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The Important Role of Melanin as Protector against Free Radicals in Skin

Keywords: Skin, melanin, UV irradiation, free radical, ESR spectroscopy

Abstract

Throughout the body, melanin is a homogenous biological polymer containing a population of intrinsic, semiquinone – like radicals. Additional extrinsic free radicals are reversibly photo-generated by UV and visible light. Melanin is the only known biopolymer containing stable free radicals. Melanin photochemistry, particularly the formation and decay of extrinsic radicals, has been the subject of numerous electron spin resonance (ESR) spectroscopy studies. Several melanin monomers exist, and the predominant monomer in a melanin polymer depends on its location within an organism. In skin melanin differs in eumelanin or pheomelanin.

Its bio-radical character and its susceptibility to UV irradiation makes melanin an excellent indicator for UV-related processes in skin.

The existence of melanin in skin is strongly correlated with the prevention of free radicals/ROS generated by UV radiation. Especially in the skin melanin (mainly eumelanin) ensures the only natural UV protection by eliminating the generated free radicals. The aim of this study was to investigate the suitability of melanin as protector of skin against UV generated free radicals.

Introduction

The sun emits a wide spectrum of electromagnetic waves of which ultraviolet light (UV) is the most aggressive towards cellular compounds. Large amounts of UVB and UVC are screened out by ozone, the major photoprotective agent formed in earth's atmosphere. Hence, solar UV radiation that reaches the earth as well as our skin, is composed of 5-10% highly energetic UVB (290-315 nm) and 90-95 % UVA (315-400 nm) which is less energetic, but penetrates the skin deeper **(Fig. 1)** due to its longer wavelength. Both UVA and UVB irradiation are very damaging to the skin. Dependent on wavelength, UV damage occurs via different mechanisms. UVA mainly produces free radicals (FR)/reactive oxygen species (ROS) though interaction with endogenous photosensitizers. These free radicals will cause indirect damage to DNA, proteins and membranes. Free radicals are believed to be involved in photodamage of dermal connective tissue cells and proteins. On the contrary, DNA with its aromatic, heterocyclic bases is a strongly absorbing chromophore for UVB (absorption maximum at 260-265nm). Direct absorption of the UVB photons leads to disruption of DNA, with cyclobutane pyrimidine dimmers (CPD) and pyrimidine pyrimidone photoproducts as a result.

Hence, both UVA and UVB play a role in the pathogenesis of photosensitive diseases such as polymorphic light eruption (PLE), sunburn, immunosuppression, photoaging and even photocarcinogenesis.

So free radical reactions in the skin are one of the most interesting subjects of skin research because they are involved in various skin diseases, including skin tumors, skin wrinkling and skin aging (1). Following UV-exposure, free radicals play a major role in producing lipid radicals ($L^•$) that seem to be responsible for the destruction of the cell membrane and ultimately the cell (2,3).

Multiple lines of defense have evolved, aimed to protect skin from oxidative stress, including prevention, interception, and repair. The first defense line against UV generated free radicals in skin is caused by melanin contained in different qualities and concentrations in human skin characterizing different skin types. Skin is characterized by their different melanin content determining the different skin types **(Table 1).**

Biopolymers from the melanin family of molecules are known to be semiconductive and photoconductive. These materials are heteropolymers of indole-

