

Protective and Regenerative Efficacy of a Plant Oil-Based Day and Night Cream: Investigated by a Novel Approach to Reveal the Impact of Blue Light Irradiation on Epidermal Barrier Integrity and Lipid Matrix

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Abstract

In recent years, the harmful effects of blue light (400 - 500 nm) as a component of visible light (400 - 700 nm) have increasingly gained attention of science, industry, and consumers. To date, only a few *in vivo* test methods for measuring the effects of blue light on the skin have been described. A direct measurement method that can detect the immediate effects of blue light on the epidermal permeability barrier (EPB) is still lacking. In this study, we present a new methodological approach that can be used to investigate both the protective and regenerative effects of cosmetic products on the EPB after blue light irradiation. In a study with 14 female volunteers, it was investigated whether the regular application of an O/W emulsion (day cream) can strengthen and protect the epidermal barrier against damaging blue light radiation of 60 J/cm² (protective study design) and also whether a disruption of the epidermal barrier caused by blue light radiation is restored faster and better by the regular application of another O/W emulsion (night cream) than in product-untreated skin (regenerative study design). The two O/W emulsions are different in plant oil, active ingredient composition and texture. The seven-day treatment with the day cream initially led to a significant increase in the normalized lipid lamellae length in the intercellular space, whereas the irradiation with blue light after 24 hours led to a significant decrease in the lipid lamellae length in the untreated test area, but not in the area pre-

viously treated with the product. Regarding the regenerative study design, a two-day treatment with the night cream was able to restore a blue-light-induced decrease in lipid lamellae length in the intercellular space. In summary, with the study designs presented here, the protective and regenerative effect of two cosmetic products could be demonstrated for the first time on the integrity of the EPB after blue light irradiation and the data showed that the Lipbarvis® method is suitable for investigating the damaging effects of blue light on the EPB *in vivo*.

Keywords

Skin Care, Blue Light Irradiation, Skin Barrier, Intercellular Lipid Lamellae, Electron Microscopy

1. Introduction

The skin is the first and most important protective layer of our body against harmful environmental influences [1]. The epidermal permeability barrier (EPB) in the stratum corneum is of central importance. The first protective layer of the skin, composed of highly organized lipids (cholesterol, ceramides, and free fatty acids) and proteins, forms an efficient permeability and moisture barrier: it protects the skin from the penetration of harmful pollutants and pathogenic microorganisms (outside-inside barrier) and simultaneously prevents the loss of water and electrolytes (inside-outside barrier) [2] [3].

Outdoors, the skin is exposed to a variety of physical, chemical, and biological noxae from environmental pollution and solar radiation [4]. In addition to the known harmful effects of ultraviolet radiation (UV) from UVB (290 - 320 nm) and UVA (320 - 400 nm), blue light (400 - 500 nm) as part of visible light (400 - 700 nm) has increasingly become the focus of discussion about negative influences in recent years. Several studies have now shown that blue light—like UV light—can cause premature skin aging (photoaging), hyperpigmentation and cancer [5] [6]. On the other hand, blue light has been used successfully for many years in the treatment of various skin diseases, such as psoriasis, atopic dermatitis, and acne vulgaris [7]. Depending on the dose, intensity, and duration, blue light can have beneficial or harmful effects on the skin similar to sunlight [8] [9]

Four reaction pathways of blue light in the skin are described in the literature. 1) In contrast to UV light, blue light penetrates much deeper into the skin. Twenty percent of blue light reaches the hypodermis [10], while UVB light is absorbed in the epidermis and only 10% of UVA light penetrates the hypodermis [11]. In the skin, the irradiated light is absorbed by various photo acceptors such as flavin and porphyrin compounds and converted into an excited state. These receptors excited by blue light can trigger a wide variety of biochemical processes [12]. The formation of reactive oxygen species that activate matrix-

degrading enzymes and thus initiate a cascade of biochemical reactions has been described many times. DNA damage, inhibited cell growth and proliferation, the generation of inflammation, increased lipid peroxidation as well as collagen and elastin degradation can result from increased ROS levels in the skin [7] [13].

