Trends in Photochemistry & Photobiology
Vol. 17, 2016

Review

Photosensitizing properties of melanin upon excitation with visible light

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ABSTRACT

When the subject is sun protection it seems that several of the basic rules of photochemistry are not being considered. If one wants to avoid a photochemical-induced reaction, the first question should be if there are and what are the absorbing molecules. This simple consideration would make clear that visible light should be considered in photo-protection strategies, since there are several photoactive molecules that are present in our skin and hair and that absorb in this wavelength region. This review will focus on the antagonist roles of melanin, which is a fundamental molecule that protect against UV-B exposure, but as any photoactive molecule, generates reactive agents, including singlet oxygen ($^1O_2$), upon photoexcitation. We aim to provide a generic overview of the reactivity of $^1O_2$ against different types of biomolecules and a critical evaluation of the recent literature dealing with the photochemistry of melanin, with emphasis on the generation of $^1O_2$ and its consequences to the health of skin and hair.

KEYWORDS: melanin, sun protection, visible light, UV-A, ROS, triplets, singlet oxygen, skin, hair, photoaging

INTRODUCTION

A myriad of intrinsic and extrinsic factors affect the health of skin and hair [1]. Without doubt, one of the major extrinsic factors is sun exposure [2, 3]. It is becoming increasingly evident that in order to improve human health, both the excess and the lack of sun exposure should be avoided [4, 5]. Aiming to have a comprehensive understanding of the effects of light, it is fundamental to pay attention to the chemical reactions that are induced by the excited states of specific biological compounds that absorb light [6]. Herein, we will give special emphasis to the effect of visible light, touching also the effects of ultraviolet (UV) to give a sense of comprehensiveness to the review. Although there is a naïve belief that only UV-B and UV-A light damage hair and skin, several literature reports show unequivocally that visible light can also damage both tissues [6-9]. Therefore, it no longer makes sense to talk about innovation in terms of sun care, without considering visible light.

The damaging mechanisms induced by UV-A and visible light are mainly due to photosensitization, a process by which molecules transform energy of light into chemical reactivity (see further information on photosensitization below) [6, 7, 8, 10]. By definition, an excited state is more reactive than its respective ground state, being capable of engaging in both electron and energy transfer reactions [10]. Hence, the key questions that should be asked to understand whether a determined light source has the potential to affect a specific tissue are: 1. Is there a chromophore light absorption in this wavelength region? and 2. Is this molecule known to participate in

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photochemical reactions? The second question is not as important as the first, because most of the excited states will engage in some sort of photochemical event [10]. Unfortunately, these questions are hardly ever being asked and almost never being answered [11]. Thus, humans still have to cope with the crude separation of ultraviolet (UV) being considered dangerous and visible light good, which is based on the way our eye photoreceptor responds. In other words, what we see should be fine, what we do not see is probably harmful!

During the evolution of *Homo sapiens* from our primate ancestor, several changes occurred in our skin, including loss of most of the fur protection, and also induction of melanin production, which is a biopolymer selected during this transformation to protect our skin against UV-B. However, melanin also produces reactive oxygen species (ROS) under exposure to UV and visible radiation [7, 8, 9, 12]. Publications of our group [7, 8] definitely showed that under UV-A and visible light excitation, melanin produces singlet oxygen ($^1O_2$), which adds to the double bonds of biomolecules and can damage hair and skin [7, 8, 10, 12]. We will start this article by reviewing the photochemistry of melanin, with emphasis on the generation of $^1O_2$. We will also review the reactivity of $^1O_2$ against different types of biomolecules and the consequences of these reactions to the health of skin and hair.

Melanocytes and melanins

The skin is the largest organ in the body and is sub-divided into three layers: epidermis, dermis and hypodermis. These layers have specific cells and extracellular structures that are important for aesthetics and for the body defense against microorganisms and sunlight [1, 2]. Melanocytes are located in the epidermal layer and its main function is the production of melanin, which is exported to keratinocytes and to hair fibers growing from the follicles (Figure 1). Melanin has been known to prevent human skin cancer in epidemiological and experimental studies and also for being an efficient protectant against UV exposure. Yet, its role in photoprotection continues to be controversial [13].

The life cycle of melanocytes is composed of several stages, including differentiation, migration, proliferation of melanoblasts and conversion to melanocytes [14]. The maturation involves the activation of melanogenic enzymes, proper

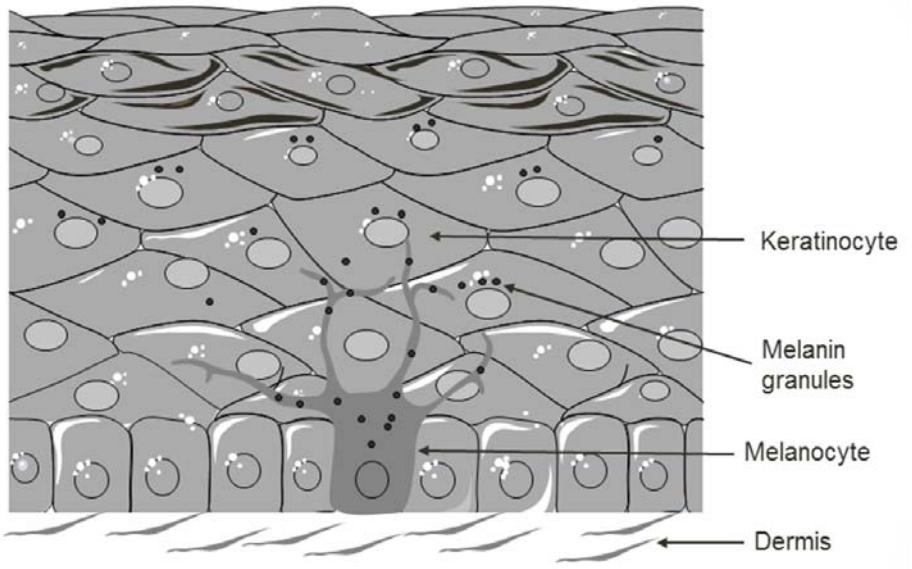

Figure 1. Schematic representation of melanocytes producing melanin: Melanin is produced by melanosomes of mature melanocytes (Melanin granules) and exported to keratinocytes in the epidermis (Servier Medical Art - adapted) [14].

structuration of melanosomes, efficient melanin synthesis and transport to keratinocytes and/or to hair shafts [15].

