends on the test considered (i.e. from the ROS involved in the

Table 9.4 Peroxyl radical hydroxyl radical ($HO^{\bullet}$), hypochlorous acid (HOCl) and peroxynitrite anion ($ONOO^{-}$) scavenging capacity of seven carotenoids and other compounds incorporated into liposomes

	Scavenging capacity[a]			
Compound	$ROO^{\bullet}$	$HO^{\bullet}$	HOCl	$ONOO^{-}$
β-Carotene	0.14	0.71	NA[b]	1.02
Zeaxanthin	0.56	1.41	3.87	0.77
Lutein	0.6	0.97	4.81	0.78
Lycopene	0.08	0.35	0.4	0.31
Fucoxanthin	0.43	1.18	6.26	NA
Canthaxanthin	0.04	0.28	0.1	NA
Astaxanthin	0.64	1.66	9.4	0.73
α-Tocopherol	0.48	1.77	NA	0.37
Quercetin	0.84	1.42	5.63	0.97
Trolox	**1.00**	**1.00**	NA	NA
Ascorbic acid	NA	NA	0.41	**1.00**
Cysteine	0.04	NA	**1.00**	0.02

The values are the mean of two independent experiments (from Rodrigues et al. 2012, a column of the original table was omitted; license conditions available at http://creativecommons.org/licenses/by/3.0/)

[a]The scavenging capacity was calculated by considering the following as references (in bold): trolox for $ROO^{\bullet}$ and $HO^{\bullet}$, cysteine for HOCl and ascorbic acid for $ONOO^{-}$

[b]NA: no activity was found for the tested concentrations

reaction), which explains the importance of introducing mixtures of different antioxidant molecules, rather than high quantities of a single compound. In addition to their specific chemical protection from ROS, many CTs are natural inhibitors of NF-kB (see Sect. 2.3.2) that governs most inflammatory reactions due to oxidative stress (Zhang et al. 2014).

Studies in vivo showed that the skin content in CTs is directly proportional to the consumption of fruit and vegetables and inversely proportional to stress factors, which is reflected in the condition of skin aging (Darvin et al. 2011a). βC and lycopene (carotenes) are the most abundant CTs in humans and constitute approximately 70% of the CTs ordinarily present in the skin (Choi et al. 2018), whereas xanthophylls are less common in the human diet. Research attention has been dedicated to ATX, which is one of the few microalgal molecules produced at the industrial scale (Spolaore et al. 2006). This powerful superoxide anion scavenger inhibits the release of MMP1 and NEP following UVA radiation upon treatment at low concentrations (Imokawa 2019). Its anti-inflammatory activity is also effective for stimuli other than photo-oxidation; for example, ATX inhibited the NF-kB activity, the expression of iNOS and COX-2 and release of TNF-α, IL-1β, IL-6 and IgE in a phthalic anhydride-induced atopic dermatitis animal model (Park et al. 2018). Camera et al. (2009) compared the protective activity of βC, CTX and ATX in FBs treated with UVA, disclosing that although βC is a strong $^1O_2$ quencher, it showed limited protective effects and resulted in phototoxicity at concentrations >2 μM. CTX did not prevent oxidative damage but increased the antioxidant enzyme HO-1, whereas ATX showed the greatest protective activity, such as reduction of Casp-3 and preservation of both the membrane integrity and the antioxidant enzymes (catalase and SOD). Nevertheless, βC showed an effective protection against damages from IR irradiation in clinical tests (Darvin et al. 2011b), thereby showing the complexity of the biological interactions caused by phytochemicals. FXT, another CT occurring in several MAs, promotes the expression of ARE genes (Fig. 9.6) via the stimulation of Nrf2 transcription factor (Berthon et al. 2017).

Overall, these data show that CTs exhibit metabolic interactions that cannot be explained with the mere ROS scavenging. Direct or indirect modulation of gene expression is often performed. More importantly, the chemical reactivity of an antioxidant compound is supposed to be independent from its isomeric conformation, but the modulation of gene expression may require molecular interactions that are isomer dependent. In this case, synthetic isomers could produce effects different from the natural blends. Studies on this topic are still limited. However, some interesting findings have been reported. For instance, Sun et al. (2016) showed that the isomer (3S,3′S)-trans-ATX, the form prevalent in *H. pluvialis*, is much more effective as a stimulant of mouse immune cells than the two other stereoisomers, i.e. (3R,3′R)-trans- and meso-trans-ATX, that contribute up to 75% of synthetic ATX. Analogously, the biological activity of the natural isomer of βC is superior to the synthetic all-trans forms (Spolaore et al. 2006).

Tocopherols and Polyphenols

Tocopherols and polyphenols are important for the protection of the skin. Polyphenols include a large family of molecules, comprising flavonoids, flavones, anthocyanidins, tannins and phlorotannins. Safafar et al. (2015) showed that both tocopherols and polyphenols are abundant in *Phaeodactylum* sp., *Nannochloropsis* sp., *Chlorella* sp., *Dunaliella* sp. and *Desmodesmus* sp. and can be efficiently extracted with methanol.

Tocopherols are a family of antioxidant molecules of which the most biologically active is α-tocopherol, namely, vitamin E, especially effective in preventing cell membrane oxidation (Marquardt et al. 2013).