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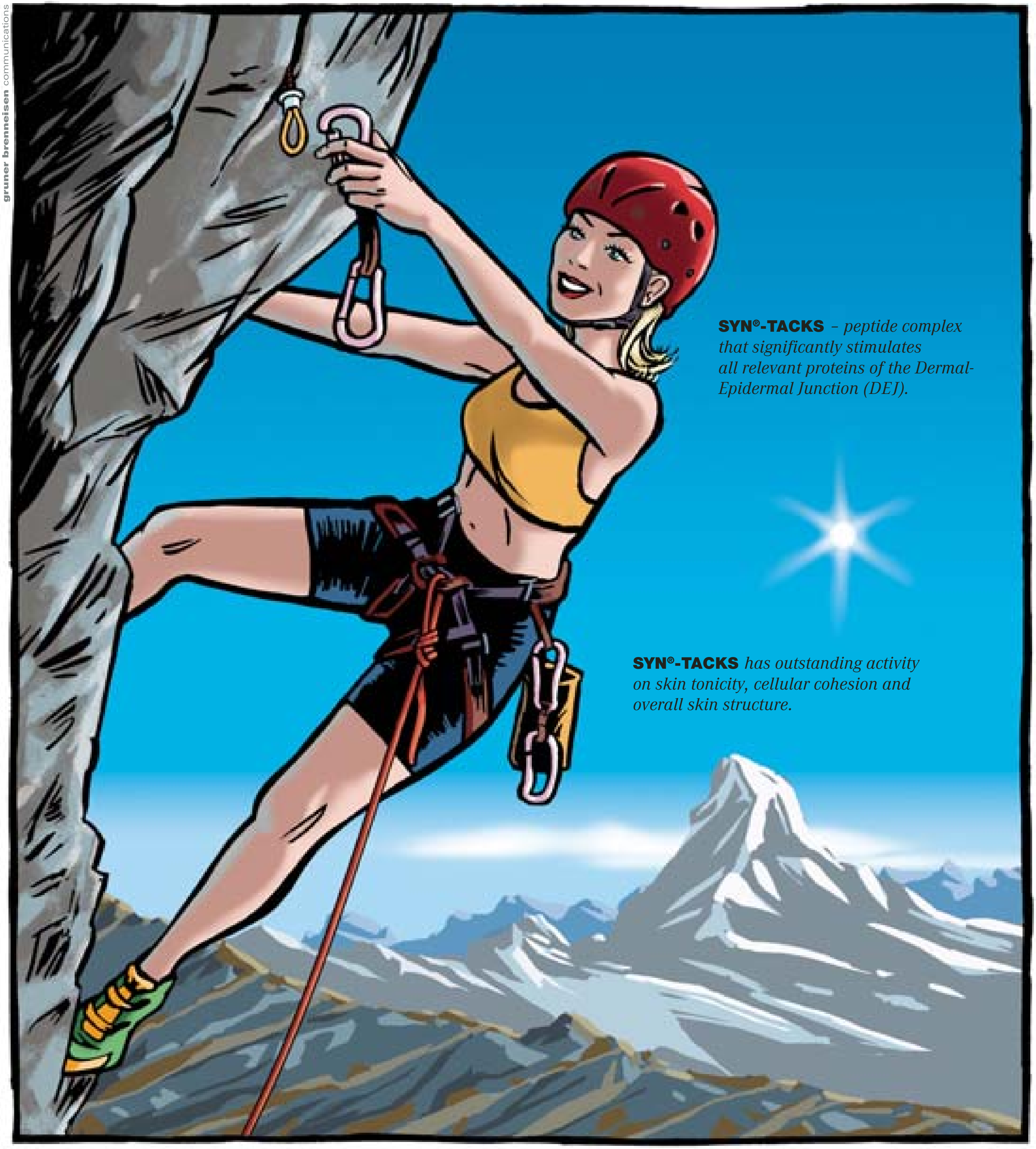

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quinones such as 5,6-dihydroxyindole (**Fig. 2**). Biologically, the melanin performs a variety of roles. Predominantly, it is a photoprotective pigment, but it is thought that it also functions as an antioxidant, free radical scavenger, and charge transport mediator.
Melanin is a biological polymer which is responsible for the pigmentation of many animals and plants.
Throughout the body, melanin is a homogenous biological polymer containing a population of intrinsic, semiquinone-like radicals. Additional extrinsic free radicals are reversibly photo-generated by UV and visible light. Melanin is the only known biopolymer containing stable free radicals. Melanin photochemistry, particularly the formation and decay of extrinsic radicals, has been the subject of numerous electron spin resonance (ESR) spectroscopy studies (4, 5, 6, 7). ESR can allow the identification of an unknown organic radical as well as provide insights in to the chemistry of radical formation and degeneration.

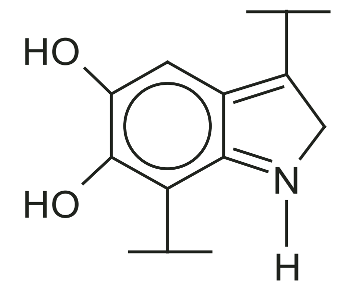

Fig. 2 Structure of 5,6-dihydroxyindole, one monomer of the melanin family.