The synthesis of melanin occurs in specific organelles called melanosomes, which are present in melanocytes in four different maturation stages (I, II, III, IV) in accordance with the structure, quality, quantity and arrangement of melanin production [16, 17]. Melanosomes in stage I are spherical and do not have enzymatic activity for melanin production. In stage II there is already small tyrosinase activity [17]. In stage III melanin is arranged evenly and in stage IV melanin granules are formed and exported in large quantities to the keratinocytes of the skin and to growing hair fibers [14].

The biochemical pathways of pigment formation in epidermal melanosomes and follicles are similar, although epidermal melanocytes are long-lived, whereas the hair melanocytes die at the end of the hair cycle [18]. Consequently, melanocytes of hair follicles are more sensitive to aging, resulting in gray hair [19, 20]. The melanin granules from skin migrate with the keratinocytes that are differentiating into corneocytes [14]. In hair, melanogenesis occurs only during the anagen (growth phase), and pigment formation is not present at the catagen (regression) and telogen (resting) phases [18, 21, 22]. Melanin granules accumulate in the cortex of the hair shaft, which is the intermediate layer between the cuticle and the medulla [11-13].

Melanin is a polymer derived from several sequential reactions of oxidation and polymerization of L-amino acid tyrosine, and it is usually divided in two types: eumelanin and pheomelanin [7, 8, 9, 14, 23-25]. Eumelanin has no sulfur in its structure and has coloration ranging from dark brown to black. Pheomelanin has sulfur in its structure and has red color (Figure 2). There is controversy in the literature regarding the control mechanisms in the synthesis of eumelanin and pheomelanin. The most accepted hypothesis considers that in the presence of cysteine the ratio of pheomelanin is larger than that of eumelanin, and when the reservoir of cysteine is depleted, eumelanin is synthesized. Subsequently, the formation of melanin granules begins by pheomelanin synthesis followed by a wrapping of eumelanin [24] (Figure 3). Both melanins are rich in conjugated double bonds and thus absorb light in the UV and in visible regions, serving as the main blockers of light, protecting against the excess of sun exposure, especially against the effects of UV-B [13, 25].

Sunlight

Solar radiation has a continuous spectrum of electromagnetic radiation, which is usually divided into ultraviolet (200 to 400 nm), visible (400 to 700 nm) and infrared (IR). IR extends from the edge of the visible red spectrum at 700 nanometers (nm) to 1 mm (Figure 4) [11, 26].

UV radiation is sub-divided into three regions: UV-C (100-290 nm), UV-B (290-320 nm) and

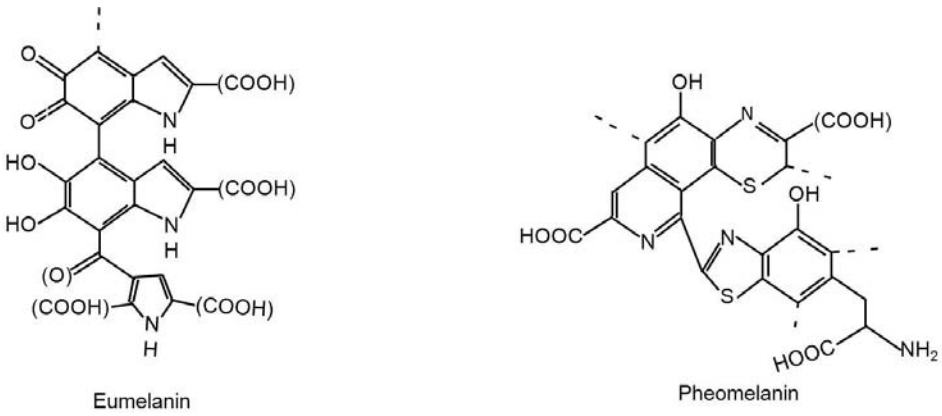

Figure 2. Schematic representation of melanins: Structures of eumelanin and pheomelanin. Adapted from Ito and Wakamatsu [24].

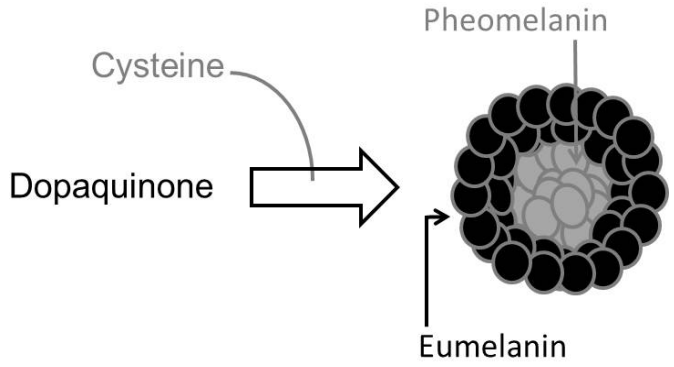

Figure 3. Scheme showing a typical melanin granule containing pheomelanin in the inside and eumelanin externally. Adapted from Ito and Wakamatsu [24].