Polyphenols, which are widespread even in the composition of higher plants, comprise a diversified class of hydrosoluble compounds that can perform an action in some way complementary to CTs and tocopherols. Goiris et al. (2014) studied six MAs from different classes and showed that they synthesise several polyphenols, which include phloroglucinol (39–81 μg/g dry weight (DW)), *p*-coumaric acid (540–7000 ng/g DW) and apigenin (7.3–13.6 ng/g DW). However, these values of concentrations are low in comparison with contents detected in many superior plants. Goiris et al. (2012) analysed CTs and the polyphenol content of hydroalcoholic extracts of some MAs and then measured their respective antioxidant capacity via three different assays: trolox equivalent antioxidant capacity, ferric reducing antioxidant potential and AAPH-induced oxidation of linoleic acid. Analysis of their findings shows that the antioxidant strength of the extracts is not always proportional to the total content of CTs and/or polyphenols and can vary with the test performed. Hence, the quantitative content in antioxidant compounds is not sufficient to establish the efficacy of microalgal preparations, because each species produces a combination of compounds with its own properties. Biological tests in ex vivo organ culture or in clinical trials are necessary to provide a reliable estimation of the efficacy of natural extracts.

Polysaccharides, Galactolipids and Lipids

Microalgal polysaccharides offer some interesting examples of antioxidant activity and other beneficial effects (see Raposo et al. 2013 for a review of their properties and applications). *P. cruentum* (Rhodophyta) produces sulphoglycolipids with important anticoagulant and antiviral properties, as well as antioxidant and anti-inflammatory activities (Plaza et al. 2009). The sulphated exopolysaccharides (SEP) produced by this MA inhibit NF-kB activity and the release of pro-inflammatory cytokines (Berthon et al. 2017). Biochemical techniques were also proposed for increasing the sulphation of these polysaccharides and their biological activity (Gersh et al. 2002).

Amongst Bacillariophyta species, the antioxidant activity of a β-D- glucan, also called chrysolaminarin or leucosin, was characterised. This glucose polymer contains β-1:3′- and β-1:6′-bonds in the ratio of 11:1 (Beattie et al. 1961). It is accumu-

lated as an energy reserve in *Odontella aurita* but also shows strong activity as a scavenger of hydroxyl radical (Xia et al. 2014). This polysaccharide was also isolated and quantified in *Cyclotella cryptica* (Roessler 1987), *P. tricornutum* (Caballero et al. 2016) and *T. pseudonana* (Hildebrand et al. 2017), and it might be present in all Bacillariophyceae species, as well as in other algal groups.

Interesting cosmetic applications connected with anti-inflammatory effects are also attributable to galactolipids (Fig. 9.7). MA extracts comprising *monogalactosyl diacylglycerol* (MGDG) and *digalactosyl diacylglycerol* (DGDG) demonstrated intense anti-inflammatory activity by reducing ear oedema after croton oil challenge in animal model, especially if the compounds had the esterified two eicosapentaenoic acid (EPA) residues (MGDG-EPA and DGDG-EPA) (Winget 1994). Their anti-inflammatory activity was showed using a *Chlorella minutissima* extract, but several other MAs were indicated as potential sources of this active compound (information worthy of confirmation), including *Chaetoceros*, *Cyclotella*, *Ellipsoidon*, *Isochrysis*, *Nannochloris*, *Nannochloropsis*, *Nitzschia*, *Phaeodactylum*, *Porphyridium*, *Skeletonema*, *Thalassiosira*, *Monochrysis* and *Monoraphidium*.

Bruno et al. (2005) showed that MGDG exerts a dose-dependent activity, which is higher than that of DGDG, and is optimised by the presence of EPA in its composition with anti-inflammatory efficacy at 20 mg/kg higher than the indomethacin control treatment (10 mg/kg). MGDGs have also been isolated from *Tetraselmis chui* and showed a strong inhibition of the release of NO by RAW264.7 macrophage cells (Banskota et al. 2013).

Intriguingly, PUFAs, of which MAs are elective sources, can exhibit anti-inflammatory effects in the skin via metabolisation to monohydroxy acids (Ziboh et al. 2000). Finally, anti-inflammatory properties were recognised to lipid mediators called resolvins (E- and D-series), which are derived from the cellular metabolism of long-chain PUFAs, such as EPA and docosahexaenoic acid (Calder 2009; Weylandt et al. 2012).

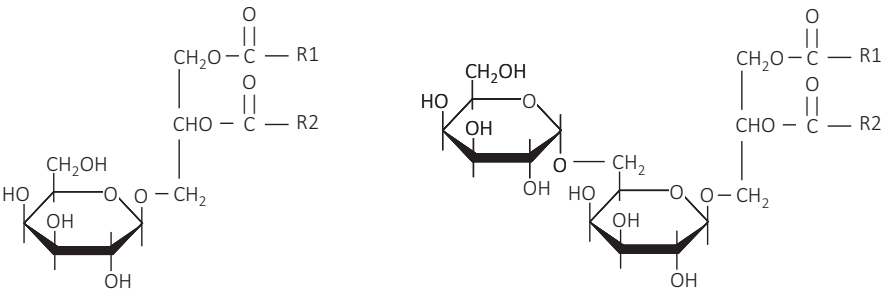

Fig. 9.7 Monogalactosyl diacylglycerol (left) and digalactosyl diacylglycerol (right). R1 and R2 are polyunsaturated fatty acids

2.4 *Issues Related with the Multifunctional Bioactivity of the Extracts*

Multifunctionality is a typical trait of microalgal extracts that has not been sufficiently appreciated. The great number of active compounds comprised in their composition makes possible to interact simultaneously with different biochemical pathways governing metabolism of cells and tissues. For example, treatments with an ethanol extract of *Chaetoceros* on ex vivo cultures of human organotypic cultures promoted hair follicle growth, modulated pigmentation, ECM composition and cell proliferation in skin, enhanced lipolysis in adipocytes and reduced sebogenesis in SGs (Zanella et al. 2012, 2016). This richness in active compounds is a trait that could be conveniently exploited in cosmetics, especially for treating multifactorial inflammatory processes at the basis of aging and other skin problems. Although MAs and other marine organisms are optimal sources of biologically active compounds (Pulz and Gross 2004; Spolaore et al. 2006; Kim 2014; Balboa et al. 2015), the added value related to the composition of their phytocomplex is still largely undervalued.