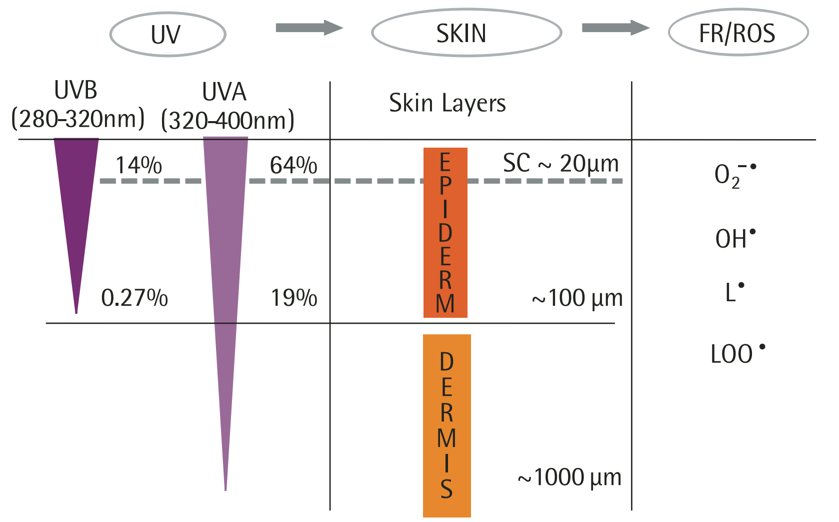

Fig. 1 Penetration of UVA and UVB radiation in the skin and the generated free radicals (FR)

Skin type	Constitutive color of un-exposed skin	Hair color	Typical Ethnicity	Exposure duration (min)
I	White	Red	Celtic	5 – 10
II	White	Blonde to light brown	Northern European, Scandinavian	10 – 20
III	White	Dark blond to brown	Average Caucasian	20 – 30
IV	Pale brown	Dark brown	Mediterranean, Oriental, Hispanic	40
V	Brown	Dark brown Black	American Indian, East Indian, Hispanic	60
VI	Dark brown Black	Black	African, Australian, South Indian, Aborigine	> 60

Table 1 Skin and hair types concerning the duration of their maximum sunlight exposure time

Several melanin monomers exist, and the predominant monomer in a melanin polymer depends on its location within an organism. In skin melanin differs in eumelanin or pheomelanin while in the eye it is exclusively eumelanin. These different melanin types are distinguishable by ESR studies, where the signals of the photo generated extrinsic radicals are distinct. ESR spectroscopy enables highly accurate and sensitive non-destructive analysis and differentiation of natural melanin and has been the most powerful method to investigate melanin.
What is the functionality of melanin in skin?
The existence of melanin in skin is strongly correlated with the prevention of free radicals generated by UV radiation. Especially in the skin melanin (mainly eumelanin) ensures the only natural UV protection by eliminating the generated free radicals.
The aim of this study was to investigate the suitability of melanin as protector/radical scavenger against free radicals in skin.

Materials and Methods

Skin testing

Skin probes

For assessing the protection a of different melanin concentrations against the generation of free radicals we used human skin biopsies of different skin types (skin type II – V; **Table 1**) supplied after cosmetic surgery. Skin strips (1x1 cm) were placed in Petri dishes (epidermal side up, in immediate contact with air) on filter paper soaked in PBS buffer containing a nitroxyl probe as the free radical trap. A punch biopsy (Ø 4 mm) was extracted immediately before UV radiation.

Test probes

UV filter formulations were applied on the horny layer (2 mg/cm²). Skin samples were kept in the dark, and UV filter formulations were allowed to treat the skin for 20 minutes. After treatment time a punch biopsy (Ø 4 mm) was extracted and exposed to UV radiation.

The used UV filters are listed in **Table 2.**

UV irradiation

The UV irradiation of skin was performed with a solar simulator SOL 2 (Hönle AG). The irradiances as integrated value over the spectral ranges were E (UVB=280-320) = 2.4 mW/cm² and E (UVA = 320-400nm) = 28.9 mW/cm². A variation of the irradiance was realized by using different transmission filters (LOT Oriel, Germany) from PF2 to PF50. Therewith the irradiance could change from 31.3 mW/cm² to 0.63 mW/cm². To test the effect of different UV doses the irradiation time was varied.

RSF method for detection of free radicals in skin

Radical trapping experiments have the potential to allow identification of the generated free radical species and were employed successfully in the detection of oxygen and carbon centered free radicals and singlet oxygen generated in skin exposed to UV radiation (8, 9, 10). We have used nitroxyl probes as traps for a quantification of free radicals. The nitroxyl probe is a suitable probe to monitor the biological redox reaction, particularly when a nitroxyl probe is localized at an area of interest. Nitroxyl probes are susceptible to oxygen concentration, reactive oxygen species (ROS), and biological redox systems, and are widely used as probes for ESR measurements. The reaction of nitroxyl probes with free radicals results in the loss of their ESR signal, indicating the possibility of using nitroxyl probes as assay for ROS detection. With the application of nitroxyl probes we measured the influence of UV radiation (280-400 nm) on the formation of free radicals in the skin. While the UVB (280-320 nm) radiation penetrates only the upper layer of the epidermis, the UVA radiation penetrates also the deep layers of the dermis. The common nitroxyl probe which was tested for their application to detect free radicals was purchased from Sigma (München, Germany). Other reagents were of the highest grade of purity commercially available.

