Light-Activated Biomedical Applications of Chlorophyll Derivatives

Macromolecular Bioscience
www.mbs-journal.de

Light-Activated Biomedical Applications of Chlorophyll Derivatives

Carlotta Pucci, Chiara Martinelli,* Andrea Degl'Innocenti,* Andrea Desii,* Daniele De Pasquale, and Gianni Ciofani**

Tetrapyrroles are the basis of essential physiological functions in most living organisms. These compounds represent the basic scaffold of porphyrins, chlorophylls, and bacteriochlorophylls, among others. Chlorophyll derivatives, obtained by the natural or artificial degradation of chlorophylls, present unique properties, holding great potential in the scientific and medical fields. Indeed, they can act as cancer-preventing agents, antimutagens, apoptosis inducers, efficient antioxidants, as well as antimicrobial and immunomodulatory molecules. Moreover, thanks to their peculiar optical properties, they can be exploited as photosensitizers for photodynamic therapy and as vision enhancers. Most of these molecules, however, are highly hydrophobic and poorly soluble in biological fluids, and may display undesired toxicity due to accumulation in healthy tissues. The advent of nanomedicine has prompted the development of nanoparticles acting as carriers for chlorophyll derivatives, facilitating their targeted administration with demonstrated applicability in diagnosis and therapy. In this review, the chemical and physical properties of chlorophyll derivatives that justify their usage in the biomedical field, with particular regard to light-activated dynamics are described. Their role as antioxidants and photoactive agents are discussed, introducing the most recent nanomedical applications and focusing on inorganic and organic nanocarriers exploited in vitro and in vivo.

1. Introduction

In organic chemistry, tetrapyrroles are loosely defined as those compounds formed by four pyrrole or pyrrole-like rings connected by single-atom carbon bridges at positions α of each ring; when these originate a closed structure, the tetrapyrrole is called macrocyclic, or more often simply cyclic. Macrocyclic tetrapyrroles tend to display high affinity to metal cations, which can be trapped at the center of the pyrrole frame forming a coordination complex.[1,2] Together with a high degree of conjugation, this fact renders tetrapyrroles pivotal molecules for biochemistry: among other things, in biological systems tetrapyrroles act as prosthetic groups, meaning that they are bound to proteins, forming with them vital functional units. Tetrapyrroles became part of the metabolism of early livings, and are still essential for the physiology of organisms from all kingdoms of life. Their overall cell roles include light harvesting, oxygen transport or storage, and electron transfer.[2]

Major types of cyclic tetrapyrroles are porphyrins, chlorins, bacteriochlorins, and corrins. While these categories generally resemble each other from a structural point of view, few variations in the degree of hydrogenation cause a relevant diversification of their biochemical properties. Porphyrins represent the least hydrogenated group; some iron (Fe)-coordinated porphyrins form the important prosthetic group heme. Hemoproteins, that is, heme-harboring proteins, serve a variety of scopes in virtually all aerobic organisms: hemoglobins, for instance, transport oxygen in the blood stream of most vertebrates, myoglobins store oxygen within the muscle tissue, and cytochromes are key components of electron-transfer chains and redox cell processes, and leghemoglobins carry oxygen within the root system of beans to symbiotic bacteria.[3,4] With a pyrrole converted to pyrroline, chlorins are a more hydrogenated variation on the tetrapyrrole archetype. They constitute the core structure of most chlorophylls, paramount pigments for photosynthesis that are widespread in plants, green algae, and cyanobacteria. Other common chlorophyll features are one or more side substitutions (including a long hydrocarbon chain), a fifth ring attached to the macrocycle, and a central magnesium (Mg) ion.[5,6] A further reduction on a

Dr. C. Pucci, Dr. A. Degl'Innocenti, Dr. A. Desii, Dr. D. De Pasquale, Prof. G. Ciofani
Istituto Italiano di Tecnologia, Smart Bio-Interfaces
Viale Rinaldo Piaggio 34, Pontedera, Pisa 56025, Italy
E-mail: carlotta.pucci@iit.it; andrea.deglinnocenti@iit.it; andrea.desii@iit.it; gianni.ciofani@iit.it
Dr. C. Martinelli
Department of Chemistry, Materials and Chemical Engineering "Giulio Natta"
Politecnico di Milano
Piazza Leonardo da Vinci 32, Milan 20133, Italy
E-mail: chiara.martinelli@polimi.it

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mabi.202100181

DOI: 10.1002/mabi.202100181

second chlorin pyrrole defines bacteriochlorins, backbone of bacteriochlorophylls—photosynthetic pigments in different taxa of autotrophic bacteria.[5,7] Corrins, finally, are more highly hydrogenated tetrapyrroles that constitute a fundamental component of cobalamins (vitamin B_{12}).[5,8]

Linear (i.e., non-cyclic) tetrapyrroles include certain luciferins, responsible for the bioluminescence of a diverse group comprising animals and protists,[9] and phycobilins, capturing photons in the photosystems of some algae and cyanobacteria.[6] Degradation can also linearize cyclic tetrapyrroles, and some of such products have a biological relevance on their own: bilirubin, a catabolite of heme, seems to have a potent physiological antioxidant activity.[10]

Breakdown products of chlorophylls, as well as any otherwise natural or artificial variation of chlorophyll, are generally termed chlorophyll derivatives. In a broad sense, the expression can refer to any chlorophyll-related compound.[11]

The chemical structure of chlorophylls prevents their direct use in drugs that need to cross the cell membrane without alteration. Purified chlorophylls are insoluble in water and poorly soluble in organic solvents; in water and biological media, they form aggregates with decreased photoactivity, unsuitable for formulation as intravenous drugs. Chemically modified derivatives have been synthesized to obtain active molecules tailored to applications as drugs, catalysts, light-harvesting compounds, and food additives.

The main structural feature of chlorophylls, the macrocycle, is preserved in most derivatives of technological interest. Electrons of the tetrapyrrole can be excited by radiation in the visible and near-infrared (NIR) part of the spectrum: depending on the chemical structure of the derivative and on the surrounding medium, excited states can relax via radiative processes (fluorescence, phosphorescence) or non-radiative processes (thermal relaxation, charge transfer, or energy transfer). The degree of reduction of the macrocycle, the coordination of a metal ion, and the presence of substituents dramatically change the chemistry of chlorophyll derivatives, from compounds acting as radical scavengers, used as antioxidants, to photosensitizers that induce light-stimulated cell damage, employed in photodynamic therapy (PDT) of tumors.[12] Regarding other biomedical applications, they have been proposed as sensitizers for vision and photothermal therapy, disinfectants, as well as promotors of wound healing.[13–16]

The activity and selectivity of chlorophyll-based photosensitizers have been improved using molecular strategies, selecting and modifying compounds with higher quantum yield (ϕ_Δ) toward photosensitization, lower dark toxicity, and absorption at the proper wavelength for the desired application. Despite the higher solubility of chlorophyll derivatives compared to their parent compound, their pharmacokinetics is still significantly unoptimized. Over the latest two decades, several solutions to improve bioavailability, accumulation in the target tissue, and cellular uptake have been proposed, many of which are based on inorganic and organic nanoparticles (NPs).[17]

Very recently, an increased interest toward chlorophyll derivatives and their applications has been registered among the scientific community, as it can be appreciated by the high number of published reviews focused on this topic. However, recent works have been mainly centered on the description of their metabolism, bioactivity, and bioavailability.[18–20] Some attention has been laid also on their applications, from food industry and medicine,[21] to the integration into organic solar cells[22] and in controlling infective diseases, such as dengue vectors.[23] Several reviews have described recent progresses in the use of chlorophyll derivatives as outstanding natural photosensitizers employed for PDT,[24,25] for instance in the treatment of psoriasis[26] and urologic malignancies.[27]

For instance, in their review focused on porphyrins and their possible application in tumor imaging and PDT, Ethirajan et al. described in a detailed way the chemical and optical properties of synthetic porphyrins and chlorophyll derivatives.[12] Rajora et al. focused on supramolecular porphyrin structures and their significant biomedical applications, especially those involving their photochemical properties; however, their biochemistry and metabolism was not discussed.[28] More recently, Mansoori et al. discussed in an extensive way the potentialities of natural products as photosensitizers in PDT against cancer.[29] The authors also introduced some chlorophyll derivatives, such as pheophorbide, chlorin e6 (Ce6), and chlorophyllin.[29] Nevertheless, it is worth noting that most of the reviews on chlorophyll derivatives focus on their applications in PDT, without discussing their potentialities as vision enhancers, as it will be discussed, instead, in this review. Moreover, an exhaustive discussion on the biomedical application of chlorophyll derivatives administered by means of ad hoc-designed NPs is currently missing in the literature, together with an overview of their use in light-activated systems both in vitro and in vivo.

Here, we aim at offering a comprehensive discussion of the chemophysical and biological properties of chlorophyll derivatives, together with a focus on their main potential light-activated biomedical applications and their recent development in nanomedicine.

2. Chemophysical Properties of Chlorophyll Derivatives

2.1. Fundamental Chemophysical Properties

Chlorophylls are cyclic tetrapyrroles carrying a characteristic isocyclic five-membered ring (**Figure 1**). The isocyclic feature is derived from the C13 propionic acid side chain of heme and protoporphyrin IX, while the C17 propionic acid is esterified with a long chain alcohol (phytol). They carry Mg^{2+} as the central metal, while hydrogen (H^+) and zinc (Zn^{2+}) ions are found in some naturally occurring derivatives. The reduction of possible double bonds at C7-C8 and C17-C18 maintains aromaticity and, in fact, most chlorophylls carry a chlorin- (single bond at C7—C8) or bacteriochlorin- (single bond in both positions) type macrocycle. Only chlorophylls *c* carries a porphyrin macrocycle, but they are not esterified at C17; structurally, they are chlorophyllides. In most chlorophyll derivatives, natural and artificial, the phytol residue is removed by hydrolysis. The phytol is useful for aggregation in micelles, for interacting with other pigments and cofactors, and as a handle in enzymatic processes. Its high lipophilicity is often undesirable in drugs, and its removal does not affect the photochemical properties of the isolated macrocycle.

The aromaticity of the tetrapyrrole moiety and the reactivity of the functional groups in the side chains define the chemistry of

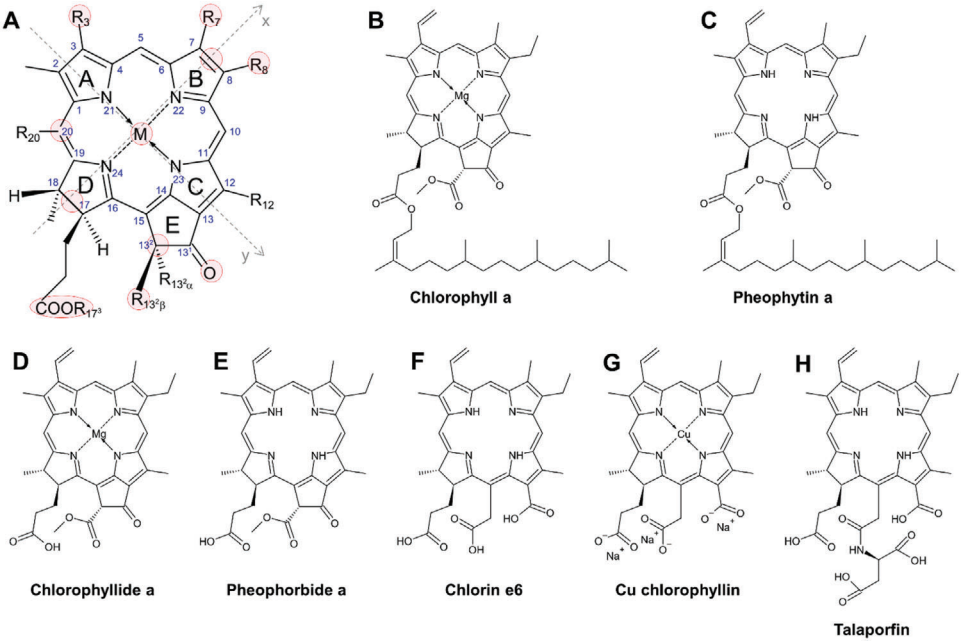


Figure 1. Structure of chlorophyll derivatives. A) Chemical structure of a generic chlorophyll (chlorin type), with the IUPAC-approved numbering of sites. The most common sites for chemical modification are highlighted in red. B) Chlorophyll *a* and some related compounds (C,E) naturally occurring derivatives, D) a precursor, and F–H) synthetic derivatives) are depicted to highlight their structural relationships and naming conventions.

chlorophylls and their derivatives (Figure 1). The meso positions along the macrocycle are susceptible to electrophilic reactions. The C7-C8 double bond in chlorin can be modified with addition and oxidation reactions without compromising the aromatic delocalization. The C3 vinyl group can be similarly modified, ester groups in $C17^3$ and $C13^2$ can be transesterified and saponified, and ring E can be modified exploiting enolization. Finally, the central metal atom can be removed and replaced.[30]

2.2. Photophysical and Photochemical Properties

Light absorption by tetrapyrroles is associated with $\pi - \pi^*$ transitions from the ground state (S_0) to short-lived excited states (S_n). The visible and NIR absorption spectrum of tetrapyrrole macrocycles is characterized by two bands: one occurring at around 400 nm, called B-band or Soret band, and another over the range 600–800 nm, the Q-band. The position of the Q-band coincides with the upper limit of the optical window for phototherapy (650–1350 nm), where the penetration of light in tissues is maximized.

Reduced tetrapyrrole macrocycles, that is, chlorin and bacteriochlorin, show a progressively red-shifted Q-band when compared to porphyrins. The Soret band is blue-shifted and split in the case of bacteriochlorins. The presence of the isocyclic ring in chlorophylls red-shifts the visible absorption of the tetrapyrrole and increases its intensity, by reducing the symmetry of the conjugated macrocycle. Substituents carrying carbonyl groups induce a red shift of the Soret band, while the effect on the Q-band depends on their position: blue shift along the *x*-axis (C7), red shift along the *y*-axis (C3, $C13^1$). The chelation of a central metal increases the symmetry of the macrocycle, inducing a blue shift of the Q-band, which decreases in intensity and collapses to two peaks.

S_n excited states rapidly decay non-radiatively to the first excited state S_1 via internal conversion. The chlorophyll derivative can return to the ground state via non-radiative vibrational relaxation, fluorescence, or passing through a triplet state (T_1) through a forbidden electronic transition (intersystem crossing, ISC). Relaxation from T_1 to S_0 can be radiative (phosphorescence, due to the longer lifetime of triplet states), or occur through interaction with surrounding chemical species in two ways: with charge transfer to molecular substrates to form radical species, which in turn react with oxygen to form reactive oxygen species (ROS) (Type I reaction), or with a direct energy transfer to the ground triplet state of molecular oxygen ($^3O_2$) to form singlet oxygen ($^1O_2$) (Type II reaction) (**Figure 2**). Type I and Type II processes are at the basis of PDT, as discussed in Section 4.1.

