Research Article
A standardized method based on pigmented epidermal models evaluates sensitivity against UV-irradiation

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Abstract
To protect the human skin from extensive solar radiation, melanocytes produce melanin and disperse it via melanosomes to keratinocytes in the basal and suprabasal layers of the human epidermis. Moreover, melanocytes are associated with pathological skin conditions such as vitiligo and psoriasis. Thus, an in vitro skin model that comprises a defined cutaneous pigmentation system is highly relevant in cosmetic, pharmaceutical and medical research. Here, we describe how the epidermal-melanin-unit can be established in vitro. Therefore, primary human melanocytes are implemented in an open source reconstructed epidermis. Following 14 days at the air liquid interface, a differentiated epidermis was formed and melanocytes were located in the basal layer. The functionality of the epidermal-melanin-unit could be shown by the transfer of melanin to the surrounding keratinocytes, and a significantly increased melanin content of models stimulated with either UV-irradiation or the melanin precursor dihydroxyphenylalanine. Additionally, an UV50 assay was developed to test the protective effect of melanin. In analogy to the IC50 value in risk assessment, the UV50 value facilitates a quantitative investigation of harmful effects of natural UV-radiation to the skin in vitro. Employing this test, we could demonstrate that the melanin content correlates with the resilience against simulated sunlight, which comprises 2.5 % UVB and 97.5 % UVA. Besides demonstrating the protective effect of melanin in vitro, the assay was used to determine the protective effect of a consumer product in a highly standardized setup.

Keywords: dermatology, reconstructed human epidermis, skin pigmentation, efficacy testing

1 Introduction

Besides guarding the inner organs from mechanic, chemical or thermal injury the skin is the first line of protection against extensive solar radiation (Alonso and Fuchs, 2003; Brenner and Hearing, 2008). The sun emits light of various wavelengths, whereby the non-visible ultraviolet (UV) part has the greatest potential to elicit tissue damage (Ullrich, 2002). While the UVB-light can solely penetrate into the upper layers of the epidermis, it is capable of directly inducing mutations. In contrast, UVA-light reaches into deeper skin layers, where it generates free radicals such as reactive oxygen species. Although the formation of reactive oxygen species is an ubiquitous process, extensive UV-radiation can overwhelm the antioxidant defense system and lead to oxidative stress in the skin (Rtitie and Fisher, 2002). Thereby, reversible and irreversible subcellular damages to nucleic acids, proteins, free amino acids and proteins of the connective tissue are caused (Kadekaro et al., 2003). In the mildest form, these damages entail sunburn features in the skin. However, when UV-radiation doses accumulate, the susceptibility of different forms of skin cancer is increased (Ullrich, 2002).

To prevent extensive UV-radiation-induced damage, the skin has developed a specialized system that protects cells in the epidermis. During embryogenesis melanoblasts migrate from the neural crest to the skin, differentiate to melanocytes and form dendritic extensions to the surrounding keratinocytes (Mayer, 1973; Wang et al., 2016). This anatomical connection was defined by Fitzpatrick and Breathnach as the “epidermal melanin unit”. It has been estimated that one melanocyte is in contact to 36 keratinocytes of the basal and suprabasal layer (Fitzpatrick and Breathnach, 1963; Nordlund, 2007). In melanocytes, the pigment melanin is produced and dispersed to the keratinocytes via melanosomes. Upon reaching the keratinocytes, melanin granules accumulate above the cell nuclei to protect the deoxyribonucleic acid (DNA) from UV-
radiation-induced damages such as pyrimidine dimer formation. Thereby, the “epidermal melanin unit” is also responsible for the color of the skin, which is called constitutive pigmentation in its basal state, and after responding to environmental stimuli such as sunlight facultative pigmentation. In addition to the direct safeguard capacity by adsorption and scattering UV-radiation, melanin protects the skin by photo-oxidizing and scavenging free radicals (Brenner and Hearing, 2008).

**In vitro**, the epidermal melanin unit could be established in tissue-engineered, reconstructed human epidermis. In addition to human keratinocytes, these models also contain melanocytes (Liu et al., 2007). Such so-called pigmented epidermal models resemble the anatomical structure of human epidermis including the interaction between human keratinocytes and melanocytes that ultimately result in macroscopically visible tanning (Bessou et al., 1995). Hence, pigmented reconstructed human epidermis may be employed as an alternative to animal testing in cosmetic, pharmaceutical and medical research, e. g. for sun protection, self-tanning agents or skin whitener. However, no study has shown a direct quantitative correlation between melanin content and the capacity to withstand natural UV-radiation *in vitro*, yet.