Sunscreens are the first defense line which should prevent the generation of primary free radicals ($^{\bullet}OH$, $O2^{-\bullet}$) caused by UVA and UVB which indirectly damage the skin. The number of generated free radicals is represented by the Radical Skin/Sun Protection Factor RSF:

$$RSF = \frac{N(\text{free radicals})\text{unprotected}}{N(\text{free radicals})\text{protected}}$$

The RSF is a factor characterizing the protection of a sunscreen against the generation of free radicals and presents the ratio between the number N of generated free radicals in the unprotected and protected skin assuming the same applied UV dose (constant irradiance, variable irradiation time) for both (11). It is also a measure for the increase of the time staying in the sun by using UV filter protection assuming the generation of the same amount N of free radicals like for the unprotected skin.

Filter	Absorption wavelength (nm)	Special Extinction E 1%/1cm	Solubility
Isoamyl p-Methoxycinnamate	260–340	> 980 (307 nm)	lipophil
Butyl Methoxydibenzoylmethane	315-390	> 1100 (355 nm)	lipophil
Tinosorb M (Bisoctrizole)	290-390	> 600 (357 nm)	microparticel, Ø 200 nm
Tinosorb S (Bemotrizinol)	290-390	> 819 (340 nm)	lipophil

Table 2 UV filter used for the different sunscreen formulations

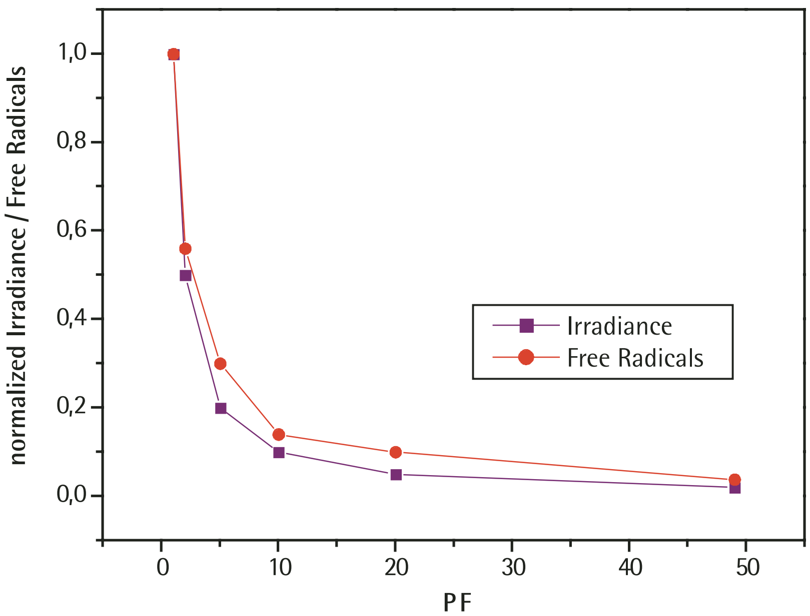

Fig. 3 Irradiance and generated free radicals in skin as a function of used transmission / density filters. Both curves are normalized to their initial value. Differences in the irradiance were realized by attenuating the exposure of the UV lamp with density filters of different transmission (PF1-100%, PF2-50%, PF10-10%, PF20-5%, PF50-2%).