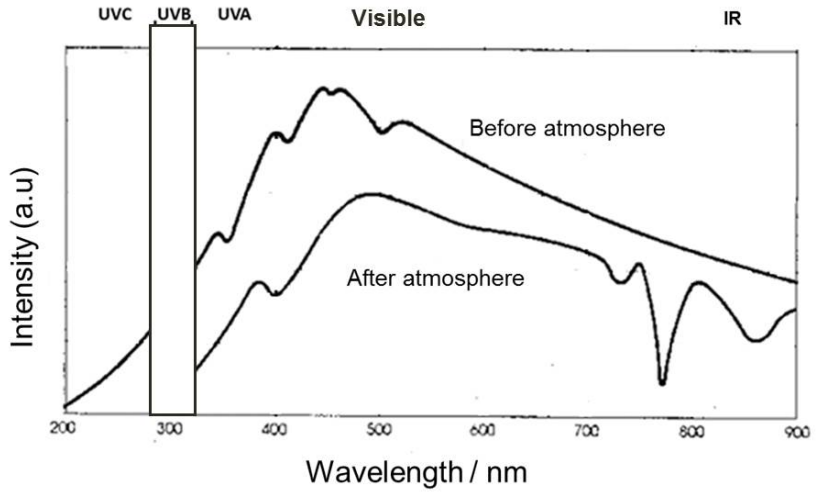

Figure 4. Spectra of solar radiation on earth. Intensity of solar radiation as a function of wavelength before and after passage through the earth's atmosphere. (www.who.int/uv/publications/UVEHeffects.pdf, with modifications).

UV-A (320-400 nm) [26]. UV-C is the most harmful, because it induces photochemical reactions in a wider range of biomolecules. Practically, all organic molecules absorb radiation in the UV-C region and react without a defined specificity. However, thanks mainly to the ozone layer, photons on this range are absorbed before reaching the surface of the earth.

UV-B radiation is absorbed by melanin, and by several compounds having conjugated double bonds such as ketones and carboxylic acids, nucleic acids (260 nm) and proteins (280 nm) [27]. Pyramidine bases (cytosine and thymine) are the main sites of UV-B absorption in the DNA. After electronic excitation, direct and specific photochemical reactions induce the formation of pyrimidine dimers and 6-4 photoproduct, as well as of other DNA adducts that usually but not always can be detected and repaired by the DNA repair systems [28]. Damages in DNA are frequently recognized by p53 that can trigger several events including senescence or apoptosis [28-30]. The worst case comes from chronic exposure to UV-B that allows for the accumulation of mutations (thymine to cytosine transversion) in repair genes (e.g., p53), with consequent malignant transformation [31]. It is worth mentioning that UV-B is also fundamental for vitamin D metabolism; in fact, it is not a good health habit to totally avoid exposing bare skin to the sun [4, 5, 32].

Unlike UV-B, which is directly absorbed by DNA, UV-A radiation is absorbed by natural chromophores and essentially act by photosensitization (see next section for a deeper explanation of the photosensitizing processes) and generates triplet species, $^1O_2$ and subsequently, other radical species [6, 12, 33]. In addition, UV-A penetrates deeper into the dermis, compared to UV-B. Consequently, UVA is responsible for tumors that develop in the deeper layers of the skin [3, 34] and for the premature aging of the skin, which is also called photoaging [2, 3, 28, 34].

The paradigm that melanin had only a protective role against sun radiation, started to be broken when it was shown that UV-A light generates reactive species by the excitation of melanin in melanoma cells [35]. High melanin content on B16-F10 melanoma cells accumulated twice as much 8-hydroxy-dGuanosina after UV irradiation, compared to cells with low melanin content [35]. Since melanin absorbs light both in the UV and in the visible regions of the solar spectrum, it is expected that the photosensitizing properties of melanin could extend to visible region. In fact, visible light is known to induce pigmentation in individuals with skin types IV and V [9, 36]. As it will be clarified with more detail below, melanin is photoactivated by visible light [7, 8, 37]. Nevertheless, before going there, it is important to review the backgrounds of the photosensitization reactions.

Photosensitization processes

Just after the absorption of a photon by a molecule in the ground state, an excited state is formed, which is a lot more reactive than its respective ground state [10, 38]. Usually, the first singlet excited state (^{1}PS*) reaches a condition of pre-equilibration and starts to decay back to the ground state by releasing heat into the surroundings, or by emitting light (fluorescence). Excited states can also have a spin inversion, which is called inter-system crossing (CIS), forming triplet excited states (^{3}PS*). Because triplets are reactive and remain in the excited state for longer periods, they are the main species responsible for the photo-oxidation reactions. Depending on its intrinsic properties as well as on its surroundings, triplets can engage either in electron transfer, or in energy transfer reactions, which are called type I and type II reactions, respectively (Figure 5). Type I occurs *via* direct electron transfer reaction with biological targets, producing radicals that can interact with molecular oxygen, which produce oxygenated products such as superoxide anion radicals ($O_2^{\cdot-}$), peroxyl radical (HOO•) and hydroxyl radical ($HO^{\cdot}$); type II reactions involve an energy transfer to molecular oxygen ($^3O_2$) forming singlet oxygen ($^1O_2$), which is highly reactive and electrophilic (Figure 5B) [38, 39]. These reactions lead to the formation of reactive species, frequently with the return of the photosensitizer to the ground state, allowing it to perform a new cycle of light absorption (Figure 5A).

It is important to emphasize that photosentization reactions are the rule and not the exception after an excited state is formed [34]. That is to say, it is actually difficult to find a chromophore that will not induce any amounts of triplets. Some may form in small amounts, but frequently that is enough to be of significance. This is symbolized by the reaction centers of photosynthetic organisms. Although these organisms survive from the direct absorption and transformation of energy from light, they also generate fair amounts of triplets and of $^1O_2$ when excited by light [40]. In fact, plants use a reasonable amount of energy to cope with the damage in the photosynthetic apparatus caused by $^1O_2$ [41].

$^1O_2$ reacts efficiently with proteins, lipids and nucleic acids. The major chemical modification in nucleic acids induced by $^1O_2$ is the generation of 8-Oxo-2'-deoxyguanosine (8-oxo-dG), which normally causes G:C to T:A transversion [42, 43]. In relation to protein oxidation, several amino acids are sensitive to oxidation by $^1O_2$, including cysteine, histidine, methionine, tryptophan and tyrosine. The oxidation of an amino acid in a protein will alter its structure and activity [44-47]. In the case of biological membranes, lipid oxidation by $^1O_2$ causes the formation of hydroperoxides, which is the initial step in the formation of peroxidation chain reactions that can cause membrane destruction [48].