2) In addition to ROS, blue light can also induce an increased formation of nitric oxide (NO) [12] [14]. In the skin, NO is an important signaling molecule that can control numerous differentiation steps of keratinocytes and anti-inflammatory processes. On the other hand, excessive NO levels caused by blue light lead to oxidative stress and thus to DNA damage [15].

3) Another reaction pathway of blue light has been described for the mitochondria. Dungal and colleagues [16] were able to prove that blue light could have a regulating function on the cytochrome C oxidase of the mitochondria. 4) The fourth mechanism of action of blue light was described by Regazzetti and colleagues [17]. They were able to show that blue light interacts with opsin-3 and thus regulates melanogenesis in melanocytes.

A direct link between blue light and a disruption of the epidermal barrier could be demonstrated, especially for ROS and NO. Nakashima *et al.* [18] were able to demonstrate increased ROS formation after blue light treatment in a similar way to UVA irradiation. An increased ROS content can lead to increased lipid oxidation in the skin and thus to a disturbed EPB [9] [10]. For UV light, Ditgen and co-workers [19] found a significant deterioration in EPB and a decrease in ceramide EOS and free fatty acids after UV light irradiation. Lohan and co-authors [20] were able to show that the ROS generated by blue light changes the composition of the lipids of the EPB and their composition.

Dang and co-workers [21] compared wild-type mice with KO mice whose nitric oxide synthase was inducible. They found that the repair of the EPB after damage was significantly faster in the wild-type mice than in the KO mice. The addition of NO donors led to improved EPB recovery kinetics and increased mRNA synthesis of proteins involved in EPB terminal protein and lipid differentiation. They concluded that NO is involved in the regulation of EPB homeostasis in mice. Gallala and colleagues found a similar result in 2004 [22]. They were able to show that NO regulates the mRNA synthesis of genes involved in ceramide metabolism. Denda and Fuziwara [23] were also able to show for mouse skin that the repair of a previously impaired EPB was delayed after blue light irradiation.

Based on these findings, a sensible approach seems to be the further development of sun protection and skin care products that can neutralize harmful blue light radiation and/or protect and repair the EPB structure and function [12]. To date, there are only a few *in vivo* studies and study designs that have investigated the efficacy of cosmetic products against the harmful effects of blue light [24]. In addition, this relatively new field of research and the few existing *in vivo* studies are characterized by a variety of wavelengths and irradiation protocols, which make it difficult and limit the comparability of the studies and their results and a ref-

erence to natural exposure to sunlight [5] [24].

We are not aware of any study that has investigated the effect of a cosmetic product on the integrity of the EPB after blue light treatment using a direct measurement method. To fill this gap, we developed a new methodological approach to investigate the effect of blue light on the integrity of the EPB. We investigated the protective and the regenerative efficacy of two vegetable oil-based skin care products (day cream, night cream) on the epidermal skin barrier after blue light irradiation.

2. Methods and Materials

2.1. Subjects, Test Products, Test Sites and Study Schedule

The study presented here was conducted as a randomized, intra-individual single study in accordance with local laws and regulations and with the standards of Good Clinical Practice of the European Community (EC-GCP, Commission Directive 2005/28/EC; exception: no ethics vote). As the products tested in this study were developed for female consumers, only women were included in the study. Subjects were selected in accordance with the recommendations of the Declaration of Helsinki and GCP Guidelines. The study included skin-healthy women aged 18 to 60 with phototype I - IV who were non-smokers. Women with known allergies, chronic diseases, women on long-term medication, severely overweight, pregnant or breastfeeding women, women with cancer in the last 10 years before the start of the study, cardiovascular disease 6 months before the study or other diseases 7 days before the study and sauna or swimming pool visits 24 hours before the start of the study were excluded. A total of 15 female subjects with a mean of 38.7 years (± 14.2 years) participated in the study, with the youngest subject being 19 years old and the oldest 57 years old. 14 test subjects completed the study. The study took place in Hamburg from 1 to 10 March 2021 (SGS INSTITUT FRESENIUS GmbH, Hamburg, Germany).