2.4.1 Chemical Antioxidant Activity Versus Signal Modulation

The mechanism of action of some MA extracts is still insufficiently elucidated. Many experimental findings cannot be explained only as effect of the chemical antioxidant activity. For example, extracts of *Isochrysis*, *Chaetoceros*, *Monodus* and *Chlorococcum* stimulate growth and prolong the anagen phase in hair follicles under ex vivo culture conditions at very low concentrations (Herrmann et al. 2012b; Zanella et al. 2012), thereby exerting a negligible antioxidant activity. Furthermore, considering that oxidative stress is not present in standard culture conditions, the mentioned extracts should affect the hair metabolism via a different mechanism, perhaps by modulating cytosolic or nuclear signals. Other case studies have shown that compounds present in MAs can modulate the genetic expression in human and animal cells, also in the absence of oxidative stress. FXT topically administered at 1% depressed the mRNA expression of COX-2, *endothelin receptor-A*, *p75 neurotrophin receptor*, *prostaglandin E receptor 1*, *melanocortin 1 receptor* and *tyrosinase-related protein 1* (Muthuirulappan and Francis 2013). An aqueous extract of *C. vulgaris* orally administered to mice modulated some immune cells by regulating the expression of IL-12 and interferon-γ (IFN-γ) with important antiallergic effects of potential cosmetic interest (Hasegawa et al. 1999). A similar preparation promoted the production of IL-1α, TNF-α, IFN-c, IL-10 and IL-6 in mouse natural killer cells following exposure to lead, thereby minimising the immune defects determined by this contaminant (Queiroz et al. 2011). An extract of *C. pyrenoidosa* inhibited the release of IL-5 and GM-CSF in mast mouse cells treated with allergenic stimuli (Kralovec et al. 2005).

These data indicate that MA active compounds can regulate several signals of the immune system. These properties deserve to be studied thoroughly, because they could alleviate problems of sensitisation, irritation and skin contact allergies, but also improve the immune defense.

2.4.2 Modulation of Fat Management

Another topic of cosmetic interest concerning MAs is the modulation of fat metabolism. The subcutis, skin and its appendages harbour three types of cells specialised in lipid metabolism, with different functions: KC (epidermis), sebocytes (SGs) and adipocytes (hypodermis or subcutis). The first two cell types are involved in the synthesis of the skin barrier and sebum, respectively, as discussed in Sect. 2.2.1. Adipocytes not only contribute to skin plumpness and provide a lipid reserve and thermal insulation but are also sources of adipokines that regulate many aspects of skin biology. Adipose tissue is a primary source of paracrine and endocrine signals with a potential secretome estimated at over 600 proteins (Fasshauer and Blüher 2015). The influence of fat secretome on skin well-being and beauty has become an increasingly important issue in cosmetics.

The anatomical contiguity of subcutis with the dermis allows the adipocytes to exert a relevant paracrine action on the dermal and epidermal tissues, thereby affecting the healing processes, hair follicle cycle and thermoregulation (Kruglikov and Scherer 2016). Chronic UV exposure inhibits the release of some adipokines, i.e. adiponectin and leptin, with increasing photo-oxidative skin damage (Kim et al. 2016). Leptin acts on skin cells via the membrane receptor *Janus kinase 2*, which transduces the signal to different secondary messengers, thereby influencing the processes of preservation and regeneration of the skin and other skin appendages (Poeggeler et al. 2010). Considering this background, the preliminary data that indicate MAs as a source of compounds active on adipocytes deserve attention. Preparations obtained from *Chromulina*, *Asterionella* and *Tetraselmis* algal cultures were proposed to inhibit different enzymes involved in fat metabolism, including *acetyl coenzyme A carboxylase*, *phosphodiesterase*, *glyceraldehyde 3-phosphate dehydrogenase*, *fatty acid synthase* and *lipoprotein lipase* (Hugues and Joel 2012). Extracts of *Chaetoceros*, *Chlorococcum*, *Monodus* and *Nannochloropsis* stimulated lipolysis in hFTS with subcutis (Zanella et al. 2012; Zanella and Pertile 2016).

Moreover, some CTs that are often included in the composition of several MA strains affect adipocyte metabolism. FXT is metabolised to fucoxanthinol and amarouciaxanthin-A, which inhibit the differentiation and development of the adipocytes (Muthuirulappan and Francis 2013). Neoxanthin, another CT, shows similar properties, whereas FXT promotes fat loss through higher expression levels of *uncoupling protein 1* and *3-adrenergic receptor* in abdominal fat tissues (Sathasivam and Ki 2018).

3 Relevance of the Adopted Experimental Model in the Development of Multifunctional Cosmetics

The previous topics have highlighted the great complexity and organisation of the skin organ with epithelial and mesenchymal tissues at close contact that exchange signals suitable to modulate the activity of their respective cells. The isolation of a cell type from this context allows the investigation of the responses to a stimulus under conditions very different from in vivo. Furthermore, skin appendages play a relevant role in the dynamic of this signalling interaction. These organs are exposed to the same cosmetic treatment of the skin, can develop specific responses and produce further signals capable of influencing the metabolism of skin cells.