Skin and hair have several molecules that absorb UV-A and produce $^1O_2$ [6, 37, 49, 50] Therefore, $^1O_2$ is mainly responsible for photoinduced skin damage by UV-A [6, 7, 49]. It is interesting that

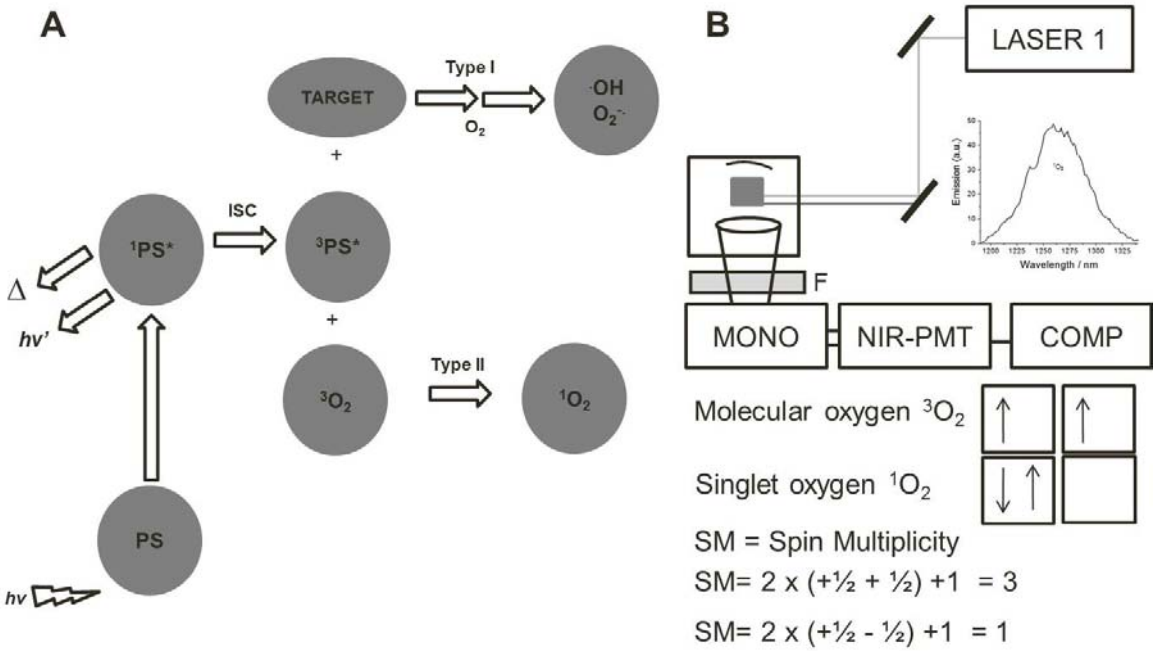

Figure 5. (A) Schematic representation of type I and type II photosensitization mechanisms: Photosensitizers (PS) are brought to the singlet excited state (^{1}PS*) and return to the ground state by emitting light (fluorescence, hυ') or go to a state excited triplet (^{3}PS*) by intersystem crossing (ICS). Triplet states can react by electron transfer with biomolecules (biological targets) (Type I reaction). Triplet states can also transfer energy to molecular oxygen ($^3O_2$) forming singlet oxygen ($^1O_2$) (type II reaction). **(B)** shows the layout of the $^1O_2$ detection equipment that consists of a laser (365 nm and 532 nm), detector (NIR PMT), and monochromator that transmits signal through the spectrum of NIR to detect 1270 nm (see the internal spectrum in Figure B). Spin Multiplicity of molecular oxygen ($^3O_2$) and singlet oxygen ($^1O_2$) are also shown.

several of these compounds that are known to absorb light in the UV-A region also absorb visible light [6]. As it will become clear in this section, the same type of photosensitization reactions are also induced by visible light.

Photosensitization of melanin and the effects of visible light on skin and hair

Melanin negatively affects the development of two very important reactions that are induced by light in the same wavelength region (UV-B), and in the same part of the human body (skin): i) the photochemical reactions after direct DNA excitation, which trigger the formation of several types of pre-mutagenic photoproducts and ii) the activation of vitamin D, which is a hormone involved in several processes that are vital for humans [4, 5, 51, 52]. The direct participation of UV-B light and the role of melanin in these two reactions have contributed as factors of human evolution, favoring the selection of dark-skinned populations in the equator and tropics, and fair-skinned populations in higher latitude places. This is the reason why fair-skinned individuals are more prone to several types of skin cancer, particularly in regions with high UV-B incidence. On the other hand, dark-skinned individuals, especially those living in high latitude places are at greatest risk of disease caused by insufficient levels of vitamin D [13, 53, 54]. As a consequence, health problems that originate from the lack of sun exposure are a lot more costly to the health system of the United States of America, than those caused by the excess of sun exposure [55].

It is clear, therefore, that melanin acts as a sun blocker, absorbing broadband UV and visible light [13, 53]. The primary biological function of melanin is to protect nuclear DNA against direct attack of UV-B and, consequently, the absorption in the UV-B is the strongest factor that favors its selection [56]. Melanin also acts as an anti-oxidant and protects mitochondrial DNA by preventing

superoxide generation by UV irradiation [57]. However, as any other chromophore, after melanin excitation there is the formation of excited states and photosensitization reactions are prone to occur. In fact, Nofsinger and colleagues observed the formation of ROS from eumelanin irradiated by UV-A. They also found that melanin granules form aggregates that produce 10 times less radical superoxide anion ($O2^{\bullet-}$) than oligomers not aggregated [58], suggesting that intermediate molecules of melanin generate reactive oxygen species [35]. There seems to be differences in the phototoxicity of the main types of melanin, i.e., eumelanin and pheomelanin; however this issue remains largely controversial [7, 24, 59]. There is evidence that redhead people have a higher prevalence of skin cancer, but this is not necessarily correlated with photo-induced reactions [60].