The new study approach presented here was conducted with two plant oil-based formulations. One was a day cream and the other a night cream. Their ingredients are listed in **Table 1** and **Table 2**.

Table 1. Composition (INCI) and specification of the test formulation (day cream).

Day Cream	Aqua (Water), Glycerin, Cetyl Alcohol, Butyrospermum Parkii (Shea) Butter, Simmondsia Chinensis (Jojoba) Seed Oil, Tocopheryl Acetate, C10-18 Triglycerides, Dextrin, Isopropyl Myristate, Olus (Vegetable) Oil, Olea Europaea (Olive) Fruit Oil, Calendula Officinalis Flower Extract, Panthenol, Citrus Aurantium Dulcis (Orange) Peel Oil Expressed, Linalool, Limonene, Alpha-Isomethyl Ionone, Coumarin, Citronellol, p-Anisic Acid, Parfum (Fragrance), Cetyl Phosphate, Arginine, Caprylyl Glycol, Stearic Acid, Palmitic Acid, Xanthan Gum, Carrageenan, Glucose, Gellan Gum, Sorbitol, Glycine Soja (Soybean) Oil, Citric Acid, Tocopherol
	Type of emulsion: O/W
	pH value: 5.0 ± 0.1 (adjusted by arginine)

Table 2. Composition (INCI) and specification of the test formulation (night cream).

Night Cream	Aqua (Water), Glycerin, Butyrospermum Parkii (Shea) Butter, Olea Europaea (Olive) Fruit Oil, Cetyl Alcohol, Tocopheryl Acetate, C10-18 Triglycerides, Dextrin, Olus (Vegetable) Oil, Simmondsia Chinensis (Jojoba) Seed Oil, Calendula Officinalis Flower Extract, Panthenol, Allantoin, Citrus Aurantium Dulcis (Orange) Peel Oil Expressed, Linalool, Limonene, Alpha-Isomethyl Ionone, Coumarin, Citronellol, p-Anisic Acid, Parfum (Fragrance), Cetyl Phosphate, Rhus Verniciflua Peel Wax, Arginine, Stearic Acid, Caprylyl Glycol, Palmitic Acid, Xanthan Gum, Carrageenan, Glucose, Sorbitol, Glycine Soja (Soybean) Oil, Citric Acid, Tocopherol
	Type of emulsion: O/W
	pH value: 5.0 ± 0.1 (adjusted by arginine)

The following study design as shown in **Table 3** was used to determine whether the day cream provides protective benefits for the epidermal skin barrier against the blue light: after the test subjects were included in the study, the right volar forearm was divided into two test areas. The untreated control and the product-treated test sites were randomized between the upper and lower forearm. A Lipbarvis[®] sample was taken from one of the two test areas as the baseline for the study (day 1, baseline). Previous studies have shown that Lipbarvis[®] values do not differ or differ only very slightly between the test fields on the forearm (unpublished data), the baseline Lipbarvis[®] sample was only taken from one of the two test fields. One test area was then treated twice daily with the day cream for 7 days. On day 8, further Lipbarvis[®] samples were taken from the treated and untreated test areas (day 8). Both test areas were then irradiated with blue light for 15 minutes and after additional 24 hours, Lipbarvis[®] samples were again taken from both test areas (day 9).

Table 3. Schedule of study procedure “protective”.

	Day Cream (study design “protective”)		
	t0 (day 1)	t1 (day 8)	t2 (day 9)
		7 days after product use ¹	24 h after blue light irradiation
Preparation			
Informed consent	X		
In-/exclusion criteria	X		
Parameter			
SC lipid matrix analysis by Lipbarvis [®] (nICCL) on both test sites	X	X	X
Blue light irradiation on both test sites		X ^{*2}	

Note: ¹: The first application of the day cream was performed after visual evaluation at the study site on one test site according to the randomisation scheme with the other one left product-untreated under supervision of a technician and after Lipbarvis[®] samples were taken. ²: Immediately after Lipbarvis[®]-sampling; SC = Stratum Corneum; nICCL = normalised intercellular lipid lamellae.

Table 4. Schedule of study procedure “regenerative”.