The study presented here was initiated to assess the influence of the melanin content in pigmented reconstructed human epidermis on the capacity of the tissue to resist UV-irradiation. Therefore, a pigmented skin model based on the open source reconstructed human epidermis (OS-REp) (Groeber et al., 2016b; Mewes et al., 2016) was generated. Following, the effect of UV-irradiation, different cell donors and the melanin precursor dihydroxyphenylalanine (DOPA) were correlated to melanin synthesis. Moreover, a test procedure that quantifies the resilience of a tissue against solar radiation was derived. Finally, the protective effect of a commercially available sun protection agent was demonstrated with the novel assay. To reflect a realistic UV-irradiation, a mixture of UVA- and UVB-radiation was used according to the global solar reference spectrum.

2 Material and methods

**Cell isolation**

Human epidermal keratinocytes were isolated from foreskin biopsies of 2 to 5 year-old donors with approval of the local ethics committee (approval number IGBZSF-2012-078) after confirmed consent of their guardians. The cell isolation procedure was based on a previously published protocol (Groeber et al., 2016a). Briefly, biopsies were washed, minced and digested with dispase (Life Technologies, Darmstadt, Germany) to dissociate the epidermis from the dermis. Thereafter, the epidermis was trypsinized (Life Technologies) to generate single-cell suspensions. Keratinocytes were cultured in EpiLife® medium supplemented with 0.2 % v/v bovine pituitary extract, 1 μg/ml recombinant human insulin-like growth factor-1, 0.18 μg/ml hydrocortisone, 5 μg/ml bovine transferrin and 0.2 ng/ml human epidermal growth factor (all from Life Technologies). Additionally, human melanocytes were obtained from the epidermis of adult skin biopsies of 19 to 61 year-old-donors and were isolated from the epidermal part by the same procedure as described for the keratinocytes. The skin phenotype of all donors was between 2 and 4. In contrast to the isolation of keratinocytes, cells were seeded and cultured in Melanocyte Growth Medium ready (PromoCell, Heidelberg, Germany).

**Generation of epidermal models**

Skin models were generated on a polycarbonate membrane of respective cell culture inserts (diameter 0.47 cm², pore size: 0.4 μm; Nunc™, Waltham, USA). To generate melanocyte-free epidermal models, keratinocytes were seeded at a cell density of 5 x 10⁶ cells/cm² in 200 μL EpiLife® medium supplemented with 0.2 % v/v bovine pituitary extract, 1 μg/ml recombinant human insulin-like growth factor-1, 0.18 μg/ml hydrocortisone, 5 μg/ml bovine transferrin, 0.2 ng/ml human epidermal growth factor (all from Life Technologies) and 1.5 mM CaCl₂ (Sigma-Aldrich, Steinheim, Germany). To ensure sufficient nutrient supply, inserts were cultured in 2 ml medium in 6-well-plates. Medium was changed after 24 hours to EpiLife® air-liquid-interface medium that additionally contains 73 μg/ml L-ascorbic acid 2-phosphate (Sigma-Aldrich). During culture, medium was replaced by fresh air-liquid-interface medium three times a week and models were cultured at 37 °C and 5 % CO₂ in a humidified incubator. For the generation of pigmented skin models, melanocytes were mingled with keratinocytes at a ratio of 1:6 applying the same culture conditions as described for the melanocyte-free tissues.

**Histological analysis**

Samples were fixed in Rotis®Histofix (Roth, Karlsruhe, Germany) for 1 hour, washed in tap water for 2 hours and embedded in paraffin. Subsequently, histological cross-sections of 3 μm were obtained. Prior to the staining, slides were deparaffinized and rehydrated. For the identification of melanin, a Fontana-Masson stain was conducted, whereby melanin is visualised by staining with silver nitrate. Counterstaining was done with nuclear fast red aluminium sulphate solution. Immunolabelling for Melan-A was performed to identify melanocytes while melanosomes were immunostained by the HMB45 antibody. Samples were exposed to citrate buffer to allow demasking of epitopes and were permeabilised with 1 % Triton X-100 (Sigma-Aldrich) in Dulbecco’s phosphate buffered saline (DPBS) (Sigma-Aldrich) for 10 minutes. Slides were then incubated with Melan-A or HMB45 antibodies (both obtained from Dako, Hamburg, Germany) at a dilution of 1:30 at 4 °C overnight, washed and subsequently stained with appropriate secondary antibodies for further 30 minutes. Subsequently, slides were washed again with DPBS and cell nuclei counterstained with 4’,6-diamidino-2-phenylindole (DAPI) at a dilution of 1:1000 (Serva Electrophoresis, Heidelberg, Germany).