An interesting result, in terms of the photo-damaging role of melanin, came out in the literature in 2010, from Mahmoud and co-workers [9]. Studies performed with human subjects with different skin types exposed to visible light showed darkening in people with skin types IV and V, but not in individuals with type II skin. They showed that darkening depended on the melanin content present in the skin before irradiation, suggesting a direct role of melanin in inducing tanning after exposure to visible light. Seeking to prove the role of photosensitizing reactions of melanin induced by visible light, we began to characterize the excited-state reactions of melanin. A possible explanation for the damage induced by visible-light excitation of melanin was the formation of $^1O_2$, which we and others have proven to occur in several experimental models [7, 8, 59].

The definitive way to prove the generation of $^1O_2$ is through its emissive properties in the near infrared, especially its characteristic spectra peaking at 1270 nm (Figure 6A). In fact, both eumelanin and pheomelanin generate $^1O_2$ after excitation in the visible (532 nm) and in the UV-A (355 nm) (Figure 6A). The amount of $^1O_2$ generated by melanin was shown to be highly dependent on the granular structure of the pigment. Agents (heat, pH, urea, oxidation) that induce opening of the biopolymer granular structure and the consequent exposure of the internal parts of the pigment, cause an expressive increase in the level of $^1O_2$ production [7, 8].

Pheomelanin generates larger amounts of $^1O_2$ than eumelanin (Figure 6B). Pheomelin is less bleached than eumelanin during photolysis (Figure 6C). Note that during 1 hour of irradiation with visible light, the absorption of eumelanin decreases by 30% compared to a 7% decrease in the absorption of pheomelanin (Figure 6C). These data indicate that pheomelanin is a better $^1O_2$ photosensitizer than eumelanin [7]. The amount of $^1O_2$ generated is larger after excitation in the UV-A compared with excitation in the visible light, as a consequence of a stronger absorption in the UV-A and also because of a higher efficiency production, which depends on the type of melanin [7, 8, 59].

The chemical reaction responsible for the photobleaching of eumelanin (Figure 6B) is the addition of $^1O_2$ to a reactive double bond and the consequent formation of a hydroperoxide in the C3 position of the indol group [8]. This type of photoproduct was detected from the photolysis of eumelanin and not from the photolysis of pheomelanin. Recent data from Ito's group in Japan indicated that $^1O_2$ is also responsible for UV-A-induced degradative oxidation of a melanin intermediate 5,6-dihydroxyindole-2-carboxylic acid (DHICA) [61].

We have shown that $^1O_2$ reactions are the main cause of photobleaching in hair by visible light [8]. $^1O_2$ generated inside hair shafts suspended in different solvents shows lifetimes a lot smaller than expected, indicating that $^1O_2$ is generated and suppressed inside hair structure. A model was proposed to explain the formation and suppression of $^1O_2$ in hair by photosensitization of melanin with visible light [8, 37].

Cells that produce higher levels of melanin suffer greater damage by visible light [7]. Phototoxicity observed in M+ melanocytic cells, which produce large amount of melanin (Figure 7A), were correlated to the absorption of visible light and the production of $^1O_2$ [7]. Control cells and pigmented M+ cells were excited with visible light (532 nm) and $^1O_2$ emission spectra were recorded. Note that only M+ cells showed measurable amounts of $^1O_2$ (Figure 7A). In order to confirm direct oxidation of DNA by melanin photosensitized oxidation,