The specific relaxation pathway is determined by the structure of the derivative and its surroundings. The selection of the central metal provides a most effective way to modify the photochemical behavior of the tetrapyrrole. Heavy metals increase ISC, resulting in the generation of more triplet electrons, promoting phosphorescence and ROS generation.[32,33] Diamagnetic ions, like Mg^{2+} and Zn^{2+}, increase the singlet state lifetime while minimizing ISC, promoting fluorescence, and energy transfer in photoreceptors. Conversely, paramagnetic ions (Mn^{3+}, gadolinium, i.e., Gd^{3+}) shorten the lifetime of excited states, promoting internal conversion and thermal relaxation.[14] The capability of the tetrapyrrole to coordinate most metals allows the introduction of ions used in imaging techniques like magnetic resonance imaging (MRI) or positron emission tomography.[34,35]

Light activation of chlorophyll derivatives depends also on intermolecular interactions. Dispersive interaction and π-π

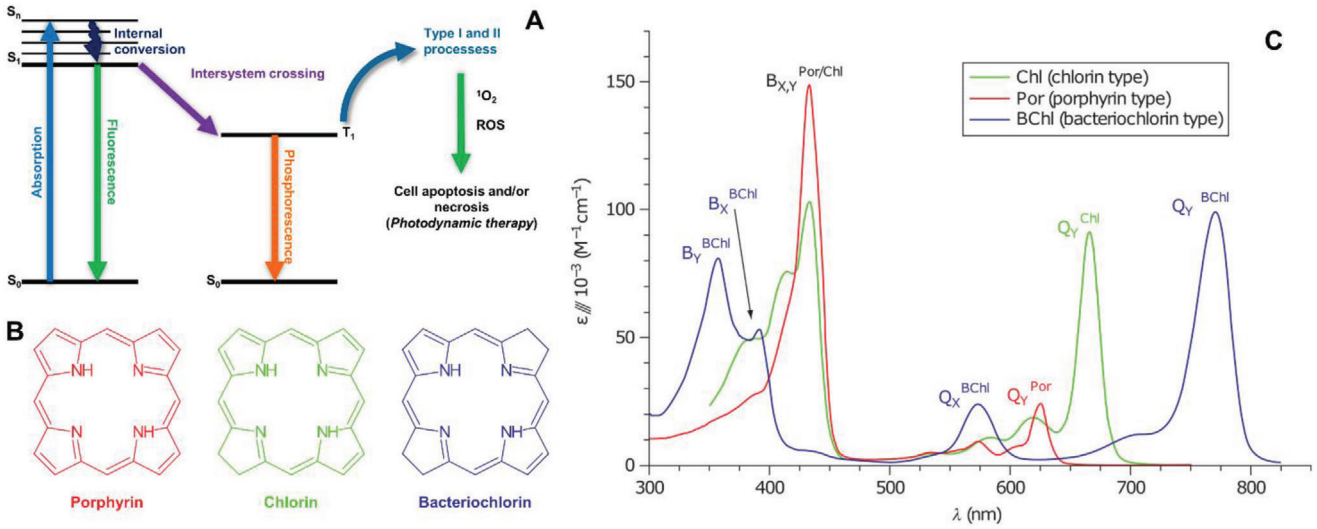


Figure 2. Photochemistry of chlorophylls. A) Jablonski diagram showing possible relaxation pathways of excited chlorophyll derivatives. B) The three photoactive compounds representing the types of chlorophylls found in nature (note that most bacteriochlorophylls are not bacteriochlorins, yet rather chlorins). C) spectra of porphyrin-, chlorin-, and bacteriochlorin-type chlorophylls. Reproduced with permission.[31] Copyright 2013, Elsevier.

stacking contribute to the formation of aggregates, which modify the position of the Q-band while decreasing its intensity. In the case of face-to-face aggregates (J-aggregation), the Q-band is red-shifted, while for side-to-side aggregates (H-aggregation) it is blue-shifted. Aggregation may be inhibited with the introduction of bulky substituents; steric hindrance distorts the macrocycle, weakening intermolecular interactions.

3. Biochemical Properties of Chlorophyll Derivatives

3.1. Metabolism

Chlorophylls are the primary photosynthetic pigments in plants, and are synthesized in the thylakoid membrane of chloroplasts.[36] Chlorophyll is located in protein complexes of photosystem I and II; its content varies during the different phases of the plant's life.[37] Chemically, chlorophylls present a tetrapyrrole porphyrin ring very similar to that of heme, even though it chelates Mg instead of Fe, and an esterified phytol. Different chlorophyll forms display peculiar pigmentation properties, correlated to their specific chemical structure.[38] Chlorophylls *a* and *b* are primarily found in edible plants, whereas chlorophylls *c*, *d*, and *f* are mainly present in cyanobacteria, diatoms, dinoflagellates, and algae.[39,40] Once removed from their physiological location, chlorophylls become very sensitive to light, high temperature, low pH, enzymes, and oxygen, both in vitro and in vivo.[41] Indeed, chlorophylls can be converted into other compounds by means of specific biochemical reactions. Catabolism of chlorophyll follows the so-called "pheophorbide *a* oxygenase (PAO)/phyllobilin" pathway, widely studied in the processes of leaf senescence and fruit ripening.[42] Currently, all known catabolic products except one are derived from chlorophyll *a*, which upon loss of the phytol chain and Mg dechelation becomes pheophorbide *a*. This reaction is catalyzed by the enzyme chlorophyllase, responsible for the hydrolyzation of the phytol chain, the creation of chlorophyllide *a*, and Mg de-chelation. While this process has been confirmedly documented during fruits ripening, some doubts have been raised on its involvement in leaves senescence.[43,44] Indeed, also pheophytinase (PPH) has been demonstrated to de-phytilate chlorophyll, originating pheophytin during leaf senescence,[45] and the so-called STAY-GREEN (SGR) enzyme has been shown to be responsible for Mg de-chelation.[46] In conclusion, SGR and PPH are involved in the formation of pheophorbide *a* in plants. PAO accounts for the formation of phyllobilins, by opening the macrocycle and producing a linear tetrapyrrole structure.[47] The resulting phyllobilin (red chlorophyll catabolite) is then transformed into a primary fluorescent chlorophyll catabolite, and exported from chloroplasts to the cytosol.[48] Here it can be modified, generating many different structures that are imported into the vacuole, thanks to an adenosine triphosphate (ATP)-binding cassette transporter.[49] In the final reaction, due to the vacuolar acidic pH, non-fluorescent chlorophyll catabolites are originated by isomerization.[50] Notably, cleavage of the isocyclic rings procure very complex structures that can be employed as photosensitizers.[18,51]

During food processing, conventional thermal treatments and acidification can induce the formation of chlorophyll derivatives. Indeed, in acidic conditions, pheophytin can be originated by displacing coordinated Mg with H. In presence of other metal ions—such as copper (Cu), cobalt (Co), Fe, and Zn—metalloporphyrins,[52] semisynthetic derivatives including sodium (Na)-Cu chlorophyllin (SCC),[53] Fe-pheophytin, and Fe-chlorins can be produced.[54,55] In order to preserve color, flavor, and nutritional properties of chlorophyll, it is essential to avoid its degradation in foods. Recently, the introduction of controlled atmosphere, non-thermal procedures, and post-harvest treatment with phytohormones has led to the retention of chlorophyll content and color in food.[56] For instance, high-pressure processing has demonstrated to be suitable for increasing cell disruption, facilitating intact chlorophyll extraction.[57]

3.2. Bioavailability

Chlorophyll is the most diffused lipophilic pigment known, and it is present in diverse quantities among different edible plants. It is well known that its amount can vary also depending on cultivation and processing strategies. Dark-green vegetables (e.g., spinach) are the main source of chlorophyll in our diet,[58] together with green fruits, olive oil, and edible seaweeds.[59–61] Currently, chlorophylls are widely employed as food additives and for monitoring agriculture and ocean productivity, thanks to their natural color. Interestingly, chlorophyll pigments have demonstrated unique bioactive properties, therefore raising interest in the scientific and medical communities.[21] Indeed, they contribute to cancer prevention, display antioxidant and antimutagenic activities, can induce apoptosis, and present antimicrobial and anti-inflammatory properties. Many studies have been performed in order to unravel the bioavailability of chlorophyll derivatives, which is strictly related to their chemical structure. Modifications in their chemistry change their ability to bind proteins[18] and their stability during circulation in the human body.[62] For instance, the presence of the phytol chain increases their hydrophobicity, while rearrangements in peripheral positions and in the isocyclic ring influence their absorption properties.[18]

Concerning the preferential metabolic paths exploited in our body, native chlorophyll follows the lipophilic absorption route due to its polarity and reactivity, while metalloderivatives (such as SCC) preferentially use the water-soluble route. As for other food ingredients, these molecules become bioavailable by release in our body thanks to the digestive processes and the subsequent interactions with gastric and intestinal juices. Successively, they are solubilized in aqueous environment (if water-soluble) or transferred to bile salt-lipidic micelles (if lipophilic), transported through enterocytes, and finally, secreted into systemic circulation.[63] Some of the most important aspects to be investigated for understanding the beneficial effects of chlorophyll and its derivatives on human health are their reactivity in the digestive tract and their bioavailability in the gut, before reaching the blood circulation. First studies identified porphyrins in urine, indicating chlorophyll bioavailability.[64] However, during digestion, in the acidic gut lumen, native chlorophyll can be significantly modified and converted into pheophytins.[65] Researches on the digestion and absorption of chlorophyll derivatives used in food industry, for instance Cu-chlorophyllins and Zn derivatives, demonstrated that Cu and Zn ions remained bound to porphyrin also after digestion, in contrast to what happens in the case of Mg.[65] This observation suggests that the pharmacokinetic properties of the derivatives depend on the central metal ion, responsible for recognition by the active transport system and by the enzymes involved in chlorophyll metabolism.[62] More recent investigations, performed in rabbits[66] and human plasma,[67] have demonstrated that chlorophyll was partially converted to pheophorbide prior to absorption, possibly by means of digestive enzymes or gut microbiome. Chlorophyllide and pheophorbide have been shown to be involved in enterohepatic circulation, behaving similarly to bile salts in the gastrointestinal tract.[66] Some in vitro digestion studies have demonstrated that different derivatives are differentially absorbed: chlorophyll *a* derivatives were micellarized more than chlorophyll *b* derivatives.[68,69] In other studies, no significant differences were found in the micellarized amounts.[70] These variations could be explained by competitive micellarization inhibition or by transport mediated by other compounds absorbed with the same mechanism.[69] Food matrix effects could also play an important role on bioavailability and would need further characterization in the next future.[68] Changes observed in the absorption routes may also be justified by structural properties, solubility, and metabolism between the different species. More lipophilic chlorophylls and pheophytins typically require micellarization,[65,69] while hydrophilic pheophorbides can be absorbed by simple diffusion and facilitated transport by ATP-binding cassette subfamily G member 2 (ABCG2) transporters,[71] favoring apical efflux and thus limiting/controlling their bioavailability.[72]

A recent work analyzed liver extracts from mice fed with a chlorophyll-rich diet, to measure the amount of chlorophyll metabolites. Authors found that the formation and/or absorption of pheophorbides, pyro-derivatives, and phytylchlorin e6 required a first-pass metabolism. Moreover, pheophorbide *a* absorption was partially dependent on scavenger receptor class B type I (SR-BI), a receptor for high-density lipoprotein. It was also hypothesized that a complementary pathway based on the de-esterification of pheophytin *a* in the liver, yielding pheophorbide *a* and phytol, could justify its origin in the liver.[73] In an interesting study, pheophorbide was found to be preferentially absorbed by myeloma cells respect to normal mouse splenocytes, indicating a better affinity for tumor cells and paving the way to its possible application as photodynamic agent for cancer treatment.[74]

SCC was characterized in human plasma[75] and studies performed in vivo allowed to determine its presence in specific tissues.[76] SCC, due to its partial hydrophilicity, can be absorbed via facilitated diffusion and a portion of Cu-chlorin e4 (Cu-Ce4), the predominant component of SCC, or its metabolites are absorbed from the human intestine.[77] Upon feeding Wistar rats with commercial Cu-Ce4, it was found in serum, liver, and kidneys, indicating absorption in its intact form. Moreover, it was observed an oxidative stress reduction in brain tissues in vivo, thus indicating protection through permeation across the blood-brain barrier.[78] Chlorophyllin, a semi-synthetic derivative of chlorophyll, was administered as a supplement to humans, and their serum was found to contain Cu-Ce4 ethyl ester as well as Cu-Ce4, which conferred a peculiar green color. This observation suggested that the two chlorin derivatives were absorbed and bioavailable in vivo.[79] Interestingly, metallochlorophyll derivatives distribution was analyzed in human blood fractions, and it was found that only a small fraction of the pigments was bound to cellular components in blood. The distribution among low-density lipoproteins, high-density lipoproteins, and high-density proteins was influenced by many chemical factors, such as polarity, presence of specific substituents, and metallic complexes.[80]

Unfortunately, systematic studies estimating the overall absorption and bioavailability are very limited. even though in vitro and in vivo researches point out that chlorophyll and its derivatives remain poorly bioavailable and present similar absorption levels. A more comprehensive study on specific tissue distribution, metabolism, and absorption kinetics will be required in the next future to complement biomedical and nutritional findings.

3.3. Antioxidant Activity

Many endogenous and exogenous factors are responsible for the formation of free radicals in our cells. Indeed, ROS are usually produced by our metabolism, in particular during mitochondrial respiration, drug metabolism, and inflammation, but their increase can also be favored by exogenous causes such as smoking, air pollution, infection, ionizing, and UV radiations. While it is essential to maintain a correct balance between production and removal of ROS, their accumulation can significatively impair proper cellular functioning and damage cellular components (cell membrane, proteins, and DNA), promoting inflammatory processes and oxidative stress. Indeed, if ROS are not correctly scavenged by endogenous enzymes or antioxidant molecules, they start accumulating, giving rise to the onset of pathologies such as cancer, premature aging, neurodegenerative diseases, and diabetes.[81] The modulation of oxidative stress can be achieved by administration of antioxidant molecules able to prevent, reduce, or favor ROS inactivation by endogenous mechanisms.[82] Even though very promising, this approach presents some limitations mainly due to their low bioavailability and solubility in water, easy degradation in physiological conditions,[83] and difficulty in crossing the blood-brain barrier, that constitutively protects our central nervous system from hazardous molecules.