All these elements should be considered when interpreting results obtained using different experimental models to achieve an appropriate characterisation of the active cosmetic ingredients. Cells in culture, 3D human skin equivalents (hSE) and cultures of human tissues ex vivo or live animals have increasing capacity to produce results that reflect interactions between cells and tissues similar to those of the human body. Each model can suitably provide useful information for characterising the biological properties of molecules and preparations, but with different predictivity values concerning the effects in vivo. This issue is of special interest in Europe, which is one of the main markets for the cosmetics industry, since the experimentation on live animals was disallowed (EC regulation No. 1223/2009).

3.1 Cell Cultures Versus Organotypic Cultures and 3D Human Skin Equivalents

In vitro cultures of skin cells are the most widely used tool for screening and characterising active ingredients, as they are easy to manage and relatively inexpensive. Generally, hFBs, hKCs and less commonly melanocytes are used. A clear distinction should be made between primary cells and immortalised line cells. Primary cells are obtained via isolation from explanted human tissues. They can survive for a limited number of generations in culture and retain some features of the donor for some time. For example, hKCs and hFBs show a proliferative capacity in vitro that decreases with the age of the donor, which also affects the maximum number of generations under culture conditions (Martin et al. 1970; Gilchrest 1983). Furthermore, hFBs isolated from elderly subjects show an unbalanced oxidative homeostasis compared with cells isolated from young donors (Boraldi et al. 2010). The primary cells, however, undergo various phenotypic changes as the passages in culture proceed and then completely lose their replicative capacity (Martin et al. 1970; Boraldi et al. 2010). Hence, the use of cells at their first passages in culture is important. Cellular alterations in the cells in culture are sometimes exploited as ‘in vitro aging model’, but important limitations occur, because these changes involve all the cell machinery instead of reflecting the typical damages of in vivo aging (Boraldi et al. 2010).

The immortalised cells (line cells) are derived from the transformation of primary cultures, which can be spontaneous or induced by viral infections, but more frequently, they are obtained via the isolation of tumour cells. Typically, these cells lack contact inhibition and have various modified biological characteristics while maintaining some basic traits of the cell type to which they belong (Jedrzejczak-Silicka 2017). Chromosomal anomalies and the loss of functionality of the p53 pro-apoptotic signal are some of the most significant anomalies (Oh et al. 2007).

The characterisation of active compounds conducted on isolated cells is affected by lack of cytokines or secondary metabolites that, in vivo, could be released from proximal different cell types exposed to the same stimulus. This limitation can be particularly relevant in the case of MA extracts, because a single cell type is unsuitable to disclose the signalling crosstalk triggered by the combined action of several active compounds (for more information on crosstalk in cellular signalling, see Vert and Chory 2011).

To overcome these problems, researchers have developed various co-culture protocols with different cell types and provided significant evidence of the relevant effect produced by cross-talking on their respective metabolism (Maas-Szabowski et al. 1999; Ghahary and Ghaffari 2007; Singh et al. 2008; Hirobe 2014). This technical approach led to the development of various hSEs, consisting of simple epidermis or full-thickness skin, with or without polymeric scaffolds for dermal matrix engineering (Stark et al. 2004, 2006; Griffith and Swartz 2006; Poumay and Coquette 2007; Li et al. 2009; Canton et al. 2010). The development of increasingly advanced hSE has substantially improved the dermatological research opportunities and led to the commercialisation of 3D models designed for different applications, such as those proposed by Episkin SA (de Brugerolle 2007; Alépée et al. 2017), MatTek Corporation (Danilenko et al. 2016) and Henkel AG & Co. KGaA (under the Phenion® brand, Mewes et al. 2017). The use of these hSEs is also important to conduct some safety tests on ingredients and cosmetic products intended for the European market, because they can no longer be performed on live animals (Nakamura et al. 2018).

As an alternative, the active compounds for cosmetics can be tested on ex vivo organotypic cultures, such as the cultures of skin, HFs, SGs and hypodermic fat. Most of these biological materials are waste tissues obtained from cosmetic or reconstructive surgery, which can be kept in culture for a few days (Fig. 9.8). The ex vivo cultures have the advantage of presenting the anatomical organisation of the tissue in vivo, including nerve endings, Langerhans and Merkel cells, and preserving some individual characteristics of the donor (e.g. sex, age and sensitivity). Xu et al. (2012) performed wound-healing studies on human skin samples and verified that they maintained biological performance similar to the skin in vivo for 6 days. Ex vivo cultures are almost irreplaceable for studies on complex annexed organs, such as HFs, because examples of in vitro reconstructed models are limited (e.g. Havlickova et al. 2009). To date, results remain far from the complexity of the human organ.

hSEs are easy to handle, available and suitable for providing replicable data (Danilenko et al. 2016), but the ex vivo skin is more representative of the in vivo