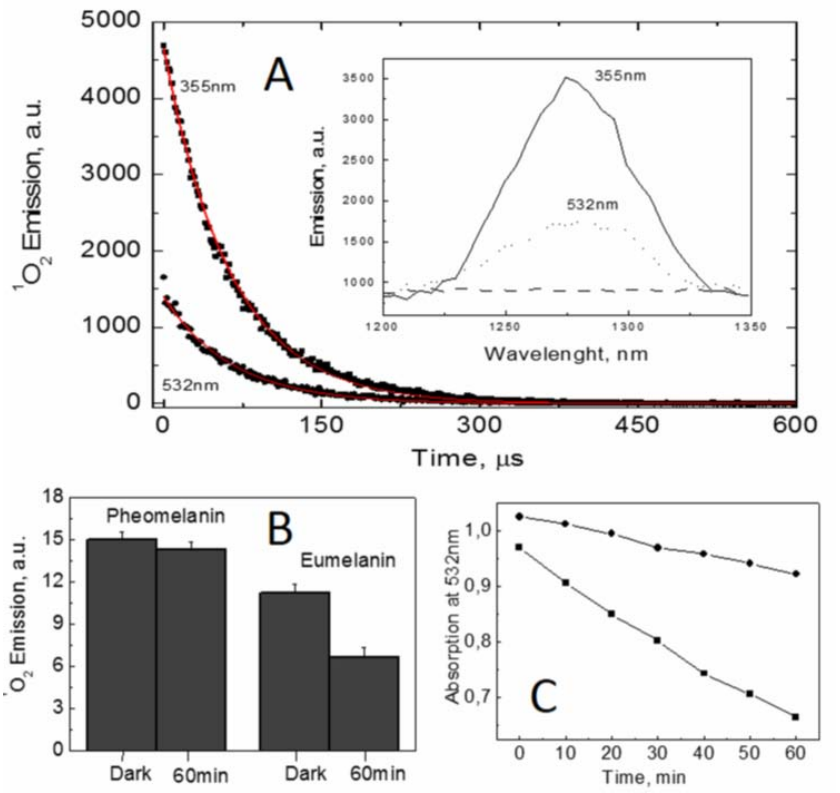

Figure 6

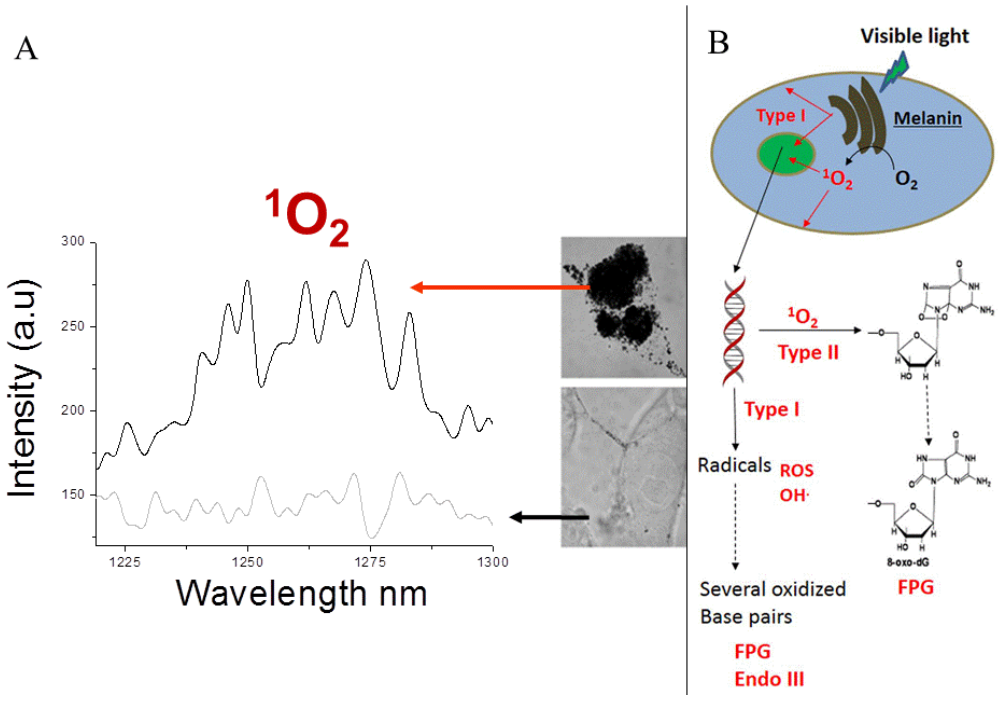

Figure 7

M+ cells were irradiated with visible light in a lower dose regime (6 J/cm^2), which is a light dose that does not induce any decrease in survival even in M+ cells. After treating these cells with endonuclease FPG and Endo III, which recognize specific oxidative lesions and cleave DNA, we observed a 5-fold increase in the comet tail by comet essay (gel electrophoresis for measuring DNA strand break in eukaryotic cell) (Figure 7B) [7]. These FPG and EndoIII-sensitive DNA lesions are pre-mutagenic alterations in DNA. These experiments showed that visible light causes the formation of pre-mutagenic DNA lesions as it has been observed for UV-A light. Additional experiments are needed to confirm the possible involvement of visible light in the induction of tumorigenesis.

The fact that melanin is able to generate $^1O_2$ by light could, in principle, be used as a tool by some pathogenic organisms to cause biological harm in others. In fact, Beltran-Garcia and co-workers have shown that melanin contributes to virulence of pathogenic fungi *Mycosphaerella fijiensis*, allowing tissue invasion and inactivation of the plant defense system [62].

Considerations on the effect of visible light on the skin and hair

Thirty years ago, photobiologists knew that UV-A radiation was able to cause oxidative damage in molecules, to cause cell death and to affect cell proliferation [63]. However, the sunscreens that were in use at that time and until few years ago, did not protect the skin against UV-A. This information, which was available for scientists, was not well propagated to the general public. Consequently, people were encouraged to expose themselves to the sun using sunscreens that only protected them against UV-B. After using UV-B sunscreens, people could stay for longer periods under the sun, because they did not feel the acute inflammatory processes characteristic of UV-B (skin erythema, for example). However, their skin was being harmed by light in other wavelength regions. In fact, we know today that most of the skin cancers that are deeper in the skin are originated from injuries caused by oxidative-type lesions, characteristic of UV-A exposure [3, 28, 34].

We and others have recently shown that visible light also induces production of reactive oxygen species, including $^1O_2$, by melanin photosensitization, causing oxidative DNA damage [7]. This finding indicates that protection against visible light should not be ignored, but rather be seriously considered by health professionals as well as by general population. Continuous exposure to visible light without proper protection can promote molecular damage that cumulates in the skin [40]. Remember also that exposure to visible light changes color of hair by photobleaching of melanin, as many perceive during summer holidays.

Good sunscreens available today allow photons in the visible range to freely penetrate the skin, causing deleterious effects similar to those caused

Legend to Figure 6. Singlet oxygen generation after melanin excitation. **(A)** Transient emission after exciting samples of eumelanin with UV-A light (355 nm) and visible (532 nm). The inset shows the emission spectra upon excitation in the UV-A and visible in the presence of sodium azide, the compound that suppresses singlet oxygen. **(B)** Pheomelanin and eumelanin before and after irradiation with visible light. **(C)** Photodegradation of melanin as a function of irradiation time in the visible. Data originally published in the article: Photosensitization and the Effect of Visible Light on Epithelial Cells. PLoS One, 2014, 9(11), e113266. doi:10.1371/journal.pone.0113266 [7].