Chlorophyll and its derivatives have been reported to prevent or decrease oxidative stress inside cells, by stimulating their physiological antioxidant pathways.[84] They effectively scavenge different free radicals species, such as hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and $^1O_2$.[85–88] Moreover, they have been demonstrated to inhibit lipid peroxidation in vitro,[87,89] in vivo,[90] and ex vivo.[87] Researches performed on these compounds as antioxidant molecules have mainly focused on chlorophyll *a*, chlorophyll *b*, and their catabolites. Nevertheless, collected observations have been partially contradictory. Some studies have suggested that chlorophyll *a* and metal derivatives exhibit greater antioxidant ability, while metal-free molecules such as pheophytins and pyropheophytins display lower antioxidant properties.[91,92] Other researches have demonstrated that porphyrin is fundamental for reducing free radicals, and chelation of Mg enhances this property.[93,94] Further works have found that chlorophyll *a* presents a reduced antioxidant ability respect to other pigments, while pheophorbide *b* and pheophytin *b* resulted to be the most potent antioxidant molecules, thanks to the presence of the aldehyde group.[89] Cu-chlorophyllin gave a more potent antioxidant capacity than natural chlorophylls, due to the specificity of the chelated metal.[90] Interestingly, the central metal ion enhanced the in vitro antioxidant activity when compared to that of metal-free chlorins, pheophytins, and pyropheophytins.[95] Chlorophyll and SCC were effective in reducing oxidative stress and influencing the redox status of human pancreatic cells.[95] Chlorophyll *a* demonstrated to be more efficient as free radical scavenger and reducing intracellular ROS production with respect to chlorophyll *b*, and pheophytins showed greater ability than chlorophylls.[95] The pigments performed their beneficial activity also reducing mitochondrial superoxide production in human pancreatic cancer cells. Hepatoma cells treated with heavy metals showed reduced oxidative stress upon treatment with SCC and chlorophyll-rich spinach extract.[96] Interestingly, metalloporphyrins have been reported to block ROS generation: an Fe-containing chlorophyllin, Fe-Ce6, was more effective than chlorophyllin (Cu-containing chlorophyll) in suppressing H_2O_2 cytotoxicity and apoptosis in human Jurkat T-cells.[96] As it has been demonstrated in the reported studies, chlorophyll and its derivatives efficiently work as powerful antioxidants, reducing ROS levels. However, it has to be noticed that, compared to other antioxidants such as carotenoids and tocopherols, they need higher concentrations to reach similar effectiveness.[97] In vitro studies have demonstrated that the antioxidant activity of chlorophyllins relies on the upregulation of heme oxygenase-1 (HO-1) and NAD(P)H quinone dehydrogenase 1 (NQO1), at least during H_2O_2-induced oxidative stress,[98,99] and on the induction of mammalian phase 2 proteins involved in cellular protection against oxidants and electrophiles.[100] Chlorophyllin prevented oxidative stress induced by ionizing radiation (γ-radiation),[87,88,101] photosensitization,[87,88] ascorbate-Fe^{2+}, NADPH-ADP-Fe^{3+}, and azobis-amidinopropane hydrochloride both in vitro and ex vivo.[87] Moreover, chlorophyllin and pheophytin *a* efficiently inhibited superoxide anion (O_2^-) generation by 12-O-tetradecanoylphorbol-13-acetate in differentiated HL-60 cells[102] and in mouse macrophage cells,[103] and the production of $^{\bullet}OH$ by Fenton reaction.[102] Wang et al. demonstrated that chlorophyll was absorbed, improving resistance to oxidative stress and promoting prolonged lifespan in *Caenorhabditis elegans*.[104]

In conclusion, data demonstrating the antioxidant activity of chlorophyll and chlorophyll derivatives can be dependent on the nature of each pigment and results can be related to the specific kind of free radical analyzed, to the experimental conditions tested, and to the fact that most of the data are obtained in vitro. Therefore, more in-depth investigations will be needed in in vivo models.

3.4. As Nutrients and Drugs

Researches performed in vitro and in vivo indicate that chlorophylls and their derivatives can have antimutagenic, antigenotoxic, and antitumorigenic effects, and can regulate the metabolism of xenobiotics, carcinogens, and mutagens in simple organisms, animals, and humans. Chlorophyll has been reported to be involved in chemoprotection processes. Upon administration to trouts as a dietary supplement, it avoided dibenzo[def,p]chrysene-induced DNA adduct formation,[105] while it efficiently prevented the genotoxic effects of 4-nitroquinoline 1-oxide in *Drosophila* upon oral administration.[106] Interestingly, administration of ascorbic acid and chlorophylls *a* and *b* protected *Drosophila* larvae from acrolein genotoxicity.[107] Chlorophyll and SCC have been shown to prevent the development of liver cancer in adults exposed to aflatoxin.[108,109] The mechanism at the basis of the antimutagenic activity has been explained by the preferential binding of mutagens in the digestive tract, able to modify their bioavailability and thus their DNA damaging effects,[110–114] and their absorption, able to modify the activity of specific enzymes.[79,115,116] It has been demonstrated that chlorophyll derivatives can inhibit cytochrome P450 enzyme and other hepatic enzymes

responsible for the bioactivation of carcinogens.[117,118] Furthermore, they can enhance the degradation of specific mutagens or promote the activity of detoxification inside cells.[112] It has to be noted that, depending on the experimental conditions tested, the function of purified chlorophyll derivatives can be hugely different, working both as tumor promoters or antitumoral agents.[119–122] Pheophorbide *a* and chlorophyllin have been demonstrated to induce growth arrest,[123] block proliferation,[124] stimulate cell differentiation, and trigger apoptosis.[125,126] Interestingly, some molecules have shown also antiviral activity: pheophytins were able to inhibit the absorption and penetration of herpes simplex virus in vitro[127] and pheophytin *a* inhibited hepatitis C virus replication.[128]

Since many years, chlorophyllin derivatives have also been used in wound healing in animals[129,130] and humans.[131–133] Some studies reported their application in different acute diseases, sepsis, infection, and ulcers.[16,132,134,135] Recently, chlorophyllin has been shown effective as inhibitor of hyaluronidase in vitro, suggesting its application as anti-aging component in cosmetic products,[136] and already demonstrated to be successful in some human pilot studies.[16,135]

Remarkable immunostimulatory and immunomodulatory effects have been proven for chlorophyllin[137,138] and pheophorbide *a*.[139] Moreover, pheophorbide *a* and pheophytin *a* exerted their anti-inflammatory effect in lipopolysaccharide-induced RAW 264.7 murine macrophages, showing a dose-dependent inhibition of nitric oxide production and suppression of inducible nitric oxide synthase.[140] Finally, chlorophyll derivatives, thanks to their photophysical properties, have been employed as photosensitizers for PDT. In this case, upon specific illumination, tetrapyrroles can generate ROS inducing oxidative stress with subsequent cell apoptosis.[141]

4. Light-Activated Biomedical Applications of Chlorophyll Derivatives

4.1. As Photoactive Agents

As shown in Section 2.2, chlorophyll derivatives have peculiar optical properties that can be exploited in several biomedical applications.[142] Among them, PDT is one of the most studied. PDT is a medical treatment that involves the use of specific light-sensitive molecules (photosensitizers) and a light source to induce damage to diseased tissues or cells. Commonly, PDT is employed to treat tumors;[143] nevertheless, it has received attention also in antimicrobial therapy[15] and to treat age-related macular degeneration.[144] As already described in Section 2.2, in PDT, a photosensitizer is excited by light at a specific wavelength from its ground state (S_0) to a short-lived excited state (S_n).[145,146] To relax back to the ground state, the photosensitizer undergoes a series of non-radiative and radiative decay processes (Figure 2). In PDT, it is pivotal that the photosensitizer can effectively populate the excited triplet state (T_1) and relax back to the ground state prevalently by Type I and Type II processes that lead to the formation of ROS and $^1O_2$. ROS and $^1O_2$ are very reactive and can damage surrounding tissues by activating several apoptotic and necrotic mechanisms in cells.[146] Excitation of chlorophyll derivatives predominantly activates Type II reactions with the formation of $^1O_2$. The efficiency of $^1O_2$ generation is measured by the $^1O_2$ ϕ_Δ, that is, the number of singlet species over the number of photons absorbed. $^1O_2$ is a short-lived, highly reactive species, whose average diffusion length is 10–55 nm in cellular media. Cellular damage induced by photosensitizers is, thus, very localized and the overall performance depends on their cellular uptake and distribution. The aggregation of the photosensitizer affects the pharmacokinetics and pharmacodynamics, both by influencing its localization in the cell and decreasing the ϕ_Δ of $^1O_2$ (shorter triplet lifetime).

The ideal photosensitizer for PDT must fulfill some specific requirements: it should be easily obtained as a pure substance with well-known chemical and optical properties; the toxicity should be triggered only by light irradiation at specific wavelengths, it should be soluble and stable in the biological milieu, it should work in the spectral range of 650–850 nm, where light penetration in tissues is at its maximum, it should have high yields of T_1 formation and $^1O_2$ and ROS production, it should selectively accumulate in the target tissue, and it should be rapidly cleared from the body after the treatment to avoid undesired phototoxicity in healthy tissues.

In this context, chlorophyll derivatives are good candidates for PDT with respect to conventional sensitizers (e.g., hematoporphirin), as they fulfill most of the requirements listed above. Chlorophyll derivatives can be extracted with high purity from natural sources or easily obtained with simple reactions from chlorophyll extracts, they show little to no dark toxicity and they are rapidly cleared from the body.[147] Their optical properties and high ϕ_Δ of ROS are also interesting for PDT. In fact, light scattering and absorption, mainly due to hemoglobin and melanin, limit the penetration of light in tissues. To stimulate photosensitizers in regions deeper than a few millimeters, their Q-band should be above 650 nm and ideally around 800 nm. A further red shift of the absorption maximum would generate a gap between T_1 and S_0 that could be lower than the energy of $^1O_2$, 0.97 eV, resulting in no singlet generation. As shown in Section 2.2, the typical Q-bands of chlorophyll derivatives fall within the correct range.

Natural chlorophyll a has a high $^1O_2$ ϕ_Δ (0.57 in CCl_4);[148] however, due to its poor solubility in water, its tendency to aggregate and its instability in the biological milieu, its application in PDT is severely hampered.[147] Pheophorbide *a* has a maximum photoactivity when irradiated at 670 nm, with ϕ_Δ of 0.6[148] and a higher dark and light stability compared to chlorophyll *a*. For this reason, its potentialities in PDT have been explored in preclinical studies.[147] For instance, Liu et al. studied the effect of pheophorbide-based PDT on human prostate cancer PC-3 cells in vitro, showing an inhibition of cell proliferation, apoptosis induction, and inhibition of tumor invasivity and mestastasis capacities.[149] A derivative of pyropheophorbide *a* was tested both in vitro and in vivo as photosensitizer for PDT against subfoveal choroidal neovascularization due to age-related macular degeneration, with promising results.[150] Purpurin-18, obtained by oxidative cleavage of pheophorbide and its esters, and chlorin p6, obtained by alkaline hydrolysis of purpurin-18, have also been investigated as potential photosensitizers in PDT.[151] Purpurin-18 is a hydrophobic molecule that absorbs light at 704 nm and has a ϕ_Δ of 0.6 (in toluene), while chlorin p6 is also stable in aqueous environments at pH 7.4, with light absorption at 656 nm and ϕ_Δ of 0.6 (measured in ethanol).[152]

Purpurin-18-mediated PDT has been recently shown to prevent the growth of triple-negative breast cancer, both in vitro and in vivo.[153]

15[1]-hydroxypurpurin-7-lactone dimethyl ester was shown to induce occlusion of capillaries and blood vessels in vivo upon appropriate light stimulation; it is, therefore, potentially suitable for the treatment of tumors and age-related macular degeneration.[154] Nevertheless, the most advanced chlorophyll derivative in clinical studies is Ce6 and its derivatives. Ce6, formed by anaerobic alkaline hydrolysis of pheophorbide *a*, is quite soluble in biological media, absorbs light between 650 and 670 nm, and has a $^1O_2$ ϕ_Δ of about 0.7 in phosphate buffer.[155] Ce6 accumulates in tumors and is cleared fast from the organism.[156] Some chlorin derivatives have been already accepted in clinical practice or are undergoing clinical trials for tumor ablation mediated by PDT.[147,156,157] For instance, Foscan, a chlorin-based photosensitizing agent known as meso-(tetrahydroxyphenyl) chlorin (*m*THPC) or Temoporfin, has been approved by the European Medical Agency in 2001[158] for the treatment of advanced head and neck squamous cell carcinoma;[159] nevertheless, strictly speaking, Temoporfin is a synthetic compound that aims at mimicking natural chlorin derivatives and it cannot be really considered a product obtained from natural sources.[160] Other chlorin derivatives proposed for PDT are mono-L-aspartyl Ce6 or Talaporfin,[161] approved in Japan in 2004 and marketed as Laserphyrin,[162] monoseryl Ce6 and Sn(IV) Ce6.[147] Mono-L-aspartyl Ce6 has also been studied as a potential photosensitizers for the treatment of age-related macular degeneration.[163] Ce6 and its derivatives were also tested as antimicrobial PDT agents. For instance, Jeon et al. demonstrated the efficacy of PDT with Ce6, stimulated with an halogen light, in inactivating several skin bacteria, and, in particular, *Propionibacterium acnes*, offering a valid alternative to conventional antibiotic therapies.[164] Uliana et al. tested the activity of Ce6 and its hydrogenated derivative (Ce6H) as antimicrobial PDT agents.[165] In particular, they synthetized both Ce6 and Ce6H starting from methyl pheophorbide *a*, obtained by methanolic extraction from the alga *Spirulina maxima*. The antimicrobial PDT activity of these two photosensitizers against a Gram-positive (*Staphylococcus aureus*) and a Gram-negative (*Escherichia coli*) bacterium, and a fungus (*Candida albicans*) was then studied. Results demonstrated that both Ce6 and Ce6H were effective in inhibiting bacteria and fungus growth; nevertheless, the derivative Ce6H showed higher efficacy against *E. coli* and *C. albicans* as compared to Ce6.[165] Several chlorin derivatives with cationic functionalization were obtained by Huang et al. from pheophytin *a* and tested as antimicrobial PDT agents against *S. aureus*, *E. coli*, and *C. albicans*.[166] The authors demonstrated that the functionalization, chemical structure, and charge of the photosensitizers have a strong impact on their activity and they should be carefully evaluated, depending on the target; for instance, a higher number of cationic charges enhances the efficacy on *C. albicans* and *E. coli*.[166] Diogo et al. compared the efficacy of Zn(II)chlorin e6 methyl ester (Zn(II)e6Me) as a photosensitizer for human dentin discs and root blocks photodynamic disifenction, with that of FotoSan, a commercial Toluidine Blue O formulation, demonstrating the higher efficacy of Zn(II)e6Me, with lower side effects.[167] Zn(II)e6Me-mediated PDT disinfection had higher efficacy also compared with 3% sodium hypochlorite (NaOCl) treatments of root blocks; however, the opposite trend was found in the treatment of dentin disks.[167]

Bacteriochlorophylls, with their high extinction coefficients (4–10 × 10^4 $M^{-1}cm^{-1}$) and longer activation wavelengths (λ_{max} = 760–780 nm), are also extremely interesting for PDT; however, their lower stability as compared to chlorophyll derivatives and their insolubilty in aqueous environments make their current application still limited.[168] Nevertheless, padeliporfin, a palladium-substituted bacteriochlorophyll derivative soluble in water, has been accepted in 2017 by EMA for PDT against prostate cancer and marketed as Tookad.[169] Clinical trials are also ongoing for the treatment of other kind of cancers and age-related macular degeneration.[170,171]Technological advances for the photostimulation of photosensitizers in PDT have been developed in the past decades. In particular, nonlinear optical phenomena provide useful tools to circumvent absorption by tissues. Two-photon absorption (TPA) allows the excitation of photosensitizers with NIR radiation. For instance, the Soret band and Q-band of Ce6 move from 380 and 650 nm to 700 and 1050 nm in the two-photon spectrum.[172] However, due to its limited absorption cross-section, photosensitization by Ce6 undergoing TPA is ineffective. To improve the photoconversion by TPA in PDT, the FRET (Förster resonance energy transfer) stimulation of Ce6 coupled with upconverting NPs (Yb^{3+} and Er^{3+} doped $NaYF_4$) has been proposed.[173] In another report, in situ photogeneration exploiting CARS (coherent anti-Stokes Raman scattering) by lipids and SHG (second harmonic generation) by collagen has been used to activate Ce6 in vitro.[174] In all these nonlinear phenomena, the probability of the absorption event is proportional to the square of the intensity, allowing for higher sensitivity. The main limitation is the use of fs lasers with small spot size, increasing the required dose tenfold compared to traditional PDT.