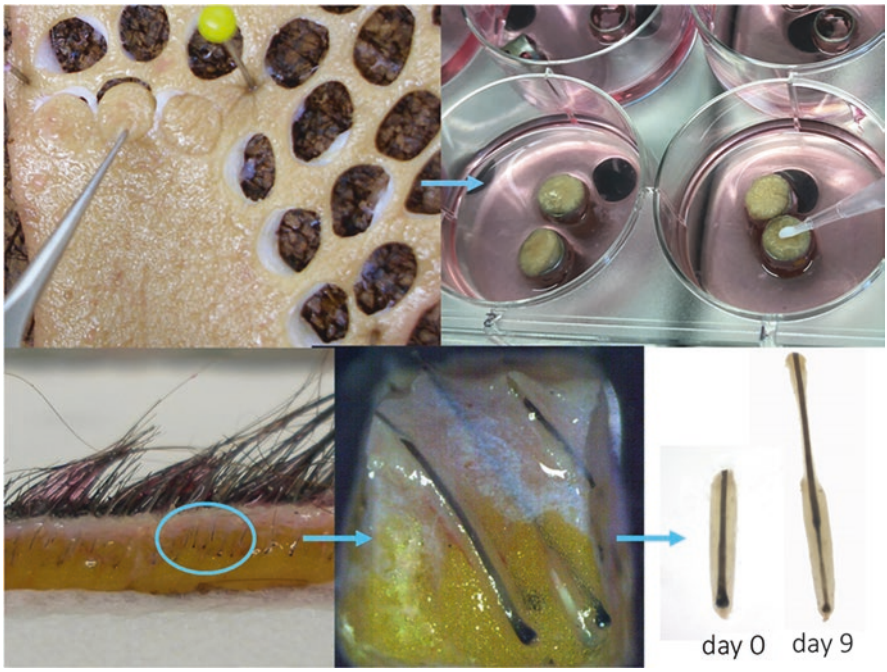

Fig. 9.8 Ex vivo models of human skin and related appendages: dissection of skin samples and cultivation (upper), scalp sample, tissue detail and hair follicles during the dissection, then the isolated hair follicles at day 0 and 10 of culture, respectively (lower) (Source: courtesy of Cutech Srl)

condition in terms of many aspects, as well as with the delivery processes following topical administration (Reus et al. 2012; Andrade et al. 2015; Sidgwick et al. 2016).

Most of the information about the biological properties of algal extracts or single compounds that they contained was obtained via experiments with cells in culture or hSEs. However, experiments conducted with the ethanol extracts of *Nannochloropsis* sp. on ex vivo organs (human skin, subcutis, sebaceous glands and hair follicles) showed that they were active in most skin compartments and appendages (Zanella and Pertile 2016). For example, the topical application of the extract to ex vivo hFTS reduced IL-1α release in response to inflammatory stimulus to a comparable extent to dexamethasone and inhibited melanogenesis to an extent comparable to retinoic acid. Besides, also skin appendages responded to systemic treatments with the same extract; reduced sebogenesis in ex vivo SGs in measure comparable or superior to benchmark compounds (e.g. Asebiol™, 5α-Avocuta® and capsaicin), stimulated growth in ex vivo HF and lipolysis in ex vivo subcutis. This combination of biological activities is not an exception but the consequence of the complex composition of many microalgal extracts, which makes them suitable for affecting the metabolism of different skin compartments.

3.2 Issues Related with Effects In Vivo

Although hESs and ex vivo organotypic cultures are suitable tools for preclinical studies, they still lack some relevant traits of animal models. The blood and lymphatic circulation is completely absent, the microbiome is altered or absent, and the stimuli due to the mechanical solicitation and variation of environmental conditions are lacking (e.g. temperature and solar radiation).

Hence, remarkable differences may occur between preclinical and in vivo findings. The clinical test is a necessary confirmation to validate the results obtained on simplified experimental models.

Skin metabolism is affected by active compounds obtained through diet (Choi et al. 2018) or topical administration, as well as with stress factors derived from lifestyle (Darvin et al. 2011). The cosmetic activity observed in a clinical trial will therefore depend on many variables that interact with the individual sensitivity exhibited by the treated subject. The active compounds applied in vivo can be ineffective in a certain number of subjects who, for unknown reasons, are not responsive. Generally, this condition does not happen with isolated cells, but can sometimes be observed in ex vivo tissues (a personal experience of one of the authors). Intriguingly, some subjects can also be non-responsive to treatments with compounds considered as gold standards (e.g. skin lighteners β-arbutin or kojic acid) as disclose a critical examination of statistical responsiveness in clinical trials (Curto et al. 1999; Solano et al. 2003). On this regard, the use of a mixture of active ingredients with synergistic activities may be advantageous to stabilise the subject's response. This strategy perfectly fits with the use of microalgal extracts. In fact, poor responsiveness or sensitivity to a particular active ingredient could be compensated by other compounds present in the extract.

As evidence of the validity of this approach, dermatologists often treat certain skin disorders, such as melasma or acne, with mixtures of several active ingredients (Kligman and Willis 1975; Lim 1999; Fabbrocini and Saint Aroman 2014; Shankar et al. 2014) to resolve the ineffectiveness of single drugs in a certain number of subjects or exploit their synergistic effects. Skin disorders are often multifactorial and could deal great benefits from multifunctional products.

Finally, even the most advanced in vitro models lack the nervous and vascular components, which affect the release of NTs and the contribution of hormones with great relevance for skin homeostasis and some important forms of inflammations (see Sect. 2.2.5). Ex vivo skin can allow some experimental options in reason of the partial conservation of some parts of the immune system and the nervous system (nerve endings, Merkel and Langerhans cells). However, studying the response of the nervous system on experimental models other than in vivo is difficult.

3.3 Product Formulation and Active Transdermal Delivery

The use of isolated cells or tissues allows researchers to perform treatments at desired concentrations for established times. Culture conditions allow cells to be placed in contact with any compound suitable to be supplemented into the medium, regardless of molecular weight. However, obtaining the same effects in vivo is difficult for at least two reasons: (1) the substance of interest does not necessarily cross the protein-lipid barrier of the SC, and (2) if this event occurs, the concentration of the substance of interest will decrease with both the diffusion through the tissue and the time elapsed since administration. Transdermal absorption is one of the most complex problems affecting the formulation of topical treatments, and only a few experimental models are available as alternatives to in vivo tests. This process consists of multiple steps that include the following: (1) partitioning of the active from the cosmetic vehicle into the SC, (2) molecular diffusion through the SC and partitioning into the epidermal viable cells and (3) diffusion through the epidermis and dermis until it eventually reaches the blood vessels (Pillai et al. 2016) that spread the compounds via a systemic way. The main route of entry is the crossing of the SC via trans- or intercellular pathways; however, some facilitating entry routes are available, such as the hair follicle infundibulum and sweat glands (the latter is preferred for hydrophilic substances) (Abd et al. 2016; Pillai et al. 2016).