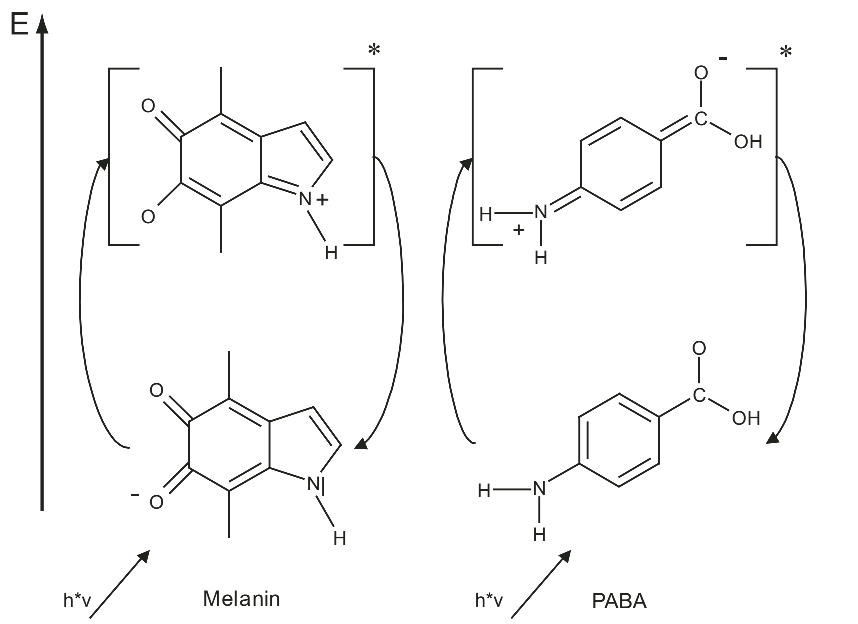

Fig. 4 Absorption of UV radiation(irradiance E) brings molecules in an excited state. Release of energy as heat and light radiation brings molecules back to the ground state and can excite again Melanin of skin or the substance PABA (first UV-filter for sunscreens).

Instrumentation for skin measurements
The measurements on skin biopsies were performed with a commercial high sensitive X-band bench top Electron Spin Resonance Spectrometer MiniScope MS200. Skin biopsies were supported in a special tissue cell. Both equipments were supplied from Magnettech GmbH Berlin, Germany.

Results

RSF determination of the effect of UV irradiation on the skin
The generation of free radicals in skin is directly correlated with the applied UV dose. **Fig. 3** shows the correlation between the variation of the irradiance and the generation of free radicals in skin. The variation of the irradiance was realized by using different transmission filters from PF 2 to PF 50. The same function of UV attenuation should be performed by the sunscreens containing UV absorbing filters.
An intrinsic defense line of the skin itself against UV generated free radicals is realized by melanin. This base protection is mainly influenced by the content of melanin in the skin classified by the skin type **(Table 1)**. Therefore the chemical structure of melanin was replicated for the first commercial UV filter PABA. The mode of action for both melanin and PABA is represented in **Fig. 4**.
Sunscreens are the first external defense line which now prevent the generation of primary free radicals ($^\bullet OH$, $O_2^{-\bullet}$) in the skin. Some modern UV filters (concentration of 1% in the same cosmetic formulation) listed in **Table 2** were compared in their radical protection with the melanin content of different skin types (skin type II – V). This comparison was performed with the measurement of the RSF of each UV filter and skin type.
The measured and calculated RSF values are listed in **Table 3** and represented in **Fig. 5**. All measured RSF values were normalized to the RSF of unprotected skin of type II. From **Table 3** it is seen that the RSF of the unprotected skins of different types is mainly lower than the RSF of the filter-protected skins. The following results are clearly seen:

- The protection against UV generated free radicals increases with higher melanin content (skin type).
- The RSF of the different skin types correlates with the corresponding exposure durations of **Table 1**.
- Only UVA and UVAB filters (concentration ≥ 1%) can clearly exceed this protection.

Conclusion

The role of melanin as protector against free radicals in skin could be impressively demonstrated with ESR spectroscopy and the applied method for the determination of the RSF.
Measurements on skin were performed with ESR spectroscopy. The amount of free radicals induced by UV radiation in skin was quantified by a new RSF (radical sun protection) Factor.
Different skin types characterized by different melanin content show different RSF values. The higher the melanin content the higher is the RSF of the corresponding skin type. The free radical protection of different UV filters (normalized to a 1 % filter concentration) were compared with the radical protection of different skin types. The results show clearly that UVA filters are more effective in reducing the free radicals than melanin. But the radical protection of skin types ≥ IV is comparable or somewhat better than that of UVB filters. Using the RSF method it is possible for the first time to compare the free radical protection of the different skin types with the different UVA and UVB filters.
ESR spectroscopy enables the direct quantification of melanin radicals and allows to analyze the functional properties of melanin as a free radical trapping agent. While the UV filters represent a first defense line, aimed to reduce the amount of UV radiation that reaches the skin structure, antioxidants constitute a second defense line by reducing the amount of free radicals generated inside the skin. The efficacy of UV filters depends mainly on their absorption spectrum. UVA filters are more efficient than UVB filters to avoid the free radical production in skin. The cosmetic formulation of the skin care product should allow the UV filters to adhere on the skin surface for a long period and the UV filters should be sufficiently photostable.