**Melanin quantification**

Skin models were digested with dispase (Life Technologies) to dissociate skin equivalent from the membrane. Epidermal layers were removed from the cell culture insert and transferred to a 1.5-ml centrifuge cap. After incubation with 0.65 ml Solvable™ (Perkin Elmer, Rodgau, Germany) for 30 minutes at 60 °C, melanin was quantified by measuring the absorbance at 405 nm using a spectrophotometer (Infinite 200M; Tecan, Maennerdorf, Switzerland). A serial dilution of synthetic melanin at a range of 0 to 50 μg/ml allowed the quantification of the melanin concentrations in the skin samples.
UV-irradiation

UV-irradiation of skin models was performed with a BIO-SUN system (Vilber Lourmat, Eberhardzell, Germany). The BIO-SUN irradiation system has integrated UV sensors and a microprocessor. Therefore, the emission of the UV light is continuously monitored and the irradiation stops automatically when the dose matches the desired set point. After the transfer of skin models to a 6-well plate without medium, samples were irradiated with UVA or UVB at different doses. Each model was irradiated three times with 24 hours in between. Following post-incubation of 48 hours after last irradiation, the melanin content was quantified. For post-incubation, tissue models were transferred to air-liquid-interface medium.

UV50 assay

In order to obtain information on the sensitivity towards solar radiation, a UV50 assay was established. The tissues were irradiated with increasing doses of simulated sunlight (0-18 J/cm²). To mimic a realistic spectrum, the used light comprised of 97.07% UVA and 2.93% of UVB. Subsequently, tissue viability was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay 3 days after the irradiation, respectively. UV50 was calculated as the respective dose, where tissue viability had decreased to 50% of the initial viability. Therefore, the percental viability (linear y axis) was plotted versus the UV dose. UV50 values were determined by a sigmoid dose-response curve showing the characteristic dose-dependent decrease of the viability. By interpolation, the dose at which the percental viability has dropped to 50% was considered the UV50 value.

DOPA treatment

25 µl DOPA was applied topically at various concentrations (0, 0.3, 1 and 3 mg/ml in DPBS) starting at day 10 of the air-liquid-interface phase for three times every 24 hours. After post-incubation for another 48 hours, the melanin content was quantified.

Characterization of sun protection agent

To assess the effect of commercially available sun lotions, skin models were treated topically with 25 µl of the respective product (Sun Dance Ultra-sensitive SPF 30, m-drogerie markt GmbH & Co. KG; Karlsruhe, Germany). Therefore, sun lotion was distributed evenly over the surface using sterile cotton swabs. Following a 30 minute incubation phase at room temperature, tissues were used for a UV50 assay as described above. Models without treatment served as control. Moreover, models that were not irradiated and models that were challenged with doses of artificial sun light of 10, 20 and 30 J/cm² were subjected to histological analysis.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) with Fisher LSD test using the appropriate control in each experiment as a reference. Values of p ≤ 0.05 were considered significant.

Fig. 1: Histological analysis of pigmented reconstructed human epidermis

(A) Fontana-Masson staining of non-pigmented (OS-REp (-) Mel) and pigmented epidermal models (OS-REp (+) Mel) and human skin (hSkin). The pigmented and non-pigmented epidermal models both showed histological features resembling human skin. Melanocytes were located in the stratum basale and melanin granules above the cell nuclei were visible in the stratum spinosum and stratum granulosum (marked with a white circle). (B) Immunofluorescence staining for Melan-A and HMB-45 of OS-REp (+) Mel and human skin (hSkin). DAPI was used for counterstaining of cell nuclei. Dendritic cellular protrusions into suprabasal epidermal layers are highlighted with an arrow. (C) Melanin content of pigmented and non-pigmented epidermal models. To determine the melanin content a spectrometric melanin quantification was performed for pigmented OS-Rep generated from cells derived from five distinct donors. Data is shown as dot plot, in which each data point represents the melanin content of one donor. Scale bar: 50 µm.
3 Results