	Night Cream (study design “regenerative”)		
	t0 (day 1)	t1 (day 2) 24 h after blue light irradiation	t2 (day 4) 2 days after prod- uct use ^{*1}
Preparation			
Informed consent	X		
In-/exclusion criteria	X		
Parameter			
SC lipid matrix analysis by Lipbarvis [®] (nICCL) on both test sites	X	X	X
Blue light irradiation on both test sites	X^{*2}		

Note: ^{*1}: The first application of the night cream was performed after visual evaluation at the study site on one test site according to the randomisation scheme with the other one left product-untreated under supervision of a technician and after Lipbarvis[®]-samples were taken. ^{*2}: Immediately after Lipbarvis[®]-sampling; SC = Stratum Corneum; nICCL = normalized intercellular lipid lamellae.

The investigation of the epidermal skin barrier regarding the regenerative properties of the night cream was conducted on the other forearm (randomized) as shown in **Table 4**: after inclusion of the test subjects in the study and Lipbarvis[®] sampling (baseline, day 1), both test areas were irradiated with blue light for 15 minutes. After one day, Lipbarvis[®] samples were taken on both test sites again (day 2). Subsequently, one test field was treated twice daily for 2 days (4 product applications in total), and the other test field remained untreated. On day 4, Lipbarvis[®] samples were again taken from both test fields.

2.2. Blue Light Exposure

The blue light exposure to the skin was carried out with a light-emitting diode device (BLE 450-MSD, developed by MSD) with a small irradiation spectrum between 420 - 470 nm and the maximum intensity at 450 nm. The primary light intensity of this light-emitting diode device is 105 lm. The volar forearm of the subjects was blue light irradiated with a distance of 5 cm between the skin and the light source to avoid thermal influences of the blue light source. The skin surface was irradiated with the 450 nm light for 15 minutes over an area of 7 cm². The total amount of light was measured using a UV Meter and the FS VIS D2 E110 sensor (Dr. Höhnle AG, Gräfeling). This resulted in a total amount of 60 J/cm² after the 15 minutes.

2.3. Lipbarvis[®] Sampling and Transmission Electron Microscopy

Lipbarvis[®] is a patented analysis method (Microscopy Services Dähnhardt GmbH,

Flintbek, Germany) for the quantitative description of the epidermal skin barrier by determining the normalized length of the lipid lamellae in the intercellular space (nICLL) of the stratum corneum. For this purpose, the uppermost layers of the stratum corneum are removed non-invasively using a special adhesive-carrier method. This method has been described several times in the literature and has already been used in many studies [25].

For sampling, a drop of Lipbarvis[®] glue was applied to a carrier and cured on the skin surface for 3 minutes. The first layers of the SC could then be removed from the skin surface using tweezers. The samples were then fixed and embedded, and the organization of the intercellular lipid lamellae (ICLL) was imaged using transmission electron microscopy (TEM CM 10, FEI, Eindhoven, Netherlands). The lipid lamellae in the intercellular space, as a marker for the integrity of the epidermal skin barrier were determined and calculated as normalized ICLL (nICLL). The values and data were used for statistical analysis.

Lipbarvis[®] samples were taken from all 14 subjects who completed the study to assess the protective and regenerative properties of the day cream and night cream. For the day cream, samples were taken at baseline, on day 8, and day 9. For the night cream, samples were taken at baseline, on day 2, and on day 4.

2.4. Statistical Analysis

The analysis of the study objectives was performed by SGS INSTITUT FRESENIUS GmbH using the computer software Microsoft EXCEL (Office 365) and SPSS Statistics 27 (SPSS Inc. an IBM Company, Chicago, IL). Microsoft EXCEL was used for the calculation of the means and the standard deviations. SPSS Statistics 27 was used for analyzing the distribution of the data (Shapiro-Wilk test) and for analyzing the significance of differences between the test regimens or points in time (ANOVA, analysis of variance, paired t-test). The hypothesis of a normal distribution was accepted when there was a p-value of >0.05 . Differences between the treatment situations and the points in time were considered statistically significant when there was a p-value < 0.05 . The original data was used for the analyses.