In general, the absorption of a compound is inversely proportional to its molecular weight and electric charge. The compounds should not exceed 500 Da (Pillai et al. 2016), although this paradigm does not represent an insurmountable limit, especially in elderly and/or very dry skin (Fields et al. 2009).

Studies conducted on hSEs, although useful, could provide results not replicable under in vivo conditions. In some tests, the absorption of hydrophilic compounds was similar to that in human skin, but lipophilic compounds were absorbed up to 800 times faster (Godin and Touitou 2007). In another hSE, a Raman spectroscopic analysis showed SC anomalies in the continuity and distribution of ceramides, fatty acids and cholesterol with important consequences on permeability (Tfayli et al. 2014). Even studies conducted on animal models showed that the permeability of the SC varies across species and with the body site in the same species consistently with skin thickness, composition of the protein-lipid matrix and density of hair follicles (Godin and Touitou 2007).

The most reliable model is probably the ex vivo human skin (Abd et al. 2016), although this model lacks blood circulation and the lymphatic vessel network, which are essential for evaluating inflammatory responses and clearance of the compounds of interest. Unfortunately, studies on MA preparations conducted on human skin in vivo or on ex vivo are still limited.

3.3.1 Lipophilic Extracts

Considering that the SC is a barrier that is rich in lipids and covered sebum, lipophilic molecules are generally absorbed more easily than hydrophilic ones. Many MAs are known to be able to accumulate CTs and lipids in high amounts, including long-chain PUFAs, so their extracts obtained with non-polar solvents are usually very rich in active compounds of cosmetic interest.

Some studies confirmed that many lipophilic compounds can be effectively absorbed but with important variations in relation to their molecular structure. For example, a good absorption of triglycerides and CTs (βC ≫ α-Carotene) was demonstrated via topical application of crude palm oil to ex vivo human skin (Sri et al. 2013).

Preparations for topical application containing FXT were tested on hairless mice ex vivo and in vivo, which showed that this compound was adsorbed and was active against inflammation and hyperplasia (Rodríguez-Luna et al. 2018).

All the compounds of the given examples are often present in lipophilic MA extracts and it can be presumed that they are being adsorbed with the same efficiency.

3.3.2 Hydrophilic Extracts

Aqueous extracts of MAs contain many small hydrophilic compounds, such as vitamins and polyphenols, as well as significant amounts of polysaccharides and polypeptides with high molecular weight (MW). Large polypeptides could remain unadsorbed, which may be advantageous, by considering the high antigenic power of some vegetable proteins. The active peptides currently designed and synthesised to modulate skin metabolism are generally made up of 3–6 amino acids and are often prepared using lipoamino acids obtained via esterification with palmitic acid in order to improve their adsorption (Fields et al. 2009).

In aqueous microalgal extracts, small peptides may be present, but their occurrence as lipoamino acids and with sequences suitable for acting as signals for human epidermal cells is unlikely. Nevertheless, Chen et al. (2011) showed that an aqueous extract of *Chlorella* containing 430–1350 kDa peptides exhibited protective effects on UVB-induced damages in FB culture.

Amongst hydrophilic compounds, significant activities may be derived from small molecules, such as glutathione, which may have topical activity (Kopal et al. 2007), and modified amino acids such as MAAs, whose characteristics have already been discussed in relation to their property to quench UVR (see Sect. 2.2.2).

The aqueous extracts of MAs also comprise vitamins, polyphenols, flavonoids, single amino acids and small glucides, which are compatible with transcutaneous absorption. Amongst these, special attention is given to the glycolipid MGDGs and DGDGs, which have anti-inflammatory activity. In vivo tests conducted on mice by using ointments of fractionated extracts of *I. galbana* rich in MGDGs confirmed their penetration through the SC and diffusion in the thickness of the skin, with strong anti-inflammatory effect (Rodríguez-Luna et al. 2017). The adsorption of

polyphenols was demonstrated ex vivo via the topical application of natural non-microalgal extracts obtained from cocoa, thereby resulting in the stimulation of the GAGs and COL synthesis in skin dermis (Gasser et al. 2008).

More difficult is reconciling the principles of the transdermal delivery with the intense anti-inflammatory activity of high-MW SEPs ($5–7 \times 10^6$ Da) obtained from *Porphyridium*, which were used by Matsui et al. (2003) in a clinical trial to treat the irritation induced with balsam of Peru. Also in this case, however, the SEP activity was remarkably increased following molecular fragmentation obtained by sonication, thereby attesting that the molecular weight and treatment efficacy are directly correlated. In a different experiment, Zhang et al. (2011) studied via a mouse model of Rosacea the anti-inflammatory activity of 5500 Da semisynthetic glycosaminoglycan ethers (SAGEs) obtained by sulphation from fermented 53 kDa HA derivatives. The croton-induced skin inflammation was effectively inhibited by topical treatment with SAGEs, whereas the native 53 kDa HA was ineffective. These data confirm that large dimensions of some polysaccharides hinder absorption. Potent absorption enhancers can be used to improve the absorption of some compounds, but they can be expensive and not always resolutive. Concerning the reported examples, it should be considered that the absorption of large molecules in inflamed skin could be due, at least partially, to the disruption of the SC integrity, which in turn is due to the application of irritants and solvent vehicles. Croton oil, for example, produces important histological alterations of the skin (Moon et al. 2001).