3.1 Pigmented reconstructed human epidermis mimics the epidermal melanin unit of human skin

Following culture at the air-liquid-interface for 14 days, the OS-REP models were highly resembling the histological architecture of human epidermis (Figure 1). The models showed a physiological differentiation pattern with a prismatic basal layer, two to three layers of stratum spinosum with flattened keratinocytes and two to three layers of stratum granulosum with keratin granules. Moreover, differentiation was reflected by a thick corneous layer composed of cell-nuclei-free corneocytes. When melanocytes were seeded together with keratinocytes, Fontana-Masson staining revealed the localization of the former in the stratum basale. This finding could also be confirmed by immunofluorescence staining with HMB-45 and Melan-A antibodies (Figure 1A+B). Additionally, Fontana-Masson staining and immunolabeling allowed visualization of dendritic melanocyte protrusions into suprabasal epidermal layers. Melanin granules were also visible above the cell nuclei in cells of the stratum spinosum and stratum granulosum that did not have direct contact with melanocytes.

Spectrometric melanin quantification revealed an increased and donor-dependent melanin content in pigmented epidermal models in comparison to melanocyte-free models (Figure 1C). The degree of pigmentation varied between 5.6 and 10.8 µg/cm², when using melanocyte populations from different donors.

3.2 Melanin content in pigmented epidermal models increases upon UV-irradiation or exposure to the melanin precursor DOPA

To test if the pigmented reconstructed human epidermis responds to increasing doses of UV-irradiation, models were irradiated with 2 or 5 J/cm² UVA and 20 or 40 J/cm² UVB (Figure 2A). Both UVA-irradiation doses of 2 and 5 J/cm² and the higher UVB dose of 40 J/cm² resulted in a statistically significant increase of the melanin content of 69% and 39% compared to the non-irradiated controls. Moreover, exposure to DOPA dose-dependently led to an increased melanin content of the epidermal models as well (Figure 2B).

![Fig. 2: Influence of UV-radiation and DOPA application on the pigmentation of epidermal models](image)

**Fig. 2: Influence of UV-radiation and DOPA application on the pigmentation of epidermal models**

(A) Determination of melanin content after UV-radiation. Pigmented models were irradiated with 2 or 5 J/cm² UVA and 20 or 40 J/cm² UVB. (B) Melanin content after topical treatment of OS-REP (+) Mel with 0, 0.3, 1 and 3 mg/ml DOPA in DPBS. (C) Viability after irradiation with increasing doses of simulated sunlight comprised of 97.07% UVA and 2.93% of UVB. Viability after irradiation with increasing doses of simulated sunlight (0-18 J/cm²) was assessed by MTT assay. Models without melanocytes (OS-REP (-) Mel), with melanocytes (OS-REP (+) Mel (-) DOPA) and after the stimulation with 3 mg/ml DOPA (OS-REP (+) Mel (+) DOPA) were tested. UV₀₅ value was determined by a sigmoid dose-response curve. (D) The correlation between the pigmentation level and the UV₀₅ value was determined. The Pearson correlation coefficient was computed and showed a strong correlation (r = 0.96). Data is shown as dot plots with mean value in (A) and (B). In (C), plots represent mean value ± standard deviation. Statistically relevant differences are indicated by stars (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, n = 3 independent experiments).
3.3 Melanin content correlates to UV-radiation and protective capacity

The primary purpose of melanin is to protect skin from extensive sunlight. To investigate the sensitivity of reconstructed human epidermis against different doses of sunlight exposure, a novel test procedure to assess responses to solar irradiation was developed (Figure 2C). To ensure realistic test conditions, we used a defined mixture of UVA (97.07%) and UVB (2.93%) light according to the global sunlight reference spectrum. The irradiation dose that led to a reduction of the viability of the models to 50% of the initial viability (UV50) proved to be an appropriate parameter for this purpose. Employing this new test method, we could show a positive dose-response relationship between the melanin content of the epidermal models and the UV50 value. Melanocyte-free models revealed a UV50 value of 6.6 J/cm², and thus a higher sensitivity against UV-radiation compared to pigmented epidermal models. Here, the presence of melanocytes, and thus melanin in the pigmented models increased the UV50 to 11.1 J/cm². Viability could furthermore be improved by exposure to DOPA, which raised the UV50 to 16.1 J/cm², respectively. The Pearson correlation coefficient (r = 0.96) revealed a strong positive correlation between both parameters (Figure 2D).