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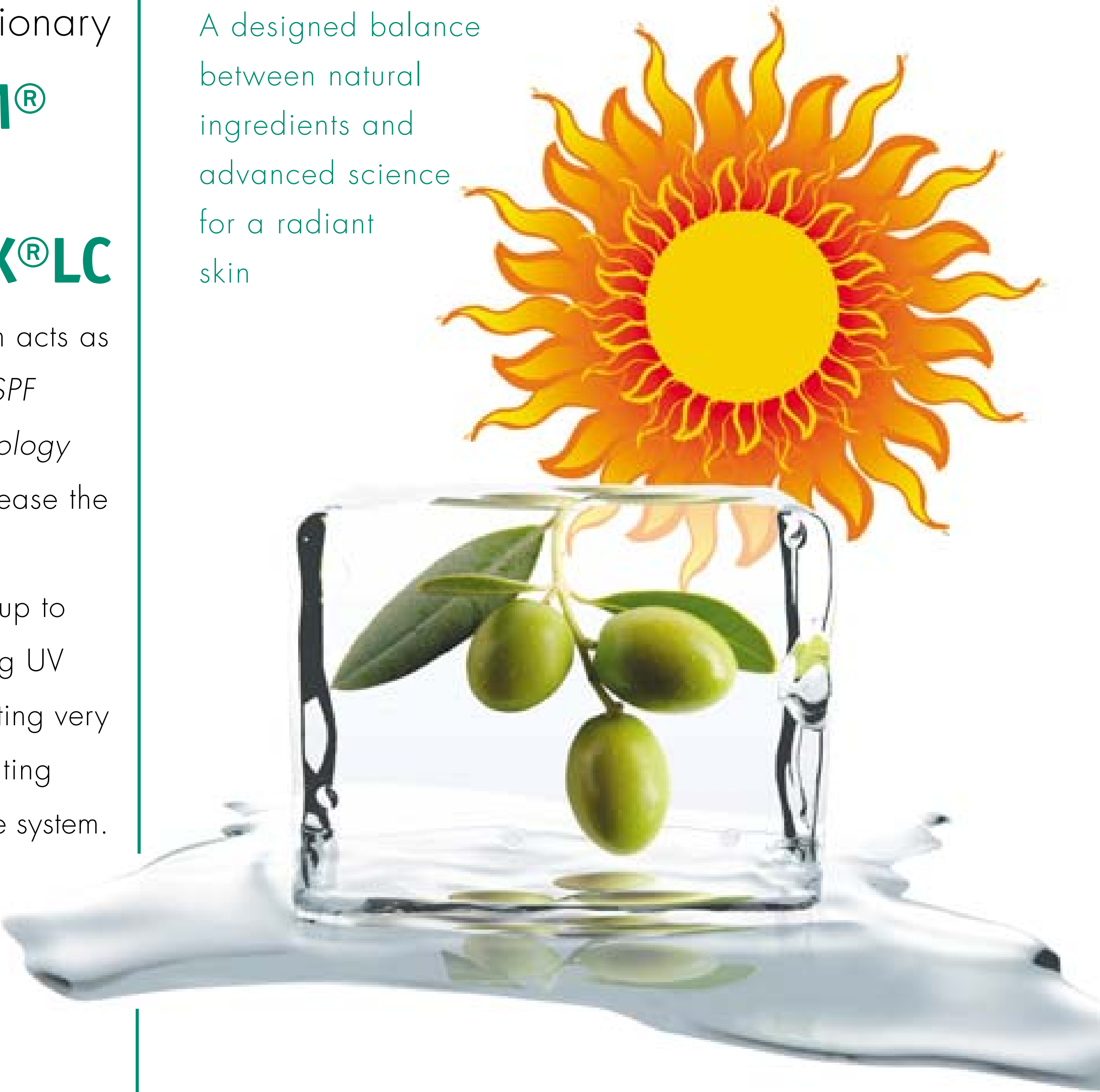

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The efficacy of antioxidants depends on several parameters. The antioxidant should be an efficient radical scavenger. Furthermore it has to be able to penetrate into the skin and to interact with the melanin. An adequate carrier system is of primary importance for this purpose.
Summarizing it is seen that ESR measurements of UV irradiated skin have shown:

- Melanin in skin works as a potent radical scavenger correlated with its role as protector against UV induced free radicals.
- Melanin in higher concentrations (skin type V) does not work as an absolute radical protection.
- An additional radical protection can be achieved by applying UVA filters.