4 Conclusions

Cosmetic applications of extracts and preparations from MAs have achieved development and progress over the past two decades due to the availability of data concerning their activities on skin. Some products have already been marketed but much less compared with their potential as sources of active ingredients (Table 9.5). Some mechanisms of action were documented, especially those related to antioxidant activities and protection from photoaging processes. Much work remains to be done, especially in applications intended for the treatment of skin appendages, modulation of fat's adipokines and effects on the skin microbiota.

The topics addressed in this review highlight how the concentration of many active compounds in a single cell, according to combinations that vary across species, allows the preparation of multifunctional extracts. This trait is not found to the same extent in other natural ingredients and is worthy to be further explored. The exploitation of MAs as a source of isolated active compounds is often economically disadvantageous compared with traditional or synthetic alternatives (see costs for carotenoids in Spolaore et al. 2006, for EPA in Molina Grima et al. 2003 and Koller et al. 2014; for a comprehensive analysis see also Barsanti and Gualtiero 2018). This condition is due to the biomass production costs, which are still relatively high despite the significant progress (Molina Grima et al. 2003; Tredici et al. 2016) and the costs of purification. However, the advisable use of microalgal extracts is to

Table 9.5 Some commercial cosmetic ingredients or products obtained from MAs

Species	Cosmetic application	Active ingredient	Commercial name	Company	Reference
Porphyridium sp.	Skin anti-wrinkle, protection from UV damage	Sulphated polysaccharide	Alguard®	Frutarom	Ryu et al. (2015), Guillerme et al. (2017)
Porphyridium cruentum	Vascular tonicity, improves the skin's aspect and helps decrease rosacea effects and redness	Extract	SILIDINE®	Greentech	Mourelle et al. (2017)
Porphyridium cruentum	Antioxidant, anti-aging and pro-healing	Extract	Cicatrol®	Greensea	Maiz (2007)
Isochrysis T-Iso	Skin tanner	Ethyl acetate extract	BIO1659	Symrise AG	Herrmann et al. (2012a)
Isochrysis T-Iso	Anti-hair loss and hair promoter	Methanol extract	BIO1631	Symrise AG	Herrmann et al. (2012b)
Tetraselmis suecica	Anti-inflammatory and anti-rheumatic	Gold-bearing extract	O^+ Gold Microalgae Extract®	Greensea	Maiz (2007)
Scenedesmus rubescens	Skin anti-photoaging and procollagen	Aqueous extract	Pepha®-Age	DSM Nutritional Products	Campiche et al. (2018)
Nannochloropsis oculata	Protection from oxidative stress, COL stimulation and skin tightening	Aqueous extract	Pepha®-Tight	DSM Nutritional Products (Pentapharm)	Stolz and Obermayer (2005)
Dunaliella salina	Stimulation of skin energy metabolism, cell proliferation and turnover, collagen synthesis	Aqueous extract	Pepha®-Ctive	DSM Nutritional Products (Pentapharm)	Stolz and Obermayer (2005)
Dunaliella salina, *Haematococcus pluvialis*	For brighter complexion, it treats uneven and dull skin, dark circles around the eye	extracts	REVEAL Color Correcting Eye Serum Brightener	Algenist	Joshi et al. (2018)

(continued)

Table 9.5 (continued)

Species	Cosmetic application	Active ingredient	Commercial name	Company	Reference
Chlorella vulgaris	Antiaging, anti-wrinkle, anti-cellulite, anti-stretch marks, anti-vascular imperfections, anti-dark circles	Extracted by alkaline hydrolysis	Dermochlorella	CODIF Recherche & Nature	Morvan and Vallee (2007)
Chlorella vulgaris	Neutralises inflammation and improves skin's natural protection	Extract	Phytomer	Phytomer	Ryu et al. (2015)
Chlorella sp.	Hydrates and beautifies skin and hair	Microalgal oil	Golden Chlorella™	Terravia Holdings	Mourelle et al. (2017)
Microalgae	Benefits for skin and hair	Microalgal oil	AlgaPür™	Terravia Holdings	Mourelle et al. (2017)
Microalgae	Skin anti-aging and rejuvenating	Polysaccharides	Alguronic Acid®	Algenist	Martins et al. (2014)

develop multifunctional ingredients, which are entirely natural and with highly sustainable ecological footprint (Fernandez et al. 2017). Their exploitation can be advantageous for treating skin disorders that have multiple causes, such as acne, dermatitis and psoriasis, but also as anti-aging and homeostasis protective agent, whose full effects require long-term application.

Moreover, the costs of biomass can be partly amortised by the high extraction yield obtained using some solvents (Table 9.6), and a great amount of product can be finalised due to their biological activity at a very low concentration (Pertile et al. 2010; Zanella et al. 2012). Consequently, the incidence of these ingredients on the production costs of high value-added products such cosmetics can be sustainable in many cases.

Unfortunately, unlike the synthetic principles, natural extracts have a composition that is partly unknown and subject to variation with the season, cultivation technique and method of extraction (Chojnacka and Kim 2015). This condition does not satisfy the practice of the cosmetic industry in terms of product standardisation and quality control. However, in order not to downplay the advantage of the multifunctional composition of microalgal extracts, a characterisation based on a metabolite fingerprinting obtained by mass spectrometry techniques may be employed, as already proposed for natural preparations intended for nutritional and phytopharmaceutical use (Mattoli et al. 2006, 2011).