Despite the potentialities of chlorophyll derivatives in PDT, their application is limited by their instability in aqueous environments and their hydrophobicity. In this sense, nanotechnological solutions have been considered to overcome this issue, as it will be later described in Section 5.

A thorough examination of alternatives to chlorophyll derivatives in PDT is beyond the scope of the current work. Apart from other tetrapyrroles, most relevant compounds include a number of clinically approved synthetic dyes and natural substances, such as phenothiazinium salts and their variations, as well as Rose Bengal, curcumin, or hypocrellin.[175] The first category contains molecules, like methylene blue and toluidine blue, that combine a good ROS production upon light exposure with an intrinsic capability of penetrating microbial cells, which makes them especially useful as antimicrobial photodynamic agents,[176] but they have also been chosen to treat diseases with non-infectious etiology.[177] Rose Bengal has been successfully utilized for the photodynamic treatment of severe cases of infectious keratitis; after all, the substance had already established its place in ophthalmology, being widely deployed to stain corneal and conjunctival damage.[178,179] At times, curcumin and hypocrellin have been used as safe photodynamic agents but, due to their poor solubility in water, they often require some kind of vector.[175]

Further candidate classes of photodynamic agents are under study. Squaraine dyes, for instance, absorb strongly in the far-red/NIR range and yield high quantities of $^1O_2$, the likely most relevant mediator of toxicity at a cell level; these dyes already gave

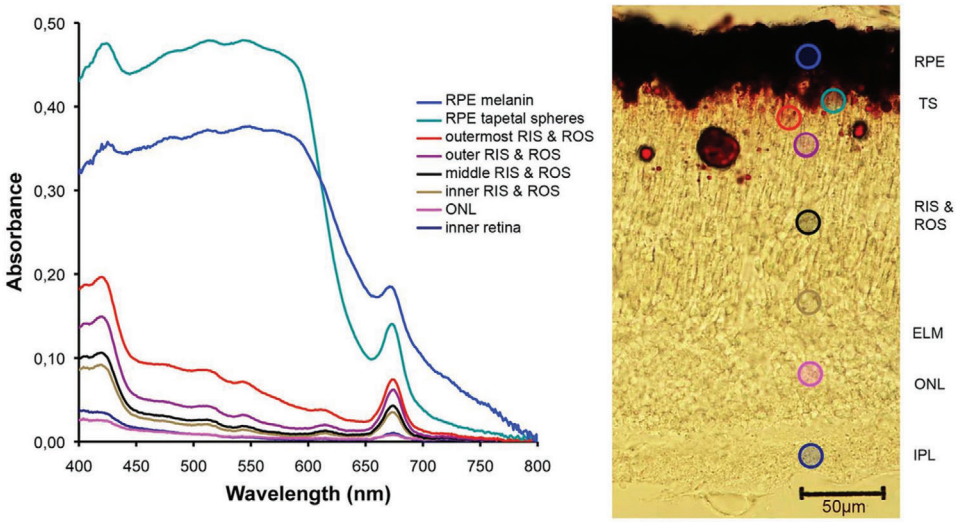


Figure 3. Absorption spectra of different regions within unstained retinal tissue (averages of five specimens) of *M. niger*, showing peaks compatible with the presence of bacteriochlotophyll. ELM = external limiting membrane; IPL = inner plexiform layer; ONL = outer nuclear layer; RIS = rod inner segment; ROS = rod outer segment; RPE = retinal pigment epithelium; TS = tapetal sphere. Reproduced under the terms of the Creative Commons CC-BY License.[188] Copyright 2016, The Authors, published by Springer Natrue.

promising results in non-clinical investigations.[175,180] Coordination complexes of transition metals are innovative photodynamic chemicals that are gradually entering clinical trials. While holding great potential for the field, major obstacles are to be faced in order to make them practically convenient: their strong points are that they tend to display particularly pronounced and conceivably hypoxia-tolerant phototoxicities, and that they could be efficiently activated via TPA modalities.[181,182] However, two-photon PDT requires comparatively higher energies, and generally needs substantial optimization; absorption wavelengths of transition metal complexes, in addition, need to be tuned to match those ideal (in terms of penetration depth) for each particular disease.[183]

4.2. As Vision Enhancers

Starting from the late '90s, a series of breakthrough discoveries from Douglas et al. defined an additional biological function for chlorophyll derivatives. Besides their well-known role in photosynthesis, in fact, they act as enhancers of vision. Specifically, the group found a first—and still the only known—natural instance of an animal deploying chlorophyll derivatives for sight: the deep-sea fish *Malacosteus niger*, also known as the black loosejaw, has a green-tuned retina, which does not allow far-red vision on its own; however, the species accumulates chlorophyll derivatives in its eye by feeding on algae-eating crustaceans. Thanks to a bioluminescent organ, *M. niger* can shine far-red light and, when this is reflected by its prey, it hits the fish retina, where it is detected by chlorophyll derivatives. Through an unclear mechanism, the energy is eventually transferred to the green-sensitive visual pigment. Ecologically speaking, such adaptation virtually guarantees stealth predation to the fish, because the majority of organisms living in its habitat (i.e., the meso-bathypelagic zone) are blind to long-wavelength light. Spectroscopic analyses showed that the type of chlorophyll derivatives accumulated by the black loosejaw is a blend of degraded, namely de-metalled and de-farnesylated, bacteriochlorophylls *c* and *d* (**Figure 3**).[184–188]

Later studies attempted to alter the spectral tuning of vertebrate visual systems by artificially providing chlorophyll derivatives. Preliminary in vitro bleaching experiments on purified bovine rhodopsin, a well-characterized vertebrate visual pigment composed by a photoreceptor protein bound to the chromophore 11-*cis*-retinal, served to select a promising chlorophyll derivative for downstream investigations on vision. Using such assay, Washington et al. screened some compounds, and determined Ce6 as the preferred choice.[189] They went on with injecting Ce6 intravenously in mice, showing that the molecule rapidly reaches the retina; by means of electroretinograms, the authors found an enriched photoreceptor response to violet and red light upon Ce6 administration.[13] Further studies focusing on PDT applications corroborate the notion that, when Ce6 or similar substances are provided systemically, in a matter of hours or even less, they accumulate in the retinal photoreceptor layer.[190] Using tiger salamanders (*Ambystoma tigrinum*), another team of researchers proved that isolated amphibian photoreceptors also respond to Ce6 treatment with an increased sensitivity toward red light.[191]

Our group demonstrated that Ce6 strengthens visual capacities of invertebrates as well, at least in the flatworm *Dugesia japonica*. Flatworms are photophobic, meaning that they naturally escape from light. Wild-type or visually impaired specimens were exposed to light, either being preventively soaked in a Ce6 solution or not. Ce6-treated individuals became more responsive to UV or red light relative to controls, and such effect disappeared whenever their sight was compromised (**Figure 4**).[192] Photoreceptor cells sustaining vertebrate vision belong to the so-called ciliary type. Invertebrate visual systems, in turn, rely on another kind of photoreceptors, termed rhabdomeric. The two typologies differ to a large extent in terms of cell shape, light-transduction machinery, and visual pigments.[193]

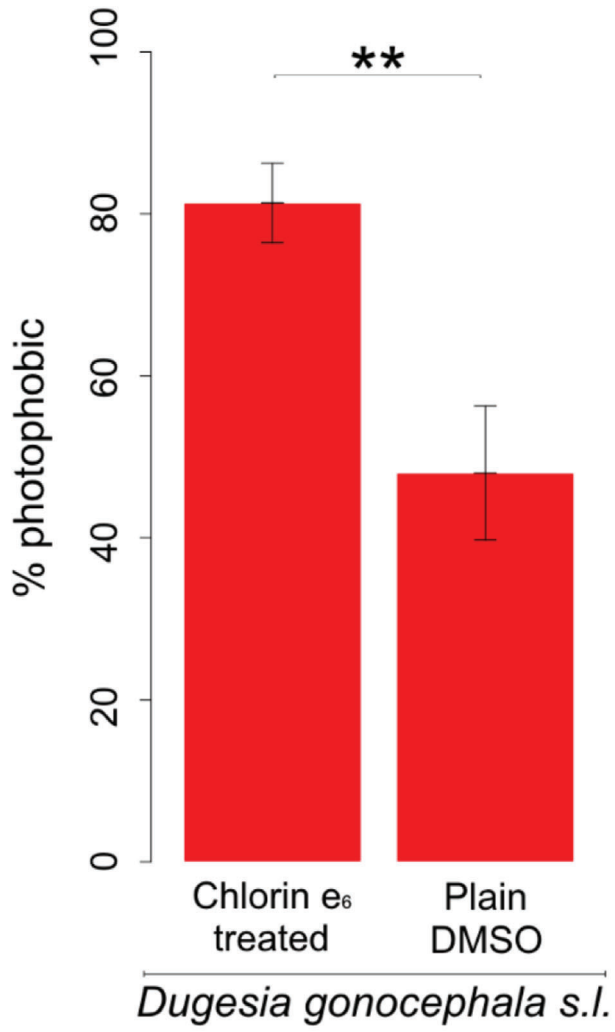


Figure 4. During behavioral assays, enhanced photophobicity is detectable in a greater proportion of flatworms (here, *D. gonocephala s.l.*) upon treatment with Ce6. DMSO = dimethyl sulfoxide, indicating controls matched for solvent composition. ** = significance for unpaired one-tailed *t*-test ($\alpha = 0.01$) and for two-tailed Mann-Whitney *U*-test ($\alpha = 0.05$). Bars represent averages of five experimental replicas, deploying 15 specimens each. Error bars indicate standard error of the mean. Reproduced under the terms of the Creative Commons CC-BY License.[192] Copyright 2017, The Authors, published by Springer Nature.

Therefore, whatever the mechanisms sustaining chlorophyll-mediated sight, the phenomenon pertains to both ciliary and rhabdomeric systems, suggesting a rather ancient origin.[192]

Consistently with the idea that Ce6 might work as a transducer for light stimuli, the visual gain of function imparted by Ce6 follows its absorption spectrum.[13,192] The current consensus is that Ce6 binds visual pigments, specifically an opsin with its retinal cofactor, by directly fitting into a pocket within the protein but other than the chromophore one. There, it may act as an allosteric modulator, perhaps via some mechanism mediated by radicals.[194–198]

Chlorophyll derivatives induce photophobicity in humans as well, at least because the condition is a known side effect of PDT.[199] There are, nevertheless, unconfirmed claims of Ce6 enhancing human scotopic (i.e., night) vision. All things considered, it looks plausible for chlorophyll-related compounds to have a biomedically relevant role in human sight one day but, prior to that, many basic aspects await clarification, including important pre-clinical and clinical notions about eye toxicity: in a bright environment, chlorophyll derivatives may trigger a ROS buildup that could damage the delicate retinal tissue.

Table 1 summarizes most relevant current and foreseeable biomedical applications of chlorophyll-related compounds.

5. Nanotechnology and Chlorophyll Derivatives

5.1. Generalities

Some of the major drawbacks of using chlorophyll derivatives in clinical applications are related to their hydrophobicity and poor stability in biological fluids. Moreover, in PDT, off-target toxicity due to accumulation in healthy tissues should be avoided as much as possible to limit side effects.

In the last decades, medical research has experienced a huge development in order to overcome limitations due to poor solubility and target specificity of many active principles. In particular, a new branch of nanotechnology known as nanomedicine has given a huge input in the quest for more effective and precise treatments.[203,204] Nanomedicine exploits the well-known peculiar physicochemical properties of NPs such as their small size (from 1 up to 1000 nm), high surface-to-volume ratio, morphology, and properties easily tuned by choosing the right components and preparation protocols. NPs offer several advantages as compared to conventional therapeutic agents: encapsulation of a high payload of active compounds, increasing their solubility, biocompatibility, bioavailability, and stability in the biological milieu; easy protocols for conjugation with specific ligands that can target the site of action, enhancing accumulation in the desired location, and reducing side effects; controlled release of active compounds.[203,204] Moreover, NPs can act themselves as therapeutic or diagnostic agents, having an active role in the therapeutic approach. In order to be used in biomedical applications, NPs must be composed of biocompatible components, their physicochemical properties must be well characterized, and their behavior in vivo must be thoroughly investigated before clinical translation.

Currently, there are a plethora of NPs that can be used for medical applications and, in particular, to deliver or to act in synergy with chlorophyll derivatives. Generally, they are divided into inorganic and organic NPs depending on their composition.[203,205]

5.2. Inorganic NPs

Inorganic NPs are usually composed of metal oxides or pure metals. Silica (SiO_2) NPs, for example, attracted a lot of attention in biomedical research as drug delivery systems, thanks to their lower toxicity and higher biocompatibility with respect to other inorganic NPs based on metal oxides. Their mesoporous structure and high surface-to-volume ratio allow the encapsulation of a significant amount of drug, and their framework is based on silicon-oxygen (Si—O) bonds that are resistant

Table 1. Summary of the main light-activated biomedical applications of chlorophyll derivatives and their current status, with references related to examples cited in this review.