Legend to Figure 7. Intracellular production of singlet oxygen by photosensitization of melanin and oxidative damage induced in DNA: **(A)** correlation between the amount of intracellular melanin and the level of singlet oxygen generation. Cells with increased production of melanin (M+) present the characteristic spectrum of singlet oxygen (black line). The basal production of melanin (CT) in the cell indicated by the black arrow is correlated with no NIR emission. B16-F10 control cells (CT) and B16-F10 pigment cells (M+) are represented besides the graph. **(B)** Simplified scheme of the damage that photosensitization of melanin cause in cells. Two main targets of damage were identified: membranes and nucleic acids. Pre-mutagenic lesions were identified by the recognition of FPG and EndoIII enzymes. Data were originally published in the article: Photosensitization and the Effect of Visible Light on Epithelial Cells. PLoS One, 2014, 9(11), e113266. doi:10.1371/journal.pone.0113266 [7].

by UV-A. Therefore, the habit of using sunscreen and staying under the sun for long periods of time can cause irreparable damages to the health of the skin, including photo-aging and possibly the formation of tumors. The ideal routine for an individual that does not have skin sensitivity is the old recipe of exposing to the sun for a short time without external protection. In doing so one gets the benefits of the sun, for example, activation of vitamin D, without suffering the risk that prolonged exposure offers, even with the use of current sunscreens. Those who have had previous hypersensitivity problems in the skin should avoid contact with sun light by physical means such as clothing, because the use of sunscreen only will not be enough. For those that also mind about the beauty of hair, excess of sun exposure should be avoided for the same reasons above-mentioned. The recently-developed hair products that have sunscreen included in its formulation will not help much, because hair bleaching is mainly caused by visible light [8]. We hope this review will help companies to develop new sunscreens that operate in a wider spectral range (including visible light), so that people can get a better protection of skin and hair.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

REFERENCES

1. Guyuron, B., Rowe, D. J., Weinfeld, A. B., Eshraghi, Y., Fathi, A. and Lamphongsai, S. 2009, Plastic and Reconstructive Surgery, 123, 1321-31.
2. Farage, M. A., Miller, K. W., Elsner, P. and Maibach, H. I. 2008, Int. J. Cosmet. Sci., 30, 87-95.
3. Agar, N. S., Halliday, G. M., Barnetson, R. S., Ananthaswamy, H. N., Wheeler, M. and Jones, A. M. 2004, Proceedings of the National Academy of Sciences of the United States of America, 101, 4954-9.
4. Holick, M. F. 2004, American Journal of Clinical Nutrition, 79, 362-371.
5. Wolpowitz, D. and Gilchrest, B. A. 2006, Journal of the American Academy of Dermatology, 54, 301-317.
6. Wondrak, G. T., Jacobson, M. K. and Jacobson, E. L. 2006, Photochemical & Photobiological Sciences: Official journal of the European Photochemistry Association and the European Society for Photobiology, 5, 215-37.
7. Chiarelli-Neto, O., Ferreira, A. S., Martins, W. K., Pavani, C., Severino, D., Faião-Flores, F., Maria-Engler, S. S., Aliprandini, E., Martinez, G. R., Di Mascio, P., Medeiros, M. H. G. and Baptista, M. S. 2014, PLoS One, 9, e113266.
8. Chiarelli-Neto, O., Pavani, C., Ferreira, A. S., Uchoa, A. F., Severino, D. and Baptista, M. S. 2011, Free Radical Biology & Medicine, 51, 1195-1202.
9. Mahmoud, B. H., Ruvolo, E., Hexsel, C. L., Liu, Y., Owen, M. R., Kollias, N., Lim, H. W. and. Hamzavi, I. H. 2010, The Journal of Investigative Dermatology, 130, 2092-7.
10. Dauben, W. G., Salem, L. and Turro, N. J. 1975, Accounts of Chemical Research, 8, 41-54.
11. Hussein, M. R. 2005, Journal of Cutaneous Pathology, 32, 191-205.
12. Vile, G. F. and Tyrrell, R. M. 1995, Free Radical Biology & Medicine, 18, 721-30.
13. Brenner, M. and Hearing, V. J. 2008, Photochemistry and Photobiology, 84, 539-549.
14. Costin, G. E. and Hearing, V. J. 2007, FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 21, 976-94.
15. Cichorek, M., Wachulska, M., Stasiewicz, A. and Tymińska, A. 2013, Postepy dermatologii i alergologii., 30, 30-41.
16. Seiji, M., Shimao, K., Birbeck, M. S. and Fitzpatrick, T. B. 1963, Annals of the New York Academy of Sciences, 100, 497-533.
17. Kushimoto, T., Basrur, V., Valencia, J., Matsunaga, J., Vieira, W. D., Ferrans, V. J., Muller, J., Appella, E. and Hearing, V. J. 2001, Proceedings of the National Academy of Sciences of the United States of America, 98, 10698-703.
18. Tobin, D. J. 2011, Pigment Cell & Melanoma Research, 24, 75-88.