3.4 The UV50 assay quantifies the protective effect of a sun protection consumer product

Epidermal models were challenged with increasing doses of artificial sun light both with and without prior topical application of a commercially available sun lotion. In the histological analysis, we could see the occurrence of strong UV-induced hydropic degeneration at a dose of 10 J/cm², when no sun protection factor was applied (Figure 3A). These features were more pronounced at 20 J/cm² but seemed to be diminished at a high dose of 30 J/cm². At this dose, cell nuclei appeared to be fragmented. In contrast, if the sun lotion was applied before the experiments, no effects could be observed at 10 J/cm². Additionally, a dose of 20 J/cm² only led to histological changes within the stratum corneum, whereas a high dose of 30 J/cm² resulted in visible changes in the viable cell layers (Figure 3A). These histological findings correlated to the measured viabilities of the tissue models (Figure 3B). Without the sun protection factor, viability was strongly decreased for low doses of artificial sun light and led to an UV50 value of 6.7 J/cm². With the sun lotion, tissues remain viable upon the treatment for higher doses and the UV50 value increased to 21.6 J/cm².

**Fig. 3: Influence of sun protection factor (SPF) and UV-irradiation**

(A) Histological analysis of reconstructed human epidermis after irradiation with different doses of simulated sunlight (0, 10, 20 and 30 J/cm²) with or without the treatment of SPF. UV-induced damage (*1: UV-induced hydropic degeneration; *2: Fragmented nuclei) of the viable cell layers was visible without SPF already at a dose of 10 J/cm² of simulated sunlight, whereas with SPF, a damage was first visible with a dose of 30 J/cm². (B) Sensitivity of epidermal models against irradiation with simulated sunlight with or without SPF. Mean value and standard deviation of tissue viability (n = 3) after irradiation with increasing doses of simulated sun light (0-30 J/cm²) without SPF (w/o SPF), and with SPF (w SPF). To quantify sensitivity, UV50 values were determined by a sigmoid dose-response curve. Models without SPF showed a UV50 value of 6.7 J/cm², whereas with SPF this value was increased to 21.6 J/cm². Scale bar: 50 µm.

4 Discussion

Due to the ability to quench the energy from photons or the scavenging of reactive oxygen species, the cutaneous pigmentation system has remarkable capacities to protect the human skin from solar radiation. In addition, melanocytes are involved in different skin diseases such as vitiligo or as indicated in recent research also in psoriasis (Arakawa et al., 2015; Wagner et al., 2015). Skin pigmentation is moreover of high social importance, since dependent on the respective cultural background, the pigmentation is thought to be either increased or decreased (Leary and Jones, 1993). Thus, there is an increasing interest to study these processes in a standardized model that mimics the in vivo situation as close as possible. To generate such a model, we seeded primary human melanocytes together with primary human keratinocytes in a ratio of 1:6 on a transwell-system. Following the culture at the air-liquid interface, the keratinocytes formed a well-differentiated epidermis. In this environment, melanocytes remained in the basal layer of the developed epidermis and established dendritic protrusions reaching to keratinocytes both in basal and suprabasal layers. Comparable to the in vivo situation and other in vitro models, these melanosomes are arranged as a protective cap over cell nuclei to prevent damage to genetic information (Kaidbey et al., 1979; Liu et al., 2007). Since melanin is detectable within the cytoplasm of keratinocytes, a physiological transfer of melanosomes to keratinocytes, and thus a functional epidermal-melanin unit in the model can be assumed.
The pigmentation system in the epidermal models showed a considerable dynamic and responded with increased melanin production upon UVA and UVB irradiation or upon exposure to DOPA. Both reactions have been well-studied in vitro and could be demonstrated in in vitro models, where comparable effects can be observed (Wolber et al., 2008; Slominski et al., 1988; Duval et al., 2001; Yoon et al., 2003; Bessou et al., 1995). In contrast to previously published models, our model was developed following an open source policy that was previously employed to generate a non-pigmented epidermal model for risk assessment (Groeber et al., 2016b; Mewes et al., 2016). Comparable to the open source concept in the information technology field, all procedures to generate the model are freely published and thus allow a broad scientific community to contribute to the further refinement of the model (Bagozzi and Dholakia, 2006; Hertel et al., 2003; Lakhani and von Hippel, 2003).