Application	Photosensitizer	Status	References
PDT for tumor ablation	Foscan (a synthetic chlorin derivative) and Tookad	Currently accepted for the treatment of advanced head and neck squamous cell carcinoma (Foscan, 2011) and prostate cancer (Tookad, 2017) by EMA. Other clinical trials are ongoing	[158,159,169–171]
	Ce6 derivatives such as mono-L-aspartyl Ce6 or Talaporfin, monoseryl Ce6, Sn(IV) Ce6	Talaporfin has been accepted in Japan (2004) and under clinical trials in other countries. Other chlorin derivatives are also under clinical trials or at research stage	[147,156,157,161,162]
	Pheophorbide *a*, purpurin-18, chlorin p6	Currently at research stage	[147,149,151,153]
PDT for antimicrobial therapy	Mainly Ce6 and its derivatives	Currently at research stage	[164–167]
PDT for age-related macular degeneration	Pyropheophorbide *a* derivatives, 15^1-hydroxypurpurin-7-lactone dimethyl ester, and Ce6 derivatives	Currently at research stage	[150,154,163]
Vision enhancement	Ce6; a natural instance of chlorophyll-aided vision deploying de-metalled and de-farnesylated bacteriochlorophylls *c* and *d*.	Currently at research stage	[13,184–188,191,192]
Optogenetics	Phytochromobilin-, phycocyanobilin-, or biliverdin-bearing phytochromes and similar (cyanobacteriochromes) have been evaluated as possible red-shifted optogenetic tools	Currently at research stage	[200–202]

to degradation in different conditions.[206] Chlorophyll extracted from leaves was successfully loaded into SiO_2 NPs and preserved its typical optical features, making this system an interesting bio-friendly approach for medical imaging applications.[207] Zhang et al. prepared mesoporous SiO_2 NPs co-loaded with Ce6 and a prodrug of cisplatin, a conventional chemotherapeutic, to treat cisplatin-resistant A549R lung cancer cells with chemo-photodynamic combination therapy.[208] In vitro tests showed promising anticancer activity, especially after irradiation with 660 nm light. Other commonly used inorganic NPs are gold (Au) NPs or Au nanoshells.[203] The latter consist of a dielectric core (generally SiO_2) coated a thin layer of Au. These systems have peculiar optical and electrical properties, low toxicity, and potential biodegradability that make them interesting for biomedical applications. For instance, it is known that they can generate a photothermal effect when irradiated with NIR light.[209] Chuang et al. synthetized Au nanorods covalently conjugated to polyethylene glycol (PEG) and polyethylenimine via S-Au bonds (APP), and subsequently loaded with Ce6 (APP/Ce6). Obtained APP/Ce6 was subsequently loaded in adipose-derived stem cells (ADSCs) to favor colon cancer uptake and homogeneous intratumor drug distribution. APP/Ce6 loaded ADSCs were exposed to light irradiation at 808 and 660 nm after tumor uptake to achieve a dual photothermal and photodynamic anticancer approach against colon cancer. This nanosystem was tested both in vitro and in vivo showing promising results (**Figure 5**).[210]

Fe-oxide NPs are also widely investigated for biomedical applications.[211] In particular, superparamagnetic Fe-oxide NPs (SPIONs) have been used both for MRI and tumor thermal ablation via magnetic hyperthermia upon stimulation with an appropriate alternated magnetic field.[212,213] SPIONs were successfully coated and stabilized in water with Ce6, forming nanoclusters about 96 nm thick. These nanoclusters proved to be promising magnetic resonance and fluorescence contrast agents and anticancer tools via PDT against breast cancer.[214]

5.3. Organic NPs

Organic NPs exist in a variety of morphologies and compositions. Liposomes are spherical vesicles composed of one or more phospholipid bilayers enclosing an aqueous core. Thanks to this peculiar morphology, they can encapsulate both hydrophobic and hydrophilic molecules.[215] Liposomes are made of phospholipids, lipids, and cholesterol, while an outer layer of PEG is often added to impart steric and stealth stability.[216] Liposomes were the first NPs approved for cancer treatment by the Food and Drug Administration.[217] Yang et al. encapsulated Ce6 in liposomes composed of dimyristoyl-sn-glycero-phosphatidylcholine and of the cationic surfactant cetyltrimethyl ammonium bromide, and demonstrated their efficacy as antimicrobial PDT agents against *C. albicans* and infected burn wounds.[218] Pyropheophorbide acid (PPa), obtained from chlorophyll degradation, was loaded into liposomes for a trimodal imaging-guided PDT of cancer. The hydrophobic nature of PPa hinders its applications in PDT; nevertheless, the encapsulation in liposomes enhanced its water solubility, blood circulation, and biodistribution in vivo. By irradiation at 690 nm, liposome/PPa NPs inhibited tumor growth in mice bearing 4T1 tumor models. Moreover, by exploiting the fluorescent features of PPa and thanks to the extra radiolabeling with iodine-125 (^{125}I), liposome/PPa demonstrated to be promising contrast agents for multimodal imaging (fluorescence, photoacoustic, and single-photon emission computed tomography/computed tomography imaging).[219] In a recent work, Nishimura et al. reported an efficient strategy to manipulate the

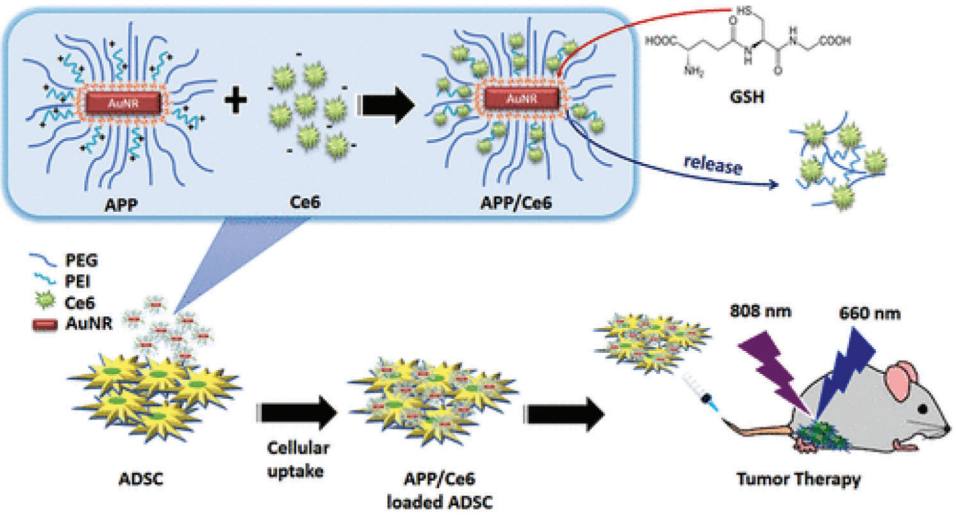


Figure 5. Scheme of the anticancer activity of APP/Ce6 loaded ADSCs nanocomplexes through combined photothermal and PDT. Reproduced with permission.[210] Copyright 2020, American Chemical Society.

aggregation state of methyl pyropheophorbide *a* (MPP) loaded in liposomes by inducing phase transitions in the lipid bilayer. They showed that below the phase-transition temperature (T_m) of the bilayer—when the bilayer is in a gel form—MPP was found to be in a dimeric state with a red-shifted absorption (wavelength from 674 to 704 nm). At temperatures higher than T_m, MPP existed in the monomeric form and the absorption at 674 nm increased. This thermochromic dimer–monomer transition was shown to be reversible. This study clearly proved how the behavior of chlorophyll derivatives is strongly affected by the external environment; therefore, environmental conditions must be carefully evaluated when using optical properties of chlorophyll derivatives for specific applications.[220]

Micelles are small spherical aggregates formed by self-assembly of phospholipids or other amphiphilic polymers. Differently from liposomes, they do not have an aqueous core; instead, their inner region is composed by the lipophilic portion of the amphiphilic molecules forming them. Therefore, micelles can encapsulate hydrophobic molecules in their inner core. Gong et al. obtained micelles by self-assembly of the amphiphilic synthetic Ce6-conjugated poly(maleic anhydride-alt-1-octadecene) (C18PMH)-PEG-Ce6. These micelles were able to encapsulate the dye IR825 for photothermal applications, while Ce6—besides acting as a fluorophore and photosensitizer—was also used as a chelating agent for Gd(III) for MRI. The obtained multifunctional nanomicelles were therefore able to be imaged by three different modalities (fluorescence, photoacoustic imaging, and MRI) and to exert an antitumor effect mediated by the combined photothermal and PDT treatments.[34]

Polymer NPs are solid nanosphere or nanocapsules composed of biocompatible and biodegradable synthetic polymers such as poly(D,L-lactide-co-glycolide) acid, poly(lactide) acid, poly(methyl methacrylate), and poly (ϵ-caprolactone), or natural polymers such as chitosan, alginate, or gelatin.[221] The hydrophobic active principle is usually loaded in the core, or conjugated directly to the polymer chain. Polymer NPs ensure higher structural integrity and stability, longer shelf life, and sustained release of their cargo, as compared to liposomes or micelles.[222] Ding et al. synthetized Ce6-loaded chitosan NPs with enhanced phototoxicity against human lung carcinoma (A549 cells) with respect to free Ce6 in vitro (**Figure 6**).[223] More recently, Yang et al. proposed polymer NPs made of a new amphiphilic polymer, namely chondroitin sulfate-g-poly (propylene sulfide), to co-load paclitaxel (PTX), a common chemotherapeutic, and Ce6 with good drug-loading efficiencies (24.31% and 14.93%, respectively). These NPs showed a strong synergetic chemo-PDT effect in vitro on human breast adenocarcinoma (MCF-7 cells). Inhibition of tumor growth was observed also in vivo.[224]

Another type of chitosan-Ce6 nanoassembly (CS-Ce6) was prepared by covalently conjugating Ce6 with chitosan. It showed, both in vitro and in vivo, light-activated antimicrobial activity against Gram-positive methicillin-resistant *S. aureus* (MRSA) and Gram-negative *Acinetobacter baumannii* (**Figure 7**).[225]

Dendrimers are peculiar polymers characterized by multiple branches extending radially and symmetrically from a central core, composed of an atom or group of atoms. Branches usually terminate with functional groups that can be used for specific conjugations. The overall morphology of dendrimers is a globular shape. Their architecture can be easily tuned to obtain well-defined and monodisperse objects with desired features and, depending on their specific composition, they can carry both hydrophobic and hydrophilic drugs. Dendrimer synthesis is extremely versatile, and can be performed starting from either synthetic or natural polymers.[226] Poly(amido amine) (PAMAM) G4.5 dendrimers were covalently conjugated with Ce6, with a minimal impact on $^1O_2$ generation and fluorescence emission of the photosensitizers. PAMAM-anchored Ce6 showed a higher photodynamic effect as compared to free Ce6 in vitro on human pharynx squamous cell carcinoma.[227] In another work, PAMAM dendrimers conjugated to Ce6 were also shown to be potent nanophotosensitizers in vitro against human cervical cancer cells, inducing photoactivated apoptosis in a more effective way than Ce6 alone.[228]

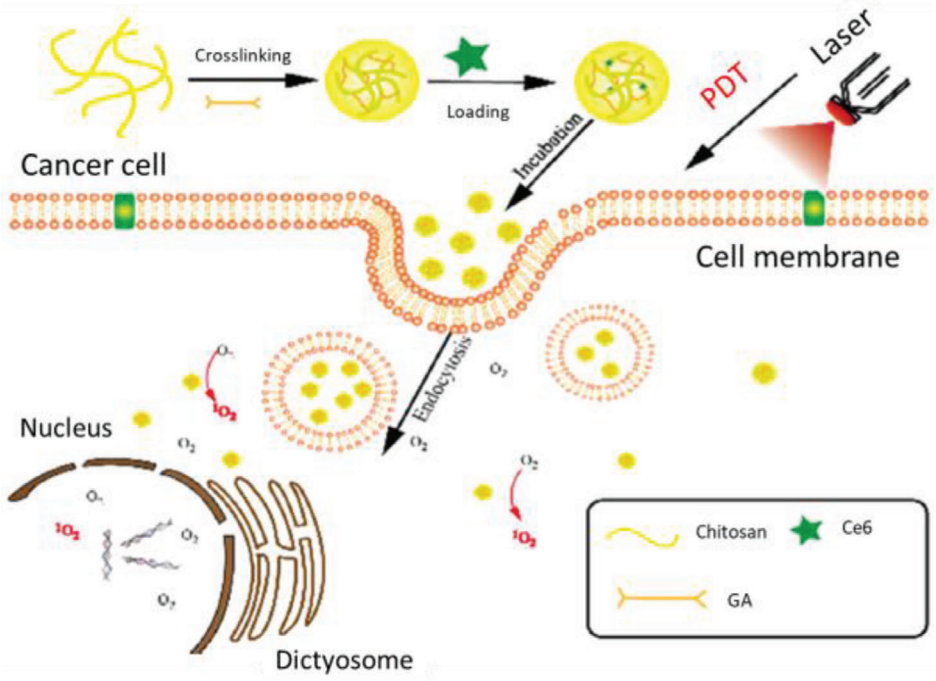


Figure 6. Scheme of Ce6-loaded chitosan NPs photodynamic action. Reproduced with permission.[223] Copyright 2018, American Chemical Society.

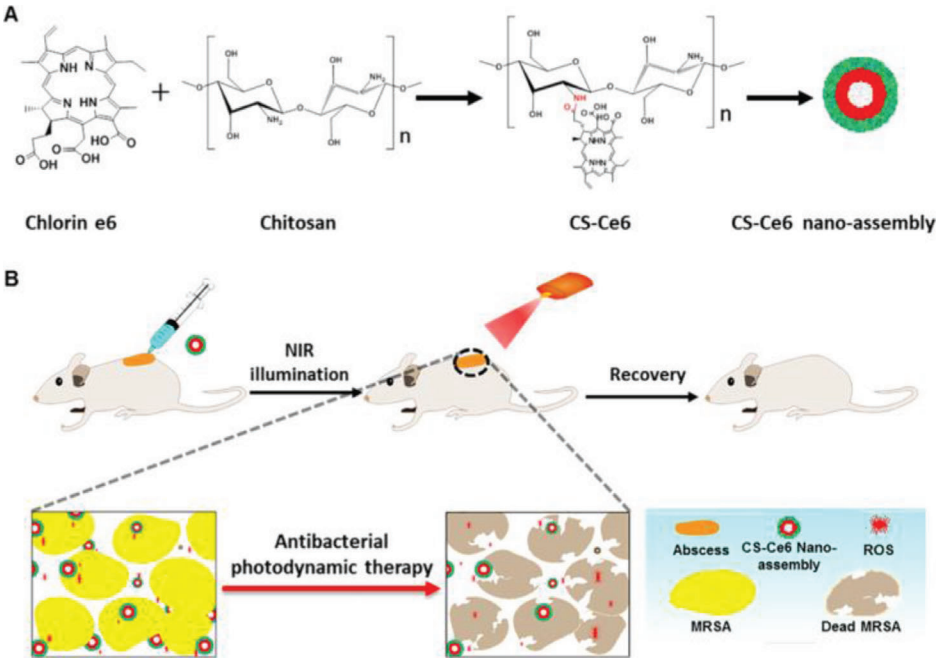


Figure 7. A) Schematic representation of CS-Ce6. B) Antimicrobial PDT protocol for the treatment of MRSA-infected subcutaneous abscess in vivo. Reproduced with permission.[225] Copyright 2019, American Chemical Society.