3. Results

3.1. Protective Study Design: TEM Investigation and Morphometric Analysis

The aim of this study was to investigate the effects of the day cream investigated here on the epidermal skin barrier after the skin had been exposed to blue light irradiation. The Lipbarvis[®] method was chosen to analyze the lipid lamellae in the intercellular space of the epidermal skin barrier. The protective study design was chosen in such a way that the day cream to be analyzed was applied by the test subjects to one test area for 7 days, while another test area was not treated. Both the treated and the untreated test area were irradiated with blue light. Lipbarvis[®] samples were taken and analyzed before application of the day cream (t_0 , base-

line), after 7 days of twice-daily treatment or non-treatment (t1), and 24 hours after blue light irradiation of the two test fields (t2). Treatment with the day cream resulted in a significant increase in normalized lipid lamellae length in the intercellular space from 204 to 230 ($p < 0.001$), while the untreated control field showed a slight, non-significant decrease in lipid lamellae from 204 to 188 after 7 days (Figure 1 and Figure 2). 24 hours after the blue light irradiation (t2), the normalized lipid lamellae length of the untreated area decreased significantly from 188 to a value of 129 ($p = 0.009$). The test area previously treated with the day cream for 7 days showed a slight, non-significant decrease in the normalized lipid lamellae length in the intercellular space from 230 to a value of 209 after blue light treatment.

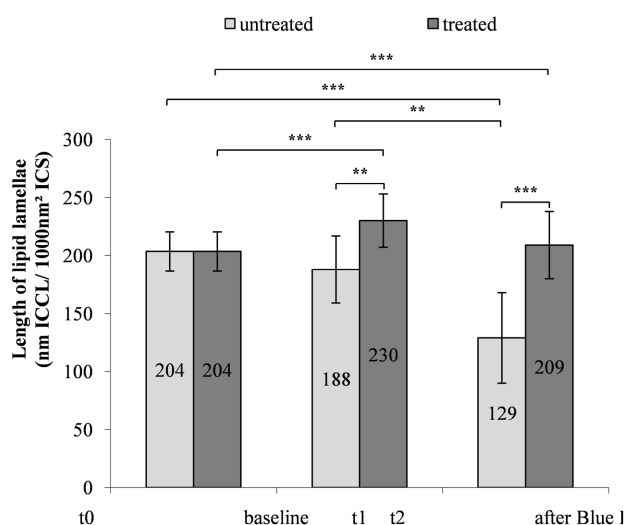


Figure 1. Normalized intercellular lipid lamellae (nICLL) before (t0, day 1) and after 7 days of product treatment with day cream (t1, day 8) and subsequent blue light exposure (protective study design). 24 hours after blue light irradiation, the length of the lipid lamellae in the intercellular space was quantified again (t2, day 9). Statistical analysis was performed by ANOVA (analysis of variance, paired t-test). ** $p < 0.01$ very significant, *** $p < 0.001$ highly significant.

3.2. Regenerative Study Design: TEM Investigation and Morphometric Analysis

For the regenerative study design, Lipbarvis[®] samples were taken at the beginning of the study (baseline), 24 hours after blue light irradiation (t1) and two days after one test field was treated with the night cream while the other test field remained untreated (t2). Before blue light irradiation, the normalized lipid lamellae length in the intercellular space showed a value of 214 (Figure 3 and Figure 4). 24 hours after blue light irradiation, the normalized length of the lipid lamellae in the intercellular space decreased significantly in both test areas, the-to-be-product-untreated test area (130, $p < 0.001$) and the to-be-treated test area (122, $p < 0.001$). The twice-daily treatment with the night cream for two days led to a significant increase in the normalized lipid lamellae length in the intercellular space to a value of 210 ($p < 0.001$) in the treated test area. The length of the lipid lamellae in the intercellular space showed virtuously no changes in the un-

treated test field.