Skin type and protected by	RSF
Skin type II and no Product	1.0
Skin type II and base with 1.0 % Butyl Methoxydibenzoylmethane (UVA – Filter)	9.2
Skin type II and base with 1.0 % Tinosorb S (UVA/B – Filter 1)	7.9
Skin type II and base with 1.0 % Isoamyl p- Methoxycinnamate (UVB – Filter)	1.6
Skin type II and base with 1.0 % Tinosorb M (UVA/B – Filter 2)	3.4
Skin type III no product	1.4
Skin type IV no product	1.8
Skin type V no product	2.5

Table 3 Radical protection (RSF) of different skin types and applied UV – filter formulations

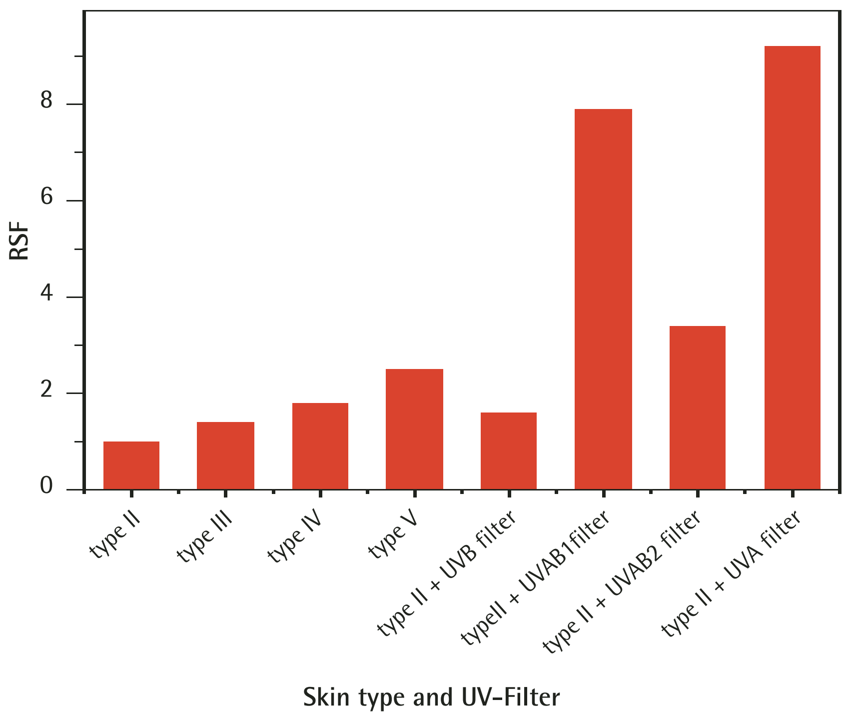

Fig. 5 RSF of different skin types protected against UV – generated free radicals by intrinsic melanin and/ or different UV – filter formulations (see **Table 3**).

References

(1) *D. Darr, I. Fridovich.* Invest Dermatol. 102 (1994):671-5. Review.

(2) *H.N. Ananthaswamy, W.E. Pierceall.* Photochem. Photobiol. 52 (1990) 1119-36. Review.

(3) *R. Ogura, M. Sugiyama, J. Nishi, N. Haramaki.* J Invest Dermatol. 97 (1991) 1044-7.

(4) *A.C. Santos Nogueira, I. Joekes.* J Photochem Photobiol B. 74 (2004) 109-17.

(5) *A. Roddick-Lanzilotta, R. Kelly.* Cosmet Sci. 55 (2004) Suppl:S113-21.

(6) *Z.D. Draelos.* Dermatol Clin. 2006 24 (2006) 81-4.

(7) *A. Valavanidis, M. Rallis, G. Papaioannou, K. Xenos, A. Katsarou.* Int. J. Cosm. Sci. 17 (1995) 157-163

(8) *Th. Herrling, L. Zastrow, J. Fuchs, N. Groth,* Skin Pharmacol. Appl. Skin Physiol. 15 (2002) 381.

(9) *Th. Herrling, J. Fuchs, J. Rehberg, N.Groth,* Free Radic. Biol. Med. 35 (2003)59.

(10) *Th. Herrling, K. Jung, J. Fuchs,* Spectrochim. Acta A: Mol. Biol. Spectrosc. 63 (2006) 840-845.

(11) *Th. Herrling, K. Jung, E. Chatelain, M. Langenauer,* SOFW-Journal 132 (2006) 24.