Solid lipid NPs (SLNs) are spherical aggregates composed of a mixture of lipids that are solid at room temperature. Usually, fatty acids, steroids, waxes, monoglycerides, diglycerides, triglycerides, and/or PEGylated lipids are used to form SLNs, while a small percentage of stabilizers or surfactants can be used during the preparation procedure to enhance the stability and reduce the final size of SLNs.[229] Hydrophobic molecules are encapsulated in the lipid core during SLNs fabrication. SLNs present several advantages with respect to other nanosystems, such as higher drug stability and prolonged release, a green preparation protocol that does not involve the use of toxic organic solvents, and stability in bodily fluids. A major drawback of SLNs is the high crystallinity of the lipid core that affects their drug-loading efficiency and release profile. To overcome these issues, a new

generation of lipid NPs, termed nanostructured lipid carriers (NLCs), has been proposed.[230] In NLCs, a small amount of a lipid that is liquid at room temperature (e.g., oleic acid) is included in the lipid mixture to give rise to amorphous or partially crystalline matrices, ensuring higher drug loading and improved release profiles.[231] SLNs loaded with *m*THPC for PDT against human breast cancer cells (MCF-7 cells) showed promising results in vitro, making SLNs good candidates for *m*THPC delivery in clinical applications.[232] NLCs co-loaded with PTX and Ce6 were also prepared and functionalized with folic acid to target human breast cancer. The combination of chemotherapeutics and PDT, together with the enhanced delivery and cellular uptake provided by the NPs, ensured a higher anticancer efficacy, both in vitro and in vivo.[233]

6. Conclusion

Chlorophyll derivatives are substances containing tetrapyrroles or tetrapyrrole-like structures derived from chlorophylls, or at least chemically resembling them. They are plentiful, and can be purified in cost-effective manners; in humans, these compounds are generally both well tolerated and effectively cleared. Per se, chlorophyll derivatives have applications as antioxidants, oncosuppressors, disinfectants, stimulators of the immune system, and promoters of wound healing. Some evidence supporting their potential use in cosmetics exists as well. With light, they cause a prompt and local overproduction of radicals. Because of that, as well as due to their safety and availability, they are widely used as photodynamic agents against cancer or other pathologies. In the future, they might be utilized as sight enhancers, especially to potentiate scotopic vision. Besides the traditional administration of chlorophyll derivatives as standalone chemicals, nanotechnological approaches have already demonstrated that delivery, safety, applicability, as well as diagnostic and/or therapeutic potential can be further refined. Indeed, thanks to their peculiar physicochemical properties, NPs have been exploited for encapsulating poorly soluble molecules with high efficiency, increasing their biocompatibility and bioavailability both in vitro and in vivo. Current research is focused on the design of inorganic and organic NPs as carriers of chlorophyll derivatives, and we expect some more clinical trials to start in the close future.

Acknowledgements

C.P., C.M., A.D., and A.D. contributed equally to this work.

Open Access Funding provided by Istituto Italiano di Tecnologia within the CRUI-CARE Agreement.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

chlorophyll derivatives, nanomedicine, photoactive agents, photodynamic therapy

Received: April 30, 2021
Revised: June 6, 2021
Published online: July 2, 2021

[1] G. P. Moss, *Pure Appl. Chem* **1987**, *59*, 779.
[2] A. R. Battersby, *Nat. Prod. Rep.* **2000**, *17*, 507.
[3] P. Ponka, *Am. J. Med. Sci.* **1999**, *318*, 241.
[4] T. Ott, J. T. van Dongen, C. Gu, L. Krusell, G. Desbrosses, H. Vigeolas, V. Bock, T. Czechowski, P. Geigenberger, M. K. Udvardi, *Curr. Biol.* **2005**, *15*, 531.
[5] M. Taniguchi, J. S. Lindsey, *Chem. Rev.* **2017**, *117*, 344.
[6] A. Tomitani, K. Okada, H. Miyashita, H. C. P. Matthijs, T. Ohno, A. Tanaka, *Nature* **1999**, *400*, 159.
[7] L. M. Ward, P. M. Shih, *PLoS One* **2021**, *16*, e0239248.
[8] B. Kräutler, *Biochem. Soc. Trans.* **2005**, *33*, 806.
[9] O. Shimomura, *J. Biolumin. Chemilumin.* **1995**, *10*, 91.
[10] R. Stocker, Y. Yamamoto, A. F. McDonagh, A. N. Glazer, B. N. Ames, *Science* **1987**, *235*, 1043.
[11] G. A. F. Hendry, in *Natural Food Colorants*, Springer, Berlin **1996**, pp. 131–156.
[12] M. Ethirajan, Y. Chen, P. Joshi, R. K. Pandey, *Chem. Soc. Rev.* **2011**, *40*, 340.
[13] I. Washington, J. Zhou, S. Jockusch, N. J. Turro, K. Nakanishi, J. R. Sparrow, *Photochem. Photobiol. Sci.* **2007**, *6*, 775.
[14] T. D. MacDonald, T. W. Liu, G. Zheng, *Angew. Chem., Int. Ed. Engl.* **2014**, *53*, 6956.
[15] K. O'Riordan, O. E. Akilov, T. Hasan, *Photodiagn. Photodyn. Ther.* **2005**, *2*, 247.
[16] M. L. Sigler, T. J. Stephens, *J. Drugs Dermatol.* **2015**, *14*, 401.
[17] X. Xue, A. Lindstrom, Y. Li, *Bioconjugate Chem.* **2019**, *30*, 1585.
[18] A. Perez-Galvez, I. Viera, M. Roca, *Curr. Med. Chem.* **2017**, *24*, 4515.
[19] M. Hayes, M. G. Ferruzzi, *Nutr. Res.* **2020**, *81*, 19.
[20] S. Zhong, A. Bird, R. E. Kopec, *Mol. Nutr. Food Res.* **2021**, *65*, 2000761.
[21] K. Solymosi, B. Mysliwa-Kurdziel, *Mini-Rev. Med. Chem* **2017**, *17*, 1194.
[22] V. Vohra, *Materials* **2018**, *11*, 2579.
[23] A. Azizullah, Z. U. Rehman, I. Ali, W. Murad, N. Muhammad, W. Ullah, D.-P. Häder, *Parasitol. Res.* **2014**, *113*, 4321.
[24] L. E. Xodo, V. Rapozzi, M. Zacchigna, S. Drioli, S. Zorzet, *Curr. Med. Chem.* **2012**, *19*, 799.
[25] J. Staron, B. Boron, D. Karcz, M. Szczygieł, L. Fiedor, *Curr. Med. Chem.* **2015**, *22*, 3054.
[26] M. L. Bruschi, J. B. da Silva, H. C. Rosseto, *Curr. Pharm. Des.* **2019**, *25*, 2279.
[27] L. Nogueira, A. T. Tracey, R. Alvim, P. Reisz, A. Scherz, J. A. Coleman, K. Kim, *Molecules* **2020**, *25*, 5417.
[28] M. A. Rajora, J. W. H. Lou, G. Zheng, *Chem. Soc. Rev.* **2017**, *46*, 6433.
[29] B. Mansoori, A. Mohammadi, M. Amin Doustvandi, F. Mohammadnejad, F. Kamari, M. F. Gjerstorff, B. Baradaran, M. R. Hamblin, *Photodiagn. Photodyn. Ther.* **2019**, *26*, 395.
[30] M. O. Senge, A. Wiehe, C. Ryppa, in *Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications* (Eds: B. Grimm, R.J. Porra, W. Rüdiger, H. Scheer), Springer Netherlands, Dordrecht **2006**, pp. 27–37.
[31] H. Scheer, *Encyclopedia of Biological Chemistry*, 2nd ed., Academic Press Inc., Waltham **2013**.
[32] Q. Cai, Y. Fei, H.-W. An, X.-X. Zhao, Y. Ma, Y. Cong, L. Hu, L.-L. Li, H. Wang, *ACS Appl. Mater. Interfaces* **2018**, *10*, 9197.
[33] A. Fercher, S. M. Borisov, A. V. Zhdanov, I. Klimant, D. B. Papkovsky, *ACS Nano* **2011**, *5*, 5499.
[34] H. Gong, Z. Dong, Y. Liu, S. Yin, L. Cheng, W. Xi, J. Xiang, K. Liu, Y. Li, Z. Liu, *Adv. Funct. Mater.* **2014**, *24*, 6492.
[35] J. Shi, T. W. B. Liu, J. Chen, D. Green, D. Jaffray, B. C. Wilson, F. Wang, G. Zheng, *Theranostics* **2011**, *1*, 363.
[36] P. Wang, B. Grimm, *Photosynth. Res.* **2015**, *126*, 189.

[37] H. K. Lichtenthaler, C. Buschmann, M. Döll, H.-J. Fietz, T. Bach, U. Kozel, D. Meier, U. Rahmsdorf, *Photosynth. Res.* **1981**, *2*, 115.
[38] *Chlorophyll* (Eds: E. Jacob-Lopes, L. Q. Zepka, M. I. Queiroz), IntechOpen, London **2017**.
[39] R. C. Dougherty, H. H. Strain, W. A. Svec, R. A. Uphaus, J. J. Katz, *J. Am. Chem. Soc.* **1970**, *92*, 2826.
[40] M. Chen, M. Schliep, R. D. Willows, Z.-L. Cai, B. A. Neilan, H. Scheer, *Science* **2010**, *329*, 1318.
[41] B. Schoefs, in *Handbook of Analysis of Active Compounds in Functional Foods* (Eds: L. Nollet, F. Toldrá), CRC Press, Boca Raton **2012**, pp. 665–685.
[42] B. Kuai, J. Chen, S. Hörtensteiner, *J. Exp. Bot.* **2018**, *69*, 751.
[43] T. Azoulay Shemer, S. Harpaz-Saad, E. Belausov, N. Lovat, O. Krokhin, V. Spicer, K. G. Standing, E. E. Goldschmidt, Y. Eyal, *Plant Physiol.* **2008**, *148*, 108 LP.
[44] N. Schenk, S. Schelbert, M. Kanwischer, E. E. Goldschmidt, P. Dörmann, S. Hörtensteiner, *FEBS Lett.* **2007**, *581*, 5517.
[45] S. Schelbert, S. Aubry, B. Burla, B. Agne, F. Kessler, K. Krupinska, S. Hörtensteiner, *Plant Cell* **2009**, *21*, 767.
[46] Y. Shimoda, H. Ito, A. Tanaka, *Plant Cell* **2016**, *28*, 2147.
[47] B. Kräutler, S. Hörtensteiner, in *Handbook of Porphyrin Science* (Eds.: G. C. Ferreira, K. M. Kadish, K. M. Smith, R. Guillard), World Scientific Publishing, Singapore **2013**, pp. 117–185.
[48] M. Hauenstein, B. Christ, A. Das, S. Aubry, S. Hörtensteiner, *Plant Cell* **2016**, *28*, 2510.
[49] M. Oberhuber, J. Berghold, K. Breuker, S. Hörtensteiner, B. Kräutler, *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 6910 LP.
[50] B. Christ, S. Schelbert, S. Aubry, I. Süssenbacher, T. Müller, B. Kräutler, S. Hörtensteiner, *Plant Physiol.* **2012**, *158*, 628.
[51] S. J. Schwartz, T. V. Lorenzo, *Crit. Rev. Food Sci. Nutr.* **1990**, *29*, 1.
[52] L. M. Moreira, A. Lima, R. R. S. Soares, V. R. Batistela, A. P. Gerola, N. Hioka, J. A. Bonacin, D. Severino, M. S. Baptista, A. E. da H. Machado, M. R. Rodrigues, L. Codognoto, H. P. M. de Oliveira, *J. Braz. Chem. Soc.* **2009**, *20*, 1653.
[53] A. Mortensen, A. Geppel, *Innovative Food Sci. Emerging Technol.* **2007**, *8*, 419.
[54] R. E. Nelson, M. G. Ferruzzi, *J. Food Sci.* **2008**, *73*, H86.
[55] Y. Nonomura, M. Yamaguchi, T. Hara, K. Furuya, N. Yoshioka, H. Inoue, *J. Chromatogr. A* **1996**, *721*, 350.
[56] J. Gross, *Pigments in Vegetables. Chlorophylls and Carotenoids*, Van Nostrand Reinhold, New York **1991**.
[57] C. Sánchez, A. B. Baranda, I. Martínez de Marañón, *Food Chem.* **2014**, *163*, 37.
[58] J. L. Roberts, R. Moreau, *Food Funct.* **2016**, *7*, 3337.
[59] R. Delgado-Pelayo, L. Gallardo-Guerrero, D. Hornero-Méndez, *Food Res. Int.* **2014**, *65*, 272.
[60] K. Chen, J. J. Ríos, A. Pérez-Gálvez, M. Roca, *Food Chem.* **2017**, *228*, 625.
[61] A. L. İnanç, *Akad. Gida* **2011**, *9*, 26.
[62] M. Szczygieł, K. Urbańska, P. Jurecka, I. Stawoska, G. Stochel, L. Fiedor, *J. Med. Chem.* **2008**, *51*, 4412.
[63] M. G. Ferruzzi, J. Blakeslee, *Nutr. Res.* **2007**, *27*, 1.
[64] J. T. Brugsch, A. Keys, *Exp. Biol. Med.* **1938**, *38*, 557.
[65] M. G. Ferruzzi, M. L. Failla, S. J. Schwartz, *J. Agric. Food Chem.* **2001**, *49*, 2082.
[66] C.-Y. Hsu, T.-H. Yeh, M.-Y. Huang, S.-P. Hu, P.-Y. Chao, C.-M. Yang, *Indian J. Biochem. Biophys.* **2014**, *51*, 388.
[67] P. Y. Chao, M. Y. Huang, W. D. Huang, K.-H. H. Lin, S. Y. Chen, C. M. Yang, *Not. Bot. Horti Agrobot. Cluj-Napoca* **2018**, *46*, 309.
[68] K. Chen, M. Roca, *J. Funct. Foods* **2018**, *41*, 25.
[69] B. Gandul-Rojas, L. Gallardo-Guerrero, M. I. Mínguez-Mosquera, *J. Agric. Food Chem.* **2009**, *57*, 5306.
[70] L. Gallardo-Guerrero, B. Gandul-Rojas, M. I. Mínguez-Mosquera, *J. Agric. Food Chem.* **2008**, *56*, 8379.
[71] R. W. Robey, K. Steadman, O. Polgar, K. Morisaki, M. Blayney, P. Mistry, S. E. Bates, *Cancer Res.* **2004**, *64*, 1242.
[72] J. W. Jonker, M. Buitelaar, E. Wagenaar, M. A. Van Der Valk, G. L. Scheffer, R. J. Scheper, T. Plosch, F. Kuipers, R. P. J. O. Elferink, H. Rosing, J. H. Beijnen, A. H. Schinkel, *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 15649.
[73] I. Viera, K. Chen, J. J. Ríos, I. Benito, A. Pérez-Gálvez, M. Roca, *Mol. Nutr. Food Res.* **2018**, *62*, 1800562.
[74] S. Chernomorsky, A. Segelman, R. D. Poretz, *Teratog., Carcinog., Mutagen.* **1999**, *19*, 313.
[75] J. W. E. Harrisson, S. E. Levin, B. Trabin, *J. Am. Pharm. Assoc. (Scientific ed.)* **1954**, *43*, 722.
[76] H. J. Henderson, E. R. Long, *Exp. Biol. Med.* **1941**, *48*, 438.
[77] M. G. Ferruzzi, M. L. Failla, S. J. Schwartz, *J. Agric. Food Chem.* **2002**, *50*, 2173.
[78] B. B. Gomes, S. B. M. Barros, E. R. S. Andrade-Wartha, A. M. O. Silva, V. V. Silva, U. M. Lanfer-Marquez, *J. Sci. Food Agric.* **2009**, *89*, 2003.
[79] P. A. Egner, K. H. Stansbury, E. P. Snyder, M. E. Rogers, P. A. Hintz, T. W. Kensler, *Chem. Res. Toxicol.* **2000**, *13*, 900.
[80] J. Dandler, B. Wilhelm, H. Scheer, *Photochem. Photobiol.* **2010**, *86*, 182.
[81] Y. Ranneh, F. Ali, A. M. Akim, H. A. Hamid, H. Khazaai, A. Fadel, *Appl. Biol. Chem.* **2017**, *60*, 327.
[82] P. P. Zandi, J. C. Anthony, A. S. Khachaturian, S. V. Stone, D. Gustafson, J. A. T. Tschanz, M. C. Norton, K. A. Welsh-Bohmer, J. C. S. Breitner, *Arch. Neurol.* **2004**, *61*, 82.
[83] Y. Liu, J. Shi, *Nano Today* **2019**, *27*, 146.
[84] V. G. Antico Arciuch, M. E. Elguero, J. J. Poderoso, M. C. Carreras, *Antioxid. Redox Signaling* **2012**, *16*, 1150.
[85] S. S. Kumar, T. P. Devasagayam, B. Bhushan, N. C. Verma, *Free Radic. Res.* **2001**, *35*, 563.
[86] Y. Nakamura, A. Murakami, K. Koshimizu, H. Ohigashi, *Cancer Lett.* **1996**, *108*, 247.
[87] J. P. Kamat, K. K. Boloor, T. P. Devasagayam, *Biochim. Biophys. Acta* **2000**, *1487*, 113.
[88] K. K. Boloor, J. P. Kamat, T. P. Devasagayam, *Toxicology* **2000**, *155*, 63.
[89] U. M. Lanfer-Marquez, R. M. C. Barros, P. Sinnecker, *Food Res. Int.* **2005**, *38*, 885.
[90] S. S. Kumar, B. Shankar, K. B. Sainis, *Biochim. Biophys. Acta – Gen. Subj.* **2004**, *1672*, 100.
[91] M. G. Ferruzzi, V. Böhm, P. D. Courtney, S. J. Schwartz, *J. Food Sci.* **2002**, *67*, 2589.
[92] Y.-R. Kang, J. Park, S. K. Jung, Y. H. Chang, *Food Chem.* **2018**, *245*, 943.
[93] R. Koníčková, K. Vaňková, J. Vaníková, K. Vánová, L. Muchová, I. Subhanová, M. Zadinová, J. Zelenka, A. Dvořák, M. Kolář, H. Strnad, S. Rimpelová, T. Ruml, R. J. Wong, L. Vítek, *Ann. Hepatol.* **2014**, *13*, 273.
[94] C. Hoshina, K. Tomita, Y. Shioi, in *Photosynthesis: Mechanisms and Effects* (Ed: G. Garab), Springer, Berlin **1998**, pp. 3281–3284.
[95] K. Vaňková, I. Marková, J. Jašprová, A. Dvořák, I. Subhanová, J. Zelenka, I. Novosádová, J. Rasl, T. Vomastek, R. Sobotka, L. Muchová, L. Vítek, *Oxid. Med. Cell. Longevity* **2018**, *2018*, 4069167.
[96] U.-J. Yang, T.-S. Park, S.-M. Shim, *J. Toxicol. Environ. Health, Part A* **2013**, *76*, 1307.
[97] C. Hsu, P. Chao, S. Hu, C. Yang, *Food Sci. Nutr.* **2013**, *4*, 1.
[98] J.-W. Yu, R. Yang, Y.-S. Kim, *Free Radic. Res.* **2010**, *44*, 655.
[99] Y. Zhang, L. Guan, X. Wang, T. Wen, J. Xing, J. Zhao, *Free Radic. Res.* **2008**, *42*, 362.
[100] J. W. Fahey, K. K. Stephenson, A. T. Dinkova-Kostova, P. A. Egner, T. W. Kensler, P. Talalay, *Carcinogenesis* **2005**, *26*, 1247.
[101] S. K. Abraham, L. Sarma, P. C. Kesavan, *Mutat. Res.* **1994**, *322*, 209.