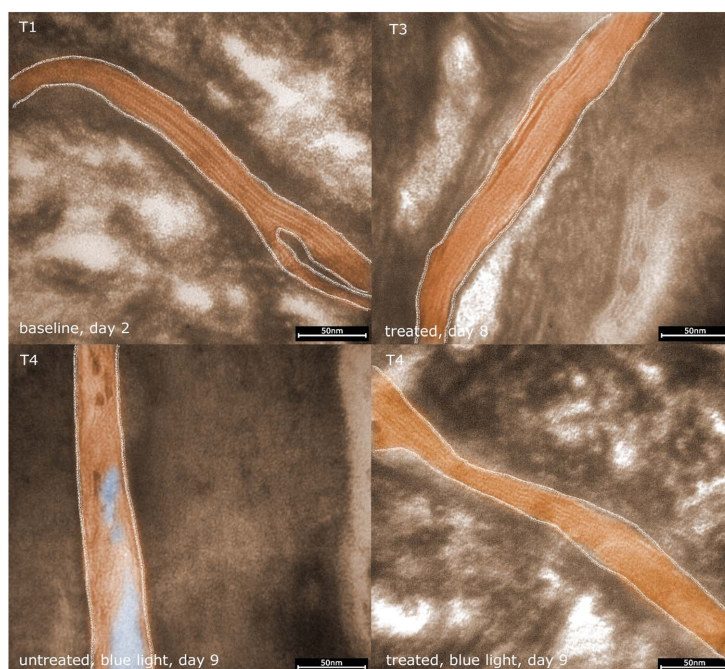


Figure 2. TEM images of the intercellular lipid lamellae in the intercellular space of the stratum corneum before (t0, day 1) and after seven days of skin care with the day cream (t1, day 8). The treated and untreated test field was then irradiated with blue light and after a further 24 hours, the lipid lamellae in the intercellular space of the previously treated test field and the untreated test field were determined (t2, day 9). The lipid lamellae in the intercellular space are colored orange, while areas with little to no lipid lamellae are colored blue. The corneocytes adjacent to the intercellular space are stained dark brown.

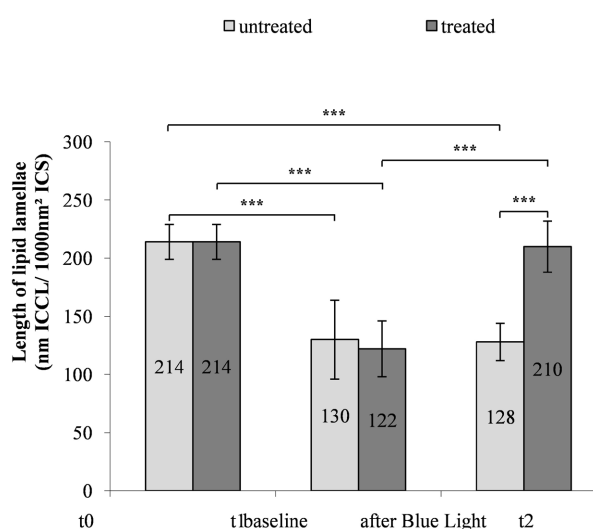


Figure 3. Normalized intercellular lipid lamellae (nICLL) before (t0, day 1) and 24 hours after blue light exposure (t1, day 2) followed by two days of twice-daily product treatment with night cream or no treatment on the control test site (regenerative study design) (t2, day 4). Statistical analysis was performed by ANOVA (analysis of variance, paired t-test). *** $p < 0.001$ highly significant.