In addition, we developed a test to quantify the resilience towards solar radiation to test if the melanin produced in the skin models is also able to protect the skin in vitro. Therefore, we irradiated tissues with increasing doses of artificial sunlight and derived a robust value, the UVs value, i.e. the value that resulted in a decrease of tissue viability to 50% that corresponds to the well-known IC50 value in risk assessment. To consider a realistic UV light exposure, a defined mixture of UVA and UVB according to the global reference spectrum of sunlight was used. Without melanocytes, the UVs value is only 6.6 J/cm² but was increased depending on the respective melanin concentration up to 1.7-fold. The strong correlation between phototype and protection from UV-irradiation has already been demonstrated in an in vivo study (Maresca et al., 2006). Hence, our study confirms that the in vitro-produced melanin has comparable protective effects as in vivo, and thus strengthens the hypothesis that a functional epidermal melanin unit is formed in the tissue-engineered epidermal models. These findings are in accordance to previous studies that showed a connection between the phototype and the production of antioxidant enzymes of keratinocytes after UVB (Bessou et al., 1995). However, from a clinical point of view, UV-radiation also triggers skin inflammation caused by radiation-induced damage to epidermal cells. The release of mediators leads to dilation of the vessels and subsequently to the typical five signs of inflamed skin: redness, swelling, heat, burning pain as well as disturbed functions (Soter, 1990). Although the UVs value simulates damage to the epidermal cells, subsequent erythema or inflammatory reactions cannot be imitated. For more precise assessment of the sun protection classes in vitro, further parameters might be considered and the model developed further in order to mimic an inflammatory reaction.

The pigmented epidermal models could be employed to investigate effects of cosmetic and pharmaceutical substances on skin pigmentation, e.g. of self-tanning agents or skin whitener. Moreover, the UVs test procedure might also be applicable to determine the sun protection factor of new cosmetic products. To circumvent critical damage to the skin, the time of direct sun exposure should be limited to a safe dose. The effect of high doses of sun light was clearly visible in our model when it was challenged with doses of 30 J/cm². Here, clear signs of UV-induced damages in the viable cell layers could be observed for a dose higher than 10 J/cm². Interestingly, the appearance of hydropic degeneration in the keratinocytes was diminished at a dose of 30 J/cm² but still fragmented nuclei could be observed. This results might indicate that doses of 10 to 20 J/cm² still allow for some cellular reactions, whereas higher doses result in immediate cell death. Using the UVs value, we could confirm the protective effect of a commercial available sun lotion. With the sun protective agent, the UVs increased 3-fold from 6.7 J/cm² to 21.6 J/cm². Interestingly this increase is less than the stated protective effect of a 30-fold prolonged time of save sun exposure. So far, the light protection factor is determined according to a standardized in vitro test method of the European cosmetics association Cosmetics Europe where test persons are exposed to UV-radiation after a standardized application of preparations (COLIPA, 2006). However, this safe time, strongly depends on multiple extrinsic and intrinsic factors. As the interplay of the intrinsic and extrinsic factors is complex, an individual safe sun exposure time cannot easily be assessed. Hence, the method presented here may help to quantify the effects of UV-doses on different skin phototypes in a highly standardized experimental setup and without the potential endangerment of test persons through the exposure to UV-radiation. In addition to the SPF analyzed in this study, UVA-PF is an important factor to quantify the protective effect of consumer products. UBV penetrates less deeply into the skin and is for the most part absorbed in the epidermis and superficial dermis. Here, it mainly interacts with keratin, melanin and connective tissue fibers. In contrast, UVA can indirectly damage DNA by inducing free oxygen radicals. It penetrates deeper into the skin and exerts its effect especially in the stratum basale of the epidermis. In further studies, the developed model will be used to determine the UVA-PF and assess damages by either UVA or UVB, which is feasible using the described experimental setup.

References


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**Conflict of interest**

The authors declare no conflict of interest.

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