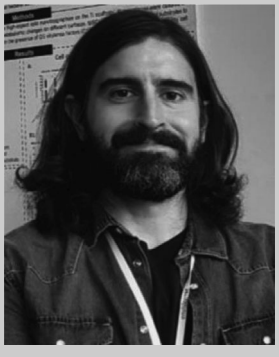
[102] K. K. Park, J. H. Park, Y. J. Jung, W. Y. Chung, *Mutat. Res.* **2003**, *542*, 89.
[103] Y. Okai, K. Higashi-Okai, *Int. J. Immunopharmacol.* **1997**, *19*, 355.
[104] E. Wang, M. Wink, *PeerJ.* **2016**, *4*, e1879.
[105] T. J. McQuistan, M. T. Simonich, M. M. Pratt, C. B. Pereira, J. D. Hendricks, R. H. Dashwood, D. E. Williams, G. S. Bailey, *Food Chem. Toxicol.* **2012**, *50*, 341.
[106] T. Negishi, H. Rai, H. Hayatsu, *Mutat. Res.* **1997**, *376*, 97.
[107] E. Demir, B. Kaya, S. K. Cenkci, *Ekoloji* **2013**, *22*, 36.
[108] C. Jubert, J. Mata, G. Bench, R. Dashwood, C. Pereira, W. Tracewell, K. Turteltaub, D. Williams, G. Bailey, *Cancer Prev. Res. (Phila).* **2009**, *2*, 1015.
[109] P. A. Egner, J. B. Wang, Y. R. Zhu, B. C. Zhang, Y. Wu, Q. N. Zhang, G. S. Qian, S. Y. Kuang, S. J. Gange, L. P. Jacobson, K. J. Helzlsouer, G. S. Bailey, J. D. Groopman, T. W. Kensler, *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 14601.
[110] V. Breinholt, D. Arbogast, P. Loveland, C. Pereira, R. Dashwood, J. Hendricks, G. Bailey, *Toxicol. Appl. Pharmacol.* **1999**, *158*, 141.
[111] T. Hayashi, M. Schimerlik, G. Bailey, *Toxicol. Appl. Pharmacol.* **1999**, *158*, 132.
[112] C.-Y. Hsu, Y.-H. Chen, P.-Y. Chao, C.-M. Chen, L.-L. Hsieh, S.-P. Hu, *Mutat. Res.* **2008**, *657*, 98.
[113] J. E. Mata, Z. Yu, J. E. Gray, D. E. Williams, R. Rodriguez-Proteau, *Toxicology* **2004**, *196*, 117.
[114] R. H. Dashwood, *Carcinogenesis* **1992**, *13*, 113.
[115] K. K. Park, Y. J. Surh, *Cancer Lett.* **1996**, *102*, 143.
[116] D. J. Castro, C. V. Löhr, K. A. Fischer, K. M. Waters, B.-J. M. Webb-Robertson, R. H. Dashwood, G. S. Bailey, D. E. Williams, *Carcinogenesis* **2009**, *30*, 315.
[117] C. H. Yun, H. G. Jeong, J. W. Jhoun, F. P. Guengerich, *Carcinogenesis* **1995**, *16*, 1437.
[118] K. Imai, T. Aimoto, M. Sato, K. Watanabe, R. Kimura, T. Murata, *Chem. Pharm. Bull.* **1986**, *34*, 4287.
[119] C. A. Blum, M. Xu, G. A. Orner, G. Darío Díaz, Q. Li, W. M. Dashwood, G. S. Bailey, R. H. Dashwood, *Mutat. Res.* **2003**, *523*, 217.
[120] R. L. Nelson, *Anticancer Res.* **1992**, *12*, 737.
[121] L. Romert, M. Curvall, D. Jenssen, *Mutagenesis* **1992**, *7*, 349.
[122] M. P. Cruces, E. Pimentel, S. Zimmering, *Mutat. Res.* **2003**, *536*, 139.
[123] K. Chimploy, G. D. Díaz, Q. Li, O. Carter, W.-M. Dashwood, C. K. Mathews, D. E. Williams, G. S. Bailey, R. H. Dashwood, *Int. J. Cancer* **2009**, *125*, 2086.
[124] L. C.-M. Chiu, C. K.-L. Kong, V. E. C. Ooi, *Int. J. Oncol.* **2003**, *23*, 729.
[125] L. C.-M. Chiu, C. K.-L. Kong, V. E.-C. Ooi, *Int. J. Mol. Med.* **2005**, *16*, 735.
[126] P. Thiyagarajan, R. Senthil Murugan, K. Kavitha, P. Anitha, D. Prathiba, S. Nagini, *Food Chem. Toxicol.* **2012**, *50*, 867.
[127] S. Sakdarat, A. Shuyprom, C. Pientong, T. Ekalaksananan, S. Thongchai, *Bioorg. Med. Chem.* **2009**, *17*, 1857.
[128] S.-Y. Wang, C.-P. Tseng, K.-C. Tsai, C.-F. Lin, C.-Y. Wen, H.-S. Tsay, N. Sakamoto, C.-H. Tseng, J.-C. Cheng, *Biochem. Biophys. Res. Commun.* **2009**, *385*, 230.
[129] L. W. Smith, A. E. Livingston, *Am. J. Surg.* **1945**, *67*, 30.
[130] L. W. Smith, A. E. Livingston, *Am. J. Surg.* **1943**, *62*, 358.
[131] J. B. Cady, W. S. Morgan, *Am. J. Surg.* **1948**, *75*, 562.
[132] B. J. Edwards, *Physiotherapy* **1954**, *40*, 177.
[133] D. C. Larato, F. R. Pfau, *N. Y. State Dent. J.* **1970**, *36*, 291.
[134] S. L. Goldberg, *Am. J. Surg.* **1943**, *62*, 117.
[135] D. B. Vasily, *J. Drugs Dermatol.* **2015**, *14*, 1157.
[136] J. P. McCook, P. L. Dorogi, D. B. Vasily, D. R. Cefalo, *Clin., Cosmet. Invest. Dermatol.* **2015**, *8*, 443.
[137] D. Sharma, S. S. Kumar, K. B. Sainis, *Mol. Immunol.* **2007**, *44*, 347.
[138] C.-H. Yun, Y. J. Jeon, Y. Yang, H. R. Ju, S. H. Han, *Int. Immunopharmacol.* **2006**, *6*, 252.
[139] N.-H. Bui-Xuan, P. M.-K. Tang, C.-K. Wong, J. Y.-W. Chan, K. K. Y. Cheung, J. L. Jiang, K.-P. Fung, *Cell. Immunol.* **2011**, *269*, 60.
[140] M. N. Islam, I. J. Ishita, S. E. Jin, R. J. Choi, C. M. Lee, Y. S. Kim, H. A. Jung, J. S. Choi, *Food Chem. Toxicol.* **2013**, *55*, 541.
[141] A. W. U. Busch, B. L. Montgomery, *Redox Biol.* **2015**, *4*, 260.
[142] J. Q. Cai, X. M. Liu, Z. J. Gao, L. L. Li, H. Wang, *Mater. Today* **2021**, *45*, 77.
[143] R. L. Yanovsky, D. W. Bartenstein, G. S. Rogers, S. J. Isakoff, S. T. Chen, *Photodermatol., Photoimmunol. Photomed.* **2019**, *35*, 295.
[144] R. Wormald, J. Evans, L. Smeeth, K. S. Henshaw, *Cochrane Database Syst. Rev.* **2007**, *pub3*, CD002030.
[145] L. B. Josefsen, R. W. Boyle, *Met.-Based. Drugs* **2008**, *2008*, 276109.
[146] B. Mansoori, A. Mohammadi, M. Amin Doustvandi, F. Mohammadnejad, F. Kamari, M. F. Gjerstorff, B. Baradaran, M. R. Hamblin, *Photodiagn. Photodyn. Ther.* **2019**, *26*, 395.
[147] A. S. Brandis, Y. Salomon, A. Scherz, in *Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications*, Springer, Dordrecht **2007**, pp. 461–483.
[148] A. A. Krasnovsky, K. V. Neverov, S. Y. Egorov, B. Roeder, T. Levald, *J. Photochem. Photobiol., B* **1990**, *5*, 245.
[149] L. Y. Liu, X. X. Man, H. X. Yao, Y. Y. Tan, *Eur. Rev. Med. Pharmacol. Sci.* **2017**, *21*, 5571.
[150] L. L. Bao, J. Bian, Y. J. Yan, L. J. Zhang, D. F. O'Shea, Z. L. Chen, *Biomed. Pharmacother.* **2017**, *88*, 1220.
[151] J. K. Hoober, T. W. Sery, N. Yamamoto, *Photochem. Photobiol.* **1988**, *48*, 579.
[152] E. Zenkevich, E. Sagun, V. Knyukshto, A. Shulga, A. Mironov, O. Efremova, R. Bonnett, S. P. Songca, M. Kassem, *J. Photochem. Photobiol., B* **1996**, *33*, 171.
[153] P. Huang, B. Zhang, Q. Yuan, X. Zhang, W. Leung, C. Xu, *Lasers Med. Sci.* **2021**, *36*, 339.
[154] S. H. Lim, P. Nowak-Sliwinska, F. A. Kamarulzaman, H. Van Den Bergh, G. Wagnières, H. B. Lee, *Photochem. Photobiol.* **2010**, *86*, 397.
[155] J. M. Fernandez, M. D. Bilgin, L. I. Grossweiner, *J. Photochem. Photobiol., B* **1997**, *37*, 131.
[156] A. Juzeniene, *Photodiagn. Photodyn. Ther.* **2009**, *6*, 94.
[157] E. S. Nyman, P. H. Hynninen, *J. Photochem. Photobiol., B* **2004**, *73*, 1.
[158] European Medicines Agency—Foscan, https://www.ema.europa.eu/en/medicines/human/EPAR/foscan#authorisation-details-section (accessed: April **2021**).
[159] K. J. Lorenz, H. Maier, *Eur. Arch. Oto-Rhino-Laryngol.* **2009**, *266*, 1937.
[160] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2020**, *83*, 770.
[161] Mono-L-aspartyl chlorin e6|Cancer—List Results—ClinicalTrials.gov, https://www.clinicaltrials.gov/ct2/results?cond=Cancer&term=mono-L-aspartyl+chlorin+e6+&cntry=&state=&city=&dist=&Search=Search (accessed: April **2021**).
[162] J. Usuda, H. Kato, T. Okunaka, K. Furukawa, H. Honda, Y. Suga, T. Hirata, T. Ohira, M. Tsuboi, T. Hirano, *J. Clin. Oncol.* **2006**, *24*, 7229.
[163] A. Kawczyk-Krupka, A. M. Bugaj, M. Potempa, K. Wasilewska, W. Latos, A. Sieroń, *Photodiagn. Photodyn. Ther.* **2015**, *12*, 161.
[164] Y. M. Jeon, H. S. Lee, D. Jeong, H. K. Oh, K. H. Ra, M. Y. Lee, *Life Sci.* **2015**, *124*, 56.
[165] M. P. Uliana, L. Pires, S. Pratavieira, T. J. Brocksom, K. T. De Oliveira, V. S. Bagnato, C. Kurachi, *Photochem. Photobiol. Sci.* **2014**, *13*, 1137.
[166] L. Huang, M. Wang, Y. Y. Huang, A. El-Hussein, L. M. Wolf, L. Y. Chiang, M. R. Hamblin, *Photochem. Photobiol. Sci.* **2018**, *17*, 638.
[167] P. Diogo, M. Mota, C. Fernandes, D. Sequeira, P. Palma, F. Caramelo, M. G. P. M. S. Neves, M. A. F. Faustino, T. Gonçalves, J. M. Santos, *Photodiagn. Photodyn. Ther.* **2018**, *22*, 205.
[168] A. S. Brandis, Y. Salomon, A. Scherz, in *Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications*, Springer, Dordrecht **2007**, pp. 485–494.