19. Slominski, A., Wortsman, J., Plonka, P. M., Schallreuter, K. U., Paus, R. and Tobin, D. J. 2005, The Journal of Investigative Dermatology, 124, 13-21.
20. Randall, V. A. 2008, Dermatologic Therapy, 21, 314-28.
21. Commo, S. and Bernard, B. A. 2000, Pigment cell research/sponsored by the European Society for Pigment Cell Research and the International Pigment Cell Society, 13, 253-9.
22. Nishimura, E. K. 2011, Pigment Cell & Melanoma Research, 24, 401-10.
23. Takahashi, T. and Nakamura, K. 2005, Journal of Cosmetic Science, 56, 47-56.
24. Ito, S. and Wakamatsu, K. 2008, Photochemistry and Photobiology, 84, 582-92.
25. Hearing, V. J. and Tsukamoto, K. 1991, FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 5, 2902-9.
26. Duthie, M. S., Kimber, I. and Norval, M. 1999, British Journal of Dermatology, 140, 995-1009.
27. Lin, J. Y. and Fisher, D. E. 2007, Nature, 445, 843-50.
28. Niida, H. and Nakanishi, M. 2006, Mutagenesis, 21, 3-9.
29. Bandyopadhyay, D. and Medrano, E. E. 2000, Annals of the New York Academy of Sciences, 908, 71-84.
30. Medrano, E. E., Yang, F., Boissy, R., Farooqui, J., Shah, V., Matsumoto, K., Nordlund, J. J. and Park, H. Y. 1994, Molecular Biology of the Cell, 5, 497-509.
31. Rouse, J. and Jackson, S. P. 2002, Science (New York, NY), 297, 547-51.
32. Lehmann, B. and Meurer, M. 2010, Dermatologic Therapy, 23, 2-12.
33. Berneburg, M., Grether-Beck, S., Kürten, V., Ruzicka, T., Briviba, K., Sies, H. and Krutmann, J. 1999, The Journal of Biological Chemistry, 274, 15345-9.
34. Halliday, G. M., Agar, N. S., Barnetson, R. S. C., Ananthaswamy, H. N. and Jones, A. M. 2005, Photochemistry and Photobiology, 81, 3-8.
35. Kvam, E. and Tyrrell, R. M. 1999, The Journal of Investigative Dermatology, 113, 209-13.
36. Liebel, F., Kaur, S., Ruvolo, E., Kollias, N. and Southall, M. D. 2012, The Journal of Investigative Dermatology, 132, 1901-7.
37. Severino, D., Pavani, C., Catellani, G. M. and Baptista, M. S. 2016, Singlet Oxygen in Hair. Chapter 38. Singlet Oxygen: Applications in Biosciences and Nanosciences, Editors, Santi Nonell and Cristina Flors, RSC.
38. Foote, C. S. 1968, Science, 162, 963-970.
39. Baptista, M. S., Cadet J., Di Mascio, P., Ghogare, A. A., Greer, A., Hamblin, M. R., Lorente, C., Nunez, S. C., Ribeiro, M. S., Thomas, A. H., Vignoni, M. and Yoshimura, T. M. 2017, Photochem. Photobiol., doi: 10.1111/php.12716.
40. Uchoa, A. F., Knox, P. P., Turchielle, R., Seifullina, N. K. and Baptista, M. S. 2008, Eur. Biophys. J., 37, 843-850.
41. Krieger-Liszkay, A. 2005, Journal of Experimental Botany, 56, 337-346.
42. Khanna, K. K. and Jackson, S. P. 2001, Nature Genetics, 27, 247-254.
43. Rouse, J. and Jackson, S. P. 2002, Science, 297, 547-551.
44. Augusto, O., Bonini, M. G., Amanso, A. M., Linares, E., Santos, C. C. X. and De Menezes, S. L. 2002, Free Radical Biology & Medicine, 32, 841-59.
45. Dean, R. T., Fu, S., Stocker, R. and Davies, M. J. 1997, Biochemical Journal, 324(Pt. 1), 1-18.
46. Davies, M. J., Fu, S. L., Wang, H. J. and Deanm R. T. 1999, Free Radical Biology and Medicine, 27, 1151-1163.
47. Gonsette, R. E. 2008, J. Neurol. Sci., 274, 48-53.
48. Itri, R., Junqueira, H. C., Mertins, O., Baptista, M. S. 2014, Biophysical Reviews, 6, 1-15.
49. Bäumler, W. 2016, Singlet Oxygen in the Skin, Chapter 36. Singlet Oxygen: Applications in Biosciences and Nanosciences, Editors, Santi Nonell and Cristina Flors, RSC.
50. Prado, F. M., Oliveira, M. C., B Miyamoto, S., Martinez, G. R., Medeiros, M. H. G., Ronsein, G. E. and Di Mascio, P. 2009, Free Radical Biology and Medicine, 47, 401-409.

51. Singh-Jagatia, P., Lee D., Knuckey, S. and Cook, G. 2012, Practising Midwife, 15, 14-16.
52. Pfeifer, G. P. 1997, Photochemistry and Photobiology, 65, 270-283.
53. Narayanan, D. L., Saladi, R. N. and Fox, J. L. 2010, International Journal of Dermatology, 49, 978-986.
54. Parra, E. J. 2007, Yearbook of Physical Anthropology, 50, 85-105.
55. Grant, W. B., Garland, C. F. and Holick, M. F. 2005, Photochem Photobiol., 81, 1276-1286.
56. Scrima, A., Koníčková, R., Czyzewski, B. K., Kawasaki, Y., Jeffrey, P. D., Groisman, R., Nakatani, Y., Iwai, S., Pavletich, N. P. and Thomä, N. H. 2008, Cell, 135, 1213-23.
57. Swalwell, H., Latimer, J., Haywood, R. M. and Birch-Machin, M. A. 2012, Free Radical Biology & Medicine, 52, 626-34.
58. Nofsinger, J. B., Liu, Y. and Simon, J. D. 2002, Free Radical Biology & Medicine, 32, 720-30.
59. Szewczyk, G., Zadlo, A., Sarna, M., Ito, S., Wakamatsu, K. and Sarna, T. 2016, Pigment Cell & Melanoma Research, 29, 669-678.
60. Mitra, D., Luo, X., Morgan, A., Wang, J., Hoang, M. P., Lo, J., Guerrero, C. R., Lennerz, J. K., Mihm, M. C., Wargo, J. A., Robinson, K. C., Devi, S. P., Vanover, J. C., D'Orazio, J. A., McMahon, M., Bosenberg, M. W., Haigis, K. M., Haber, D. A., Wang, Y. and Fisher, D. E. 2012, Nature, 491, 449-53.
61. Ito, S., Kikuta, M., Koike, S., Szewczyk, G., Sarna, M., Zadlo, A., Sarna, T. and Wakamatsu, K. 2016, Pigment Cell and Melanoma Research, 29, 340-351.
62. Beltrán-García, M. J., Prado, F. M., Oliveira, M. S., Ortiz-Mendoza, D., Scalfo, A. C., Pessoa, A., Medeiros, M. H. G., White, J. F. and Di Mascio, P. 2014, PLoS One, 9, e91616.
63. Hawk, J. L. M. 1990, Journal of Photochemistry and Photobiology B: Biology, 5, 267.