The change from a 'one active → one claim' approach to natural phytocomplexes suitable to produce a combination of desirable effects leads to the problem on the functional characterisation of the ingredient. Establishing a quantitative correlation between a biological effect and the responsible active agent is difficult and sometimes impossible, because the ingredient formulation should be standardised. As mentioned for chemical characterisation, to determine the advantages offered by very active complex mixtures, a different standard of product characterisation should be developed. For example, each extract might be evaluated in terms of con-

Table 9.6 Extract yield obtained from some MAs using different solvents

Strain \ solvent	hydrophilic → lipophilic					
	water	methanol	ethanol	isopropanol	ethyl acetate	hexane
Tetraselmis suecica	32%	17%	15%	3%	7%	5%
Chaetoceros calcitrans f. pumilus	64%	54%	22%	10%	9-12%	
Thalassiosira pseudonana	22%	39%	27%	15%	18%	
Monodus subterraneus	20%	20%	7-9%	2%	4%	
Chlorococcum minutum	22%	24%	16%	10%	5-8%	

Values expressed as percentage of the dried biomass (data from Pertile et al. 2010 and Zanella et al. 2016)

centration and activity according to an efficacy ratio by comparison with a panel of reference compounds, which are selected as golden standards for each application of interest. Figure 9.9 shows an exemplificative diagram of functional quantification

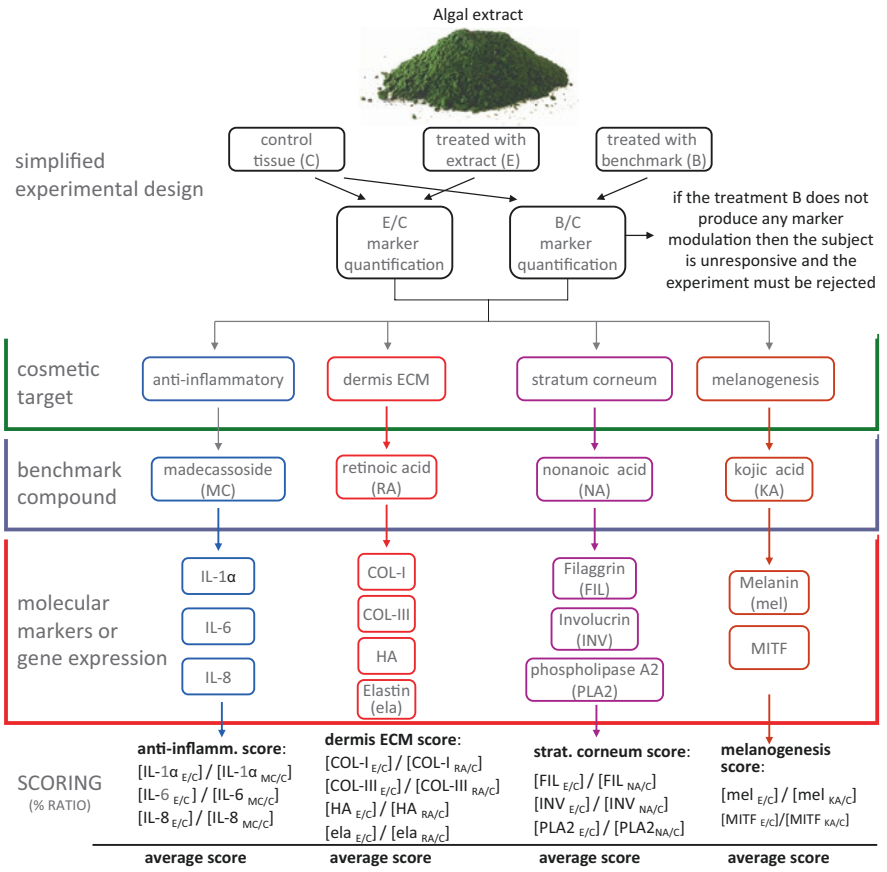

Fig. 9.9 Example of test panel for functional characterisation of a MA extract. The extract (E) is tested on an advanced experimental model (e.g. hSE or hFTS) in comparison with golden standard actives, namely, benchmarks (B), here arbitrarily selected for exemplification. The "simplified" experiment requires the comparison of the effectiveness between E and B treatments. The response to the B treatment in comparison to C defines subject's responsiveness; in case of no marker variation the experiment should be abandoned. Below the experimental design, possible markers and benchmarks (that cosmetic operators should define in a shared way) are schematised. For each application, the variation of the marker in response to treatments E and B is estimated as a ratio on sample C, then the values obtained are used to calculate the % ratio between the marker variation produced by treatment E on that produced by treatment B (i.e. $\text{marker}_{E/C} / \text{marker}_{B/C} \times 100$). This approach would allow the characterisation of multifunctional extracts using the response to B as a normalising factor, thereby making the results comparable between different subjects with different sensitivities (from which depend the range of variation in response to treatments). At least three responsive subjects need to be tested for each application. (*MITF*: microphthalmia-associated transcription factor)

as a result of a panel of comparative tests aimed to specific applications (to be differently arranged with the targets of the cosmetic product category). A similar 'report sheet' could be compiled following tests on ex vivo human skin cultures and averaging the results obtained from no less than three responsive subjects. This approach can allow to determine in which application the extract is more active and the related ranking of effectiveness.

The proposals above, which are intended as representative hypotheses, imply important changes in the current business model used for cosmetic products, which is a significant obstacle. Nevertheless, this issue should be framed in the context of rapid aging experienced by populations in advanced economies. Health costs will grow at unsustainable rate. Hence, both cosmetics and nutrition science can and should play an increasingly important role in the promotion of the well-being and prevention of metabolic disorders. The development of multifunctional cosmeceuticals is in line with this approach, and the related costs should be assessed considering the connected substantial healthcare savings.

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