[169] Tookad |. E. M. Agency, https://www.ema.europa.eu/en/medicines/human/EPAR/tookad (accessed: May **2021**).
[170] Search of: tookad—List Results—ClinicalTrials.gov, https://clinicaltrials.gov/ct2/results?cond=&term=tookad&cntry=&state=&city=&dist= (accessed: May **2021**).
[171] EudraCT, Clinical Trials register—Search for Tookad, https://www.clinicaltrialsregister.eu/ctr-search/search?query=tookad (accessed: May **2021**).
[172] H. Gattuso, A. Monari, M. Marazzi, *RSC Adv.* **2017**, *7*, 10992.
[173] C. Wang, H. Tao, L. Cheng, Z. Liu, *Biomaterials* **2011**, *32*, 6145.
[174] A. V. Kachynski, A. Pliss, A. N. Kuzmin, T. Y. Ohulchanskyy, A. Baev, J. Qu, P. N. Prasad, *Nat. Photonics* **2014**, *8*, 455.
[175] H. Abrahamse, M. R. Hamblin, *Biochem. J.* **2016**, *473*, 347.
[176] G. P. Tegos, M. R. Hamblin, *Antimicrob. Agents Chemother.* **2006**, *50*, 196.
[177] H. H. Jajarm, F. Falaki, M. Sanatkhani, M. Ahmadzadeh, F. Ahrari, H. Shafaee, *Lasers Med. Sci.* **2015**, *30*, 1475.
[178] G. Amescua, A. Arboleda, N. Nikpoor, H. Durkee, N. Relhan, M. C. Aguilar, H. W. Flynn Jr., D. Miller, J.-M. Parel, *Cornea* **2017**, *36*, 1141.
[179] A. Naranjo, A. Arboleda, J. D. Martinez, H. Durkee, M. C. Aguilar, N. Relhan, N. Nikpoor, A. Galor, S. R. Dubovy, R. Leblanc, *Am. J. Ophthalmol.* **2019**, *208*, 387.
[180] D. Ramaiah, I. Eckert, K. T. Arun, L. Weidenfeller, B. Epe, *Photochem. Photobiol.* **2002**, *76*, 672.
[181] S. Monro, K. L. Colón, H. Yin, J. Roque, III, P. Konda, S. Gujar, R. P. Thummel, L. Lilge, C. G. Cameron, S. A. McFarland, *Chem. Rev.* **2018**, *119*, 797.
[182] L. K. McKenzie, H. E. Bryant, J. A. Weinstein, *Coord. Chem. Rev.* **2019**, *379*, 2.
[183] C. Imberti, P. Zhang, H. Huang, P. J. Sadler, *Angew. Chem.* **2020**, *132*, 61.
[184] R. H. Douglas, J. C. Partridge, K. Dulai, D. Hunt, C. W. Mullineaux, A. Y. Tauber, P. H. Hynninen, *Nature* **1998**, *393*, 423.
[185] R. H. Douglas, J. C. Partridge, N. J. Marshall, *Prog. Retinal Eye Res.* **1998**, *17*, 597.
[186] R. H. Douglas, J. C. Partridge, K. S. Dulai, D. M. Hunt, C. W. Mullineaux, P. H. Hynninen, *Vision Res.* **1999**, *39*, 2817.
[187] R. H. Douglas, C. W. Mullineaux, J. C. Partridge, *Philos. Trans. R. Soc., B* **2000**, *355*, 1269.
[188] R. H. Douglas, M. J. Genner, A. G. Hudson, J. C. Partridge, H.-J. Wagner, *Sci. Rep.* **2016**, *6*, 39395.
[189] I. Washington, C. Brooks, N. J. Turro, K. Nakanishi, *J. Am. Chem. Soc.* **2004**, *126*, 9892.
[190] T. Nakashizuka, K. Mori, N. Hayashi, K. Anzail, K. Kanail, S. Yoneya, D. M. Moshfeghi, G. A. Peyman, *Retina* **2001**, *21*, 493.
[191] T. Isayama, D. Alexeev, C. L. Makino, I. Washington, K. Nakanishi, N. J. Turro, *Nature* **2006**, *443*, 649.
[192] A. Degl'Innocenti, L. Rossi, A. Salvetti, A. Marino, G. Meloni, B. Mazzolai, G. Ciofani, *Sci. Rep.* **2017**, *7*, 3374.
[193] D. Arendt, *Int. J. Dev. Biol.* **2003**, *47*, 563.
[194] F. Balem, N. Yanamala, J. Klein-Seetharaman, *Photochem. Photobiol.* **2009**, *85*, 471.
[195] N. Yanamala, J. Klein-Seetharaman, *Pharmaceuticals* **2010**, *3*, 3324.
[196] K. N. Woods, J. Pfeffer, J. Klein-Seetharaman, *Front. Mol. Biosci.* **2017**, *4*, 85.
[197] M. Marazzi, H. Gattuso, A. Giussani, H. Zhang, M. Navarrete-Miguel, C. Chipot, W. Cai, D. Roca-Sanjuán, F. Dehez, A. Monari, *J. Phys. Chem. Lett.* **2019**, *10*, 7133.
[198] J. Mitchell, N. Yanamala, Y. L. Tan, E. E. Gardner, K. C. Tirupula, F. Balem, M. Sheves, D. Nietlispach, J. Klein-Seetharaman, *Photochem. Photobiol.* **2019**, *95*, 787.
[199] C. Kurachi, V. S. Bagnato, *Opt. Photonics News* **2016**, *27*, 32.
[200] R. Narikawa, K. Fushimi, M. Ikeuchi, *Biochem. Biophys. Res. Commun.* **2015**, *461*, 390.
[201] R. Narikawa, T. Nakajima, Y. Aono, K. Fushimi, G. Enomoto, S. Itoh, M. Sato, M. Ikeuchi, *Sci. Rep.* **2015**, *5*, 7950.
[202] Y. Uda, Y. Goto, S. Oda, T. Kohchi, M. Matsuda, K. Aoki, *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 11962.
[203] C. Martinelli, C. Pucci, G. Ciofani, *APL Bioeng.* **2019**, *3*, 011502.
[204] C. Pucci, C. Martinelli, G. Ciofani, *ecancer* **2019**, *13*, 961.
[205] A. Qidwai, Annu, B. N., S. Kotta, J. K. Narang, S. Baboota, J. Ali, *Photodiagn. Photodyn. Ther.* **2020**, *30*, 101782.
[206] C. Bharti, N. Gulati, U. Nagaich, A. Pal, *Int. J. Pharm. Invest.* **2015**, *5*, 124.
[207] I. Mitra, N. Manna, J. S. Manna, M. K. Mitra, *Procedia Mater. Sci.* **2014**, *6*, 770.
[208] W. Zhang, J. Shen, H. Su, G. Mu, J. H. Sun, C. P. Tan, X. J. Liang, L. N. Ji, Z. W. Mao, *ACS Appl. Mater. Interfaces* **2016**, *8*, 13332.
[209] X. Wu, C.-M. Dong, in *Photonanotechnology for Therapeutics and Imaging*, Elsevier, Amsterdam **2020**, pp. 83–104.
[210] C. C. Chuang, Y. N. Chen, Y. Y. Wang, Y. C. Huang, S. Y. Lin, R. Y. Huang, Y. Y. Jang, C. C. Yang, Y. F. Huang, C. W. Chang, *ACS Appl. Mater. Interfaces* **2020**, *12*, 30021.
[211] A. Hervault, N. T. K. Thanh, *Nanoscale* **2014**, *6*, 11553.
[212] C. Tapeinos, A. Marino, M. Battaglini, S. Migliorin, R. Brescia, A. Scarpellini, C. De Julián Fernández, M. Prato, F. Drago, G. Ciofani, *Nanoscale* **2019**, *11*, 72.
[213] C. Pucci, D. De Pasquale, A. Marino, C. Martinelli, S. Lauciello, G. Ciofani, *ACS Appl. Mater. Interfaces* **2020**, *12*, 29037.
[214] A. Amirshaghaghi, L. Yan, J. Miller, Y. Daniel, J. M. Stein, T. M. Busch, Z. Cheng, A. Tsourkas, *Sci. Rep.* **2019**, *9*, 2613.
[215] M. Gulati, M. Grover, S. Singh, M. Singh, *Int. J. Pharm.* **1998**, *165*, 129.
[216] M. L. Immordino, F. Dosio, L. Cattel, *Int. J. Nanomed.* **2006**, *1*, 297.
[217] Y. Barenholz, *J. Controlled Release* **2012**, *160*, 117.
[218] Y. T. Yang, H. F. Chien, P. H. Chang, Y. C. Chen, M. Jay, T. Tsai, C. T. Chen, *Lasers Surg. Med.* **2013**, *45*, 175.
[219] H. Zhou, L. Xia, J. Zhong, S. Xiong, X. Yi, L. Chen, R. Zhu, Q. Shi, K. Yang, *Nanoscale* **2019**, *11*, 19823.
[220] N. Nishimura, S. Nakayama, A. Horiuchi, M. Kumoda, T. Miyatake, *Langmuir* **2019**, *35*, 7242.
[221] A. Nasir, A. Kausar, A. Younus, *Polym.-Plast. Technol. Eng.* **2015**, *54*, 325.
[222] C. Martinelli, C. Pucci, M. Battaglini, A. Marino, G. Ciofani, *Adv. Healthcare Mater.* **2020**, *9*, 1901589.
[223] Y. F. Ding, S. Li, L. Liang, Q. Huang, L. Yuwen, W. Yang, R. Wang, L. H. Wang, *ACS Appl. Mater. Interfaces* **2018**, *10*, 9980.
[224] X. Yang, X. Shi, Y. Zhang, J. Xu, J. Ji, L. Ye, F. Yi, G. Zhai, *J. Controlled Release* **2020**, *323*, 333.
[225] R. Zhang, Y. Li, M. Zhou, C. Wang, P. Feng, W. Miao, H. Huang, *ACS Appl. Mater. Interfaces* **2019**, *11*, 26711.
[226] P. Kesharwani, K. Jain, N. K. Jain, *Prog. Polym. Sci.* **2014**, *39*, 268.
[227] E. Bastien, R. Schneider, S. Hackbarth, D. Dumas, J. Jasniewski, B. Röder, L. Bezdetnaya, H.-P. Lassalle, *Photochem. Photobiol. Sci.* **2015**, *14*, 2203.
[228] S. R. Lee, Y. J. Kim, *Nanomaterials* **2018**, *8*, 445.
[229] W. Mehnert, K. Mäder, *Adv. Drug Delivery Rev.* **2012**, *64*, 83.
[230] I. Chauhan, M. Yasir, M. Verma, A. P. Singh, *Adv. Pharm. Bull.* **2020**, *10*, 150.
[231] F. Tamjidi, M. Shahedi, J. Varshosaz, A. Nasirpour, *Innovative Food Sci. Emerging Technol.* **2013**, *19*, 29.
[232] F. P. Navarro, G. Creusat, C. Frochot, A. Moussaron, M. Verhille, R. Vanderesse, J. S. Thomann, P. Boisseau, I. Texier, A. C. Couffin, M. Barberi-Heyob, *J. Photochem. Photobiol., B* **2014**, *130*, 161.
[233] Q. Zhang, J. Zhao, H. Hu, Y. Yan, X. Hu, K. Zhou, S. Xiao, Y. Zhang, N. Feng, *Int. J. Pharm.* **2019**, *569*, 118595.

Carlotta Pucci was awarded her Ph.D. in Chemical Sciences at the University of Rome "La Sapienza" (Italy) working on the synthesis of liposomes and on the study of their interactions with biomacromolecules. She is currently a postdoctoral fellow in the Smart Bio-Interfaces Research Line of the Italian Institute of Technology in Pontedera, Italy. She is specialized in the fabrication and characterization of different kinds of biocompatible and multifunctional nanomaterials for the treatment of central nervous system diseases.

Chiara Martinelli received her Ph.D. in Medical Nanotechnology from the European School of Molecular Medicine in Milan, Italy. She worked as a postdoctoral fellow in the Smart Bio-Interfaces Research Line of the Italian Institute of Technology in Pontedera (Italy), where she developed antioxidant nanovectors for space medicine applications and for the treatment of central nervous system diseases. Her research interests focus on multifunctional nanomaterials, drug delivery, biological interactions, and cellular responses to microgravity.

Andrea Degl'Innocenti is a postdoctoral researcher at Italian Institute of Technology. He obtained his B.Sc. and M.Sc. (with honors) in molecular biology from the University of Pisa. He then pursued his doctoral studies in Germany, working on the genetics of smell at the Max Planck Institute for Brain Research. After spending some more time at the institute as a postdoc, he returned to Italy joining Italian Institute of Technology. Currently, he studies space-elicited oxidative imbalance with omic approach. Other interests include neurobiology and senses. Regarding chlorophyll derivatives, he investigates their role in vision and as photodynamic agents against brain tumors.



Andrea Desii is currently a researcher at the Center for Materials Interfaces of Italian Institute of Technology. After receiving his Ph.D. at Scuola Superiore Sant'Anna in 2013, he moved to Milan (Italy), in the group of Prof. GuglielmoLanzani at Italian Institute of Technology (CNST@PoliMi), working on semiconducting polymeric photosensitizers for artificial vision. In 2018, he joined Prof. Gianni Ciofani first at Politecnico di Torino (Italy), then in its current position in the Smart Bio-Interfaces group, working on multifunctional nanodrugs for the treatment of brain cancer.

Gianni Ciofani, Ph.D., is Research Director at the Italian Institute of Technology, where he is Principal Investigator of the Smart Bio-Interfaces Research Line and Coordinator of the Center for Materials Interfaces (Pontedera, Italy). His main research interests are in the field of smart nanomaterials for nanomedicine, bio/non-bio interactions, and biology in altered gravity conditions. He is coordinator or unit leader of many grants/projects, and in 2016 he was awarded a European Research Council (ERC) Starting Grant.