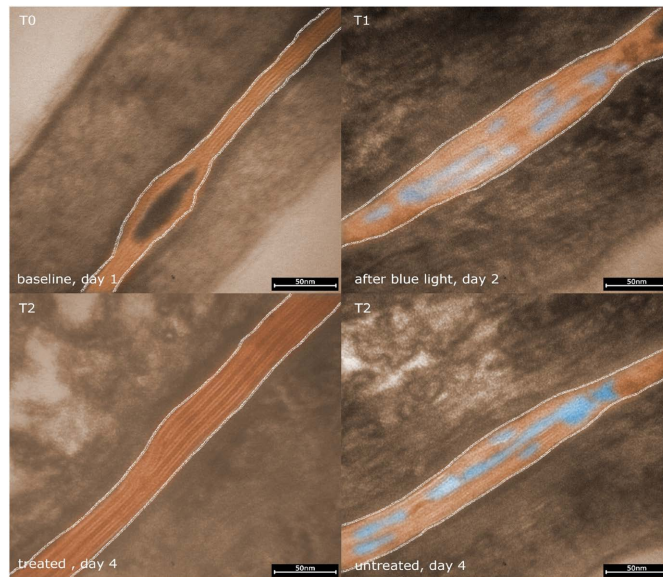


Figure 4. TEM images of the intercellular lipid lamellae in the intercellular space of the stratum corneum before (t0, day 1) and after blue light exposure (t1, day 2) followed by two product-untreated days as well as two days of twice-daily product treatment with night cream (t2, day 4) (regenerative study design). The lipid lamellae in the intercellular space are colored orange, while areas with little to no lipid lamellae are colored blue. The corneocytes adjacent to the intercellular space are stained dark brown.

4. Discussion

In recent years, the negative effects of blue light as a component of the visible light of solar radiation on the skin have been increasingly discussed [4] [25]. The changes to the skin induced by blue light, such as premature skin aging, are becoming more concerning to consumers and the cosmetics industry. The number of studies documenting the harmful effects of blue light on the skin and describing protective ingredients is increasing [24]. However, these studies can only be compared to a very limited extent, as the available study data were generated using various protocols and different irradiation intensities [26]. Following Campiche *et al.* [5], we treated the skin with a radiation intensity of 60 J/cm² in the study presented here. In Central Europe, this dose corresponds to the blue light intensity that can be measured on a clear summer day at midday after one hour of sun exposure [5].

We are not aware of any studies that can show the protective or regenerative effect of a cosmetic product on the integrity of the EPB after blue light treatment using a direct measurement method. Against this background, in the present study, we investigated whether and what damage blue light causes to the epidermal barrier and whether the regular application of a vegetable oil-based O/W emulsion (day cream) can protectively strengthen the epidermal barrier against the effects of visible light. In the second part of the study, it was investigated whether a disruption of the epidermal barrier caused by blue light is restored faster and better by the regular application of a plant oil-based O/W emulsion (night cream) compared to untreated skin.

We used the Lipbarvis[®] method to analyze the integrity of the EPB. This method determines the normalized length of the lipid lamellae in the intercellular space in the middle stratum corneum (SC) [27]. A healthy EPB shows values of 195 nICLL and higher, dry skin values of 100 nICLL and lower while atopic and very dry skin shows values of 50 nICLL [28]. In contrast to biophysical measurement methods such as transepidermal water loss (TEWL) measurements, the Lipbarvis[®] method is more sensitive and can indicate even minor changes in EPB [19] [27] [29].

In a preliminary study, we were able to show that TEWL and skin moisture measurements are not suitable parameters to prove the influence of blue light on the EPB under the study conditions chosen here (data unpublished). The results of our study have shown that the blue light dose used here has a measurable effect on the EPB 24 hours after irradiation of the skin surface. In both study designs, we demonstrated a significant decrease in the normalized length of the lipid lamellae in the intercellular space from 188 to a mean value of 129 (protective study design) and from 214 to 130 (regenerative study design) for the untreated skin, thus demonstrating blue light-induced damage to the EPB for the first time using a direct measurement method. The integrity of the EPB after irradiation with blue light corresponds to slightly dry skin [27].

The damage to the EPB caused by the blue light dose used here is accompanied by an impairment of homeostasis. This result could be due to an increased formation of nitric oxide from the photolytic release of NO from nitrosated proteins [8] [15]. A direct correlation between blue light irradiation, a higher level of NO, and a disruption of the EPB has been described several times in the literature and could explain the damage to the EPB in the data presented here [7] [15]. The effect of NO on the epidermal skin barrier is highly concentration-dependent. Gallala *et al.* [22] and Dang *et al.* [21] were able to show that the gene expression of proteins involved in ceramide metabolism and the terminal differentiation of the EPB, and the lipid metabolism is increased at low NO concentrations, while it is inhibited at higher NO concentration in the cell.