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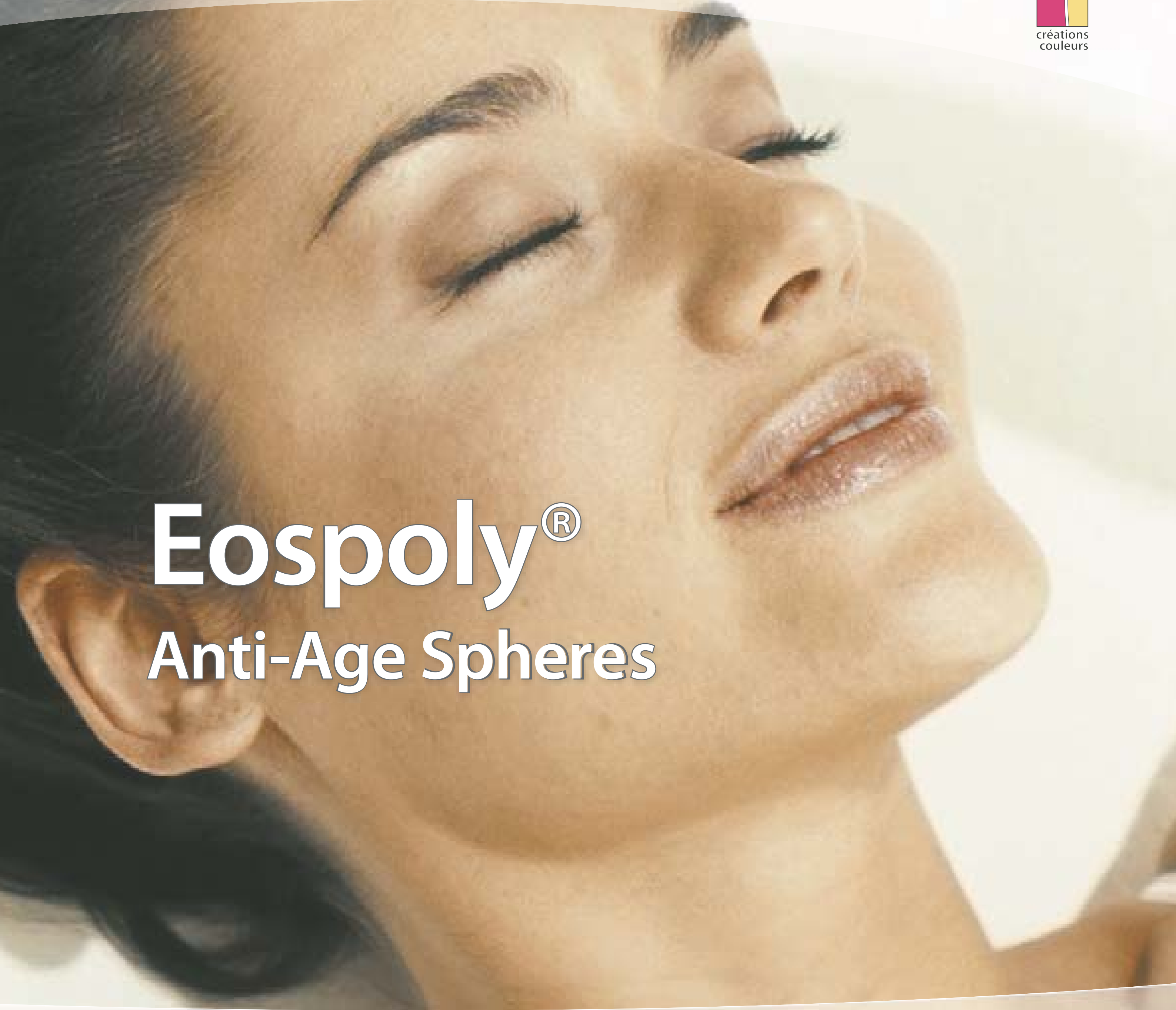

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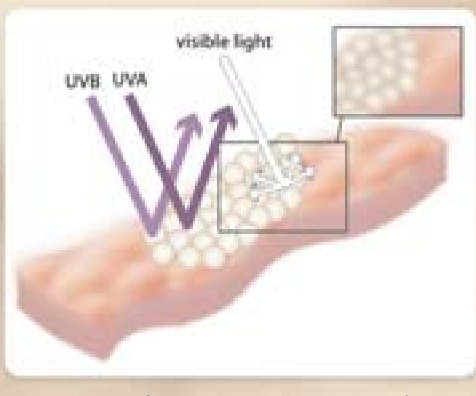

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