In addition to NO, the damage to the EPB shown could also be due to the increased formation of ROS. Similar to NO, an increased concentration of ROS leads to damage to lipids, proteins, and DNA [10] [12]. This can result in damage to the EPB and other cellular dysfunctions, which can lead to wrinkles, age spots and loss of skin elasticity [10] [11]. Nakashima and colleagues [18] found increased ROS formation in human keratinocytes and mouse skin after blue light irradiation in a similar way to UVA light but with lower efficiency. Tsuchida and Sakiyama [25] confirmed the results of Nakashima, but they were also able to show that the lipid oxidation induced by blue light irradiation differs from the UVA-induced reaction.

The skin naturally possesses various protective systems against oxidative stress. In addition to enzymes such as catalase, glutathione peroxidase or superoxide dismutase, the skin's protective system includes substances that cannot be syn-

thesized by the human organism. These include tocopherol (vitamin E), vitamin C, carotenoids, flavonoids, and phytoestrogens [30] [31]. Carotenoids, in particular, have a central role in neutralizing free radicals in the skin [32] after blue light irradiation at a dose comparable to that in the study presented here [33]. The study data presented here were able to show the EPB-protecting properties of a daycare product against blue light. The 7-day care treatment prevented a significant decrease in lipid lamellae in the intercellular space after blue light treatment and thus showed a protective effect of the product. It can be assumed that the tocopherol contained in the formulation, the various plant oils and in particular panthenol as primary active ingredients accompanied with the product given pH of 5.0 supported the skin's natural protective systems against oxidative stress after blue light irradiation [34]. Our study data also showed that the blue light-induced damage to the EPB could be quickly repaired by applying the night cream. Skin homeostasis was restored after only 2 days of treatment with the night cream, while the untreated skin continued to show EPB damage. We conclude that the lipophilic components of the night cream penetrated the intercellular space of the stratum corneum and created an environment favorable to the regeneration of the EPB.

5. Conclusion

In summary, the data from the study demonstrate that the Lipbarvis[®] method is suitable for investigating the damaging effects of blue light on the EPB *in vivo*. One limitation of the study presented here is that no ethics vote was obtained. Nevertheless, with the study design presented here, the protective and regenerative effect of two cosmetic products could be demonstrated for the first time on the integrity of the EPB after blue light treatment using a direct measurement method. In light of this, topical cosmetic formulations can play a crucial role in mitigating the negative effects of blue light irradiation on epidermal EPB function and structure, thereby preserving skin health. In the ongoing discourse regarding the expansion and customization of sunscreens and skin care products, the methodological approach introduced here may represent an innovative means to extend and substantiate cosmetic product claims.

Authors' Contributions

Conceptualization: D.D. (Dorothee Dähnhardt), S.D.-P., I.H., I.S., P.S. and D.S.; methodology: D.D. (Dorothee Dähnhardt), S.D.-P., D.S. and D.D. (Dana Ditgen); investigation: D.D. (Dorothee Dähnhardt), S.D.-P., D.S. and D.D. (Dana Ditgen); resources: S.D.-P., D.S. and P.S.; writing—original draft preparation: D.D. (Dorothee Dähnhardt), S.D.-P., I.S. and I.H.; writing—review and editing: D.S., D.D. (Dana Ditgen), P.S., I.S., D.D. (Dorothee Dähnhardt) and S.D.-P.; supervision: P.S.; project administration: I.H., I.S., D.S. and D.D. (Dorothee Dähnhardt). All authors have read and agreed to the published version of the manuscript.

Informed Content Statement

Informed consent was obtained from all subjects.

Statement of Ethics

The present human intervention study was conducted in accordance with the Revised Declaration of Helsinki, and in line with the European Community Good Clinical Practice (EC-GCP) standards.

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Conflicts of Interest

D.D. (Dorothee Dähnhardt) and S.D.-P. are employees of the Microscopy Services Dähnhardt GmbH in Flintbek, Germany. The authors I.S., I.H. and P.S. are employees of Kneipp GmbH in Würzburg, Germany. D.S. and D.D. (Dana Ditzgen) are employees of SGS INSTITUT FRESENIUS GmbH in Hamburg, Germany. The authors have no conflicts of interest to declare.

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